

INVITED REVIEW

Root Structure and Functioning for Efficient Acquisition of Phosphorus: Matching Morphological and Physiological Traits

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• **Background** Global phosphorus (P) reserves are being depleted, with half-depletion predicted to occur between 2040 and 2060. Most of the P applied in fertilizers may be sorbed by soil, and not be available for plants lacking specific adaptations. On the severely P-impoorished soils of south-western Australia and the Cape region in South Africa, non-mycorrhizal species exhibit highly effective adaptations to acquire P. A wide range of these non-mycorrhizal species, belonging to two monocotyledonous and eight dicotyledonous families, produce root clusters. Non-mycorrhizal species with root clusters appear to be particularly effective at accessing P when its availability is extremely low.

• **Scope** There is a need to develop crops that are highly effective at acquiring inorganic P (P_i) from P-sorbing soils. Traits such as those found in non-mycorrhizal root-cluster-bearing species in Australia, South Africa and other P-impoorished environments are highly desirable for future crops. Root clusters combine a specialized structure with a specialized metabolism. Native species with such traits could be domesticated or crossed with existing crop species. An alternative approach would be to develop future crops with root clusters based on knowledge of the genes involved in development and functioning of root clusters.

• **Conclusions** Root clusters offer enormous potential for future research of both a fundamental and a strategic nature. New discoveries of the development and functioning of root clusters in both monocotyledonous and dicotyledonous families are essential to produce new crops with superior P-acquisition traits.

Key words: Actinorhizal, capillaroid roots, carboxylates, Casuarinaceae, cluster roots, Cyperaceae, dauciform roots, exudation, Fabaceae, Proteaceae, proteoid roots, Restionaceae.

INTRODUCTION

Phosphorus (P) is an essential inorganic nutrient for all living organisms. It is required as a structural component in nucleic acids and phospholipids, as an element in intermediates in carbon metabolism, and to allow (in) activation of a wide range of enzymes. After nitrogen (N), P is quantitatively the most important inorganic nutrient for plant growth, and often limits primary productivity in natural systems as well as cropping systems, unless supplied as fertilizer (Vance *et al.*, 2003). P is a non-renewable resource, unlike N, which can be assimilated from N_2 into NH_3 by free-living and symbiotic N_2 -fixing microorganisms, or converted into NH_3 , NO_3^- or urea industrially. Moreover, global P reserves are rapidly being depleted; depending on the assumed scenario, current P reserves will be halved (relative to the reserves at the turn of the twentieth century) by 2040 or, more likely, by 2060 (Steen, 1998). Whilst our global P reserves are being depleted, P levels in many agricultural soils are building up, because 80–90% of P applied as fertilizer is sorbed by soil particles, rendering it unavailable for plants that lack specific adaptation to access sorbed P (Gerke *et al.*, 1994; Jones, 1998a). With decreasing global P reserves, P-fertilizer prices are bound to increase. There is an urgent need to develop crops that are more efficient at acquiring

inorganic P (P_i) from soil and/or at using P more efficiently. Equally, it is becoming increasingly important to use crops that reduce the off-site effects of P fertilization, thus reducing the risks of pollution of streams and rivers.

Unlike nitrate, which readily moves in soil towards the roots via both mass flow and diffusion, phosphate (P_i) is highly immobile. Mass flow typically delivers as little as 1–5% of a plant's P demand, and the amount intercepted by growing roots is only half of that (Lambers *et al.*, 1998). The rest of all required P_i must reach the root surface via diffusion; diffusion coefficients for phosphate in soil are typically very low compared with those for other nutrients: $0.3\text{--}3.3 \times 10^{-13} \text{ m}^2 \text{ s}^{-1}$ (Clarkson, 1981). Diffusion is particularly slow in dry soil (e.g. Turner and Gilliam, 1976; Bhadoria *et al.*, 1991). Increasing P_i delivery to roots via mass flow can be achieved by enhanced transpiration rates, but this cannot have a major effect, and would be at the expense of a plant's water-use efficiency. Root interception of P_i can be increased by root proliferation, increased frequency and length of root hairs, a modified root architecture that enhances allocation to shallow soil horizons, and mycorrhizal symbioses. Diffusion of P_i toward the root can be increased by increasing the moisture content of dry soil, or by increasing the P_i concentrations in the soil solution through release of P_i from complexed, sorbed or organic forms of P. This review focuses on structural and

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functional root traits that enhance P_i acquisition from soil with a low availability of P_i , i.e. soils with a reasonable amount of total P, but where diffusion of P_i towards the root limits plant growth. In particular, it deals with traits of native species naturally occurring on soils with a low P_i availability, to explore the potential of these traits for future crop plants. The focus will be on species native to south-western Australia and the Cape region in South Africa, two of the world's 25 biodiversity hotspots. Both regions were once part of the southern-hemisphere super-continent Gondwanaland, and their soils are ancient and deeply weathered, especially those in Western Australia, some of which are estimated to over be 3 billion years old (White, 1986). As soils weather over thousands to millions of years, both the total P levels and the availability of P_i decline (Walker and Syers, 1976; Crews *et al.*, 1995; Richardson *et al.*, 2004). The decline in availability results from the main initial soil P-containing mineral, calcium apatite, being utilized by organisms to form organic P, and by sorption of P onto the surfaces of other minerals. This mineral-sorbed P is labile and can be desorbed in response to diffusion gradients as a result of P_i uptake by plant roots, or can be chemically displaced by root exudates. Over (geological) time, mineral-sorbed P can also be surrounded (occluded) by Fe and Al, rendering it essentially unavailable to plants. The evolutionary consequence of this decline in P levels and P_i availability is an incredibly diverse array of plant species with present-day root adaptations with remarkable ability to acquire sparingly available soil P, and to use internal P efficiently, and that could be explored for future use in crops.

COMMON ROOT TRAITS TO ENHANCE P_i ACQUISITION

Root architecture

This denotes the spatial configuration of roots of different order and age, with the implication that the overall configuration has some functional significance (Lynch, 1995). In terms of P nutrition, the root architecture of *Phaseolus vulgaris* when grown at low P_i supply is significant for immobile nutrients such as P because more laterals are produced in shallow soil horizons, where most of the P is located (Lynch and Brown, 2001). Fitter *et al.* (2002) showed that root architecture had a major effect in *Arabidopsis thaliana*, both in a pot experiment and in a field experiment conducted under natural conditions, on the fitness of a mutant with a reduced number of lateral roots relative to its isogenic wild-type when P was the limiting nutrient. As expected, when the more mobile nitrate was the limiting nutrient for plant growth, there was no difference in fitness between mutant and wild-type.

Root biomass

Most species allocate more biomass to roots when P_i is limiting for their growth (Brouwer, 1963, 1983). Some of the observed difference in biomass allocation pattern between plants grown with a high vs. a low supply of P_i

may be ontogenetic, owing to comparisons of plants at different sizes, rather than a truly plastic response (Kemp and Blair, 1994; Niklas, 1994). However, there is also clear evidence that P_i supply has a direct effect on biomass partitioning, independent of ontogeny (Ryser *et al.*, 1997; De Groot *et al.*, 2001). Interestingly, many *Lupinus* species, some known to be highly P-efficient, show little change in biomass partitioning to roots as dependent on P_i supply (Keerthisinghe *et al.*, 1998; Pearse *et al.*, 2006; S. J. Pearse, unpubl. data). This low plasticity has been found in both cluster-root-bearing and non-cluster-root-forming *Lupinus* species (S. J. Pearse, unpubl. data). Effects of P_i supply on biomass partitioning between roots and shoots are thought to involve a decreased production in and export of cytokinins from roots at a low P_i supply, possibly associated with a decreased rate of uptake and metabolism of nitrogen (Kuiper *et al.*, 1989). In the case of *Lupinus* species, would that mean that they show no change in cytokinin production and export as dependent on their P status? Or might they have a limited response to cytokinins? Do they respond to variation in N supply? Given that we do know that *L. albus* and *L. mutabilis* do increase their biomass partitioning to roots (increased root mass ratio) under water stress (Carvalho *et al.*, 2004), a low plasticity in root mass ratio supply is either not typical for all species in this genus or is restricted to effects of P. Is the lack of response to P_i as found for some *Lupinus* species linked to the capacity of some species in this genus to produce cluster roots? Interestingly, cytokinins play a role in both biomass partitioning (Kuiper *et al.*, 1989) and cluster-root formation, as antagonists of auxins (Neumann *et al.*, 2002). Would that mean that the *Lupinus* species lacking the capacity to produce root clusters can relatively easily be modified into cluster-forming plants? So far, there are no data in the literature to provide satisfactory answers to many of the questions raised here.

Root length

In field plots of *Beta vulgaris*, total root-length production over the entire growing season was 3–4 times the size of the living root system at harvest (120 km m^{-2}) in high-P plots, and five times (200 km m^{-2}) in low-P plots (Steingrobe, 2001). The author calculated a 25% increase in P_i uptake at low P supply as a result of this enhanced root-length production compared with that at the root production of high-P plants. A similarly enhanced root-length production at a low P supply has been observed for *Hordeum vulgare* (Steingrobe *et al.*, 2001). Increased root production, without a proportional increase in living-root biomass, i.e. enhanced root turnover, allows greater amounts of uptake of immobile soil resources, such as P. Fast root turnover is a very important trait of cluster-root-producing species, as discussed below (Shane and Lambers, 2005a).

Specific root length

In the cases where plants were found to respond to P_i supply with a change in specific root length (SRL), their

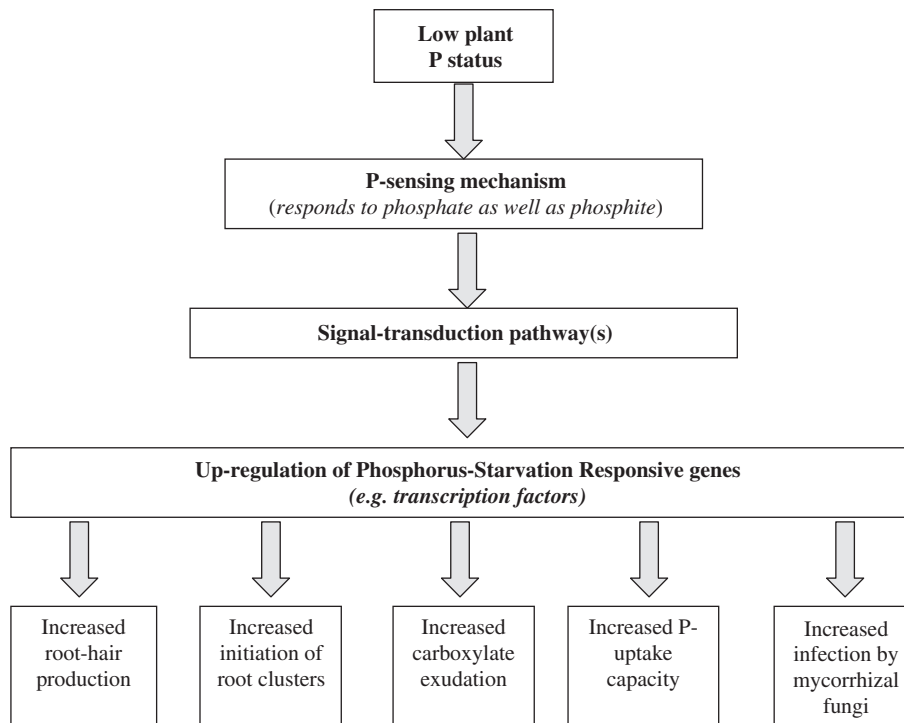


FIG. 1. Plant responses to inorganic phosphorus (P_i) limitation. A low external P_i availability decreases the plant's internal P status. When the plant senses a low P status, P-starvation responses are up-regulated. P-starvation responses, depending on the species, include increased root-hair formation, root-cluster initiation and development, carboxylate exudation, P-uptake capacity, and mycorrhiza formation. Phosphite acts as an analogue of phosphate, and hence suppresses the typical P-starvation responses shown here. Based on Abel *et al.* (2002) and other references cited in the text.

SRL increased with decreasing P_i supply (Powell, 1974; Christy and Moorby, 1975; Schroeder and Janos, 2005). The increase in SRL is associated with a decrease in root diameter (Powell, 1974), especially for the apical regions of the root system (Mollier and Pellerin, 1999). However, a decrease in root diameter is by no means a universal response to a low P_i supply (e.g. Borch *et al.*, 1999; Schroeder and Janos, 2005).

Root hairs

Root hairs are a fairly common root structure, and increased root-hair length and numbers are considered to be an adaptation that enhances P_i acquisition and a plant's competitive advantage when soil P_i is limiting for growth (Bates and Lynch, 2001). Species that develop more and/or longer root hairs, e.g. *Lolium perenne*, are far more efficient at accessing P_i from soils, and thus show less of a growth response in P-fertilized soils than do species that lack these traits, e.g. *Podocarpus totara* (Clarkson, 1981). This point was elegantly demonstrated in a comparison of genotypes of *Hordeum vulgare*; genotypes with the capacity to form longer root hairs (about 1 mm) took up more P, and tended to yield better when P_i was limiting crop growth compared with genotypes having roots hairs half the length (about 0.5 mm) (Gahoonia and Nielsen, 2004). Root-hair abundance and length is enhanced by P deficiency (Schmidt, 2001). The increased growth of root hairs observed for plants grown at low P_i availability

can be mimicked in plants grown at high P_i supplies by adding an ethylene precursor to 'high-P' roots. Similarly, root-hair growth can be inhibited by adding the ethylene inhibitor 1-amino-cyclopropane-1-carboxylate (ACC) to the medium of 'low-P' roots (Zhang *et al.*, 2003). This suggests that ethylene plays a major role in modulating the growth of root hairs in response to plant P nutrition. A recent detailed anatomical analysis in *A. thaliana* has shown that the effects of low P_i availability and ethylene on root-hair development differ, with low P status leading to a decreased size and increased number of cortical cells, and increased numbers (approximately double) of root-hair-bearing epidermal cell files, whereas ethylene does not (Zhang *et al.*, 2003). Split-root experiments have established that a shoot-derived signal is required for root hairs to increase in length; the signal is translocated to the roots only when the shoot senses a low P status, and root-hair length is even further enhanced by a low P status in the roots (Jungk, 2001). The signal is unknown but given that auxin is also involved in root-hair formation (Schmidt, 2001), the shoot-derived signal might be an auxin. Increased length and abundance of root hairs is one of the typical adaptive P-starvation-induced plant responses (Fig. 1).

Mycorrhizal associations

The vast majority (82%) of all higher plant species have the capacity to form a symbiotic association with

a mycorrhizal fungus (Brundrett, 2002). It is widely accepted that the ancestral mode of P_i acquisition in higher terrestrial plants was through an association with arbuscular mycorrhizal (AM) fungi; more recent mycorrhizal associations include ectomycorrhizas and orchidaceous mycorrhizas (Brundrett, 2002). At low P_i availability, the mycorrhizal symbiosis often enhances plant P_i uptake and growth, especially when the species has a root system that is relatively coarse with few root hairs, e.g. in *Citrus* species (Graham and Eissenstat, 1994). However, even when there is no effect on net P_i uptake or growth, there can be a major down-regulation of the roots' high-affinity P_i transporters (Smith *et al.*, 2003), possibly in response to an improved plant P status, owing to rapid P_i uptake by the external mycelium. Further research is required to confirm this contention. For further information on the role of mycorrhizas for plant P_i uptake, the reader is referred to recent reviews (Brundrett, 2002; Söderström, 2002). Present-day non-mycorrhizal species must have lost their ability to form an association with AM fungi. Of the minority of species that do not form a mycorrhizal symbiosis, some have specialized root clusters such as proteoid and dauciform root clusters, and specialized physiology associated with rapid rates of carboxylate exudation, as discussed below. However, it should be noted that these specialized adaptations are not restricted to non-mycorrhizal species. There are, indeed, several mycorrhizal species that also have the capacity to produce root clusters, e.g. *Casuarina* species (Casuarinaceae), *Myrica* species (Myricaceae) and *Viminaria viminalis* (Fabaceae) (Neumann and Martinoia, 2002; Shane and Lambers, 2005a). Mycorrhizal associations can even be formed with the root clusters of *Hakea verrucosa*, a species naturally occurring on soils containing high levels of nickel (Boulet and Lambers, 2005). Furthermore, some non-mycorrhizal species (e.g. Brassicaceae) lack morphological adaptations like root clusters, but show rapid rates of carboxylate exudation (Hoffland *et al.*, 1989). Finally, some species that lack mycorrhizal associations, e.g. Chenopodiaceae and Urticaceae (Lambers *et al.*, 1998), would appear to have no specialized morphological adaptations, and these species tend to be restricted to relatively nutrient-rich sites and habitats with low competitive pressure (Olsson and Tyler, 2004).

High-affinity P_i transporters

Much remains to be discovered about the expression of high-affinity P_i transporters as dependent on plant P status. Recent discoveries have revealed that sugars are integrally related to P-deficiency-induced expression of one of these transporters in *L. albus* (Liu *et al.*, 2005). Exogenous sugars stimulate accumulation of transcripts of a high-affinity transporter in dark-grown, P-sufficient seedlings. Conversely, in intact P-deficient plants, expression of this transporter in cluster roots was reduced in girdled plants, and in dark-grown plants in which expression was rapidly restored upon re-exposure to light. Similar results were also obtained for a gene encoding acid phosphatase and a third gene, both being P-deficiency induced. There is

obviously cross-talk between phosphorus acquisition and carbon metabolism, similar to that between nitrogen and sulfur uptake and carbon metabolism (Lejay *et al.*, 2003). The promoters of the genes encoding the high-affinity transporter and the acid phosphatase contain a short sequence that is identical to the binding site for a transcription activator for P-deficiency-induced genes in *A. thaliana* (Rubio *et al.*, 2001).

Enhanced expression of high-affinity, plasma-membrane-bound P_i transporters in roots, and a concomitantly increased P-uptake capacity, is a typical P-starvation response (Burleigh and Harrison, 1999; Dong *et al.*, 1999) (Fig. 1). This response is usually interpreted as an acclimation to a low availability of P_i in soil. However, diffusion of P_i in soil is the key limiting factor for P_i uptake, and changes in kinetic parameters of the roots' P-uptake system, including an increase in I_{max} (maximum P_i inflow rate), have little effect on a plant's capacity to acquire P_i from soil (Silberbush and Barber, 1983; Raghothama and Karthikeyan, 2005). This is not to say that plants do not need a high-affinity system for P_i uptake; rather, it shows that enhancing the expression of this P_i transport system does not have a proportional effect on P_i uptake, and may have no effect at all. This may explain, partly, why over-expression of a high-affinity phosphate transporter in transgenic *Hordeum vulgare* had no effect on P_i uptake from soil (Rae *et al.*, 2004). What might be the adaptive significance of differential expression of high-affinity P_i transporters? Species that have a very low capacity to adjust their P_i uptake capacity in response to changes in P_i supply in the root environment show signs of P toxicity at elevated P_i supply (Shane *et al.*, 2004a, b; Shane and Lambers, 2006). Therefore, we suggest that the capacity to *down-regulate* P_i transporters at *high* P_i supply is the trait that has adaptive significance, rather than the capacity to *up-regulate* P_i transporters at a *low* P_i supply. Although over-expression of high-affinity P_i transporters may not enhance P_i acquisition from soil, we envisage that it might improve internal P utilization. P-starved *A. thaliana* plants have been found to express high-affinity P_i transporters in roots as well as in developing flowers and fruits (Karthikeyan *et al.*, 2002). If expression of high-affinity P_i transporters can be reduced in reproductive organs of grain crops, accumulation of P in seeds may be decreased, allowing P to be utilized in photosynthetic tissues, which could lead to increased grain production, and greater P return to the soil in organic form.

Effects of phosphite on P-starvation responses

A low plant P status induces the P-starvation responses as discussed above. Many of these responses are suppressed by phosphite, which acts as an analogue of phosphate. Typically suppressed P-starvation responses include increased allocation to root biomass (Varadarajan *et al.*, 2002), enhanced root-hair formation (Ticconi *et al.*, 2001), up-regulation of the high-affinity phosphate transporters and acid phosphatases (Varadarajan *et al.*, 2002), and cluster-root formation (Gilbert *et al.*, 2000).

Phosphite inhibits mycorrhiza formation in *Zea mays* (Seymour *et al.*, 1994), but not in *Allium cepa* (Sukarno *et al.*, 1998), *Eucalyptus marginata*, *E. globulus* or *Agonis flexuosa* (Howard *et al.*, 2000). The contrasting results for mycorrhization might be due to the fact that phosphite inhibits the expression of high-affinity phosphate transporters (Varadarajan *et al.*, 2002), which would lower the plant P status, and thus indirectly enhance mycorrhization. Phosphite is also used as a fungicide, e.g. to combat the soil-borne plant pathogen *Phytophthora cinnamomi* in natural ecosystems (Hardy *et al.*, 2001). Considering the effect of phosphite on P-starvation responses, the use of phosphite as a fungicide in pristine ecosystems clearly needs further scrutiny.

Rhizosphere alteration

As discussed above, enhanced root production is an adaptive response to acquire poorly mobile soil resources. An alternative strategy is to enhance the availability of P_i in soil. There are two fundamentally different mechanisms to enhance P_i availability. First, in superficial, dry soil horizons, where most of the P will be located, the mobility of P_i can be enhanced by the release of water into that dry soil. The released water would originate from moister regions in the soil, and be transported inside the root system in a process termed 'hydraulic redistribution' (Burgess *et al.*, 1998, 2000). When this process was first described for desert plants that took up water from deep soil layers and released it, at night, into superficial layers, it was termed hydraulic lift. However, it has since been established that water can flow downwards as well as upwards, and it is envisaged that it will also move horizontally, from roots in moist superficial patches via the stem to the roots in drier soil, where the water can be released. Secondly, the concentration of P_i , the only form of P that is taken up by roots, can be enhanced by the release of root exudates, particularly carboxylates and phosphatases (Raghothama and Karthikeyan, 2005). These two strategies to enhance P_i availability are discussed in the next two sections.

ENHANCED P_i UPTAKE ASSOCIATED WITH HYDRAULIC REDISTRIBUTION?

When some roots are in contact with moist soil, while others on the same plant are in dry soil, water may move from moist to dry patches. This was first discovered in desert shrubs, where water can move from moist deep soil layers into shallower dry soil, and was termed 'hydraulic lift' (Caldwell and Richards, 1989). The water that is transported from deeper and moist layers into shallower dry soil is available for the species that exhibited hydraulic lift as well as for neighbouring plants (Caldwell and Richards, 1989). Hydraulic lift usually occurs at night, in C_3 species, but can also occur during the day in CAM species (Yoder and Novak, 1999). However, water can also move from roots in shallow layers, moistened after rain, to roots deep in the profile (Burgess *et al.*, 1998). Equally, water can move horizontally, via the stem, depending on soil water

content (Hultine *et al.*, 2003), and hence the term 'hydraulic redistribution' is more appropriate (Burgess *et al.*, 1998, 2000). Hydraulic redistribution is not restricted to woody species, but has also been demonstrated in the crop species *Pennisetum americanum* (Vetterlein and Marschner, 1993). Water uptake by roots requires the expression of water-channel proteins (aquaporins); these proteins are expressed in a diurnal pattern, with low expression levels at night and increasing expression early in the day (Vandeleur *et al.*, 2005). One would expect that water release also requires expression of water-channel proteins, but this has not yet been investigated.

Because diffusion of P_i in dry soil is very slow (Amijee *et al.*, 1991; Bhadoria *et al.*, 1991), P_i uptake declines with decreasing soil moisture content (Turner and Gilliam, 1976; Vig and Singh, 1983; Mouat and Nes, 1986). Therefore, nutrient uptake from dry shallow patches in soil is expected to increase when the soil is moistened due to hydraulic redistribution (Vetterlein and Marschner, 1993; Horton and Hart, 1998; Huang, 1999), and this could be especially significant for poorly mobile nutrients such as P. Equally, when deeper soil layers contain abundant P reserves, hydraulic redistribution down the profile might enhance the uptake of P_i (McCulley *et al.*, 2004). This interesting concept of enhancing P_i availability by hydraulic redistribution is, however, particularly difficult to approach experimentally, and hence there is little convincing evidence to support it. Valizadeh *et al.* (2003) found that P banded in dry topsoil was accessed by *Triticum aestivum* with access to moist subsoil, as a result of to the release of hydraulically lifted water.

In summary, there is a wealth of information on the occurrence of hydraulic redistribution and the use of hydraulically lifted water by neighbouring plants. It is highly likely that hydraulic redistribution enhances P_i acquisition from P-enriched, dry soil patches. However, further research is required to establish the extent to which hydraulic redistribution may favour P_i acquisition.

ENHANCED P_i UPTAKE ASSOCIATED WITH THE RELEASE OF ROOT EXUDATES

In addition to increasing the diffusion coefficient of P_i in soil by hydraulic redistribution as discussed above, root activity can also enhance the concentration of P_i in soil, owing to the release of exudates.

Carboxylates

Carboxylates (e.g. citrate, malate) can be major components of exudates released by roots, especially under P deficiency (Gardner *et al.*, 1983; Hoffland *et al.*, 1989; Keerthisinghe *et al.*, 1998). However, some high-exuding plant species, e.g. *Cicer arietinum*, appear to release carboxylates (mainly malonate) constitutively (Wouterlood *et al.*, 2004). Carboxylates mobilize both inorganic P and organic P (P_o), because they complex metal cations that bind phosphate and displace phosphate from the soil matrix by ligand exchange (Fig. 2) (Gerke

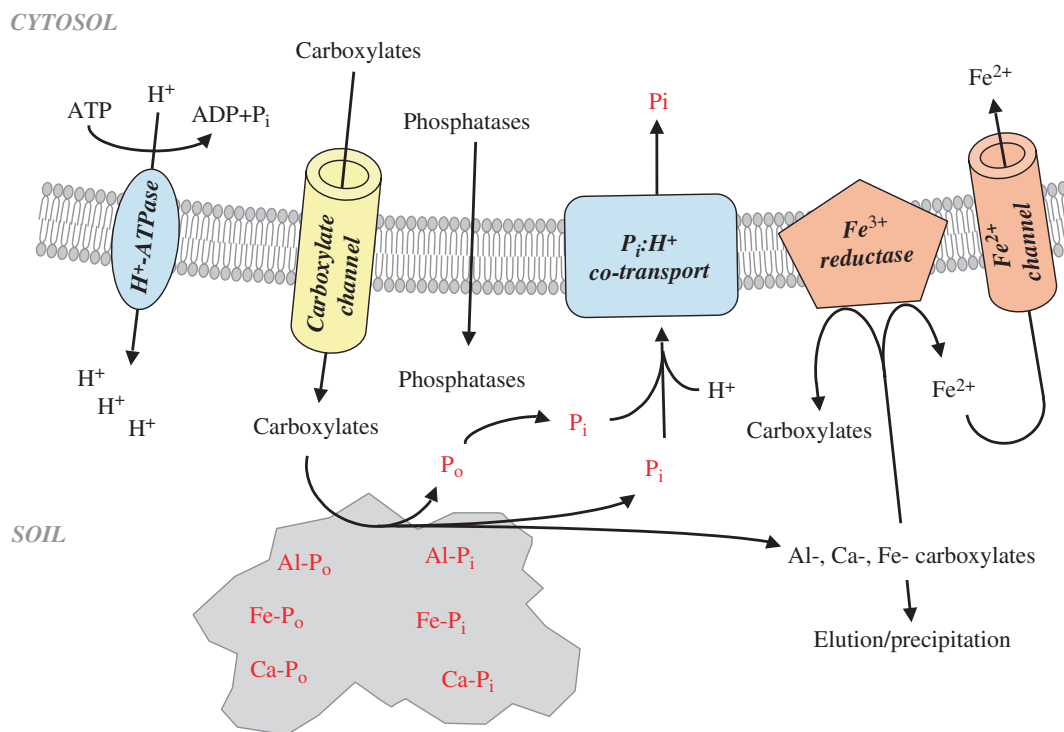


FIG. 2. Effects of carboxylates (and other exudates) on inorganic phosphorus (P_i) and organic P (P_o) mobilization in soil. Carboxylates are released via an anion channel. The exact way in which phosphatases are released is not known. Carboxylates mobilize both inorganic and organic phosphorus. Phosphatases hydrolyse organic phosphorus compounds, once these have been mobilized by carboxylates. Carboxylates will also mobilize some of the cations that bind P. Some of these cations (especially Fe) move to the root surface for uptake by the roots. Others move down the soil profile, where they are thought to give rise to the formation of laterite (in the case of Fe) (Pate *et al.*, 2001; Verboom and Pate, 2003) or other precipitates (in the case of Al or Ca) (Verboom and Pate, 2006).

et al., 2000; Hayes *et al.*, 2000; Jones *et al.*, 2003). The cations excreted together with the carboxylates to maintain charge balance may be protons, leading to rhizosphere acidification (Hinsinger, 2001; Hinsinger *et al.*, 2003). However, other cations, especially K^+ , are at least as important (Y Zhu *et al.*, 2005), and carboxylate exudation is not invariably associated with acidification (Roelofs *et al.*, 2001). Transport of carboxylates in the anionic form from the cytosol (pH ≈ 7.2 – 7.5) into a more acidic rhizosphere is likely to result in protonation of the carboxylates in the rhizosphere. This is likely to contribute to scavenging of H^+ from the rhizosphere, and hence increase the rhizosphere pH. In fact, unless the soil pH is initially alkaline, acidification does not enhance P_i availability; rather, acidification immobilizes P_i at low pH due to the formation of Fe and Al complexes (Lambers *et al.*, 1998). In addition to P immobilization, acidification can influence the extent of ionization of carboxylates (Jones, 1998b; Hinsinger *et al.*, 2003), which can reduce their chelating ability, potentially rendering them ineffective in acidified soil (Pearse *et al.*, 2006)

Phenolics and mucilage

Exudation of phenolics may also increase under P deficiency (Neumann and Römheld, 2001; Juszczuk *et al.*, 2004), but this has received less attention in the literature.

Similarly, release of mucilage can be enhanced under P deficiency, and this can also enhance P_i availability in soil (Nagarajah *et al.*, 1970; Gaume *et al.*, 2000; Grimal *et al.*, 2001). Phenolics and mucilage act in the same way as carboxylates (Guppy *et al.*, 2005), but tend to be less effective than carboxylates (Neumann and Römheld, 2001). In addition, release of phenolics may serve a fungistatic role (Weisskopf *et al.*, 2006), as further discussed in the section below dealing with root clusters.

Phosphatases

Organic P typically accounts for 30–80% of total P in soil (Pederson, 1953; Tarafdar and Claassen, 1988; Adams, 1992). Soil organic P compounds (mainly phosphate mono- and di-esters, Sumann *et al.*, 1998), after having been mobilized by carboxylates, must first be hydrolysed, to release P_i for plant uptake (George *et al.*, 2002) (Fig. 2). Acid phosphatases can hydrolyse a range of organic P compounds (Tarafdar and Claassen, 2001), and both expressed sequence tags for phosphatase (Uhde-Stone *et al.*, 2003) and these enzymes are more abundant in the rhizosphere when plants are P starved (e.g. Li *et al.*, 1997; Gilbert *et al.*, 1999; Yun and Kaeppler 2001; Wasaki *et al.*, 2003). Phytases are required to hydrolyse phytate (= *myo*-inositol penta- and hexa-phosphates), which is fairly resistant to other phosphatases (Hayes *et al.*, 2000).

Phytate can be a major component of the soil organic P pool (Pederson, 1953; McKercher and Anderson, 1968). Phosphatases and phytases in soil may be of microbial origin (Tarafdar and Claassen, 2001), but roots also exude phosphatases (Tarafdar and Claassen, 2001, 2005), and roots of some species also release significant amounts of phytases (Li *et al.*, 1997). Most plants have a very limited capacity to access phytate in the rhizosphere, except in the presence of micro-organisms that can dephosphorylate phytate (Richardson *et al.*, 2001). Transgenic plants of *A. thaliana*, exhibiting enhanced exudation of extracellular phytase (derived from *Medicago truncatula*) from their roots, have greater access to phytate than their wild-type (Xiao *et al.*, 2005). Similarly, transgenic plants of *Trifolium subterraneum*, exhibiting enhanced, constitutive expression and exudation of a phytase derived from *Aspergillus niger*, had better access to phytate than wild-type plants (George *et al.*, 2004). However, this effect was only pronounced when plants were grown in non-sorbing, sterile laboratory media, and much less so when plants were grown in soil where phytase is rapidly immobilized, limiting its ability to interact with phytate (George *et al.*, 2005). This suggests that phytate can only be dephosphorylated by phytase after it has been mobilized into the soil solution by, for example, carboxylates (Fig. 2).

Exudation as dependent on soil moisture

Phosphate-starvation responses are controlled systemically, via signals originating in the shoot (Abel *et al.*, 2002; Fig. 1). This explains why a low soil moisture content, which reduces the mobility of P_i in soil (e.g. Turner and Gilliam, 1976; Bhadoria *et al.*, 1991), and hence tends to lower the plant's P status, enhances root exudation (Liebersbach *et al.*, 2004). As a consequence, P_i uptake is affected much less by water shortage than expected on the basis of the effect of soil moisture on P_i mobility in soil.

In summary, roots of many species release an array of exudates (e.g. carboxylates, phenolics, protons and other cations, phosphatases, water, mucilage), and thus enhance the availability of P_i in the rhizosphere. The nature and effectiveness of the exudates depends on species as well as environmental conditions. A low plant P status tends to enhance exudation.

SPECIALIZED ROOT STRUCTURES: ROOT CLUSTERS

The specialized roots discussed here, collectively called 'root clusters', combine a specialized structure and specialized physiology (see below) to maximize P_i acquisition from soils of low fertility, especially when P is present in 'sorbed' or insoluble sources (e.g. rock phosphate and iron phosphate). Proteoid (e.g. Keerthisinghe *et al.*, 1998) and dauciform (Shane *et al.*, 2005; Playsted *et al.*, 2006) root clusters are induced by P deficiency and occasionally by Fe deficiency (reviewed in Shane and Lambers, 2005a). P deficiency induces a wide range of genes in cluster roots of *L. albus*, including genes

involved in carbon metabolism, secondary metabolism, P scavenging and remobilization, plant hormone metabolism, and signal transduction, when compared with P-sufficient and P-deficient non-cluster roots (Uhde-Stone *et al.*, 2003).

There are several 'types' of root clusters, occurring in both monocotyledonous and in dicotyledonous species (Fig. 3). The best known examples are the 'bottlebrush-like' proteoid roots (Fig. 3A, B) described by Purnell (1960) for woody species of Proteaceae. Proteacean taxa are distributed primarily in Australia and South Africa, but proteoid-like roots have also been described in a range of other species from several families, e.g. in Fabaceae [*Lupinus albus* (white lupin) and *Aspalathus linearis* (rooibos)] (Fig. 3E, F) (Dinkelaker *et al.*, 1995; Shane and Lambers, 2005a). Monocotyledonous families containing rushes (Restionaceae from the southern hemisphere) and sedges (Cyperaceae with a worldwide distribution) form root clusters termed 'dauciform' roots (in sedges; Fig. 3C, D) and 'capillaroid' roots (in rushes; Fig. 3G–I).

Root cluster morphologies involve formation of compact clusters of (determinate) branch roots (rootlets), or root hairs, in a small soil volume which markedly increases the surface area of the root system (Fig. 3). Moreover, root clusters are ephemeral; even in the woody species that develop them, rootlets remain in the primary state of growth until they senesce, and although we know little about their turnover, it is becoming apparent that these fine roots are physiologically active for little more than a few weeks (Shane *et al.*, 2004c, 2005b; Playsted *et al.*, 2006). The root architecture of field-grown, root-cluster-forming species is patterned toward strongly soil-binding root-cluster development in upper soil layers where levels of nutrients are often enriched. Briefly, the basic root-cluster structures are as follows (the reader is referred to Lamont, 2003, for more details). Proteoid roots formed by species in Proteaceae are either 'simple' or 'compound', but occasionally both types are found on single root systems (e.g. in South African genera of *Leucadendron* and *Protea*; Lamont, 1983). Only a few genera within Proteaceae (e.g. Australian *Banksia* and *Dryandra* and South African *Orothamnus*; Lamont 1982, 1983) produce 'compound' proteoid root clusters (e.g. *Banksia grandis*; Fig. 3A). The 'compound' proteoid roots are essentially multiples of the 'simple' root clusters, but there are likely to be distinct ecophysiological reasons for the differences between the simple cluster roots and the compound clusters (Fig. 3), which tend to form dense root-mats in natural systems. Many proteacean genera (e.g. Australian *Hakea* and South African *Serruria*), and genera of other families [e.g. Fabaceae including the South African *Aspalathus* (rooibos) and the crop species *Lupinus albus*] produce 'simple' proteoid roots (Fig. 3B, E, F) that include numerous short, determinate rootlets, and each simple proteoid root is separated by unbranched regions (Fig. 3B). The main difference between the simple root clusters of Proteaceae (e.g. *Hakea*; Fig. 3B) and some species in the Fabaceae (e.g. *Aspalathus linearis*; Fig. 3F) is most notably the density of rootlets produced per unit root axis, which is far greater in the Proteaceae. Within

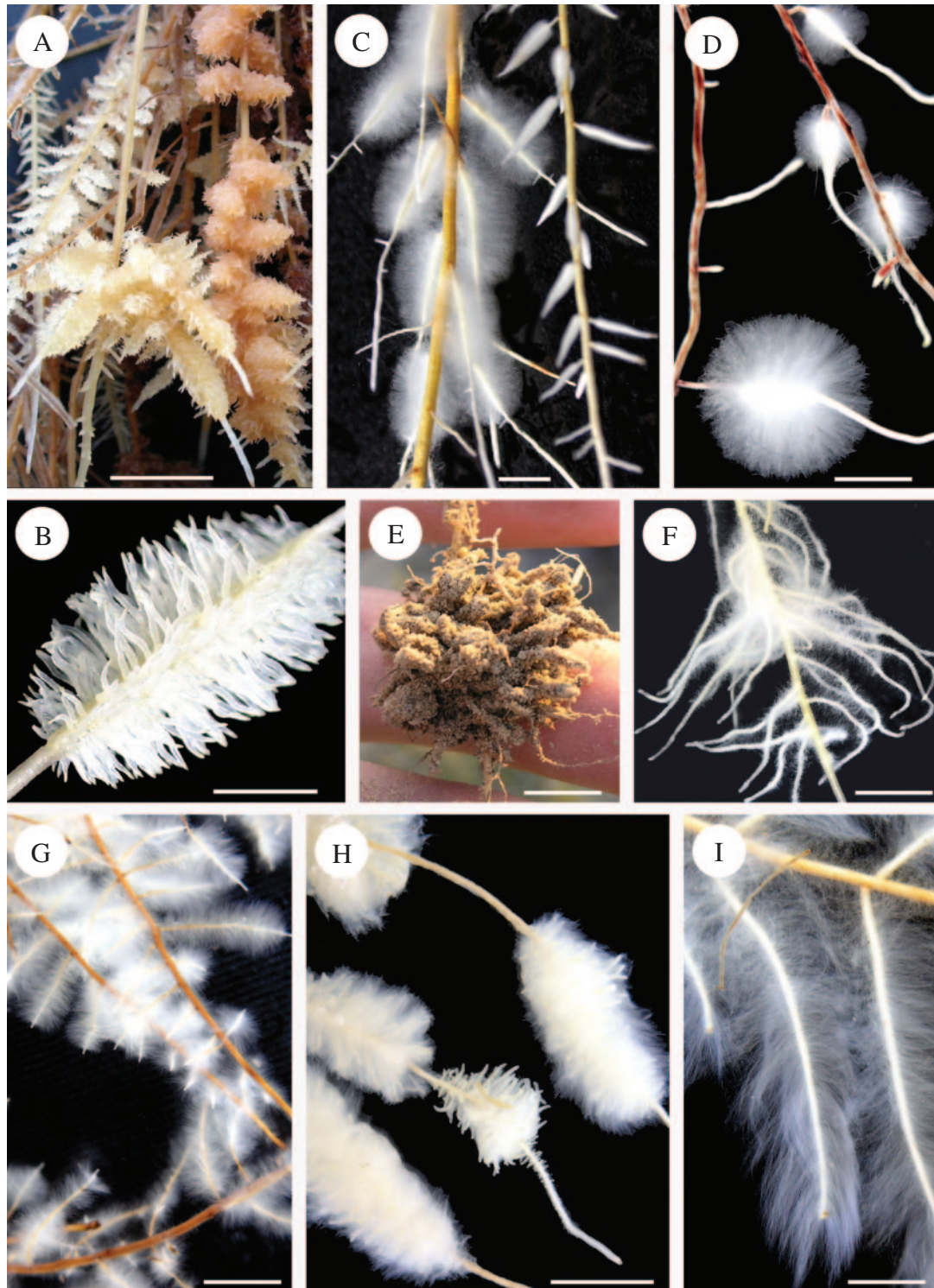


FIG. 3. Root-cluster morphologies induced in Proteaceae, Restionaceae, Cyperaceae and Fabaceae by a low supply of phosphorus. All species are well adapted to soils of extremely low P concentrations and endemic to the South West Botanical Province of Western Australia (WA), or the Cape Floristic Region of South Africa (SA). Plants were raised from seed (Proteaceae and Fabaceae) or cuttings (Restionaceae and Cyperaceae) collected from natural habitats, and then grown in nutrient solutions (glasshouses at the University of Western Australia or University of Cape Town) containing $\leq 1 \mu\text{M}$ [P] (except for the roots of a plant shown in E, which were collected in soil containing $\leq 10 \mu\text{g P g}^{-1}$ in Suid Bokkeveld, SA). (A) Proteoid roots (compound type) of *Banksia prionotes*; acorn banksia (Proteaceae, WA); scale bar = 13 mm. (B) Proteoid root (simple type) of *Hakea prostrata*; harsh hakea (Proteaceae, WA); scale bar = 4 mm. (C) Dauciform roots of *Lepidosperma squamatum* (Cyperaceae, WA); scale bar = 2 mm. (D) Dauciform roots of (*Tetraria* sp. (Cyperaceae, SA); scale bar = 2 mm. (E) Cluster root of *Aspalathus linearis*; rooibos (Fabaceae, SA); scale bar = 12 mm. (F) Cluster root of the same species shown in E grown in nutrient solution; scale bar = 4 mm. (G) Capillaroid roots of *Thamnochortus fracternus* (Restionaceae, SA); scale bar = 4 mm. (H) Capillaroid roots of *Mastersiella digitata* (Restionaceae, SA); scale bar = 6 mm. (I) Capillaroid roots of *Chondropetalum tectorum* (Restionaceae, SA); scale bar = 5 mm.

the genus *Lupinus*, some species, e.g. *L. albus*, produce easily recognizable root clusters, whereas others form structures that are distinct from non-cluster roots and have been termed ‘cluster-like’ roots by Hocking and Jeffery (2004), who showed that their physiology (below) is rather similar to that of the root clusters in *L. albus*.

Dauciform root clusters were first described by Russian plant scientists for Cyperaceae (sedges) (Selivanov and Utemova, 1969, and references cited therein). They were subsequently found in cyperacean species in Great Britain (Davies *et al.*, 1973; Ballard, 2001), continental Europe (Bakker *et al.*, 2005; Güsewell, 2005), and in many parts of Australia (Lamont, 1974; Phillips and Weste, 1984; Shane *et al.*, 2005a; Playsted *et al.*, 2006) and New Zealand (Powell, 1973). Lamont (1974) named them ‘dauciform’ roots, because of the carrot-shape of the dauciform root axis (Fig. 3C, D). Dauciform roots often occur in groupings of 20–30 individuals (Lamont, 1974) and each dauciform root may be as short as 2 mm, e.g. in *Carex* (cosmopolitan) species, or much longer, e.g. up to 12 mm in *Lepidosperma* (Western Australian) and *Tetrasia* (South African) species (Fig. 3C, D, respectively) (Lamont, 1974; Shane *et al.*, 2005a; Neumann and Römheld, 2006). Instead of the usual formation of dense clusters of short rootlets, the mature axis of a dauciform root is covered with dense clusters of long (approx. 2 mm; Fig. 3C, D) root hairs. The tips of the dauciform root axis are either indeterminate (and may form additional dauciform roots in sequence along the main axis; see Fig. 3C), or the dauciform root tip is determinate, and the entire dauciform root senesces (cf. figure 1 in Shane *et al.*, 2005b).

The monocotyledonous family of the Restionaceae has a Gondwanan distribution (Pate and Meney, 1999). Approximately 486 species are located mainly in Africa (over 300 species in South Africa) and in mainland Australia and Tasmania (approx. 150 species), and a few species are found in New Zealand, South America (Chile) and South East Asia (Indochina). Approximately half of the Australian taxa develop root clusters, especially species adapted to arid environments, where their development begins only after the onset of seasonal rains (figure 1.3 in Meney and Pate, 1999). These ‘capillaroid’ roots were discovered and named by Lamont (figure 4 in Lamont, 1982), and are characterized by clumps of roots or rootlets, densely covered with exceptionally long root hairs (Fig. 3G–I). Their name (capillaroid) stems from the sponge-like properties on holding soil water (Lamont, 1980, 1982). Little is known about their structure and development in species of Restionaceae and how these specialized roots contribute to plant nutrition and water balance. We have recently found root clusters in South African Restionaceae that are remarkably ‘proteoid-like’ in their morphology (Fig. 3H), and produce distinct (ephemeral) clusters separated by unbranched main root axis. However, most species observed thus far have the morphology typical of that shown in Fig. 3G, I. We hypothesize that the physiology and functioning of capillaroid roots is similar to that of proteoid roots.

Most physiological information about root-cluster functioning has been derived from studies of *L. albus*, but much has also been discovered about root-cluster functioning in native plants adapted to soils of extremely low P_i concentration. One of the most important aspects is the importance of the influence of the stage of development on root-cluster functioning. The finding that carboxylate (e.g. citrate) release in *L. albus* (Watt and Evans, 1999) and in proteacean species such as *Hakea* (*H. prostrata*, Shane *et al.*, 2004c; *H. undulata*, Dinkelaker *et al.*, 1997) occurs in an exudative burst strongly supports the view that root development and physiological activity are closely linked as the components of root systems grow and mature (McCully, 1999). Studies of the ‘compound’ (mat-forming) proteoid roots of *Banksia integrifolia* have also shown that carboxylates, such as citrate, are released into the rhizosphere (Grierson, 1992; Roelofs *et al.*, 2001), but there are no reports on the time course of carboxylate exudation in taxa that produce compound roots. It has now become apparent that dauciform roots in Cyperaceae, although morphologically and anatomically very distinct, also release carboxylates (citrate) in large quantities during a developmentally programmed exudative burst, thus functioning in a way very similar to proteoid roots (Shane *et al.*, 2005b, 2006; Playsted *et al.*, 2006). Another parallel between dauciform and proteoid roots is that both are suppressed when plants have a high P status. Like proteoid roots, dauciform roots release a variety of other compounds, as further discussed below. There is no physiological information on capillaroid roots.

In summary, root clusters differ greatly in their anatomy and morphology, but are rather similar with respect to their physiology. Our knowledge on capillaroid roots is restricted to their anatomy and morphology; their physiology remains to be investigated. The release of carboxylates from root clusters of Proteaceae and Fabaceae, and dauciform roots of Cyperaceae in an exudative burst is bound to be vital for their function, as further discussed in the section ‘Root clusters: combining structure and functioning’.

PHYLOGENY OF ROOT-CLUSTER-FORMING SPECIES

Proteoid root clusters were perhaps once considered as a curiosity associated with many proteacean species (Purnell, 1960) and *L. albus* (Gardner *et al.*, 1983). However, proteoid roots have since been described for a wide range of species in families that are not at all closely related to Proteaceae (Shane and Lambers, 2005a). Although very few species in the genus *Lupinus* produce the kind of clusters that are found in *L. albus* (Clements *et al.*, 1993; Skene and James, 2000), many others produce ‘cluster-like roots’, which function in a similar way to the ‘true cluster roots’ (Hocking and Jeffery, 2004). Within the Cyperaceae (sedges), dauciform roots are restricted to two tribes: Cariceae and Rhynchosporeae (Lamont, 1981). Outside the Cyperaceae, dauciform

roots have only been reported for *Juncus pauciflorus* and *J. squarrosus* (Juncaceae, reeds) (Powell, 1973). Capillaroid roots have been reported exclusively in the Restionaceae (rush) (Lamont, 1982). Viewed in this way, it is obvious that root clusters (a term we use here to refer to all types of clusters: simple and compound proteoid or cluster roots, dauciform roots, capillaroid roots, cluster-like roots; Fig. 3) are actually more widespread in the plant kingdom than considered before.

Root clusters are found in two large monocotyledonous families: Cyperaceae (dauciform roots) and Restionaceae (capillaroid roots) (Fig. 4). Root clusters also occur in several dicotyledonous families. Proteoid roots were first discovered in the Proteaceae (Purnell, 1960), which belong to the Proteales (Eudicots). This accounts for the name 'proteoid' roots. There are no records of root clusters in other families within the Proteales; while growing over a year in low-P nutrient solution in the glasshouse, *Platanus hybrida* (Platanaceae, Proteales) never produced any root clusters (H. M. Stace and H. Lambers, unpubl. data). Apart from the Proteales, within the eudicots, root clusters have been described in Core Eudicots (Rosids) only; that is, they occur in several families that are phylogenetically very distantly related to the Proteaceae (Fig. 4) (Skene, 2000; Shane and Lambers, 2005a). Within the Rosids, root clusters occur in four orders belonging to the eurosids I (fabids): Fagales (Betulaceae, Casuarinaceae, Myricaceae), Cucurbitales (Cucurbitaceae), Rosales (Elaeagnaceae, Moraceae) and Fabales (Fabaceae). Root clusters have obviously evolved several times. What is very striking is that actinorhizal species belonging to different orders and families, namely Betulaceae (Fagales), Casuarinaceae (Fagales), Eleagnaceae (Rosales) and Myricaceae (Fagales), all have the capacity to produce root clusters. Why is there an association between root-cluster-bearing habit and being actinorhizal? Does a plant's capacity to recognize and form an association with *Frankia* species have something in common with its capacity to develop clusters? Further investigations of the signal-transduction pathways involved in the actinorhizal symbioses and root-cluster formation may lead to fascinating new discoveries. In addition, a careful study of species belonging to the four other actinorhizal families, Rosaceae, Rhamnaceae (both Rosales), Coriariaceae and Datisceae (both Cucurbitales) (Swensen, 1996; Vessey et al., 2005), might well reveal more records of cluster-root-bearing species.

Root clusters are no longer the curiosity restricted to plants from 'down under', but occur in many distantly related families throughout the plant kingdom. Many species are used as crops, for nuts (*Macadamia* species), a source of protein (*Lupinus* species), tea (*Aspalathus linearis*), timber and pulpwood (*Grevillea* species) (Shane and Lambers, 2005a). Others may be used in pastures; for example, in eastern Canada and western North America some sedges (*Carex* sp.) are recognized for their potential for use as forage for grazing (Uresk, 1986; Catling et al., 1994), and *Kennedia* species for introduction as food (Rivett et al., 1983) or pasture plants (Cocks, 2001). Considering our dwindling P reserves,

these cluster-bearing species need to receive greater emphasis in future research.

RELATIONSHIPS BETWEEN P-ACQUISITION STRATEGIES AND SOIL TYPES

Having discussed the structure and functioning of root clusters in different plant families, we now explore where root-cluster-bearing species fit in the landscape. The Western Australian flora offers a unique opportunity to explore that question. Both non-mycorrhizal, cluster-bearing species belonging to the Cyperaceae and Proteaceae, and mycorrhizal species without root clusters are common in south-western Australia, a global biodiversity hotspot (Myers et al., 2000). In addition, there are several species that are both mycorrhizal and cluster-bearing (e.g. Casuarinaceae and Fabaceae). Are the different nutrient acquisition adaptations distributed randomly or linked to certain habitat factors? We can address this question in some detail using McArthur's (1981) detailed descriptions of soil and vegetation for 150 reference sites in south-western Western Australia. Non-mycorrhizal, cluster-bearing Proteaceae predominate on the most P-impooverished soils in the region, whereas mycorrhizal Myrtaceae without root clusters predominate on soils that have somewhat higher P levels (Fig. 5). Whereas Myrtaceae in Western Australia generally dominate forests and tall woodlands, and Proteaceae generally dominate shrublands/heaths and low woodlands, the occurrences of species of the two families are by no means mutually exclusive. Proteaceae understorey species in eucalypt woodlands and in highly diverse mixed heaths of Proteaceae/Myrtaceae are very common. The region offers unique opportunities to study specialization to soil types and coexistence of species with different nutrient acquisition strategies. This flora will allow us to discover the relative advantages of root adaptations as dependent on different soil conditions (Fig. 5), but further work is needed to appreciate the more intricate relationships between soils and roots. Very little information is available for Casuarinaceae, which are mycorrhizal as well as cluster-bearing (Reddell et al., 1997). However, the scarce available data indicate an intermediate position for this group between the cluster-bearing Proteaceae of lower-P soils and the mycorrhizal Myrtaceae of higher-P soils. Solid data for the distribution of cluster-bearing Cyperaceae, Fabaceae and Restionaceae as dependent on soil P levels are lacking, and hence we can only speculate where they fit in Fig. 5.

In summary, the vegetation of Western Australia's global biodiversity hotspot, located on ancient, heavily weathered soils, offers unique opportunities to study the intricate relationships between soils and vegetation, discussed in this review. This flora will allow us to discover the relative advantages of root adaptations as dependent on different soil conditions (Fig. 5), but further work is needed to appreciate the more intricate relationships between soils and roots.

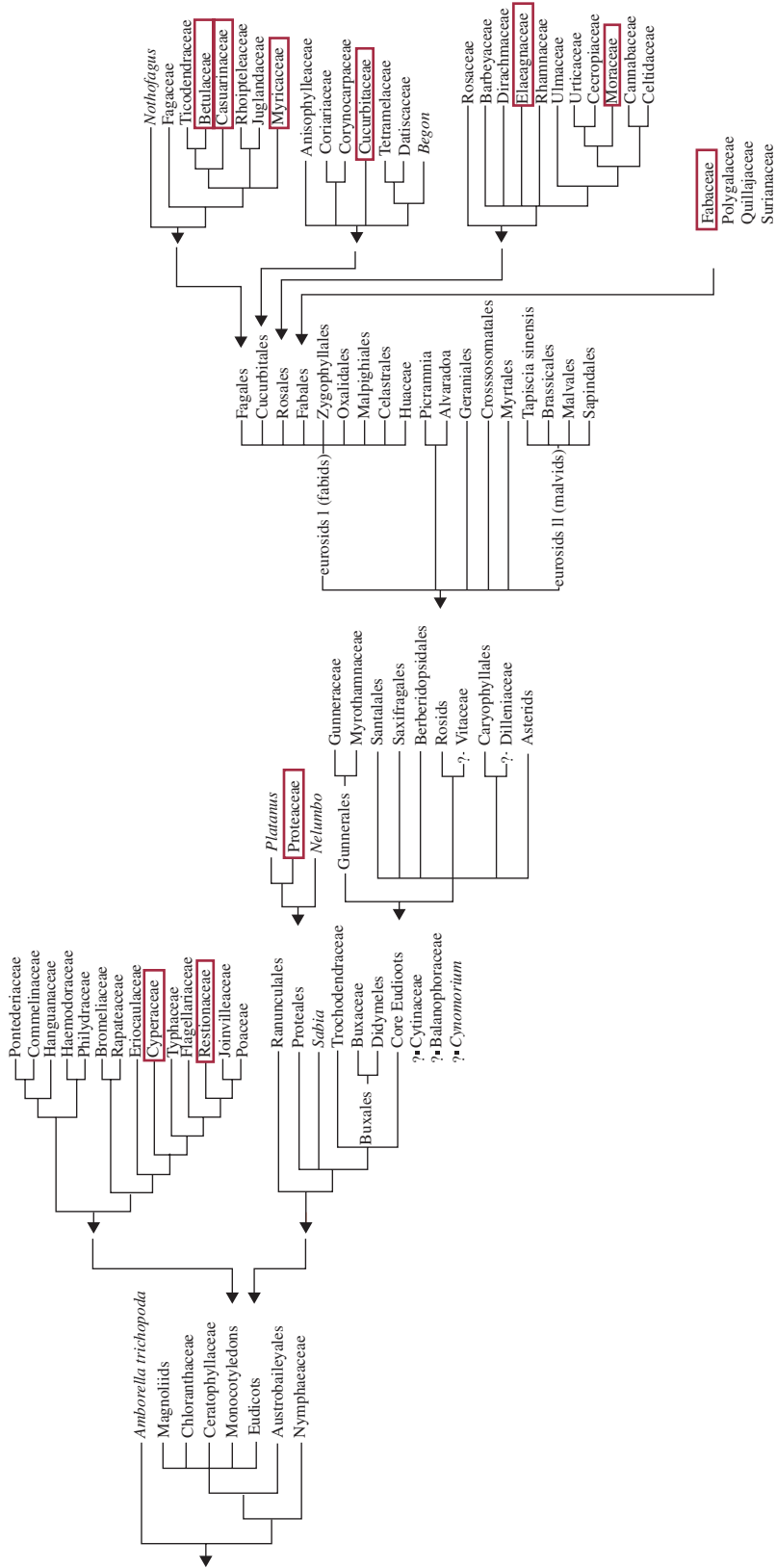


FIG. 4. Phylogeny of root clusters, based on references cited in the text and on <http://itolweb.org/tree/?group=angiosperms>. Root clusters occur in two monocotyledonous families and eight dicotyledonous families belonging to the Proteales and the Core Eudicots.

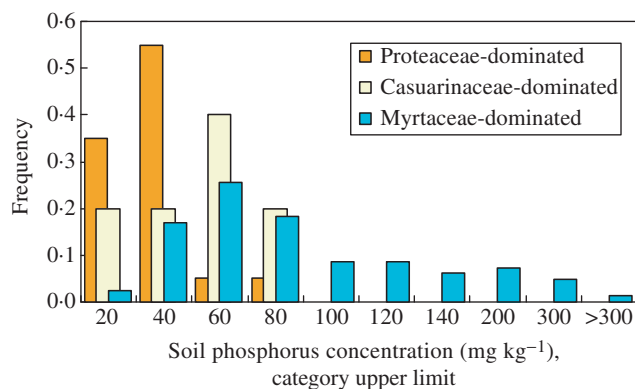


FIG. 5. Distribution of species belonging to Proteaceae, Casuarinaceae and Myrtaceae, three major plant families in south-western Australia, as dependent on soil P concentration. The figure is based on information in McArthur (1981) and was prepared as follows. All reference sites where the main species (trees in the case of forests and woodlands, shrubs in the case of shrublands) were either Proteaceae ($n = 20$ sites), Myrtaceae ($n = 82$) or Casuarinaceae ($n = 5$) were selected, and the total P concentration of the A₁ horizon was tabulated. Total P was analysed by the Kjeldahl method (Murphy and Riley, 1962). Then, for each family group, the relative frequency of occurrence of P concentration classes was calculated. Total P in the A₁ horizon correlated well with total P in the B₁ horizon and with bicarbonate-extractable P in the A₁ horizon (data not shown).

ROOT CLUSTERS: MATCHING STRUCTURE AND FUNCTIONING

Roots of many species release exudates under low-P conditions, but most would not survive in the ancient and severely weathered soils where proteoid roots are so common (Fig. 5). What exactly determines the success of these non-mycorrhizal Proteaceae on the world's most P-impooverished soils? We propose that it is their 'specialized' morphology and anatomy (structure), which is matched by their 'specialized' physiology and biochemistry (functioning) (Shane and Lambers, 2005a). It should be added, however, that those specializations are merely matters of programming aspects of cellular structures and functions that are *not* unique to root clusters. Lateral-root formation is a universal process in plants, but only the co-ordinated development of hundreds of lateral roots can give rise to the structures we call cluster roots (Fig. 3). Similarly, production of carboxylates (e.g. citrate and malate) occurs in all plant cells; however, their release in an exudative burst has only been described for root clusters.

In a comparative study of a number of crop species, many of which are known for their release of relatively large quantities of carboxylates, we found that the species that combined carboxylate release with root-cluster formation out-performed other species when grown at severely limiting P_i supply (Fig. 6). An even more convincing case has been made by Paul Reddell (pers. comm.), who compared a number of rainforest proteacean species from north-eastern Australia. Most of these were non-mycorrhizal and cluster-bearing, as expected. However, one of these species was mycorrhizal and did not produce root clusters. Most significantly, this non-

cluster-bearing mycorrhizal species was outperformed in terms of biomass production at the lowest soil P_i levels, which confirms our hypothesis on the significance of the cluster-root habit. Bolland *et al.* (2000) carried out field experiments, using three high-exuding *Lupinus* species, two with root clusters (*L. albus* and *L. luteus*) and another without root clusters (*L. angustifolius*) (Hocking and Jeffery, 2004). Their results further confirm that root clusters confer a distinct advantage when soil P_i levels are severely limiting for growth in plants that lack these specialized structures.

Carboxylates are a major component of the exudates released by root clusters, and probably the ones most effective at mobilizing phosphorus, but they are not the only component. As briefly discussed above, root clusters often also release phenolics. Although these phenolics may mobilize some phosphorus, it is more likely that their ecophysiological role is to inhibit microbial breakdown of exuded carboxylates (Neumann and Römheld, 2001). Weisskopf *et al.* (2006) point out three distinctive mechanisms to inhibit microbial breakdown of exudates released from *L. albus*. First, acidification would slow down breakdown by bacteria. Secondly, excretion of phenolics (mainly isoflavonoids) during the exudative burst slows down fungal metabolism because it leads to fungal sporulation. Finally, release of antifungal cell-wall-degrading enzymes (chitinase and glucanase) prior to the exudative burst would inhibit fungal growth. The fact that exudative bursts result in very high concentrations of carboxylates in the rhizosphere will in itself maximize the effect of the exuded carboxylates, because it allows them to act before microbial populations build up. Additional protection of exuded carboxylates by the mechanisms discovered by Weisskopf *et al.* (2006) in *L. albus* further enhances their efficiency. It is therefore highly unlikely that micro-organisms play a significant role in mobilizing phosphorus in the rhizosphere of *L. albus* cluster roots (Weisskopf *et al.*, 2006). In Proteaceae (e.g. *Banksia attenuata*; Marschner *et al.*, 2005) and *L. albus* (Marschner *et al.*, 2002) different bacterial communities are associated with different age classes of proteoid roots, and with proteoid and non-proteoid roots. Furthermore, phosphate-solubilizing bacteria associated with proteoid roots (but not with the non-proteoid roots) of *Teloplia speciosissima* (waratah) increase the solubility of calcium phosphate (Wenzel *et al.*, 1994). However, what remains to be investigated is whether the differences in microbial communities emerged before or after the exudative burst. The bacterial communities may function in P cycling in the ecosystem, but whether they actually enhance the availability of P for the plant remains to be demonstrated. Alternatively, they might increase the rate of decomposition of root clusters after these have depleted most of the P that has been made available by carboxylates and phosphatases.

Some ectomycorrhizas also release carboxylates (e.g. oxalate) and protons, and this will enhance the availability of P_i (e.g. Arvieu *et al.*, 2003). However, as with non-cluster-bearing proteacean and *Lupinus* species, which are less effective at accessing P_i when soil levels are very low

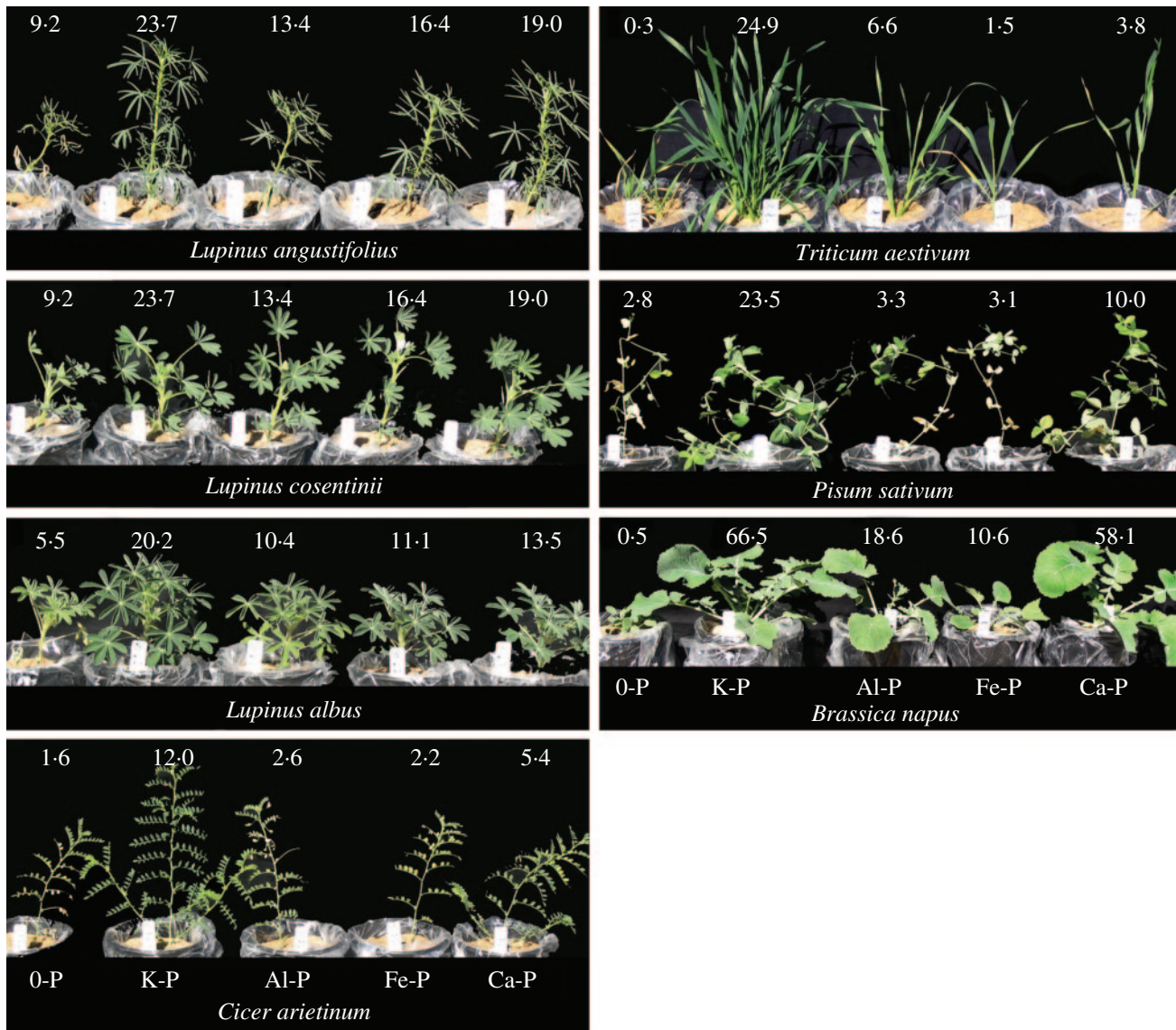


FIG. 6. Shoot fresh weights (g) of several crop species grown for 7.5 weeks supplied with basal nutrients and 0 (0-P) or 40 mg P kg⁻¹ dry sand in the form of soluble KH₂PO₄ (K-P), or sparingly soluble AlPO₄ (Al-P), FePO₄ (Fe-P), or Ca₅OH(PO₄)₃ (Ca-P) (n = 14). When P_i is supplied in sparingly soluble forms, *Lupinus albus* and *L. cosentinii*, which combine high carboxylate release with the formation of cluster roots, performed best.

than their cluster-bearing counterparts, ectomycorrhizal species without root clusters are expected to be less effective than non-mycorrhizal species with clusters.

While compound proteoid roots of, for example, *Banksia* (Grierson, 1992) species appear to function in much the same way as the simple clusters of, for example, *Hakea* (Shane *et al.*, 2004c) and *Lupinus* (Watt and Evans, 1999), their morphology is strikingly different (Fig. 3). The greater complexity of compound cluster roots, however, is only due to one extra branching order. Both in the field and in hydroponics, the rootlets of compound clusters develop synchronously, very similar to simple cluster roots. Simple clusters are not necessarily smaller than compound clusters: Lamont (2003) reported simple clusters of *Hakea prostrata* of 200 mm long and 70 mm wide. Both simple and compound root clusters are

concentrated in surface soils (A₀ and A₁ horizons), but compound clusters tend to form root mats (Lamont, 2003). In ancient, nutrient-impoverished soils, the extremely low concentration of P_i in the soil may result in extremely competitive P recycling. In an ecosystem comprising many species similarly constrained by available P, variation in the strategies for acquisition of P_i as a limiting resource may be important for coexistence. Functionally, mats of surface cluster roots may be similar to root mats found in other nutrient-poor ecosystems with tight nutrient cycles, e.g. tropical rain forests on white sands (Cuevas and Medina, 1988). Such root mats minimize nutrient losses by scavenging of nutrients directly from decomposing litter, or even through contributing to litter decomposition (i.e. release of phosphatases, Grierson and Comerford, 2000; possibly peptidases, Schmidt *et al.*,

2003). Although proteoid roots may form a virtually continuous fabric near the soil surface due to the persistence of senesced cluster roots (Gould, 1998), our field observations suggest that root clusters in their active mature state represent only patches within this mat. The information currently available does not allow us to identify any clear functional difference between simple and compound root clusters. We propose that their functioning as clusters is similar, but that simple and compound clusters have different implications for root-system architecture that may place different constraints or create different opportunities for optimal placement in soil horizons and nutrient-enriched patches. The carbon costs of construction and functioning of cluster roots are high (see below) and dense mats of compound cluster roots are likely to be especially costly. Compound clusters may be more suitable for placement within an existing network of roots (as in a root mat) wherever a favourable (humus-rich) patch appears. Thus, in *Banksia* woodlands of Western Australia where leaf litter accumulates, proliferation of mats of particularly compound cluster roots just below the leaf litter may facilitate competitive recycling of a resource that is distributed fairly homogeneously (Lamont, 1982). By contrast, in other environments where leaf litter accumulation is less, the resource may be distributed more patchily within the soil and justify the formation of opportunistic simple cluster roots. This is probably more the case in the fire-prone serotinous proteacean 'fynbos' of the Cape area of South Africa where leaf litter production is low (78 g m^{-2}) compared with the Australian 'kwongan' ($194\text{--}409 \text{ g m}^{-2}$) (Stock and Allsopp, 1992). In this system fire plays a major role in recycling P. Soil levels of P_i increase significantly after fire and allow early growth of seedlings, following release of seeds from serotinous structures; however, soil P_i rapidly returns to pre-fire levels as the vegetation regrows (Stock and Allsopp, 1992). Simple cluster roots may be more compatible with more explorative root-foraging strategies in which clusters are formed on long axes, which can continue to grow when the clusters senesce. It would be interesting to determine whether compound cluster roots differ from simple cluster roots in their ability to mobilize organic P, and whether the biogeography and ecology of species producing these differing roots are distinct.

The effectivity of both simple and compound clusters is based on a short and intensive 'extraction' of soil P_i in a confined volume of soil. This is accomplished by high rootlet density and synchronous exudation of P-mobilizing and antimicrobial compounds (in *L. albus*), and followed by fast uptake of P_i . Changes of rhizosphere pH and moisture content may assist in P mobilization and uptake, as described above, resulting in divergent root-cluster specializations. The morphological distinction between compound and simple cluster roots is obvious, but there are probably many other, less obvious distinctions in the composition of exudates from cluster roots of species growing in different habitats. For example, although growing in the same area (mere metres apart), *Protea obtusifolia* and *Leucadendron meridianum* occur exclu-

sively in shallow pockets of limestone-derived soils, while *Protea susannae* and *Leucadendron coniferum* occur exclusively on adjacent, uniformly deep colluvial sands (Mustart *et al.*, 1994). It would be interesting to explore the capacities of the cluster roots of these species to exploit the soils on which they occur. In the Cyperaceae and species from other families, it has been found that calcifuge species exude more acetic acid whereas calcicole species exude more citric and oxalic acid, possibly because of the differential capacity of these carboxylates to solubilize Fe and P_i from the respective soils (Ström *et al.*, 1994). In environments where cluster-root-forming Proteaceae, capillaroid-root-forming Restionaceae and dauciform-root-forming Cyperaceae co-occur, it would be intriguing to establish whether these diverse structures are truly functional analogues, or whether functional distinctions contribute to coexistence. It is likely that these structures all rely on carboxylate exudation for mobilizing inorganic and organic P, as shown for Proteaceae (Shane *et al.*, 2004c) and Cyperaceae (Shane *et al.*, 2005b, 2006; Playsted *et al.*, 2006). However, the composition of those carboxylates (Lambers *et al.*, 2002) and the accompanying enzymes (e.g. Gilbert *et al.*, 1999) and phenolics (Weisskopf *et al.*, 2006) may vary with the nutritional niche that the species occupy.

In summary, current evidence shows that the success of cluster-bearing, non-mycorrhizal species in low-P soils is based on a combination of root structure (root clusters) and root functioning (production and release of carboxylates and other exudates). 'Root clusters' as a collective term for cluster roots (e.g. Proteaceae, Fabaceae), capillaroid roots (Restionaceae) and dauciform roots (e.g. Cyperaceae) have diverse anatomical structures, but most probably are all an adaptation to the constraint of low concentration and sparingly soluble P. However, it is possible that there are diverse variations on the 'root-cluster' strategy to deal with diverse environments.

EFFECTS OF FAST-EXUDING SPECIES ON NEAREST NEIGHBOUR AND SPECIES IN CROP ROTATIONS

Provided roots of other species are positioned close enough to active root clusters of their neighbours, they are expected to benefit from the activity exhibited by these clusters. Such a beneficial effect of *L. albus* on the growth and P content of *Triticum aestivum* was demonstrated by Horst and Waschkies (1987). Cu *et al.* (2005) subsequently showed that *L. albus* monocultures preferentially depleted a citric-acid-leachable soil P_i pool, whereas *T. aestivum* monocultures preferentially depleted the water-leachable soil P_i pool. The mixed cultures depleted both pools. The *L. albus* monocultures lowered the soil pH by 0.3 pH units, whereas the *T. aestivum* monocultures raised it by 0.8 pH units; the mixed cultures gave a soil pH intermediate between the two monocultures. Thus, plants of one species may partially offset the effect on soil pH caused by the other. Cluster-root-bearing crop plants

such as *L. albus* as well as high-exuding non-cluster-bearing species would appear to have enormous potential as intercrops (L. Li *et al.*, 2003; Zhang and Li, 2003; S. M. Li *et al.*, 2004; Hauggaard-Nielsen and Jensen, 2005). However, there is also competition between intercropped species, and the effects on growth and yield are therefore not invariably positive (Dessougi *et al.*, 2003). We are unaware of any experimental results for native species in their natural habitats, but envisage that very positive interactions also occur between plants of cluster-root-bearing species and their non-cluster-root-forming neighbours.

Beneficial effects of species with a large capacity to mobilize soil P are not restricted to neighbouring plants, but may extend to the following crop (Kamh *et al.*, 1999). Plants of *Zea mays* grown after *Brassica napus* or *Beta vulgaris* took up more P_i in the presence of a preceding crop's residue (Dessougi *et al.*, 2003). Similar positive effects were observed for both *Z. mays* (Kamh *et al.*, 2002) and *Triticum aestivum* (Nuruzzaman *et al.*, 2005) grown after a legume; the legume was grown with sufficient nitrogen, and the effect was ascribed to P mobilization and independent of the N₂-fixing potential of the legumes. Little *et al.* (2004) showed that Olsen-extractable P in plots 8 weeks after sowing potatoes was enhanced after growing *L. albus* or a combination of *L. albus* and *B. napus* as a cover crop relative to that after *Avena sativa* or *B. napus* alone. These results provide evidence that cover crops containing *L. albus* potentially enhance the P_i availability for the following crop. However, it is very unlikely that the carboxylates persist long enough to be of benefit to the next crop; we hypothesize that *L. albus* roots cause a shift from less available to more available P_i pools.

In summary, high-exuding species, and especially those with root clusters, can have a positive effect on neighbouring crop plants as well as on the following crop. The potential of these P-mobilizing species has been studied in some detail, but much is still to be discovered, especially on native plants in natural systems.

PERSPECTIVES FOR CROP PLANTS IN A WORLD WHERE P RESERVES ARE BEING DEPLETED

As discussed above, many species occurring on severely P-impoorished soils in south-western Australia and the Cape region in South Africa exhibit adaptive root specializations (root clusters) that enhance the availability of P_i in the rhizosphere. Root clusters are a combination of adaptive structures with adaptive physiology. Root clusters are not restricted to species from these Mediterranean regions, but also occur in a large number of species elsewhere in the world (e.g. Cyperaceae) as well as in several crop species. Given the remarkable similarity of form and function among root clusters from distant families, indicating that the structures evolved independently a number of times, the evolution of these traits appears to be a result of intensification of certain common

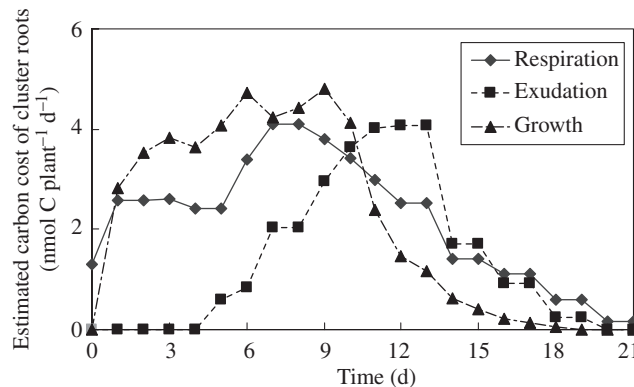


FIG. 7. Estimated carbon costs of cluster roots in *Hakea prostrata* (total plant fresh mass was approximately 600 g). While the clusters are active, 52–100% of daily produced photosynthate is utilized in growth, respiration and carboxylate exudation. On an annual basis, these figures should be divided by 4–6, because the clusters of *H. prostrata* are only active in the wet season (2–3 months). The calculations are based on primary data presented on growth, respiration and carboxylate exudation in Shane *et al.* (2004c) and on leaf photosynthesis, total leaf area and total root cluster in Shane and Lambers (2005b), assuming that half of the clusters harvested by Shane and Lambers (2005b) were older than 21 d, and hence dead.

existing elements; therefore, incorporating these traits in new crop species seems to be a reasonable proposal (Skene, 2003). Considering that P reserves are rapidly being depleted, while vast amounts are present in soils that have been fertilized for decades, we should consider options for incorporating root clusters in new crop species. The situations where this strategy would be favourable occur where considerable amounts of P_i can be mobilized that would otherwise remain unavailable. It is important that the soil physicochemical and biological processes that support plant growth remain in place. Because the superior P_i acquisition of high-exuding plant species is based on a localized chemical extraction of soil, certain risks, including decreased pH and excessive P_i depletion, will need to be assessed.

Several advantages of a large capacity to mobilize P in the rhizosphere, especially by root clusters, have been discussed above. Are there also downsides that need to be considered before aiming to introduce root clusters in new crops? In the next paragraphs we discuss possible disadvantages: high carbon costs of root clusters, mobilization of potentially toxic ions in the rhizosphere, and increased risks of P leaching from soil.

There are obviously costs involved in P mobilization through root-cluster production and functioning. We have used information on leaf photosynthesis, total leaf area and root-cluster production of *Hakea prostrata* (Shane and Lambers 2005b), combined with data on growth, respiration and carboxylate exudation of the same species (Shane *et al.*, 2004c), to estimate these costs. Our very rough estimates based on these two papers indicate that well over half of all the carbohydrates produced in photosynthesis are required for the growth, respiration and carboxylate exudation of cluster roots in *H. prostrata* (Fig. 7). This strategy is therefore not one that should be

adopted for new crops because it would seriously compromise crop growth; however, this value needs to be qualified. First, cluster roots of *H. prostrata*, which naturally occurs in a Mediterranean (winter rainfall) environment, are only produced during the wet season (Lamont, 1982) and function only at very low plant P status (Shane *et al.*, 2003). That is, the calculated value should be divided by 4–6 to express the result per annum, as the cluster roots are only active for 2–3 months per year. That brings the estimate much closer to the only other, equally rough, estimate for *L. albus*: 23 % of whole-plant photosynthate production (Dinkelaker *et al.*, 1989). These estimates are also fairly similar to the estimated cost of 7–20 % to sustain mycorrhizal symbioses (Lambers *et al.*, 1998). Secondly, the root clusters are not only involved in uptake of P, but acquire most other nutrients as well, in particular micronutrients (Shane and Lambers, 2005b) and nitrogen (Schmidt *et al.*, 2003; Hawkins *et al.*, 2005).

Carboxylates not only mobilize nutrients in the rhizosphere needed for growth, but may also enhance the availability of toxic ions, e.g. heavy metals. In a field experiment on an acidic, lateritic ironstone gravel sand, Brennan and Bolland (2003) found greater uptake of cadmium in *L. luteus* (with root clusters) than in *L. angustifolius* (without clusters). Similarly, Brennan and Bolland (2005) found greater uptake of cadmium in *Brassica napus* (high exudation, no root clusters) than in *Triticum aestivum* (low exudation, no clusters). This is obviously a risk that needs to be considered carefully, especially in view of the fact that many sources for P fertilizer are contaminated with cadmium.

Species that effectively mobilize P in the rhizosphere might do this to the extent that P is leached from superficial soil layers down the profile, and hence contribute to pollution of streams and rivers, contributing to their eutrophication (Djordjic *et al.*, 2004; Fortune *et al.*, 2005). This risk should be managed by measuring both agronomic and environmental soil P saturation, and fertilizing accordingly (Maguire and Sims, 2002). We consider this to be a risk only on soils with a very low capacity to sorb P and that are heavily fertilized; that is, on soils where the ability of crop species to mobilize P would not confer a yield advantage. These are rarely the soils used for agriculture; if they are, (Weaver and Prout, 1993), and if P-mobilizing species are used, then the P-fertilization regime obviously has to be very closely monitored.

Provided the risks of enhanced cadmium uptake and eutrophication are carefully managed, P-acquisition efficient new crops, especially high-exuding, cluster-bearing crops, offer tremendous potential. New crops with improved traits as listed above under ‘Common root adaptations to enhance P_i acquisition’ can be developed using an analysis of quantitative trait loci (QTL) (e.g. Hu *et al.*, 2001; Yan *et al.*, 2004; J. Zhu *et al.*, 2005; Su *et al.*, 2006). Rather than restricting ourselves to crop species that are currently commonly used, we could explore the potential of as yet non-domesticated species with superior P-acquisition traits or use species that are frequently

referred to as ‘lost crops’; that is, species that were commonly used several decades ago, but are no longer mainstream species today. It is also likely that breeding for the modern cultivars of high-yielding crops has selected against efficient P-acquisition strategies, which in early selections in a high-P environment might have compromised yield. Crossing modern cultivars with ‘heritage’ crops is an obvious potential strategy. Wide crosses offer possibilities to introduce root clusters into *Lupinus* species that lack this trait. We have a good understanding of the phylogeny of the genus *Lupinus* (Ainouche and Bayer, 1999) and of the species within this genus that develop ‘true clusters’ (Clements *et al.*, 1989) or ‘cluster-like roots’ (Hocking and Jeffery, 2004). This should offer perspectives to develop new lupin crops with root clusters. Although root clusters are clearly efficient adaptations for P_i acquisition, in some crops selection for single characters such as longer root hair length may be sufficient to enhance access to P.

Finally, further investigations of the developmental processes involved in cluster-root formation should identify key genes that allow the production of root clusters. Such a molecular approach appears to be most promising for the incorporation of cluster roots in crops that currently lack them, and the tools for such bioengineering are at hand, including the ability to clone root-specific genes with root-tissue-specific promoters that are regulated by nutritional demands (Bucher, 2002). In order to increase P-acquisition efficiency of non-cluster-rooted species, alteration of the architecture of the root system, secretion/exudation of chemical compounds and enzymes into the rhizosphere, and enhanced uptake of P_i would be required. Some progress has been made towards bioengineering enhanced P_i acquisition into plant roots. Selection for longer root hairs may be possible (Rengel and Marschner, 2005). The *eto1* mutant plants of *A. thaliana*, which synthesize more ethylene and have longer root hairs than wild-type plants (Pitts *et al.*, 1998), provide a possible mechanism for altering root architecture. Enhanced uptake of insoluble P (hydroxyapatite) and improved growth has been reported for tobacco constitutively expressing a heterologous citrate synthase (López-Bucio *et al.*, 2000). Over-expression of P_i transporters has been shown to increase P_i uptake by suspension cultures, although there has been no success in increasing whole-plant P_i uptake in barley, probably due to regulation of the homologous P_i transporter utilized (Rae *et al.*, 2004). *A. thaliana* transformed with heterologous phytase secreted the enzyme only from roots when grown on medium containing low P_i concentrations, enabling growth on phytate as a sole P source (Mudge *et al.*, 2003). However, as discussed above, none of the structures and pathways associated with growth and functioning of root clusters is unique for root clusters. What determines if a cluster will be formed or not is the programming of the structures and processes. Thus, modification of regulatory elements may be a more appropriate mechanism than introduction of heterologous genes. It is envisaged that root-cluster-forming species will soon be used to study species-specific development for which *A. thaliana*

is not a good model (Van Lijsebettens and Van Montagu, 2005).

If we wish to apply information gleaned from native plants for cropping and pasture systems, we should not just be thinking of new crop species and bioengineering. We should also consider new cropping systems, where combinations of species in intercropping systems and ideal rotations are used to maximize the acquisition of P_i from low-P soils. Equally, and although not the focus of this review, there are possibilities to enhance P-use efficiency, in addition to improved P-acquisition efficiency. These approaches should lead to more sustainable cropping systems with less off-site risks of eutrophication of streams and rivers.

CONCLUDING REMARKS

Global P reserves are rapidly being depleted, whilst agricultural soils that have been fertilized for decades contain substantial amounts of P that cannot be accessed by plants lacking specific root adaptations. To acquire soil P_i more efficiently, new crops need to be developed, and there should be a strong focus on species with root clusters, as these represent a combination of form and function that is highly desirable in a world where P will be harder to obtain. There is still much to be learned regarding the role of root clusters in natural systems, and it is envisaged that new knowledge based on investigations of such systems will further enhance our potential to develop new crops and cropping systems, which use P more efficiently.

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