

Leaf manganese accumulation and phosphorus-acquisition efficiency

Hans Lambers¹, Patrick E. Hayes¹, Etienne Laliberté¹, Rafael S. Oliveira^{1,2}, and Benjamin L. Turner^{3,1}

¹ School of Plant Biology, The University of Western Australia, Stirling Highway, Crawley (Perth), WA 6009, Australia

² Departamento de Biologia Vegetal, Universidade Estadual de Campinas, Rua Monteiro Lobato 255, Campinas 13083-862, Brazil

³ Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Republic of Panama

Plants that deploy a phosphorus (P)-mobilising strategy based on the release of carboxylates tend to have high leaf manganese concentrations ([Mn]). This occurs because the carboxylates mobilise not only soil inorganic and organic P, but also a range of micronutrients, including Mn. Concentrations of most other micronutrients increase to a small extent, but Mn accumulates to significant levels, even when plants grow in soil with low concentrations of exchangeable Mn availability. Here, we propose that leaf [Mn] can be used to select for genotypes that are more efficient at acquiring P when soil P availability is low. Likewise, leaf [Mn] can be used to screen for belowground functional traits related to nutrient-acquisition strategies among species in low-P habitats.

Phosphorus-acquisition strategies

Here we explore the idea of using leaf [Mn] to indicate a carboxylate-releasing P-acquisition strategy. The rationale behind this contention is that the availability of both P and Mn are increased when roots release carboxylates into the rhizosphere [1] (Figure 1; see Glossary). The availability of some other micronutrients is also enhanced, but most of these do not lead to a signal as strong as that provided by Mn. The release of carboxylates into the rhizosphere is important for P acquisition, because they mobilise not only inorganic P, but also organic P, which can be a major fraction of soil P, especially when P availability is low [2].

Addressing this topic is timely, because there is a growing interest among plant ecologists in belowground functional traits, to complement the suite of 'easy-to-measure' aboveground traits [3]. Furthermore, because of the gradual decline in phosphate rock that is used to produce P fertilisers [4], there is an increasing need for more P-efficient cropping systems [5]. Therefore, a simple tool to screen for P-acquisition efficiency in crop species would be welcomed by agronomists and plant breeders.

Corresponding author: Lambers, H. (hans.lambers@uwa.edu.au).

Keywords: carboxylates; exudation; manganese; phosphorus; phosphorus-acquisition efficiency.

1360-1385/

© 2014 Elsevier Ltd. All rights reserved. <http://dx.doi.org/10.1016/j.tplants.2014.10.007>

Manganese as a plant nutrient

The significance of Mn as an essential plant nutrient was firmly established in 1922 [6]. More recent work has revealed the role of Mn in redox processes, as an activator of a large range of enzymes, and as a cofactor of a small number of enzymes, including proteins required for light-induced water oxidation in photosystem II [7,8]. Crop plants that contain 50 $\mu\text{g Mn g}^{-1}$ dry weight (DW) in their

Glossary

Arbuscular mycorrhiza: a type of mycorrhizal association that forms arbuscules or coiled hyphae (highly branched exchange structures) within cortical cells of the root.

Carboxylate: an organic anion, which is the organic acid minus the proton(s). For example, citrate is the carboxylate released from the deprotonation of the organic acid, citric acid.

Chelate: a compound that combines reversibly, usually with high affinity, with a metal ion (e.g., iron, copper, or manganese).

Cluster roots: bottle brush-like or Christmas tree-like structures in roots with a dense packing of root hairs, releasing carboxylates into the rhizosphere, thus solubilising poorly available nutrients (e.g., P) in the soil.

Ectomycorrhiza: mycorrhizal association, mostly in woody species, in which a fungal mantle covers fine roots.

Heavy metal: a metal with a mass density exceeding 5 g ml^{-1} .

Hyperaccumulating plant species: plants that typically accumulate 100 times more of a specific heavy metal than the concentrations that occur in nonaccumulating plants growing in the same substrates. For most elements, including Mn, the threshold concentration is 1000 $\mu\text{g g}^{-1}$ DW, except for zinc (10 000 $\mu\text{g g}^{-1}$), gold (1 $\mu\text{g g}^{-1}$), and cadmium (100 $\mu\text{g g}^{-1}$).

Iron-regulated transporter (IRT): associated with the uptake of iron from the rhizosphere into root cells. It is not highly specific and transports other micronutrients.

Micronutrient: inorganic nutrients that a plant requires in relatively small quantities, such as copper, iron, Mn, molybdenum, and zinc.

Mycorrhiza: a structure arising from a symbiotic association between a mycorrhizal fungus and the root of a higher plant [from the Greek words for fungus and root, respectively; the Greek plural would be mycorrhizas, but the Latin plural (mycorrhizae) is also used].

Natural resistance associated macrophage protein (NRAMP): a divalent cation transporter associated with the uptake of transition metals, such as copper, iron, Mn, and zinc.

Nonmycorrhizal plant family: a plant family whose members predominantly are unable to establish a symbiotic association with a mycorrhizal fungus.

Rhizosphere: the zone of soil influenced by the presence of a root.

Scleromorphic: containing a relatively large amount of tough structures (sclerenchyma).

Sorption: the process referring to the binding of, for example, phosphate onto the surface of (i.e., adsorption) and inside (i.e., absorption) soil particles. The term was coined by McBain in 1909 [79]. In soil science, the noncommittal term 'sorption' is used to indicate all processes that result in the transfer of material from the soil solution to the solid phase.

Transition metal: any metal in the d-block of the periodic table, which includes groups 3–12 of the periodic table; the f-block lanthanide and actinide series are also considered transition metals and are referred to as 'inner transition metals'.

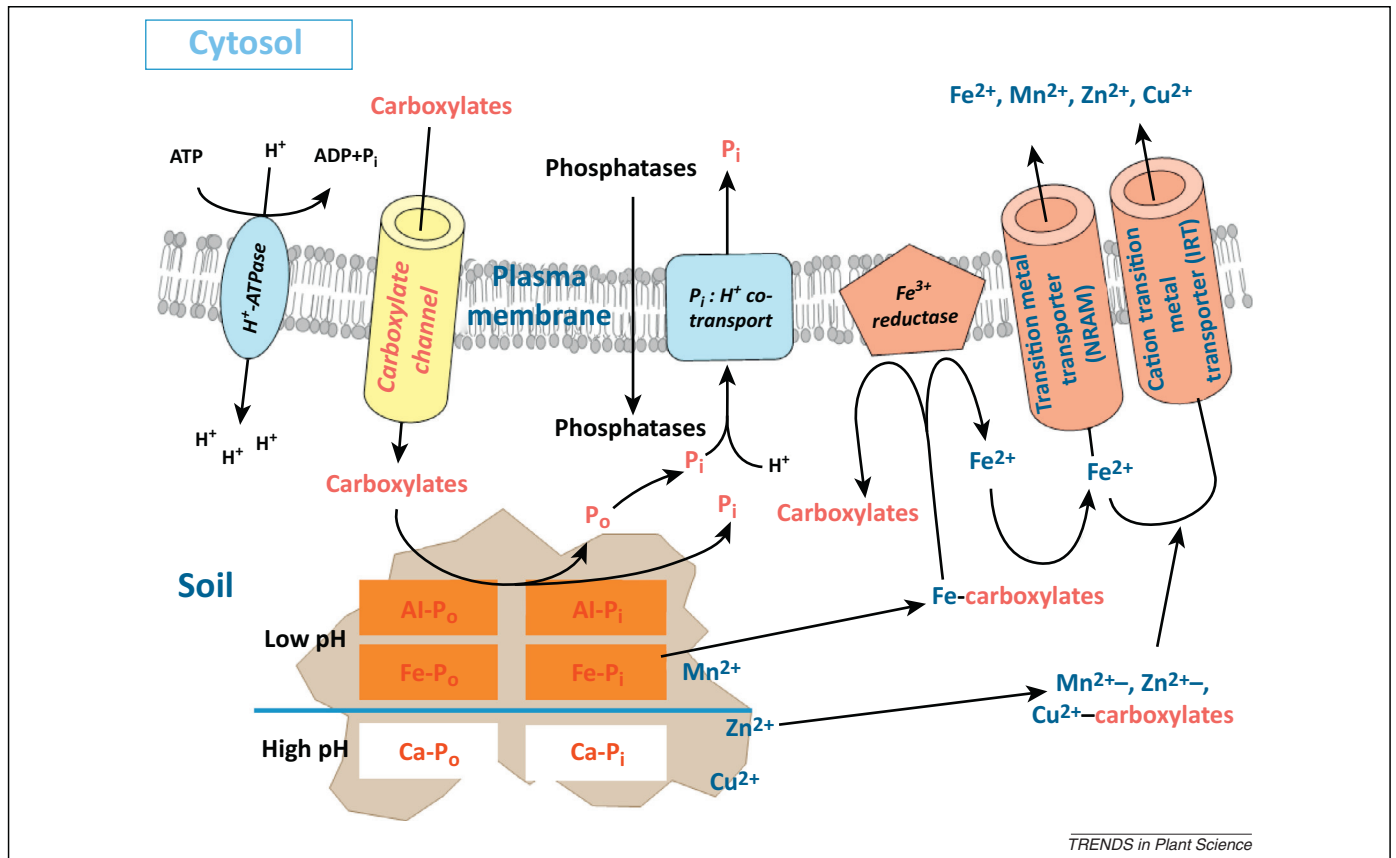


Figure 1. Effects of carboxylates (and other exudates with similar effects, e.g., polygalacturonate [80]) on mobilisation of phosphorus (P) and transition metals. Carboxylates (organic anions) are released via a carboxylate channel. The manner in which phosphatases are released is unknown. Carboxylates mobilise both inorganic (P_i) and organic (P_o) P, which are both sorbed onto soil particles. At acid pH, P_i and P_o bind to oxides and hydroxides of iron (Fe) and aluminium (Al); at alkaline pH, these compounds are precipitated by calcium (Ca). The carboxylates effectively take the place of P_i or P_o, thus pushing this into solution. The released phosphatase enzymes hydrolyse P_o compounds after they have been mobilised by carboxylates. Carboxylates also mobilise some of the transition metal cations, especially Fe, manganese (Mn), zinc (Zn), and copper (Cu). Chelated Fe moves to the root surface, where it is reduced, followed by uptake via a Fe²⁺ transporter [iron-regulated transporter (IRT)]. This transporter is not specific and also transports other micronutrients, such as Mn, Cu, and Zn, which have been mobilised by carboxylates in soil. Alternatively, these transition metals can be taken up by a transporter referred to as natural resistance associated macrophage protein (NRAMP). For further explanation, see the main text. Modified from [13].

leaves are considered to have sufficient Mn for maximum growth and yield [9]. Conversely, Mn toxicity can occur when plants are grown at moderately low soil pH or in flooded soils, when Mn availability is increased [10–13] and its uptake is not tightly regulated. Critical toxicity concentrations in leaves range from 200 to 3500 μg Mn g⁻¹ DW [14], but some hyperaccumulators, such as Proteaceae species in New Caledonia, may contain >10 000 μg Mn g⁻¹ DW without harmful effects [14,15]. Mechanisms that counter Mn toxicity in plants involve Mn export from the cytoplasm, across the tonoplast for sequestration into the vacuole, or across the plasma membrane out of the cell [16]. The *Arabidopsis AtMTP* family of genes encode proteins of the cation diffusion facilitator family, some of which have a role in metal tolerance [17]. Expression of cation diffusion facilitators in a Mn-hypersensitive yeast mutant restored Mn tolerance to wild type levels, showing the importance of this transport system for Mn tolerance [18].

High leaf [Mn] in nonmycorrhizal species with cluster roots

Relatively high leaf [Mn] are typically found in species that produce cluster roots, particularly Proteaceae species,

which are almost all nonmycorrhizal and occur on soils with very low P availability [19–22]. Cluster roots also occur in actinorrhizal species and in many Fabaceae [1,23]. These specialised roots release large amounts of carboxylates in an ‘exudative burst’ to mobilise P, and this also mobilises Mn [23,24]. For a large number of Proteaceae, the range of [Mn] is 126–10 000 μg Mn g⁻¹ DW [20,25–29]. In New Caledonia, no Proteaceae species exhibit leaf [Mn] <100 μg Mn g⁻¹ DW [20]. Likewise, in Fabaceae species with cluster roots, relatively high leaf [Mn] have been observed: 7370 μg Mn g⁻¹ DW in *Lupinus albus*, which is also nonmycorrhizal [30], and 120 μg Mn g⁻¹ DW in *Aspalathus linearis* [31]. These high concentrations can be explained by the ability of cluster roots to mobilise Mn as well as P [24,32,33]. For example, in a glasshouse experiment with *Hakea prostrata* (Proteaceae), variation in leaf [Mn] was positively correlated with investment in cluster roots [34], and similar results were found for *L. albus* [35]. Given that a concentration of 50 μg Mn g⁻¹ DW is considered sufficient for maximum growth of crop plants [9], concentrations >100 μg Mn g⁻¹ DW are considered ‘high’, especially for species with scleromorphic leaves (e.g., many Proteaceae). However, the exact concentrations will also depend on Mn availability in the soil

(which is strongly pH dependent) and, hence, the concentration in leaves should be compared with that of other species growing at the same location [22].

Mn is taken up as Mn^{2+} by roots from the rhizosphere, partly involving broad-specificity transporters [16,36] (Box 1). The broad specificity of these transporters accounts for Mn accumulation and toxicity in plants where soil Mn availability is high. The availability of soil Mn increases with decreasing soil pH, until approximately pH 5, at which point the availability declines again [13,37]. Importantly, Mn availability is also increased by root exudation of carboxylates, which chelate Mn and reduce Mn^{4+} to Mn^{2+} in either acidic or alkaline soils [38].

The literature reviewed for nonmycorrhizal, cluster-rooted species discussed above leads to the hypothesis that leaf [Mn] can be used as a proxy for the carboxylate-releasing P-mobilising strategy and as a screening tool for P-acquisition efficiency when soil P availability is low. Here, we explore this hypothesis.

High leaf [Mn] in other nonmycorrhizal species

A high leaf [Mn] has been found in *Phytolacca acinosa* and *Phytolacca americana*, with up to 19 300 $\mu\text{g Mn g}^{-1}$ DW [39–43]; these Mn-hyperaccumulating species belong to Phytolaccaceae, a nonmycorrhizal and non-cluster-rooting family [44,45]. Mn-hyperaccumulating species contain about 100 times more Mn than nonaccumulator species, reaching at least 1000 $\mu\text{g Mn g}^{-1}$ DW [46]. There is no information in the literature on the rhizosphere chemistry of *Phytolacca* species and, thus, it is unknown whether Mn accumulation depends on the release of carboxylates, protons or both. Based on the high oxalate concentration in leaves of *P. americana* [47], we surmise that this species releases protons generated in the production of oxalic acid, and that the high internal concentration of carboxylate anions (oxalate) is used to internally chelate and detoxify Mn.

Polygonum perfoliatum and *Polygonum hydropiper* (Polygonaceae) are also Mn-hyperaccumulating herbaceous species, with shoot concentrations up to 18 340 μg

Mn g^{-1} DW [42]. Polygonaceae species lack cluster roots and are considered nonmycorrhizal [44]; however, *Polygonum viviparum* has been found to be ectomycorrhizal [48]. No mechanism(s) accounting for their Mn hyperaccumulation are known.

Similar to the cluster-root forming *L. albus*, the non-cluster-root-producing nonmycorrhizal *Lupinus angustifolius* also accumulates high concentrations of Mn in its leaves: 1108 $\mu\text{g Mn g}^{-1}$ DW [49]. Such Mn accumulation is thought to be due to reducing conditions in the rhizosphere, allowing for the increased availability of Mn [49]. Both *L. albus* and *L. angustifolius* are nonmycorrhizal [1] and release relatively large amounts of carboxylates and protons into their rhizosphere [50]. Therefore, high leaf [Mn] is not restricted to species producing cluster roots.

Across a coastal dune chronosequence in Jurien Bay in Western Australia, leaf [Mn] is consistently greater in nonmycorrhizal species compared with co-occurring mycorrhizal species, with most nonmycorrhizal species known to release carboxylates [22]. Interestingly, this occurs across all soils along this 2-million year dune chronosequence, despite the soils showing a wide range of pH values (~5–9). At the community level and within individual nonmycorrhizal species, leaf [Mn] also increases with increasing soil age and associated declines in soil P availability (Figure 2A) and pH, but is not influenced by total or exchangeable soil [Mn] [22]. One species with high leaf [Mn], *Conostylis candidans* (100 $\mu\text{g Mn g}^{-1}$ DW), has been subsequently shown to release a range of carboxylates in its rhizosphere (F. Albornoz and E. Laliberté, unpublished data 2014) in soils spanning a wide range of pH (6–8; determined in 10 mM CaCl_2) and total [P] of 20–430 mg P kg^{-1} . Other noncluster-rooted nonmycorrhizal species with high leaf [Mn] along the same chronosequence remain to be further investigated.

High shoot [Mn] (451–1156 $\mu\text{g Mn g}^{-1}$ DW) have been observed in *Discocactus placentiformis*, a nonmycorrhizal cactus species, abundant in low-P soils in the campos rupestres of Central Brazil (Figure 2B) [51]. When grown

Box 1. Mn transport from the rhizosphere into roots

Plants use transition metal transporters to take up metals such as iron (Fe), copper (Cu), Mn, nickel (Ni), zinc (Zn), and cadmium (Cd), which are generally found at low concentration in the soil [81–83]. In *Arabidopsis thaliana* [36,84], *Solanum lycopersicum* (tomato) [85], and *Oryza sativa* (rice) [86], a cation transporter (IRT1), has a broad substrate range (e.g., Fe, Zn, Mn, Ni, and Cd) [83]. *Oryza sativa*, a Strategy II plant, which takes up Fe as a chelate, has several yellow stripe-like (YSL) genes. Among them, OsYSL2 transports Fe(II)-nicotinamine as well as Mn(II)-nicotinamine [83]. Strategy II of Fe uptake is found in Poaceae [87]. In *Citrus aurantium* (Seville orange), a Strategy I species, increasing the Zn or Mn concentration in the nutrient solution decreases plant Fe concentrations; likewise, Fe inhibits the uptake of Zn and Mn [88]. In *Ulmus laevis*, leaf [Mn] increases fivefold when plants are grown under Fe-deprived conditions in nutrient solution [89].

The broad specificity of transition metal transporters [90,91] may partly account for accumulation of Mn (and, to a lesser extent, Zn and Cu) when Mn is mobilised by exuded carboxylates [34]. However, there are also more specific Mn transporters, which are essential for Mn uptake from soil with low Mn availability, such as natural resistance associated macrophage protein 1 (NRAMP1) in *A. thaliana* [92] and

NRAMP5 in *O. sativa*, deployed for constitutive uptake of Fe and Mn as well as Cd [93,94]. Citrus root stocks differ in their Fe-deficiency tolerance. *Murraya exotica* (orange jasmine) is more tolerant than *Poncirus trifoliata* var. *monstrosa* (flying dragon) [95]. The Mn concentration in *M. exotica* is independent of Fe availability: whereas the Zn concentration in its roots doubles, that in *P. trifoliata* var. *monstrosa* increases fourfold. *Murraya exotica* appears to have mechanisms for regulating uptake of Mn and, to a lesser extent, Zn, in response to Fe deficiency [95]. We conclude that even closely related species differ in the specificity of transition-metal uptake from the rhizosphere. Manganese uptake appears to be the most tightly linked with Mn availability in the rhizosphere and, thus, offers the best tool to screen for a P-mobilising strategy based on carboxylate release.

Recently, some of the genes involved in transition metal transport in plants have been identified, but Mn^{2+} transport pathways are only just beginning to be unravelled at the molecular level [16]. Several transporter gene families have been implicated in Mn^{2+} transport into root cells, NRAMP transporters in *O. sativa* [96] and *A. thaliana* [92], and ZIP transporters in [67]. In addition, the characterization of Mn hyperaccumulator plants allows the identification of genes that confer this trait [67].

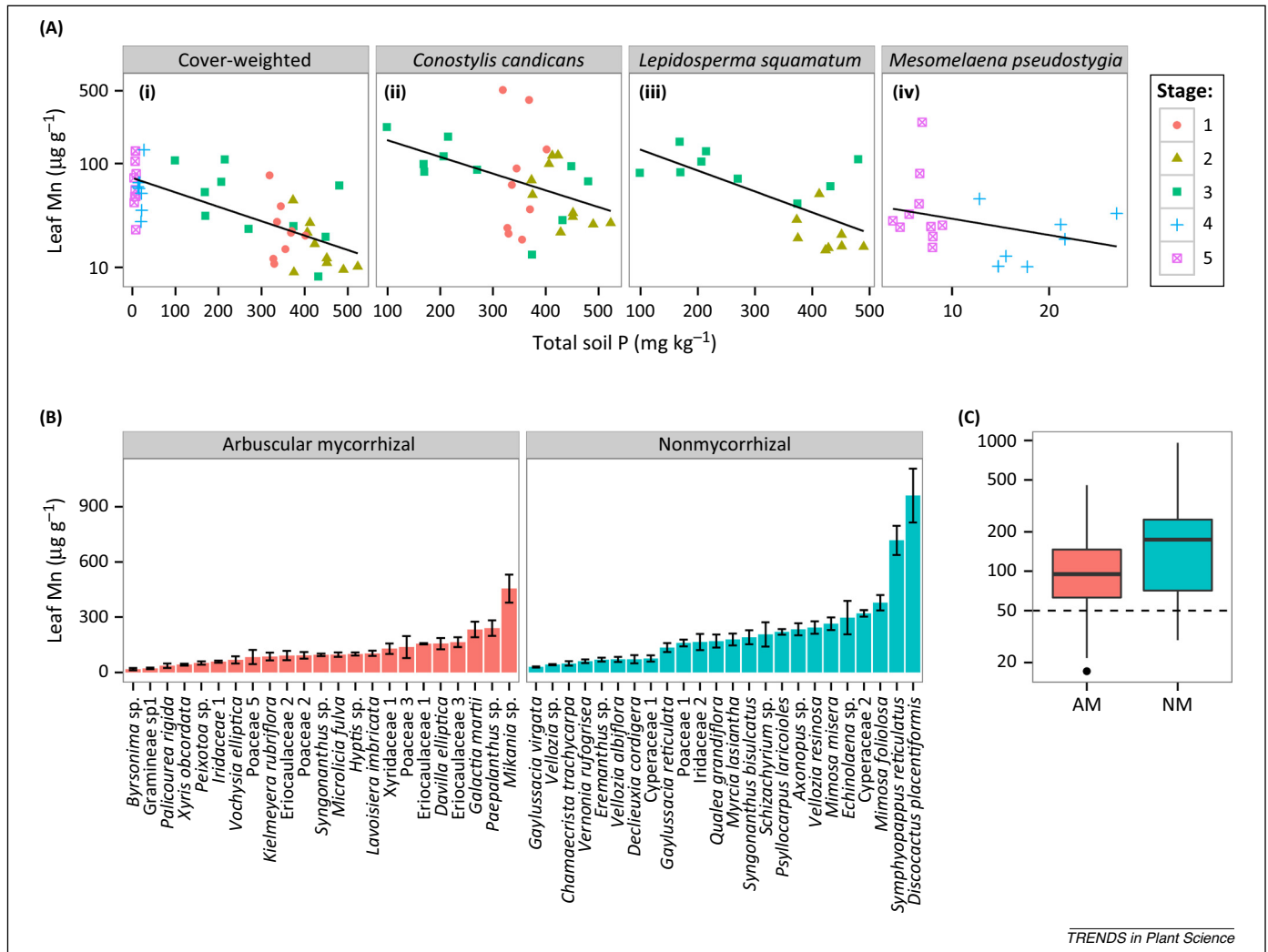


Figure 2. (A) Relations between leaf manganese (Mn) concentration and total soil phosphorus (P) concentration along the Jurien Bay dune chronosequence, Western Australia [22]. Leaf Mn concentrations are calculated either at the community level (i.e., cover-weighted) or individually for the three nonmycorrhizal species that occurred across at least two chronosequence stages. Lines of best fit are shown for each panel. The increase in cover-weighted leaf [Mn] with declining soil [P] partly reflects the greater relative cover of nonmycorrhizal Proteaceae (which had high leaf [Mn]) in these soils. Differences in cover-weighted leaf [Mn] were only accounted for by differences in soil [P] [22], not by differences in soil pH or soil Mn availability, which is low on all dunes [97]. Soil [P] was also the strongest predictor of intraspecific differences in leaf [Mn] for the three nonmycorrhizal species shown in (i–iv), although pH also had a significant (but smaller) effect. (B) Leaf Mn concentrations of campos rupestres species of central Brazil with different nutrient-acquisition strategies. Each bar represents average values for three to five individual plants. See [103] for details on characteristics of campos rupestres soils where these species were collected. (C) Leaf Mn concentrations of arbuscular mycorrhizal (AM) and nonmycorrhizal (NM) campos rupestres species of central Brazil. The central vertical bar in each box represents the median, the box represents the interquartile range, and the whiskers represent the most extreme data points that are still within 1.5 of the upper or lower quartiles. The circles outside the whiskers are values that are more than 1.5 from the upper and lower quartiles. The notches represent confidence intervals around the median. The dashed line indicates the leaf [Mn] adequate for crop growth.

in a low-P nutrient solution, the roots of this cactus increase their exudation of carboxylates, predominantly oxalate, but also malate and citrate. Other nonmycorrhizal species in the campos rupestres also show high leaf [Mn] when compared with mycorrhizal species in the same communities (Figure 2B). We speculate that the high leaf [Mn] of nonmycorrhizal campos rupestres species is accounted for by root carboxylate release.

High leaf [Mn] in species of typically mycorrhizal families

Thirty one *Alyxia* species (Apocynaceae) from New Caledonia all contain high leaf [Mn] [15], with no information on their Mn-uptake mechanisms. *Alyxia* species are considered arbuscular mycorrhizal [52]. *Cupania tenuivalvis* (Sapindaceae) is a species native on acidic soils in cerradão

vegetation in Brazil, with leaf [Mn] of $3300 \mu\text{g Mn g}^{-1} \text{ DW}$ [53]. We can only speculate that the mechanism accounting for Mn hyperaccumulation in *Alyxia* and *Cupania* species are similar to those we discuss for other species in this section. If so, carboxylates would be released by the plant, because there is no conclusive evidence that arbuscular mycorrhizal fungi access significant amounts of insoluble P or Mn sources in soil [54].

Within the genus *Eucalyptus* (Myrtaceae), the two subgenera *Symphomyrtus* and *Monocalyptus* differ nutritionally, based on [Mn] in their leaves, stem and bark; [Mn] are consistently greater in symphyomyrts than in monocalypts [55]. These two subgenera are likely either arbuscular mycorrhizal or both arbuscular mycorrhizal and ectomycorrhizal [52]. There is no evidence that these differences in foliar [Mn] are accounted for by differences in soil [Mn]

Opinion

and, hence, it is hypothesised that they are most likely due to differences in Mn mobilisation in the rhizosphere. Leaf [Mn] in eucalypts are so high ($800 \mu\text{g Mn g}^{-1}$ DW in green leaf-fall and up to $2800 \mu\text{g Mn g}^{-1}$ DW in leaves of glass-house-grown seedlings) that they are within the toxic range for most plants [14]. The mechanism for Mn accumulation is unknown for these and other eucalypts [56], but we do know that *Eucalyptus gummifera* can access poorly soluble forms of inorganic P (aluminium phosphate and iron phosphate) [57], presumably through the releases of carboxylates.

Austromyrtus bidwillii (Myrtaceae), a tropical rainforest tree in north-eastern Australia, hyperaccumulates Mn in its leaves (up to $19\,200 \mu\text{g Mn g}^{-1}$ DW) and bark (up to $26\,500 \mu\text{g Mn g}^{-1}$ DW) [58]. Given that its leaves contain about three times more carboxylates (up to $123\,000 \mu\text{g g}^{-1}$ DW) than required to chelate all Mn, it is likely that, as in the *Phytolacca* species discussed above, it releases predominantly protons and not carboxylates, thereby acidifying the rhizosphere and consequently mobilising soil Mn [58].

Gossia bidwillii (Myrtaceae) is a Mn-hyperaccumulating eastern Australian subtropical rainforest tree [59,60]. There is no published information on the mechanism accounting for the Mn accumulation of this species. In another Australian Mn-hyperaccumulating species, *Denhamia fournieri* (previously known as *Maytenus fournieri*) (Celastraceae), Mn appears to be associated with carboxylates in leaves [61], suggesting that Mn accumulation is associated with proton release, as in the *Phytolacca* species discussed above.

Chengiopanax sciadophylloides (synonyms: *Eleutherococcus sciadophylloides* and *Acanthopanax sciadophylloides*) (Araliaceae) is a Mn-hyperaccumulating Japanese tree; Mn accumulation is based on acidification of the rhizosphere, but not carboxylate release, as in the other species discussed above [62,63]. Hyperaccumulation in this species occurs in noncontaminated forest soils and is specific to Mn, not other metals [64–66]. A zinc-regulated transporter/iron-regulated transporter (ZRT/IRT1)-related protein (ZIP) gene analogue (Box 1) encodes a protein with 65% or less sequence identity with ZIPs of other herbaceous species. Expression of this gene is induced in the callus of Mn-deficient *C. sciadophylloides*, but it does not show Zn or Fe transport [67].

Schima superba (Theaceae) is a Mn-accumulating subtropical tree species native to China [68], without known mechanisms accounting for its Mn accumulation.

In summary, Mn hyperaccumulation in mycorrhizal species does not reflect soil [Mn], [P] or pH, but is associated with Mn mobilisation in the rhizosphere, most likely due to the release of protons and subsequent acidification of the rhizosphere. The carboxylates generated to produce the protons released into the rhizosphere are used internally to bind Mn inside plant tissues, thus reducing the toxic effects of Mn hyperaccumulation.

Variation in leaf [Mn] or root [Mn] as dependent on P treatments

In barley (*Hordeum vulgare*), an elevated P supply reduces Mn acquisition [69], suggesting a role for carboxylates in mobilising both P and Mn in this species. For example,

under a high P supply, carboxylate release would be suppressed, thus reducing Mn uptake. It has been shown that mycorrhizal plants of subclover (*Trifolium subterraneum*) grown at a limiting P supply had higher leaf [P], but a relatively lower leaf [Mn] compared with their nonmycorrhizal counterparts [70], indicating alternative strategies to acquire P, depending on the presence of mycorrhizal inoculum [71]. Following a pulse of P, the root [Mn] declined, suggesting a role for P- and Mn-mobilising exudates, as indicated by the reduced uptake of Mn at a high P supply.

In summary, plants that exhibit a P-mobilising strategy dependent on the release of carboxylates or protons, show lower leaf [Mn] when supplied with sufficient P compared with that of P-limited plants.

Effects of carboxylate-releasing plants on leaf [Mn] in neighbouring plants

When wheat (*Triticum aestivum*) is grown in a cropping situation together with the nonmycorrhizal cluster-rooted *L. albus*, its leaves contain higher leaf [Mn] [30]. This shows that wheat can enhance its Mn uptake when neighbouring white lupins mobilise Mn in soil. Similarly, when grown in pots together with *Banksia attenuata* (Proteaceae), leaf [Mn] and growth of the ectomycorrhizal species *Scholzia involucreata* (Myrtaceae) are enhanced, indicating facilitation of Mn uptake by a cluster-rooted species [72].

Concluding remarks

Plants that release relatively large amounts of carboxylates tend to have relatively high leaf [Mn], for example, Proteaceae and some lupin species [20]. However, some species that hyperaccumulate Mn may not release carboxylates, but strongly acidify their rhizosphere instead, such as *C. sciadophylloides* and *Phytolacca* species [62,63]. Therefore, we suggest that high leaf [Mn] in an environment with a low P availability should be taken only as a strong indication of root carboxylate exudation, primarily for nonmycorrhizal species. High leaf [Mn] by itself does not provide firm evidence for carboxylate exudation. Results showing high leaf [Mn] must be followed up with analyses of rhizosphere carboxylates, or possibly other exudates, before inferring that the studied species must have a specialised P-mobilising strategy. High leaf [Mn] is possibly only associated with carboxylate release into the rhizosphere in nonmycorrhizal species of the ‘Proteaceae type’, sensu [73] and in mycorrhizal species that can switch to a carboxylate-releasing strategy [71]. These are typically associated with soils of low P availability, such as along the Jurien Bay chronosequence [22] and on the sandplains of the Brazilian cerrado [51]. By contrast, high leaf [Mn] in species of the ‘Brassicaceae type’, which occur on nutrient-rich soils, might be associated with release of protons and internal carboxylate accumulation. Acidification of the rhizosphere at low soil pH will render P less available, rather than more [74]. This holds for both inorganic and organic P [75].

We propose leaf [Mn] as a valuable tool to screen for P-efficient crop genotypes in a common environment with low soil P availability, provided the promising genotypes are subsequently further investigated, focussing on

Box 2. Leaf [Mn] as a proxy for the exudation of P-mobilising carboxylates: a tool to screen for efficient crop cultivars and belowground functional traits

Phosphorus is a macronutrient that is limiting for plant productivity in many natural and managed ecosystems [98]. To sustain crop productivity requires a continuous input of P fertilisers, which are produced from mined phosphate rock, a nonrenewable resource that is gradually being depleted [4]. Therefore, there is a growing need to develop crops that are better at acquiring soil P. One such strategy is based on mycorrhizal associations; another, which is particularly effective when soil P availability is very low, is based on the exudation of P-mobilising carboxylates [21]. Measuring carboxylates in the rhizosphere is laborious. In addition, carboxylate exudation depends on climatic conditions and can occur as relatively short pulses, thus complicating sampling. We propose to use leaf [Mn] as a first step to obtain information on the carboxylate-releasing P-mobilising strategy in a range of genotypes screened for variation in P-acquisition efficiency [1]. If the results provide an indication for rhizosphere carboxylates, this can then be followed up by measurements of exuded carboxylates [99].

Plant ecologists are increasingly interested in functional traits, that is, traits that allow grouping of species that have similar roles in an ecosystem [3,100,101]. Most of these traits pertain to aboveground plant characteristics, because these are easiest to measure.

rhizosphere exudates, primarily, carboxylates (Box 2). Within a plant community on soils with low P availability, leaf [Mn] also provides a strong indication of specific species utilising P-mobilising carboxylate-releasing strategies [22] (Box 2). When plants that lack such a strategy are analysed, as dependent on neighbouring species, some of which do and others that do not depend on the P-mobilising carboxylate-releasing strategy, some evidence might be obtained for facilitation of nutrient acquisition, as shown in a mixed cropping situation [30] and a pot experiment [72]. The approach is not advocated for use between sites, because these may differ in soil pH and Mn availability [53].

We also propose leaf [Mn] as a useful trait for within-site comparisons to add to the standard set of traits typically considered in trait-based community ecology studies and comparative functional ecology [3]. In these two fields of study, a small number of traits are typically measured across a range of co-occurring species. Consequently, easily measured aboveground traits (e.g., specific leaf area or leaf dry matter content) are favoured, with the underlying (but generally untested) assumption that above- and belowground traits are coordinated among species [76]. Therefore, most trait-based community ecology and comparative functional ecology studies are currently biased towards aboveground traits. Leaf [Mn] could provide an easily measured aboveground trait that reflects belowground functioning as a time-integrated proxy for P acquisition via carboxylate release. This is especially important because carboxylate release can occur in pulses over a short period of time [77,78].

In conclusion, we propose that leaf [Mn] analysis is a valuable screening tool, both in breeding crops for a high P-acquisition efficiency and in identifying species in a community that use a P-mobilising strategy.

Acknowledgements

H.L. was supported by the Australian Research Council (ARC; DP0985685 and DP110101120); P.E.H. was supported by an Australian

Belowground functional traits are mostly restricted to mycorrhizal status, the capacity to symbiotically fix nitrogen, and morphological traits [3,102]. Traits related to nutrient acquisition are considered desirable to include, but hard to measure [3]. We propose to use leaf [Mn] as a first step to obtain information on the carboxylate-releasing P-mobilising strategy in natural environments where the P availability is low [22].

The proposed approach is expected to be particularly promising for plants growing in alkaline soil, where carboxylate release is an effective strategy to release P and mobilise Mn [13]. It should be equally effective on slightly acidic soils, as demonstrated along the Jurien Bay dune chronosequence in Western Australia [22]. On more acidic soils in campos rupestres in Brazil, below pH 5 [51,103], Mn availability declines with decreasing pH [74]. Here, carboxylate release is expected to be associated with increasing Mn availability only if the accompanying cations are not protons, which is a possibility [104,105].

In summary, the proposed approach looks promising for a range of soil conditions where P is a major limiting nutrient, but further experimental work is required to determine the exact soil conditions for which the approach is most useful.

Postgraduate Award through the University of Western Australia; E.L. was supported by a DECRA (DE120100352) from the ARC; and R.S.O. was supported by São Paulo Research Foundation (Fapesp 2010/172040 and Fapesp 2011/520720).

References

- Lambers, H. *et al.* (2013) How a phosphorus-acquisition strategy based on carboxylate exudation powers the success and agronomic potential of lupines (*Lupinus*, Fabaceae). *Am. J. Bot.* 100, 263–288
- Turner, B.L. *et al.* (2013) Soil microbial biomass and the fate of phosphorus during long-term ecosystem development. *Plant Soil* 367, 225–234
- Pérez-Harguindeguy, N. *et al.* (2013) New handbook for standardised measurement of plant functional traits worldwide. *Aust. J. Bot.* 61, 167–234
- Scholz, R.W. and Wellmer, F.-W. (2013) Approaching a dynamic view on the availability of mineral resources: what we may learn from the case of phosphorus? *Global Environ. Change* 23, 11–27
- Simpson, R.J. *et al.* (2011) Strategies and agronomic interventions to improve the phosphorus-use efficiency of farming systems. *Plant Soil* 349, 89–120
- McHargue, J.S. (1922) The role of manganese in plants. *J. Am. Chem. Soc.* 44, 1592–1598
- Broadley, M. *et al.* (2012) Function of nutrients: micronutrients. In *Marschner's Mineral Nutrition of Higher Plants* (3rd edn) (Marschner, P., ed.), pp. 191–248, Academic Press
- Stout, P.R. and Arnon, D.I. (1939) Experimental methods for the study of the role of copper, manganese, and zinc in the nutrition of higher plants. *Am. J. Bot.* 26, 144–149
- Epstein, E. and Bloom, A.J. (2005) *Mineral Nutrition of Plants: Principles and Perspectives*, Sinauer
- El-Jaoual, T. and Cox, D.A. (1998) Manganese toxicity in plants. *J. Plant Nutr.* 21, 353–386
- Horiguchi, T. (1988) Mechanism of manganese toxicity and tolerance of plants. *Soil Sci. Plant Nutr.* 34, 65–73
- Foy, C.D. *et al.* (1978) The physiology of metal toxicity in plants. *Annu. Rev. Plant Physiol.* 29, 511–566
- Lambers, H. *et al.* (2008) *Plant Physiological Ecology* (2nd edn), Springer
- Krämer, U. (2010) Metal hyperaccumulation in plants. *Annu. Rev. Plant Biol.* 61, 517–534
- Brooks, R.R. *et al.* (1981) Studies on manganese-accumulating *Alyxia* species from New Caledonia. *Taxon* 30, 420–423
- Pittman, J.K. (2005) Managing the manganese: molecular mechanisms of manganese transport and homeostasis. *New Phytol.* 167, 733–742

- 17 Delhaize, E. *et al.* (2007) A role for the *AtMTP11* gene of *Arabidopsis* in manganese transport and tolerance. *Plant J.* 51, 198–210
- 18 Peiter, E. *et al.* (2007) A secretory pathway-localized cation diffusion facilitator confers plant manganese tolerance. *Proc. Natl. Acad. Sci. U.S.A.* 104, 8532–8537
- 19 Lambers, H. *et al.* (2006) Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Ann. Bot.* 98, 693–713
- 20 Jaffré, T. (1979) Accumulation du manganèse par les Protéacées de Nouvelle Calédonie. *C.R. Acad. Sci. Paris D* 289, 425–428
- 21 Lambers, H. *et al.* (2008) Plant nutrient-acquisition strategies change with soil age. *Trends Ecol. Evol.* 23, 95–103
- 22 Hayes, P. *et al.* (2014) Foliar nutrient concentrations and resorption efficiency in plants of contrasting nutrient-acquisition strategies along a 2-million-year dune chronosequence. *J. Ecol.* 102, 396–410
- 23 Shane, M.W. and Lambers, H. (2005) Cluster roots: a curiosity in context. *Plant Soil* 274, 101–125
- 24 Gardner, W.K. *et al.* (1982) The acquisition of phosphorus by *Lupinus albus* L. I. Some characteristics of the soil/root interface. *Plant Soil* 68, 19–32
- 25 Fernando, D.R. *et al.* (2009) Foliar Mn accumulation in eastern Australian herbarium specimens: prospecting for 'new' Mn hyperaccumulators and potential applications in taxonomy. *Ann. Bot.* 103, 931–939
- 26 Denton, M.D. *et al.* (2007) *Banksia* species (Proteaceae) from severely phosphorus-impooverished soils exhibit extreme efficiency in the use and re-mobilization of phosphorus. *Plant Cell Environ.* 30, 1557–1565
- 27 Fernando, D.R. *et al.* (2006) Manganese accumulation in the leaf mesophyll of four tree species: a PIXE/EDAX localization study. *New Phytol.* 171, 751–758
- 28 Rabier, J. *et al.* (2007) Characterization of metal tolerance and accumulation in *Grevillea exul* var *exul*. *Int. J. Phytoremediation* 9, 419–435
- 29 Child, R. and Smith, A.N. (1960) Manganese toxicity in *Grevillea robusta*. *Nature* 186, 1067
- 30 Gardner, W.K. and Boundy, K.A. (1983) The acquisition of phosphorus by *Lupinus albus* L. IV. The effect of interplanting wheat and white lupin on the growth and mineral composition of the two species. *Plant Soil* 70, 391–402
- 31 Morton, J.F. (1983) Rooibos tea, *Aspalathus linearis*, a caffeineless, low-tannin beverage. *Econ. Bot.* 37, 164–173
- 32 Grierson, P.F. and Attiwill, P.M. (1989) Chemical characteristics of the proteoid root mat of *Banksia integrifolia* L. *Aust. J. Bot.* 37, 137–143
- 33 Dinkelaker, B. *et al.* (1995) Distribution and function of proteoid roots and other root clusters. *Bot. Acta* 108, 193–200
- 34 Shane, M.W. and Lambers, H. (2005) Manganese accumulation in leaves of *Hakea prostrata* (Proteaceae) and the significance of cluster roots for micronutrient uptake as dependent on phosphorus supply. *Physiol. Plant* 124, 441–450
- 35 Moraghan, J. (1992) Iron-manganese relationships in white lupine grown on a calciaquoll. *Soil Sci. Soc. Am. J.* 56, 471–475
- 36 Korshunova, Y.O. *et al.* (1999) The IRT1 protein from *Arabidopsis thaliana* is a metal transporter with a broad substrate range. *Plant Mol. Biol.* 40, 37–44
- 37 Godo, G.H. and Reisenauer, H.M. (1980) Plant effects on soil manganese availability. *Soil Sci. Soc. Am. J.* 44, 993–995
- 38 Jauregui, M.A. and Reisenauer, H.M. (1982) Dissolution of oxides of manganese and iron by root exudate components. *Soil Sci. Soc. Am. J.* 46, 314–317
- 39 Xue, S.G. *et al.* (2004) Manganese uptake and accumulation by the hyperaccumulator plant *Phytolacca acinosa* Roxb. (Phytolaccaceae). *Environ. Pollut.* 131, 393–399
- 40 Xue, S.G. *et al.* (2005) Manganese uptake and accumulation by two populations of *Phytolacca acinosa* Roxb. (Phytolaccaceae). *Water Air Soil Pollution* 160, 3–14
- 41 Xu, X. *et al.* (2006) Distribution and mobility of manganese in the hyperaccumulator plant *Phytolacca acinosa* Roxb. (Phytolaccaceae). *Plant Soil* 285, 323–331
- 42 Liu, P. *et al.* (2010) Manganese tolerance and accumulation in six Mn hyperaccumulators or accumulators. *Plant Soil* 335, 385–395
- 43 Min, Y. *et al.* (2007) Accumulation and uptake of manganese in a hyperaccumulator *Phytolacca americana*. *Miner. Eng.* 20, 188–190
- 44 Gerdemann, J.W. (1968) Vesicular-arbuscular mycorrhiza and plant growth. *Annu. Rev. Phytopathol.* 6, 397–418
- 45 Janos, D.P. (1980) Vesicular-arbuscular mycorrhizae affect lowland tropical rain forest plant growth. *Ecology* 61, 151–162
- 46 Brooks, R.R. *et al.* (1998) Phytomining. *Trends Plant Sci.* 3, 359–362
- 47 Dou, C.M. *et al.* (2009) Accumulation and detoxification of manganese in hyperaccumulator *Phytolacca americana*. *Plant Biol.* 11, 664–670
- 48 Massicotte, H.B. *et al.* (1998) Anatomical aspects of field ectomycorrhizas on *Polygonum viviparum* (Polygonaceae) and *Kobresia bellardii* (Cyperaceae). *Mycorrhiza* 7, 287–292
- 49 Reay, P.F. and Waugh, C. (1981) Mineral-element composition of *Lupinus albus* and *Lupinus angustifolius* in relation to manganese accumulation. *Plant Soil* 60, 435–444
- 50 Pearse, S.J. *et al.* (2006) Carboxylate release of wheat, canola and 11 grain legume species as affected by phosphorus status. *Plant Soil* 288, 127–139
- 51 Abrahão, A. *et al.* (2014) Convergence of a specialized root trait in plants from nutrient-impooverished soils: phosphorus-acquisition strategy in a nonmycorrhizal cactus. *Oecologia* 176, 345–355
- 52 Wang, B. and Qiu, Y.-L. (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16, 299–363
- 53 Viani, R.A.G. *et al.* (2014) Soil pH accounts for differences in species distribution and leaf nutrient concentrations of Brazilian woodland savannah and seasonally dry forest species. *Perspect. Plant Ecol. Evol. Syst.* 16, 64–74
- 54 Smith, S.E. and Read, D.J. (2008) *Mycorrhizal Symbiosis*, Academic Press and Elsevier
- 55 Hill, J. *et al.* (2001) Does manganese play a role in the distribution of the eucalypts? *Aust. J. Bot.* 49, 1–8
- 56 Grigg, A.M. *et al.* (2008) Water relations and mineral nutrition of closely related woody plant species on desert dunes and interdunes. *Aust. J. Bot.* 56, 27–43
- 57 Mullette, K.J. *et al.* (1974) Insoluble phosphorus usage by *Eucalyptus*. *Plant Soil* 41, 199–205
- 58 Bidwell, S.D. *et al.* (2002) Hyperaccumulation of manganese in the rainforest tree *Austromyrtus bidwillii* (Myrtaceae) from Queensland, Australia. *Funct. Plant Biol.* 29, 899–905
- 59 Fernando, D.R. *et al.* (2006) In vivo localization of manganese in the hyperaccumulator *Gossia bidwillii* (Benth.) N. Snow & Guymer (Myrtaceae) by cryo-SEM/EDAX. *Plant Cell Environ.* 29, 1012–1020
- 60 Fernando, D.R. *et al.* (2007) Variability of Mn hyperaccumulation in the Australian rainforest tree *Gossia bidwillii* (Myrtaceae). *Plant Soil* 293, 145–152
- 61 Fernando, D.R. *et al.* (2008) Foliar manganese accumulation by *Maytenus founieri* (Celastraceae) in its native New Caledonian habitats: populational variation and localization by X-ray microanalysis. *New Phytol.* 177, 178–185
- 62 Mizuno, T. *et al.* (2008) Age-dependent manganese hyperaccumulation in *Chengiopanax sciadophylloides* (Araliaceae). *J. Plant Nutr.* 31, 1811–1819
- 63 Mizuno, T. *et al.* (2006) Continual pH lowering and manganese dioxide solubilization in the rhizosphere of the Mn-hyperaccumulator plant *Chengiopanax sciadophylloides*. *Soil Sci. Plant Nutr.* 52, 726–733
- 64 Memon, A.R. and Yatazawa, M. (1984) Nature of manganese complexes in manganese accumulator plant – *Acanthopanax sciadophylloides*. *J. Plant Nutr.* 7, 961–974
- 65 Memon, A.R. *et al.* (1979) Absorption and accumulation of iron, manganese and copper in plants in the temperate forest of central Japan. *Soil Sci. Plant Nutr.* 25, 611–620
- 66 Mizuno, T. *et al.* (2013) Manganese hyperaccumulation from non-contaminated soil in *Chengiopanax sciadophylloides* Franch. et Sav. and its correlation with calcium accumulation. *Soil Sci. Plant Nutr.* 59, 591–602
- 67 Mizuno, T. *et al.* (2008) Cloning of ZIP family metal transporter genes from the manganese hyperaccumulator plant *Chengiopanax sciadophylloides*, and its metal transport and resistance abilities in yeast. *Soil Sci. Plant Nutr.* 54, 86–94
- 68 Yang, S.X. *et al.* (2008) Manganese uptake and accumulation in a woody hyperaccumulator, *Schima superba*. *Plant Soil Environ.* 54, 441–446
- 69 Pedas, P. *et al.* (2011) Elevated phosphorus impedes manganese acquisition by barley plants. *Front. Plant Sci.* 2, 37

- 70 Nazeri, N. *et al.* (2013) Do arbuscular mycorrhizas or heterotrophic soil microbes contribute toward plant acquisition of a pulse of mineral phosphate? *Plant Soil* 373, 699–710
- 71 Ryan, M.H. *et al.* (2012) Carbon trading for phosphorus gain: the balance between rhizosphere carboxylates and mycorrhizal symbiosis in plant phosphorus acquisition. *Plant Cell Environ.* 35, 2061–2220
- 72 Muler, A.L. *et al.* (2014) Does cluster-root activity of *Banksia attenuata* (Proteaceae) benefit phosphorus or micronutrient uptake and growth of neighbouring shrubs? *Oecologia* 174, 23–31
- 73 Lambers, H. and Teste, F.P. (2013) Interactions between arbuscular mycorrhizal and non-mycorrhizal plants: do non-mycorrhizal species at both extremes of nutrient-availability play the same game? *Plant Cell Environ.* 36, 1911–2070
- 74 Lindsay, W.L. (1979) *Chemical Equilibria in Soils*, John Wiley & Sons
- 75 Celi, L. and Barberis, E. (2005) Abiotic stabilization of organic phosphorus in the environment. In *Organic Phosphorus in the Environment* (Turner, B.L. *et al.*, eds), pp. 113–132, CAB International
- 76 Reich, P.B. (2014) The world-wide ‘fast-slow’ plant economics spectrum: a traits manifesto. *J. Ecol.* 102, 275–301
- 77 Watt, M. and Evans, J.R. (1999) Proteoid roots. Physiology and development. *Plant Physiol.* 121, 317–323
- 78 Shane, M.W. *et al.* (2004) Developmental physiology of cluster-root carboxylate synthesis and exudation in harsh hakea. Expression of phosphoenolpyruvate carboxylase and the alternative oxidase. *Plant Physiol.* 135, 549–560
- 79 Nagarajah, S. *et al.* (1970) Competitive adsorption of phosphate with polygalacturonate and other organic anions on kaolinite and oxide surfaces. *Nature* 228, 83–85
- 80 Turner, B.L. and Laliberté, E. (2014) Soil development and nutrient availability along a two million year coastal dune chronosequence under species-rich Mediterranean shrubland in southwestern Australia. *Ecosystems* (in press)
- 81 Guerinot, M.L. (2000) The ZIP family of metal transporters. *Biochim. Biophys. Acta Biomembranes* 1465, 190–198
- 82 Hall, J.L. and Williams, L.E. (2003) Transition metal transporters in plants. *J. Exp. Bot.* 54, 2601–2613
- 83 Kobayashi, T. and Nishizawa, N.K. (2012) Iron uptake, translocation, and regulation in higher plants. *Annu. Rev. Plant Biol.* 63, 131–152
- 84 Connolly, E.L. *et al.* (2002) Expression of the IRT1 metal transporter is controlled by metals at the levels of transcript and protein accumulation. *Plant Cell* 14, 1347–1357
- 85 Eckhardt, U. *et al.* (2001) Two iron-regulated cation transporters from tomato complement metal uptake-deficient yeast mutants. *Plant Mol. Biol.* 45, 437–448
- 86 Bughio, N. *et al.* (2002) Cloning an iron-regulated metal transporter from rice. *J. Exp. Bot.* 53, 1677–1682
- 87 Römheld, V. and Schaaf, G. (2004) Iron transport in plants: future research in view of a plant nutritionist and a molecular biologist. *Soil Sci. Plant Nutr.* 50, 1003–1012
- 88 Martínez-Cuenca, M-R. *et al.* (2013) Effects of high levels of zinc and manganese ions on Strategy I responses to iron deficiency in citrus. *Plant Soil* 373, 943–953
- 89 Venturas, M. *et al.* (2014) Root iron uptake efficiency of *Ulmus laevis* and *U. minor* and their distribution in soils of the Iberian Peninsula. *Front. Plant Sci.* 5, Published online March 25, 2014. (<http://dx.doi.org/10.3389/fpls.2014.00104>)
- 90 Conte, S.S. and Walker, E.L. (2011) Transporters contributing to iron trafficking in plants. *Mol. Plant* 4, 464–476
- 91 Baxter, I.R. *et al.* (2008) The leaf ionome as a multivariable system to detect a plant’s physiological status. *Proc. Natl. Acad. Sci. U.S.A.* 105, 12081–12086
- 92 Cailliatte, R. *et al.* (2010) High-affinity manganese uptake by the metal transporter NRAMP1 is essential for *Arabidopsis* growth in low manganese conditions. *Plant Cell* 22, 904–917
- 93 Ishimaru, Y. *et al.* (2012) OsNRAMP5, a major player for constitutive iron and manganese uptake in rice. *Plant Signal. Behav.* 7, 763–766
- 94 Ishimaru, Y. *et al.* (2012) Characterizing the role of rice NRAMP5 in manganese, iron and cadmium transport. *Nat. Commun.* 2, 286
- 95 Wulandari, C. *et al.* (2014) Effect of iron deficiency on root ferric chelate reductase, proton extrusion, biomass production and mineral absorption of citrus root stock orange jasmine (*Murraya exotica* L.). *J. Plant Nutr.* 37, 50–64
- 96 Sasaki, A. *et al.* (2012) Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. *Plant Cell* 24, 2155–2167
- 97 McBain, J.W. (1909) The mechanism of the adsorption (‘sorption’) of hydrogen by carbon. *Philos. Mag. Ser. 6* 18, 916–935
- 98 Vitousek, P.M. *et al.* (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. *Ecol. Appl.* 20, 5–15
- 99 Veneklaas, E.J. *et al.* (2003) Chickpea and white lupin rhizosphere carboxylates vary with soil properties and enhance phosphorus uptake. *Plant Soil* 248, 187–197
- 100 Han, W.X. *et al.* (2011) Biogeography and variability of eleven mineral elements in plant leaves across gradients of climate, soil and plant functional type in China. *Ecol. Lett.* 14, 788–796
- 101 Mitchell, P.J. *et al.* (2008) Using multiple trait associations to define hydraulic functional types in plant communities of south-western Australia. *Oecologia* 158, 385–397
- 102 Van der Heijden, M.G.A. and Scheublin, T.R. (2007) Functional traits in mycorrhizal ecology: their use for predicting the impact of arbuscular mycorrhizal fungal communities on plant growth and ecosystem functioning. *New Phytol.* 174, 244–250
- 103 Oliveira, R.S. *et al.* (2015) Mineral nutrition of *campos rupestres* plant species on contrasting nutrient-impoorished soil types. *New Phytol.* <http://dx.doi.org/10.1111/nph.13175> (in press)
- 104 Roelofs, R.F.R. *et al.* (2001) Exudation of carboxylates in Australian Proteaceae: chemical composition. *Plant Cell Environ.* 24, 891–904
- 105 Zhu, Y. *et al.* (2005) A link between citrate and proton release by proteoid roots of white lupin (*Lupinus albus* L.) grown under phosphorus-deficient conditions? *Plant Cell Physiol.* 46, 892–901