Opinion

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Leaf manganese accumulation and phosphorus-acquisition efficiency

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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.

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 μg 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 nonaccumulator plants growing in the same substrates. For most elements, including Mn, the threshold concentration is 1000 μg g^{-1} DW, except for zinc (10 000 μg g^{-1}), gold (1 μg g^{-1}), and cadmium (100 μg 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'.
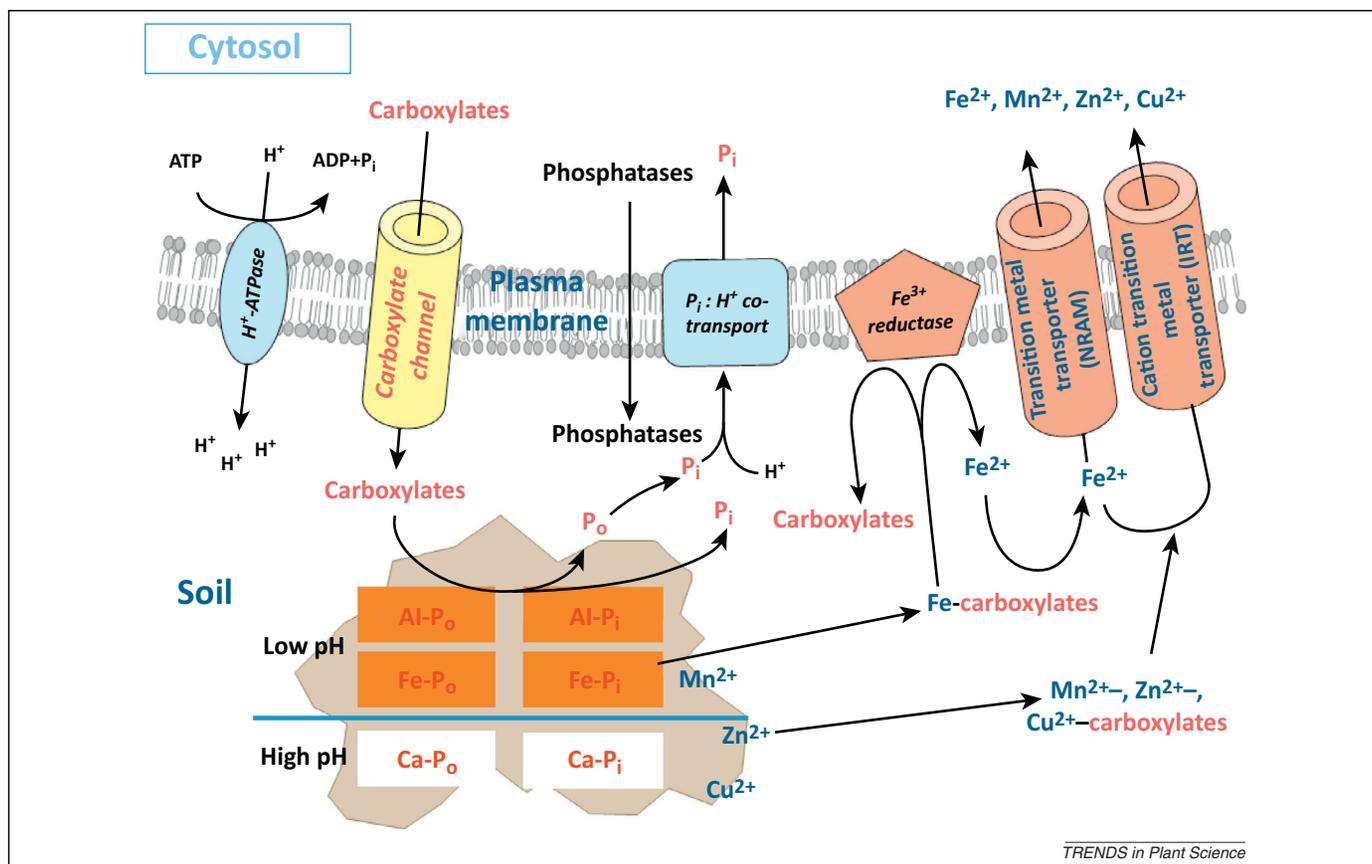
Figure 1. Effects of carboxylates (and other exudates with similar effects, e.g., polygalacturonate [80]) on mobilisation of phosphorus (P) and transition metals. Carboxylates (organic anions) are released via a carboxylate channel. The manner in which phosphatases are released is unknown. Carboxylates mobilise both inorganic (Pi) and organic (Po) P, which are both sorbed onto soil particles. At acid pH, Pi and Po 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 Pi or Po, thus pushing this into solution. The released phosphatase enzymes hydrolyse Po 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 Fe^{2+} 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 actinorhizal 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 µg 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 µg 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 µg 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 candicans* (100 µg 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 µg Mn g^{-1} DW) have been observed in *Discocatus placentiformis*, a nonmycorrhizal cactus species, abundant in low-P soils in the campos rupestres of Central Brazil (Figure 2B) [51]. When grown

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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].
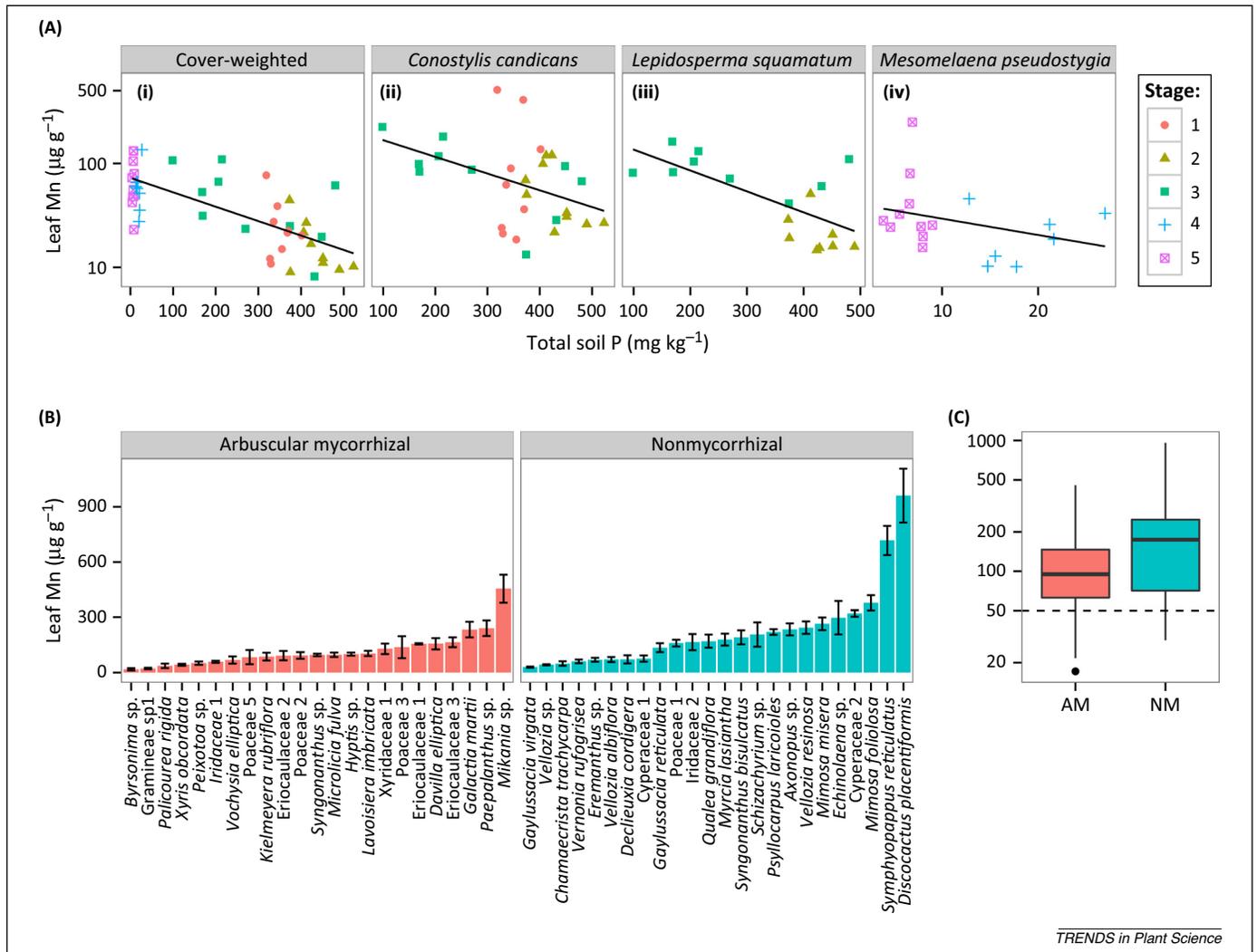
Figure 2. **(A)** Relations between leaf manganese (Mn) concentration and total soil phosphorus (P) concentration along the Jurien Bay dune chronosequence, Western Australia [22]. Leaf Mn concentrations are calculated either at the community level (i.e., cover-weighted) or individually for the three 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 µg Mn g^{-1} 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 *Symphyomyrtus* 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]

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 μg Mn g^{-1} DW in green leaf-fall and up to 2800 μg Mn g^{-1} DW in leaves of glasshouse-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 μg Mn g^{-1} DW) and bark (up to 26 500 μg Mn g^{-1} DW) [58]. Given that its leaves contain about three times more carboxylates (up to 123 000 μg 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 founieri*) (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 involucrata* (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 cerradão [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

Opinion

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.

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.

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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 below-ground 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

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