

Sensitivity of seedling growth to phosphorus supply in six tree species of the Australian Great Western Woodlands

Article

Accepted Version

Williams, A., George, S., Birt, H., Daws, M. and Tibbett, M.
ORCID: <https://orcid.org/0000-0003-0143-2190> (2019)
Sensitivity of seedling growth to phosphorus supply in six tree species of the Australian Great Western Woodlands. *Australian Journal of Botany*, 67 (5). pp. 390-396. ISSN 0067-1924 doi: <https://doi.org/10.1071/BT18247> Available at <https://centaur.reading.ac.uk/85122/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.1071/BT18247>

Publisher: CSIRO

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online



Sensitivity to phosphorus supply of six tree seedlings of the Australian Great Western Woodlands

Journal:	<i>Australian Journal of Botany</i>
Manuscript ID	BT18247.R1
Manuscript Type:	Research paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Williams, Andrea; Jim's Seeds, Weeds & Trees George, Suman ; University of Western Australia Faculty of Science Daws, Matt; University of reading , School of Agriculture, Policy and Development Birt, Henry; University of Queensland School of Agriculture and Food Sciences Tibbett, Mark; University of Reading,
Keyword:	Phosphorus, Eucalypts, Acacia spp., Seedling development, Growth

SCHOLARONE™
Manuscripts

Phosphorus is routinely applied to soils in tree-based restoration schemes, despite the balance between beneficial and deleterious effects being poorly understood. For six woody species from the Great Western Woodlands of Western Australia, growth increased in response to low concentrations of applied phosphorus. At higher phosphorus concentration, growth either plateaued or declined. Applied phosphorus may have different and unpredictable effects on native species suggesting caution when applying high rates of phosphorus in a field-setting.

For Review Only

1

2 **Sensitivity of seedling growth to phosphorus supply in six tree species of the Australian**3 **Great Western Woodlands**

4

5

6 Andrea Williams^a, Suman George^b, Henry W. G. Birt^{c,d}, Matthew I. Daws^d, Mark Tibbett^{d,e*}

7

8 ^aJim's Seeds, Weeds & Trees, Boulder, Western Australia9 ^bSchool of Agriculture and Environment, University of Western Australia, Crawley, WA,

10 Australia

11 ^cSchool of Agriculture and Food Sciences, The University of Queensland, Qld, Australia.12 ^dCentre for Agri-Environmental Research and Soil Research Centre, School of Agricultural

13 Policy and Development, University of Reading, Berkshire RG6 6AR, UK

14 ^eSchool of Biological Sciences, University of Western Australia, Crawley, WA, Australia

15

16 *Author for correspondence

17

18 Email: m.tibbett@reading.ac.uk

1 **Abstract**

2 Many Australian native plants from regions with ancient, highly weathered soils have
3 specialised adaptations for acquiring phosphorus (P) and can exhibit negative effects of
4 excess P supply on growth and survival. Despite this, fertiliser (including P) is routinely
5 applied in post-mining and other restoration schemes. In this study we investigated the effect
6 of a range of applied P on the growth and tissue P concentrations for six woody species from
7 the Great Western Woodlands (GWW) of Western Australia, a region that it not only
8 biodiverse, but that has experienced significant levels of mining related activities. Our data
9 from a pot-based experiment show that all six species exhibited greater growth with increased
10 P application up to 15 mg kg sand⁻¹. However, at P concentrations in excess of 15 mg kg⁻¹,
11 dry mass accumulation did not increase further for three of the species tested. For the other
12 three species, dry mass accumulation declined as the P concentration increased above 15 mg
13 kg⁻¹. For all of the study species, root and shoot P concentrations increased as the
14 concentration of applied P increased. The internal shoot P concentration, at which dry matter
15 accumulation either plateaued or started to decline, was in the range 1.95 to 3.2 mg P g⁻¹ DM.
16 This was approximately 2 to 4 times the concentration found in natural vegetation. These data
17 suggest that in a restoration context, there is a potential risk that, excess P application may
18 decrease plant growth rates for some species. Consequently, the addition of fertiliser to
19 restored sites may have unpredictable impacts on the plant community by directly reducing
20 the growth of some species while increasing the growth of others. We suggest that careful
21 consideration should be given to designing appropriate fertiliser regimes for land restoration
22 schemes in ancient P deplete landscapes to avoid the risk that fertiliser addition has the
23 unwanted outcome of decreasing growth and survival of the target native species whilst
24 increasing the abundance of unwanted weeds or aggressive pioneer species.

25 **Key words:** fertiliser, P accumulation, P-toxicity, P-use efficiency, rehabilitation

1 **Introduction**

2 The Great Western Woodlands (GWW) of Western Australia is the largest intact temperate
3 woodland left on earth (Morton 2014). The region hosts a plethora of biodiversity including
4 3,300 flowering plants, approximately 30 % of Australia's *Eucalyptus* species and 138 reptile
5 species (Booth 2013). Whilst being a biodiversity hotspot, the GWW also contains rich
6 mineral resources and has been subjected to mining: the GWW have significant geographical
7 overlap with the Western Australian goldfields (Watson *et al.* 2015). As a result, there is a
8 need for a greater understanding of the growth, physiology and nutrition of native tree species
9 for use in post-mining restoration.

10 The addition of fertiliser to increase plant growth in newly established post-mining
11 restoration is a common feature of mine restoration programmes both in Australia and
12 elsewhere (e.g. George *et al.* 2006; Worrall *et al.* 2007; Standish *et al.* 2010; Tibbett 2010;
13 Williamson *et al.* 2011; Zipper *et al.* 2011; Ortiz *et al.* 2012; Spain *et al.* 2019). However, an
14 increasing number of studies suggest that there may be contrasting effects of fertiliser
15 addition on different functional groups of species. For example, addition of phosphorus
16 (henceforth, P-fertiliser) to mine restoration in the Cape Region of South Africa and in SW
17 Western Australia increased the growth of both native and non-native annual species, but
18 either increased mortality or reduced the establishment of slower growing species such as
19 Proteaceae (Holmes 2001; Daws *et al.* 2013, 2015).

20 The Cape Floristic Region and SW Western Australia have soils depleted in nutrients,
21 particularly P, due to a preponderance of ancient, highly weathered soils (Handreck 1997;
22 Lambers *et al.* 2008;). Consequently, many plant species in these environments have
23 specialised adaptations, such as root clusters, mycorrhizal symbioses and exudation of
24 carboxylases and phosphatases, for acquiring P (Lambers *et al.* 2006, 2008). Furthermore, a
25 number of Australian plant species occurring on nutrient impoverished soils have a limited

1 ability to regulate P-uptake when the P-supply is increased. These species are sensitive to P-
2 toxicity when supplied with P concentrations above those that they naturally experience in
3 soil (Shane *et al.* 2004a; Handreck 1991; Lambers *et al.* 2002; Pang *et al.* 2010; de Campos *et*
4 *al.* 2013), due potentially to the loss of low affinity transporter systems (Huang *et al.* 2011).
5 Consequently, when undertaking environmental restoration in environments with naturally
6 nutrient deficient soils, there may not just be little benefit to growth from adding (P-)
7 fertiliser, but also potential negative effects on growth and survival for a range of species.
8 However, many of these studies on responses to applied-P have either focused on Proteaceae
9 or on species from the naturally P-deficient soils of SW Western Australia (or both). As a
10 result, there is a need to investigate plant responses to applied-P in other nutrient deficient
11 regions.

12 The soils of the GWW are also typically deficient in P and consequently, the
13 application of fertiliser, particularly P, to increase plant growth is viewed as a requirement to
14 increase growth and establishment in post-mining restoration in this region (e.g. KCGM
15 2015). However, there is minimal available data of the effects of P-addition, on species from
16 the GWW, particularly with regard to potential problems such as P-toxicity.

17 Consequently, our current study was established to determine how tree seedlings
18 native to the Australian GWW respond to a wide range of P concentrations. For six species
19 we tested, (1) their response to low-P (P-response efficiency), (2) their capacity to grow and
20 regulate P-uptake at high-P, where toxicity might depress growth, and (3) the response of root
21 and shoot tissue P concentrations to applied P.

1 **Materials and methods**

2 *Plant material and experimental design*

3 Six tree and shrub species from three genera, all native to the GWW of Western Australia,
4 were used in the current study (see Table 1). All six species are used in post-mining
5 restoration in this region. This study was carried out in a shade house established in
6 Kalgoorlie in the South Western Interzone in the Coolgardie Botanical district in WA,
7 Australia (30°44'56'' S, 121°27'57'' E; Beard 1990) for the study. All seeds used were
8 subjected to appropriate pre-treatments (e.g. application of smoke water) to enhance
9 germination, following which the seeds were planted into a sand substrate.

10 Seeds were sown in October 2006 with seedling growth occurring from November
11 2006 to April 2007 for a total period of 136 days. This time of year was chosen for the
12 experiment as it is the optimal time for growth in this region. Temperatures during this period
13 ranged from 13.6 to 36.9 °C. Temperature in the shade house was not regulated and reflect
14 the range of temperatures typical for the GWW area.

15 For each of the six species, four replicate pots containing 5 kg of yellow river sand
16 were established at each of seven phosphorus concentrations (0, 4, 8, 15, 30, 90, 150 mg P
17 kg⁻¹ sand). The sand contained 1 mg kg⁻¹ Colwell P and N was below detection limits. P was
18 added in the treatments as KH₂PO₄. Additional nutrients were provided in two aliquots
19 throughout the experiment to give a nutrient concentration of: (mg kg⁻¹ soil) K 62.8, S 39.4,
20 Ca 40.9, Mg 7.9, Cu 0.53, Zn 2.86, Mn 3.29, B 0.12, Co 0.09, Na 0.04, Mo 0.08 and N 33. N
21 was added in the form of NH₄NO₃ and sulphur in MgSO₄7H₂O. Soil moisture was
22 maintained throughout the experiment by adding deionised water to match weight loss. Plants
23 were checked regularly for pests and signs of infection.

24

25 *Plant measurements*

1 At the end of the growth period (136 days after the start of the experiment), plants were
2 carefully removed from the growing medium. Roots were washed with water; the plants were
3 then separated into roots and shoots. Morphological features were noted and then samples
4 were then oven dried at 70°C to a constant weight. Sub samples were digested using nitric
5 perchloric acid (1:3) and P was measured using the heteropoly-molybdenum method (Ames
6 1966).

7 For each species, growth responses (total plant dry mass) to applied-P were calculated
8 at P supplies of 4, 8, 15, 30, 90 and 150 mg P kg⁻¹. Following the methodology in Pang *et al.*
9 (2010), these growth responses were calculated for the following ranges of P application: 1)
10 subtracting total dry mass for the 0 mg P kg⁻¹ treatment from dry mass at 4 mg P kg⁻¹; 2)
11 subtracting dry mass at 4 mg P kg⁻¹ treatment from dry mass at 8 mg P kg⁻¹; 3) subtracting
12 dry mass at 8 mg P kg⁻¹ treatment from dry mass at 15 mg P kg⁻¹; 4) subtracting dry mass at
13 15 mg P kg⁻¹ treatment from dry mass at 30 mg P kg⁻¹; 5) subtracting dry mass at 30 mg P
14 kg⁻¹ from dry mass at 90 mg P kg⁻¹, and 6) subtracting dry mass at 90 mg P kg⁻¹ from dry
15 mass at 150 mg P kg⁻¹. The increments in plant dry mass were then divided by the difference
16 between amounts of P supplied to provide P-response efficiency values (dry mass produced
17 per unit of applied P in each of the six ranges of P application, such that for each incremental
18 increase in applied P:

19

20
$$\text{P response efficiency} = \Delta \text{ P in dry mass} / \Delta \text{ applied soil P.}$$

21

22 *Statistical analysis*

23 This investigation followed a two factor (concentration of applied-P and species) randomised
24 block design. Two-way ANOVA implemented in Minitab 14 was used to assess, for each
25 species, whether there were effects of increasing external P concentration on either tissue

- 1 specific (root versus shoot) dry mass or P concentration. Data did not require transformation
- 2 prior to analyses as the assumptions of ANOVA with respect to normality and homogeneity
- 3 of variances were met.

For Review Only

1 **Results**

2 *Plant biomass*

3 Plant growth in the absence of applied-P varied between species with total dry mass (root
4 plus shoot) ranging from 0.14 to 1.42 g for *Acacia acuminata* and *Eucalyptus torquata*,
5 respectively (Fig. 1). Across the six species there was no correlation between seed mass and
6 maximum plant dry mass in the absence of applied-P ($P > 0.05$). Root biomass of *Atriplex*
7 *nummularia*, *Atriplex vesicaria* and *Maireana triptera* increased significantly as external P
8 increased from 0 to 15 mg kg⁻¹ and then exhibited no further change as P increased further up
9 to 150 mg kg⁻¹ (Fig. 1 c,d,f). For *Acacia acuminata*, *Acacia hemiteles* and *Eucalyptus*
10 *torquata* there was little change in root biomass as soil P concentration increased (Fig. 1
11 a,b,e).

12 For all six species, shoot biomass increased as the applied P concentration increased
13 from 0 to 15 mg kg⁻¹ (Fig. 1). However, at P concentrations greater than 15 mg kg⁻¹ there was
14 either no further increase in shoot biomass (*Atriplex nummularia*, *Atriplex vesicaria* and
15 *Acacia acuminata*; Fig. 1a,c,d) or biomass declined with increasing P (*Maireana triptera*,
16 *Eucalyptus torquata* and *Acacia hemiteles*; Fig. 1b,e,f). For each of the six individual species
17 the effect of increasing external P on overall dry mass was significant (Two-way Anova, $P <$
18 0.05). In addition for all six species shoot biomass was significantly higher than root biomass
19 (Two-way Anova, $P < 0.05$).

20

21 *Tissue P concentration*

22 For all six species there was a significant effect of increasing concentrations of external P on
23 root and shoot P concentrations (Two-way Anova, $P < 0.001$; Fig. 2), with little exogenous P
24 was required to give foliar P concentration considerably above level found in the natural
25 flora. For *Acacia acuminata*, *Acacia hemitelata* and *Eucalyptus torquata*, tissue P

1 concentrations exhibited little response to P concentration in the growing medium until the P
2 concentration was approximately 15 mg kg⁻¹. Beyond this external concentration, both root
3 and shoot P concentrations continued to increase with increasing applied-P (Fig. 2a,b,e). In
4 contrast, for *Atriplex nummularia*, *Atriplex vesicaria* and *Maireana triptera*, tissue P
5 concentrations increased in response to the lowest level of applied P (4 mg kg⁻¹). There were
6 also significant differences in P concentrations between root and shoot tissues (Two-way
7 Anova, $P \leq 0.008$). The interaction between tissue type and external P-concentration was
8 significant (Two-way Anova, $P \leq 0.001$) for all species, except *Atriplex nummularia*,
9 indicating differences in the response of root and shoot P concentration to increasing external
10 P. For two of the species (*Atriplex nummularia* and *Atriplex vesicaria*), P concentrations were
11 significantly higher in shoot than root tissue (Fig. 2a,d). For *Acacia acuminata*, root P
12 accumulation was significantly higher than that of its shoot P, while limited significant
13 differences in root and shoot P were observed for *Acacia hemiteles*, *Eucalyptus torquata* and
14 *Maireana triptera*. At maximum total dry mass (approximate external P concentration of 15
15 mg kg⁻¹), shoot P concentrations ranged from 1.95 (*Atriplex nummularia*) to 3.72 mg g⁻¹ DM
16 (*Acacia acuminata*). For *Acacia hemitelata*, *Eucalyptus torquata* and *Maireana triptera* a
17 decline in biomass with increasing applied P coincided with shoot P-concentrations in excess
18 of 2.47, 2.1 and 3.2 mg g⁻¹ DM, respectively.

19

20 *P*-response efficiency

21 P-response efficiency differed among the 6 species. *Atriplex nummularia* and *Eucalyptus*
22 *torquata* showed the highest apparent P-response efficiency as P increased from 0 to 4 mg kg⁻¹
23 sand while *Acacia hemiteles* showed the lowest apparent P-response efficiency (Fig. 3).
24 When applied P increased from 4 to 8 mg kg⁻¹, *Atriplex nummularia* still had the highest P-
25 response efficiency, but the order for the other species changed: *Maireana triptera* now had

1 the second highest P-response efficiency whilst *Eucalyptus torquata* was ranked fourth of the
2 six species. When applied P increased further, P-response efficiency declined in all species
3 and was either close to zero or negative at the highest P concentrations (Figure 3).

4

For Review Only

1 Discussion

2 An increase in external P-supply resulted in an increased biomass for our study species only
3 when the P supply was relatively low. At higher P supply, there was either no further increase
4 in biomass or biomass declined. Similar patterns have been observed previously for a range
5 of Australian species from severely nutrient-impooverished environments (Grundon 1972;
6 Groves and Keraitis 1976; Handreck 1997; Pang *et al.* 2010; de Campos *et al.* 2013).

7 Maximum dry mass of our study species was generally observed at an external P
8 concentration of 15 mg kg⁻¹ sand. In addition, for all the study species P-response efficiency
9 values were positive at low P-application rates (≤ 15 mg P kg⁻¹) indicating a continuing
10 growth response to increasing P application. However, above 15 mg P kg⁻¹, values were
11 either close to zero or negative suggesting that there is no further incremental benefit to
12 growth from applying P at rates greater than 15 mg kg⁻¹. Similarly, Ryan *et al.* (2009)
13 recorded maximum root and shoot dry mass for the Australian native *Ptilotus polystachys* at
14 15 mg P kg⁻¹ soil. Pang *et al.* (2010), also observed maximum dry mass for four perennial
15 legumes at an external P concentration of 12 mg kg⁻¹ soil and for their remaining 7 species,
16 maximum dry mass was achieved at 24 mg kg⁻¹ soil. In addition, Pang *et al.* (2010) reported
17 that for 8 of their study species, when the external P concentration was greater than that
18 required to obtain maximum dry mass, total dry mass was reduced, even though most of the
19 species did not exhibit obvious visual symptoms of P-toxicity.

20 Significantly higher P-concentrations were observed in roots than shoots of *Acacia*
21 *acuminata*. This may reflect an ability to allocate and store excess P within the root tissue.
22 Such a mechanism has been shown in several species of Proteaceae (Jeschke and Pate 1995;
23 Shane *et al.* 2004b; Shane and Lambers 2006) and may serve to buffer the leaves and shoots
24 from the effects of excess P as well as enabling P remobilisation for subsequent growth
25 thereby contributing to highly efficient P use.

1 For the three species that exhibited a negative effect of elevated levels of external-P
2 on biomass accumulation, the shoot P concentrations at which these negative effects occurred
3 were at the lower end of the range of leaf and shoot P concentrations for P-toxicity compiled
4 previously (0.9 to 47 mg P g⁻¹ dry mass; Shane *et al.* 2004b). However, the range of values
5 for shoot P at which negative effects were observed (2.1 to 3.2 mg g⁻¹ DM) are in close
6 agreement with those reported for P-toxicity in *Eucalyptus marginata* (1.8 to 5.5mg P g⁻¹
7 DM) by Kariman *et al.* (2014). One possible explanation for the difference between our
8 current values and those reported in Shane *et al.* (2004b) is that many of the values reported
9 by Shane and co-workers are for the onset of *visible* symptoms of P-toxicity (e.g. necrosis):
10 our results indicate the onset of a negative effect on plant growth.

11 It is also interesting to note that the values of shoot P that we report at the lowest
12 external P application rates were consistent with concentrations recorded for plants in the
13 same families harvested from the natural environment (Foulds 1993). In fact, over a wide
14 range of habitats, Foulds (1993) only very occasionally found tissue P concentration over 1
15 mg g⁻¹ P. In the current study, we found only small levels of fertilisation caused P
16 concentrations to rise above values found in natural ecosystems, with peak concentrations,
17 after P application approximately 2-4 times that found in native habitats.

18 Although initial seedling growth rates across species are often positively related to
19 seed mass (e.g. Westoby *et al.* 1996), this was not the case over the 136 days of growth in our
20 current study, possibly because seedling growth was no longer relying on stored seed
21 reserves. Indeed, despite having a seed mass approximately one third of that of the largest
22 seeded species, *Atriplex nummularia* achieved the greatest dry mass. In addition, *Atriplex*
23 *nummularia* had the highest P-response efficiency values of our six study species, and a
24 higher P-use efficiency than all 11 perennial herbaceous legumes that were investigated by
25 Pang *et al.* (2010) as potential pasture legumes for use conditions of under low P availability.

1 While high P-use efficiency between soil P concentrations of 0 and 4 mg kg⁻¹ might relate to
2 seed P concentrations, P-use efficiency was also high between 4 – 8 and 8 – 15 mg P kg⁻¹.
3 Thus, *Atriplex nummularia* appears to have an ability to acquire P and use it efficiently for
4 biomass production even in low P environments. Consequently, this species has potential for
5 rapid growth and establishment in the low P environments of post mining restored sites.

6 Three of the study species (the two *Acacia* species and *Eucalyptus torquata*) were
7 able to maintain their internal root and shoot P-concentrations at a constant level at low, but
8 not at high, external P concentrations. This is similar to the findings by de Campos *et al.*
9 (2013) who reported the ability of two *Acacia* species (*Acacia truncata* and *Acacia xanthina*)
10 to regulate internal P-concentrations at low external P-concentrations. However, at higher
11 external P concentrations root and shoot P-concentrations increased in all six of our study
12 species. Similar to our current study, de Campos *et al.* (2013) also reported a mixed result in
13 terms of the effect of increasing P on biomass accumulation: *Acacia truncata* was
14 unresponsive in terms of biomass to high P, whilst *Acacia xanthina* exhibited declining
15 biomass as P increased. These results suggest that the growth response to elevated P, even
16 within co-occurring members of a genus, is unpredictable as also reported for the genus
17 *Banksia* (de Campos *et al.* 2013).

18 Our data suggest that even low concentrations of soil P can have negative effects on
19 plant growth for a range of woody species from the GWW. Based on a soil dry bulk density
20 of 1 g cm⁻³ and applied P being limited to the top 100 mm of soil, the concentrations at which
21 either negative effects were observed, or growth showed no further response (15 or 30 mg P
22 kg⁻¹ soil dependent on species), are equivalent to field application rates of 15 or 30 kg P ha⁻¹.
23 These application rates are at the low end of those used in many mine site restoration
24 programmes: 25-80 kg P ha⁻¹ are commonly applied (e.g. Holmes 2001; Spain *et al.* 2015;
25 Standish *et al.* 2015; KCGM 2015). Consequently, the rates of P applied to mine restoration

1 have the potential to reduce, rather than increase, growth of some species. While fungal
2 associations can moderate some of the negative effects of applied-P for some species
3 (Kariman *et al.* 2014), the application of P has the potential to change the outcome of
4 competitive interactions by differentially affecting growth of individual species.

5 Applied P can also result in an increased abundance of weeds in restored sites
6 (Holmes 2001; Daws *et al.* 2013, 2015). For example, in five-year-old restored jarrah forest
7 in Western Australia weed density increased from close to zero to more than 5 stems m⁻² as
8 the P-application rate increased from 0 to 80 kg ha⁻¹ (Daws *et al.* 2015). Negative effects of
9 applied-P on plant growth have also been reported in previous studies of mine restoration
10 where applied-P increased mortality or reduced abundance for longer-lived, slow growing
11 species (e.g. Holmes 2001; Daws *et al.* 2013, 2015). However, in these field-based studies it
12 was not possible to determine if the reduced performance of native species resulted from
13 increased competition with weed species, direct negative effects of applied-P on growth, or
14 both. Nonetheless, our current results support the hypothesis that fertiliser application may
15 negatively affect the competitiveness of some species by actually reducing their growth rates.
16 When combined with applied-P increasing the growth and competitiveness of other species
17 (e.g. weeds and annuals), this introduces an important consideration for the use of applied-P
18 in the restoration of natural plant communities.

19

20 **Conclusions**

21 For six plant species from the GWW we found that while low levels of applied-P increased
22 plant growth, above an application rate of 15 mg kg⁻¹ effects on plant growth became
23 unpredictable: growth either declined or showed no further response to increasing external P
24 concentrations. Overall our data suggest that careful consideration should be given to
25 designing appropriate fertiliser regimes for restored sites to avoid the risk that fertiliser

1 addition has the unwanted outcome of decreasing growth and survival of the target native
2 species whilst increasing the abundance of weeds.

3

4 **Acknowledgements**

5 The completion of this work was supported by the Building Outstanding Impact Support
6 Programme H&F38: Restoring biodiversity to phosphorus sensitive forests. we are grateful
7 for the seeds and facilities provided by Jim's Seeds Weeds & Trees Pty Ltd.

8

9 **Conflicts of Interest**

10 The authors declare no conflict of interest.

11

12 **References**

13 Ames BN (1966) Assay of inorganic phosphate, total phosphate and phosphatases. *Methods in*
14 *Enzymology* **8**, 115–118.

15 Beard JS (1990) Plant life of Western Australia. *Annual Review of Plant Physiology* **24**, 225–
16 252.

17 Booth C (2013) Conserving the Great Western woodlands. *Wildlife Australia* **50**, 26.

18 Daws MI, Standish RJ, Koch JM, Morald TK (2013) Nitrogen and phosphorus fertiliser
19 regime affect jarrah forest restoration after bauxite mining in Western Australia. *Appl.*
20 *Vegetation Science* **16**, 610–618.

21 Daws MI, Standish RJ, Koch JM, Morald TK, Tibbett M, Hobbs RJ (2015) Phosphorus
22 fertilisation and large legume species affect jarrah forest restoration after bauxite mining.
23 *Forest Ecology and Management* **354**, 10–17.

24 de Campos MCR, Pearse SJ, Oliveira RS, Lambers H (2013) Downregulation of net
25 phosphorus-uptake capacity is inversely related to leaf phosphorus-resorption proficiency

- 1 in four species from a phosphorus-impooverished environment. *Annals of Botany* **111**,
2 445–454.
- 3 Groves RH, Keraitis K (1976) Survival of seedlings of three sclerophyll species at high levels
4 of phosphorus and nitrogen. *Australian Journal of Botany* **24**, 681–690.
- 5 Grundon NJ (1972) Mineral nutrition of some Queensland heath plants. *The Journal of*
6 *Ecology* **60**, 171–181.
- 7 Handreck KA (1991) Interactions between iron and phosphorus in the nutrition of *Banksia*
8 *ericifolia* L. f. var. *ericifolia* (Proteaceae) in soil-less potting media. *Australian Journal*
9 *of Botany* **39**, 373–384.
- 10 Handreck KA (1997) Phosphorus requirements of Australian native plants. *Australian*
11 *Journal of Soil Research* **35**, 241–289.
- 12 Holmes PM (2001) Shrubland restoration following woody alien invasion and mining: effects
13 of topsoil depth, seed source, and fertilizer addition. *Restoration Ecology* **9**, 71–84.
- 14 Huang CY, Shirley N, Genc Y, Shi S, Langridge P (2011) Phosphate utilization efficiency
15 correlates with expression of low-affinity phosphate transporters and noncoding RNA,
16 *IPSI*, in barley. *Plant Physiology* **156**, 1217–1229.
- 17 Jeschke DW, Pate JS (1995) mineral nutrition and transport in xylem and phloem of *Banksia*
18 *prionotes* (Proteaceae), a tree with dimorphic root morphology. *Journal of Experimental*
19 *Botany* **46**, 895–905.
- 20 Kariman K, Barker SJ, Finnegan PM, Tibbett M (2014) Ecto- and arbuscular mycorrhizal
21 symbiosis can induce tolerance to toxic pulses of phosphorus in jarrah (*Eucalyptus*
22 *marginata*) seedlings. *Mycorrhiza* **24**, 501–509.
- 23 KCGM (2015) Mine closure plan. [http://www.superpit.com.au/wp-](http://www.superpit.com.au/wp-content/uploads/2015/05/ESR_ENV_REP321_KCGM-Mine-Closure-Plan-2015_03312015.pdf)
24 [content/uploads/2015/05/ESR_ENV_REP321_KCGM-Mine-Closure-Plan-](http://www.superpit.com.au/wp-content/uploads/2015/05/ESR_ENV_REP321_KCGM-Mine-Closure-Plan-2015_03312015.pdf)
25 [2015_03312015.pdf](http://www.superpit.com.au/wp-content/uploads/2015/05/ESR_ENV_REP321_KCGM-Mine-Closure-Plan-2015_03312015.pdf)

- 1 Lambers H, Juniper D, Cawthray GR, Veneklaas EJ, Martinez-Ferri E (2002) the pattern of
2 carboxylate exudation in *Banksia grandis* (Proteaceae) is affected by the form of
3 phosphate added to the soil. *Plant and Soil* **238**, 111–122.
- 4 Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ (2006) Root structure and
5 functioning for efficient acquisition of phosphorus: matching morphological and
6 physiological traits. *Annals of Botany* **98**, 693–713.
- 7 Lambers H, Raven JA, Shaver GR, Smith SE (2008) Plant nutrition-acquisition strategies
8 change with soil age. *Trends in Ecology and Evolution* **23**, 95–103.
- 9 Morton S (2014) 'Ten Commitments Revisited: Securing Australia's Future Environment.'
10 (CSIRO Publishing: Collingwood).
- 11 Ortiz O, Ojeda G, Espelta JM, Alcaniz JM (2012) Improving substrate fertility to enhance
12 growth and reproductive ability of a *Pinus halepensis* Mill. afforestation in a restored
13 limestone quarry. *New Forests* **43**, 365–381.
- 14 Pang JY, Tibbett M, Denton MD, Lambers H, Siddique KHM, Bolland MDA, Revell CK,
15 Ryan MH (2010) Variation in seedling growth of 11 perennial legumes in response to
16 phosphorus supply. *Plant and Soil* **328**, 133–143.
- 17 Ryan MH, Ehrenberg S, Bennett RG, Tibbett M (2009) Putting the P in *Ptilotus*: a
18 phosphorus-accumulating herb native to Australia. *Annals of Botany* **103**, 901–911.
- 19 Shane MW, Szota C, Lambers H (2004a) A root trait accounting for the extreme phosphorus
20 sensitivity of *Hakea prostrata* (Proteaceae). *Journal of Experimental Botany* **27**, 991–
21 1004.
- 22 Shane MW, McCully ME, Lambers H (2004b) Tissue and cellular phosphorus storage during
23 development of phosphorus toxicity in *Hakea prostrata* (Proteaceae). *Journal of*
24 *Experimental Botany* **55**, 1033–1044.

- 1 Shane MW, Lambers H (2006) Systemic suppression of cluster-root formation and net P-
2 uptake rates in *Grevillea crithmifolia* at elevated P supply: a proteacean with resistance
3 for developing symptoms of 'P toxicity'. *Journal of Experimental Botany* **57**, 413–423.
- 4 Spain AV, Tibbett, M., Hinz, D.A., Ludwig J.A., Tongway, D.J. (2015) The mining-restoration
5 system and ecosystem development following bauxite mining in a biodiverse environment
6 of the seasonally dry tropics, Northern Territory, Australia. In: *Mining in Ecologically*
7 *Sensitive Landscapes* (Ed M. Tibbett), pp. 159-227. CRC Press, Netherlands.
- 8 Standish RJ, Daws MI, Gove AD, Didham RK, Grigg AH, Koch JM, Hobbs RJ (2015) Long-
9 term data suggest jarrah-forest establishment at restored mine sites is resistant to climate
10 variability. *Journal of Ecology* **103**, 78–89.
- 11 Standish RJ, Tibbett M, Vlahos S, Stokes BA & Hobbs RJ (2010) The effect of fertiliser on
12 floristic diversity and composition of early-successional jarrah forest restored after bauxite
13 mining in south-western Australia. In: *Proceedings of Fifth International Conference on*
14 *Mine Closure, Santiago, Chile* (Eds. A. B. Fourie, M. Tibbett & J. Wiertz), pp 387-395.
15 Australian Centre for Geomechanics, Perth.
- 16 Tibbett, M. (2010) Large-scale Mine Site Restoration of Australian Eucalypt Forests After
17 Bauxite Mining: Soil Management and Ecosystem Development. In: *Ecology of Industrial*
18 *Pollution* L.C. Batty & K. Hallberg, (Eds), pp. 309-326. Cambridge University Press, UK.
- 19 Watson, AW, Judd S, O'Sullivan W & Watson JE (2015). A collaborative approach for Mining,
20 Environment Organisations and Traditional Owners to manage and conserve biodiversity in
21 Australia's Great Western Woodlands. In: *Mining in Ecologically Sensitive Landscapes* (Ed
22 M. Tibbett), pp. 251-263. CRC Press, Netherlands.
- 23 Westoby M, Leishman M, Lord J (1996) Comparative ecology of seed size and dispersal.
24 *Philosophical Transactions of the Royal Society London B* **351**, 1309–1318

- 1 Williamson JC, Rowe EC, Hill PW, Nason MA, Jones DL, Healey JR (2011) Alleviation of
2 both water and nutrient limitations is necessary to accelerate ecological restoration of
3 waste rock tips. *Restoration Ecology* **19**, 194– 204.
- 4 Worrall, R. C., Spain, A. V. & Tibbett, M. (2008) Establishment of Native Tree Species on
5 Coal Tailings — Lessons from Ebenezer Mine, Queensland, Australia. *In*: Fourie, A.,
6 Tibbett, M. & Weiersbye, I. & Dye P. (Eds.). *Proceedings of the Third International*
7 *Seminar on Mine Closure, Johannesburg, South Africa*. Pp 739 – 750 Australian Centre for
8 Geomechanics, Perth.
- 9 Zipper CE, Burger JA, McGrath JM, Rodrigue JA, Holtzman GI (2011) Forest restoration
10 potentials of coal-mined lands in the eastern United States. *Journal of Environmental*
11 *Quality* **40**, 1567–1577.

12

13

1 Table 1. Details of the six study species used in the pot-based experiment of the effect of
 2 applied-P on plant growth.

3

Species	Genus	Seed mass (mg)	Growth form
<i>Acacia acuminata</i> Benth.	Fabaceae	15.2	Shrub or tree
<i>Acacia hemiteles</i> Benth.	Fabaceae	22.1	Shrub
<i>Atriplex nummularia</i> Lindl.	Chenopodiaceae	6.86	Shrub
<i>Atriplex vesicaria</i> Heward ex Benth.	Chenopodiaceae	5.77	Shrub
<i>Eucalyptus torquata</i> Luehm.	Myrtaceae	5.18	Tree
<i>Maireana triptera</i> (Benth.) Paul G. Wilson	Chenopodiaceae	12.54	Shrub

4

5

1 **Figure legends:**

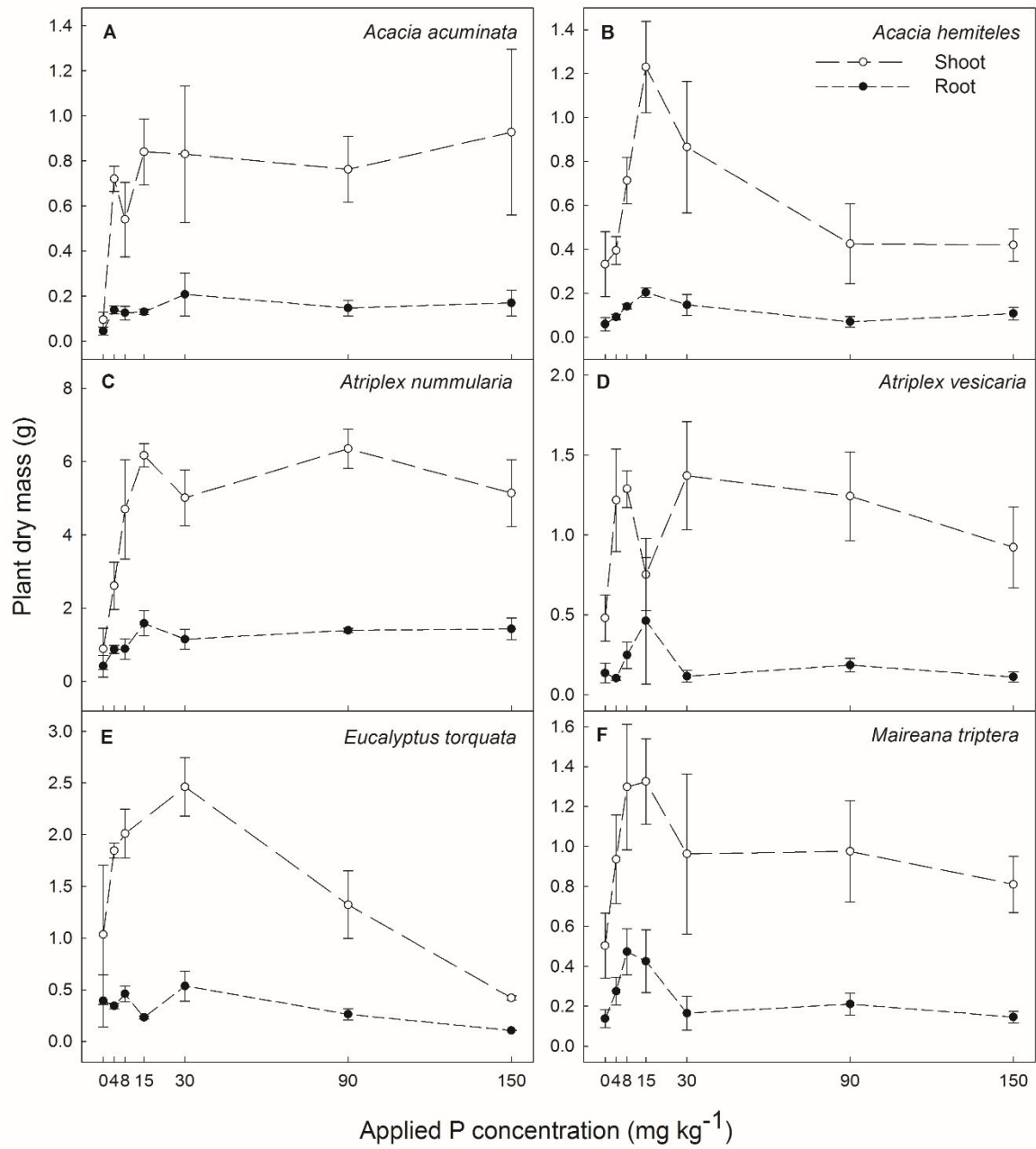
2 Fig. 1. The effect of a range of external P concentrations on dry mass of six woody species
3 from the Great Western Woodlands grown for 136 days in washed river sand at a range of
4 external soil P concentrations, n=4 +/- standard error of the mean.

5

6 Fig.2. Comparison of root and shoot P concentrations for six woody species from the Great
7 Western Woodlands grown for 136 days in washed river sand at a range of external P
8 concentrations, n=4 +/- standard error of the mean. Dotted lines parallel to the x-axis
9 represent phosphorus concentrations in shoot tissue for the plant families as reported in the
10 natural flora of south west Western Australia (Foulds 1993).

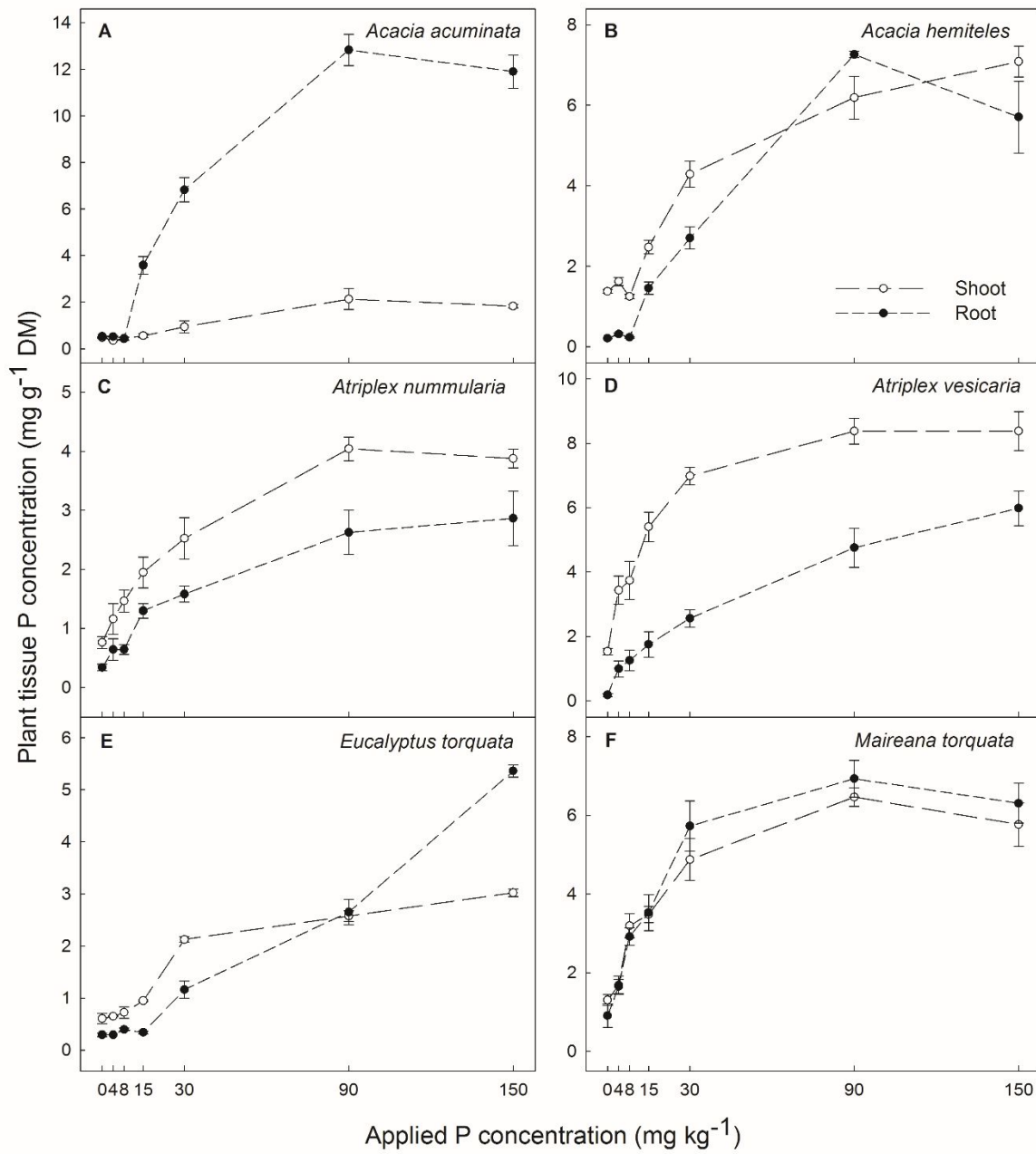
11

12 Fig.3. Phosphorus-response efficiency (yield response per unit of applied P) measured for six
13 P-application ranges (0-4, 4-8, 8-15, 15-30, 30-90 and 90-150 mg P kg⁻¹ sand) for six woody
14 species from the Great Western Woodland, n=4 +/- standard error of the mean.

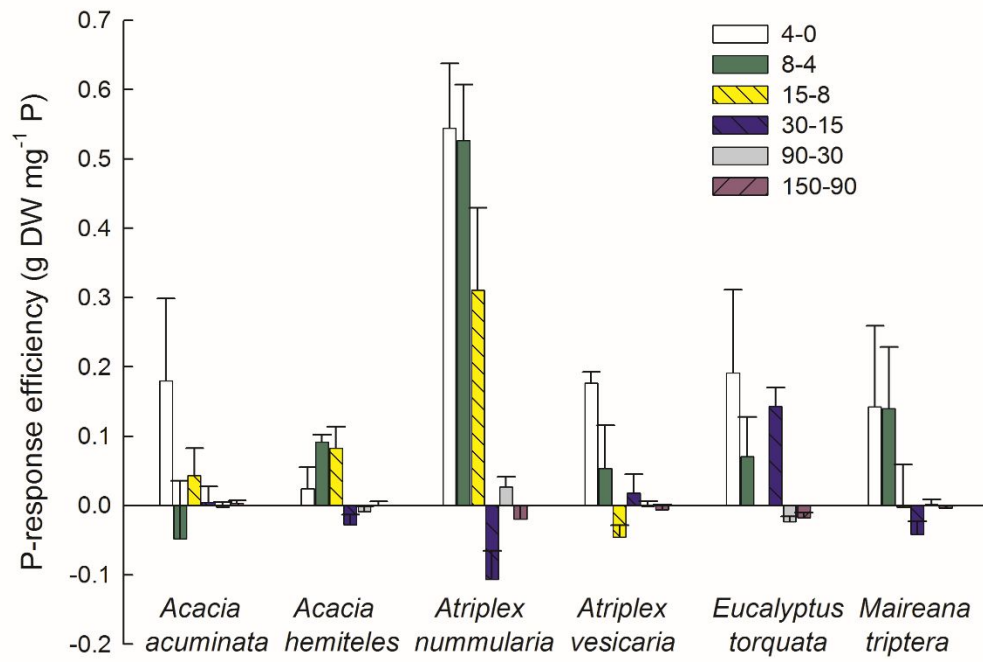
1 **Figure 1.**

2

1 **Figure 2.**



2

1 **Figure 3.**

2

view Only

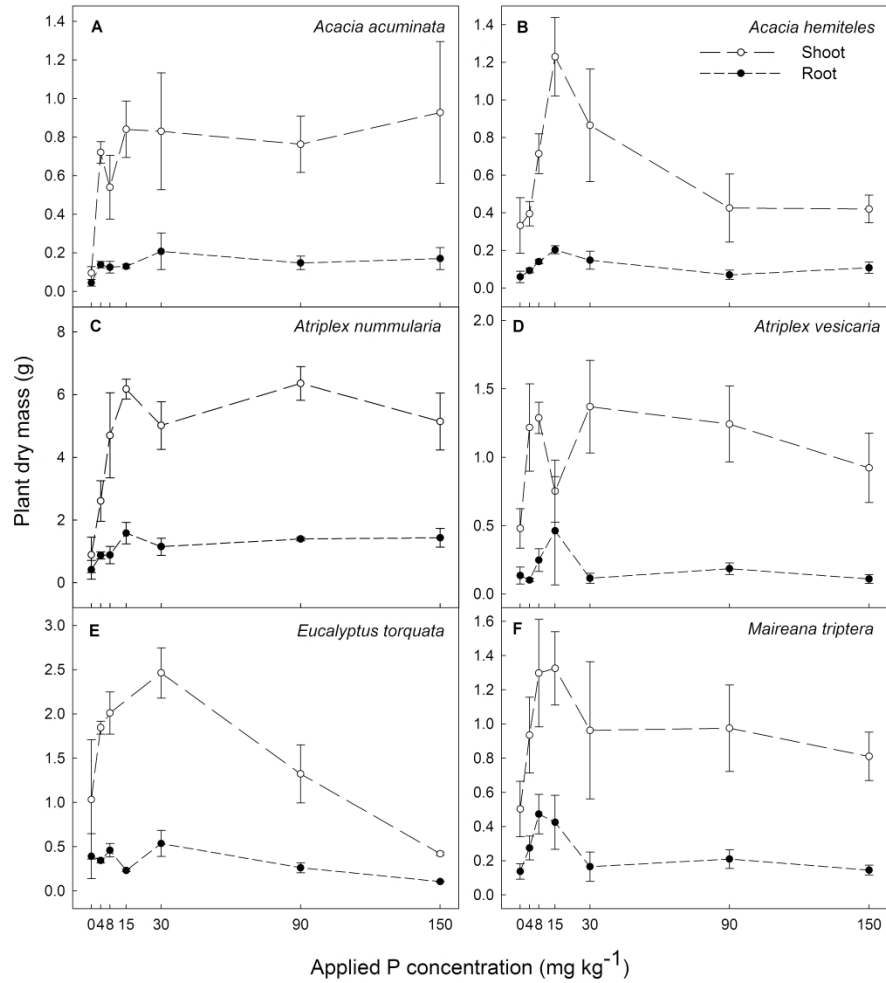


Fig. 1. The effect of a range of external P concentrations on dry mass of six woody species from the Great Western Woodlands grown for 136 days in washed river sand at a range of external soil P concentrations, n=4 +/- standard error of the mean.

863x1117mm (150 x 150 DPI)

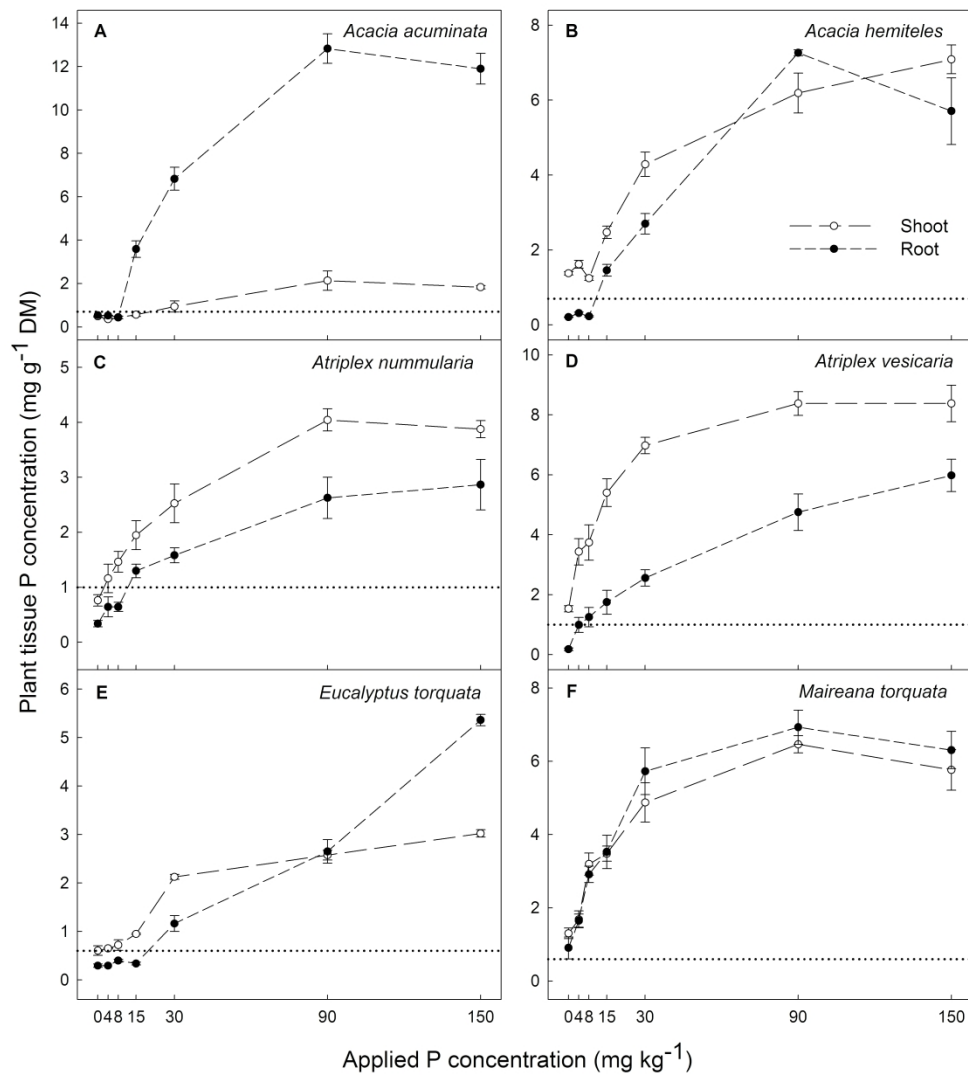


Fig.2. Comparison of root and shoot P concentrations for six woody species from the Great Western Woodlands grown for 136 days in washed river sand at a range of external P concentrations, $n=4 \pm$ standard error of the mean. Dotted lines parallel to the x-axis represent phosphorus concentrations in shoot tissue for the plant families as reported in the natural flora of south west Western Australia (Foulds 1993).

849x956mm (150 x 150 DPI)

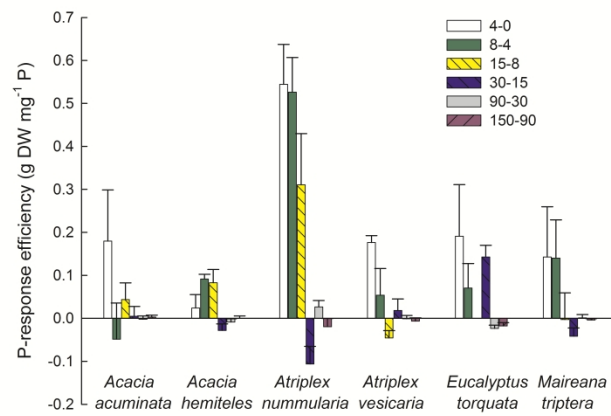


Fig.3. Phosphorus-response efficiency (yield response per unit of applied P) measured for six P-application ranges (0-4, 4-8, 8-15, 15-30, 30-90 and 90-150 mg P kg⁻¹ sand) for six woody species from the Great Western Woodland, n=4 +/- standard error of the mean.

846x1095mm (150 x 150 DPI)

Species	Genus	Seed mass (mg)	Seed P concentration (mg g⁻¹ DM)	Growth form
<i>Acacia acuminata</i> Benth.	Fabaceae	15.2	2.1	Shrub or tree
<i>Acacia hemiteles</i> Benth.	Fabaceae	22.1	1.9	Shrub
<i>Atriplex nummularia</i> Lindl.	Chenopodiaceae	6.86	0.7	Shrub
<i>Atriplex vesicaria</i> Heward ex Benth.	Chenopodiaceae	5.77	0.7	Shrub
<i>Eucalyptus torquata</i> Luehm.	Myrtaceae	5.18	0.6	Tree
<i>Maireana triptera</i> (Benth.) Paul G. Wilson	Chenopodiaceae	12.54	0.7	Shrub



112x61mm (150 x 150 DPI)