Micronutrient transport in mycorrhizal symbiosis; zinc steals the show

Peer-reviewed author version

RUYTINX, Joske; Kafle, Arjun; Usman, Muhammad; CONINX, Laura; Zimmermann, Sabine D. & Garcia, Kevin (2020) Micronutrient transport in mycorrhizal symbiosis; zinc steals the show. In: FUNGAL BIOLOGY REVIEWS, 34 (1) , p. 1 -9.

DOI: 10.1016/j.fbr.2019.09.001
Handle: http://hdl.handle.net/1942/31110

Micronutrient transport in mycorrhizal symbiosis; zinc steals the show.

Joske Ruytinx, Arjun Kafle, Muhammad Usman, Laura Coninx, Sabine Dagmar Zimmermann, Kevin Garcia

▶ To cite this version:

Joske Ruytinx, Arjun Kafle, Muhammad Usman, Laura Coninx, Sabine Dagmar Zimmermann, et al.. Micronutrient transport in mycorrhizal symbiosis; zinc steals the show.. Fungal Biology Reviews, Elsevier, In press, 34 (1), pp.1-9. 10.1016/j.fbr.2019.09.001 . hal-02394154

Micronutrient transport in mycorrhizal symbiosis; zinc steals the show

Joske Ruytinx[1,2], Arjun Kafle[3], Muhammad Usman[4], Laura Coninx[2], Sabine D. Zimmermann[4] and Kevin Garcia[3*]

[1]Research group Microbiology, Department of Bioengineering Science, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussel, Belgium.

[2]Centre for Environmental Sciences, Hasselt University, Agoralaan building D, 3590 Diepenbeek, Belgium.

[3]Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC 27695-7619, USA.

[4]BPMP, Univ Montpellier, CNRS, INRA, SupAgro, Montpellier, France.

[*]Corresponding authors:

Kevin Garcia, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC 27695-7619, USA.

Phone: +1-919-515-2040; Email: kgarcia2@ncsu.edu

Joske Ruytinx, Research group Microbiology, Department of Bioengineering Science, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussel, Belgium. Centre for Environmental Sciences, Hasselt University, Agoralaan building D, 3590 Diepenbeek, Belgium.

Phone: XYZ; Email: XYZ

Abstract

Mycorrhizas are mutual beneficial associations between soil-borne fungi and plant roots. Mycorrhizal fungi provide their host plant with essential minerals in exchange for sugars and/or lipids. Traditionally, transport and translocation of macronutrients, including nitrogen and phosphorus, throughout the fungal mycelium and towards the host plant are well studied. However, the regulation of nutrient exchange and their contribution in the morphogenesis and development of mycorrhizas remains unclear. In this Opinion, we argue that including micronutrients in the current models of symbiotic transport is essential to fully understand the establishment, maintenance, and functioning of mycorrhizal associations. Homeostatic mechanisms at the cellular level and the first transport proteins involved have been recently documented for zinc (Zn) in arbuscular mycorrhizal, ectomycorrhizal, and ericoid mycorrhizal fungi. Mycorrhizal plants benefit from an improved Zn status in control conditions and are better protected from the devastating effect of low or high environmental Zn availability. These recent progresses are paving the way for a better understanding of micronutrient allocation in mycorrhizas. Revising our vision on the role of micronutrients, particularly of Zn, in these interactions will allow a better use of mycorrhizal fungi in sustainable agriculture and forestry, and will increase management practices in waste land, as well as in agricultural and natural ecosystems.

Keywords

Arbuscular mycorrhizal symbiosis, Cation Diffusion Facilitator, Ectomycorrhizal symbiosis, Fungal homeostasis, Nutrient transport, Zinc-iron permease.

Introduction

The conquest of land by plants, around 450 million years ago, was facilitated by the recruitment of fungal root symbionts, forming the premise of mycorrhizal symbiosis (Field *et al*., 2015; Strullu-Derrien *et al*., 2018). Today, the vast majority of land plants still associate with mycorrhizal fungi and rely on them for their nutrient supply (Brundrett and Tedersoo, 2018). Different types of mycorrhiza can be distinguished. The most widespread and studied types are arbuscular mycorrhizal (AM) and ectomycorrhizal (EcM) symbioses. Less common types are restricted to particular plant hosts and include ericoid mycorrhizal (ErM) symbiosis. Regardless of their striking morphological divergence and evolutionary independence, all types ensure reciprocal exchanges between host plant and fungus. Plants allocate photosynthetic carbon (C) to their fungal symbionts in exchange for water and essential nutrients (Garcia *et al*., 2016; Martin *et al*., 2016; Rich *et al*., 2017). Regulation of these exchanges and the mechanisms by which nutrients impact the establishment and maintenance of mycorrhiza remain largely unclear (Carbonnel and Gutjahr, 2014; Garcia *et al*., 2015).

Recent evidence indicates that reciprocity in C/phosphorus (P) or C/nitrogen (N) exchanges determines sustainability in the symbiosis (Hammer *et al*., 2011; Kiers *et al*., 2011; Fellbaum *et al*., 2012; Bogar *et al*., 2019; Kafle *et al*., 2019). In mycorrhizal fungi, glucose availability triggers the degradation of soil organic N sources, induces N assimilation pathways, and alters the transcription of various N transporters (Fellbaum *et al*., 2012; Rineau *et al*., 2013). However, N provision towards the host plant does not always explain the amount of C a particular fungal partner receives (Corrêa *et al*., 2015), and reduction of N supply by EcM fungi results in host defense response rather than in restricted C allocation (Hortal *et al*., 2017). This suggests that the regulation of nutrient exchange and its impact on maintenance of the symbiosis must be more complex than originally thought and might involve multiple elements. Experiments with *Oryza sativa* colonized by the AM fungus *Rhizophagus irregularis*, pointed to changes in C/N exchange ratio depending on Zn conditions (Corrêa *et al*., 2014). This indicates the synergy of N and Zn to be important for mycorrhizal plant responses. Therefore, the bioavailability and plant needs in micronutrients, particularly in Zn, seem to play a more important role in the maintenance of the AM symbiosis and C allocation than previously thought.

Micronutrients, such as the metals iron (Fe), manganese (Mn), copper (Cu), and Zn are essential for fungal and plant growth but become toxic when present in excess. Their homeostatic concentration ranges in most plants are narrow (Pilon *et al*., 2009; Sinclair and Krämer, 2012; Connorton *et al*., 2017). Unlike macronutrients N, P, potassium (K), magnesium (Mg) or sulfur (S), micronutrients needed in low amounts yet fulfill indispensable roles in cellular metabolism as structural or catalytic compound of many proteins thanks to their specific chemical properties (Hänsch and Mendel, 2007). In particular, Zn homeostasis is of great interest since its non-optimal soil bioavailability is estimated to impact on agricultural production of 40% of cultivated lands (Alloway, 2008). Low Zn bioavailability results in compromised growth and inferior grain quality for major crops (*e.g.* Johnson-Beebout *et al*., 2016), whereas its excess inhibits plant growth and results in waste lands (*e.g.* Chowdhary *et al*., 2018; He *et al*., 2019). Gathering additional knowledge on fungal Zn homeostasis, its regulation, and its impact on mycorrhizal symbiosis has the potential to lead to impactful applications in agriculture and waste land management.

The dual effect of mycorrhizal fungi on plant micronutrient status

To balance micronutrient concentration in their tissues, plants develop various strategies, including modification of root architecture, change in the chemistry of root exudates, or interaction with soil microbes like AM and EcM fungi (Rasouli-Sadaghiani *et al*., 2011; Ferrol *et al*., 2016; Nanda and Wissuwa, 2016). These fungi play a dual role on plant micronutrient status, either by improving their acquisition under limiting conditions, or by preventing their accumulation in plant tissues in contaminated soils. Besides, mycorrhizal symbiosis may result in alleviation of plant stress, induced by environmental micronutrient excess, through activation of detoxification mechanisms (Bui and Franken, 2018; Cicatelli *et al.*, 2010; Merlos *et al.*, 2016). This results in considerable plant growth, fitness, and crop quality improvement (Watts-Williams *et al.*, 2013), making possible the use of these fungi in phytoremediation/mycoremediation, as well as biofortification practices (*e.g.* Abu-Elsaoud *et al*., 2017).

Examples of mycorrhizal fungi contributing to Zn, Cu, Fe and Mn acquisition under deficient conditions and survival under potentially toxic conditions include both EcM and AM symbioses (*e.g.* Adriaensen *et al*., 2005; Canton *et al.*, 2016; Liu *et al.*, 2000, 2018). However, the involvement of mycorrhizal fungi in these responses greatly depends on the plant species or even cultivars. *R. irregularis* AM colonization increases Cu tolerance of particular maize cultivars whereas it has no effect on growth in other cultivars (Merlos *et al.*, 2016). Similar results were obtained when comparing plants growing on soils from Mexican Mn-mining sites, with AM fungi decreasing Mn uptake in native vegetation (*Ambrosia psilostachya*) but not in maize (Rivera-Becerril *et al.*, 2013). This differential Mn response might ultimately have supported environmental adaptation of the particular native plant species. Facilitation of adaptation towards high environmental Cu by AM symbiosis was also described for *Elsholzia spledens* (Li *et al.*, 2017), a Cu hyperaccumulating plant and indicator species of Chinese Cu mining sites (Lou *et al.*, 2004).

Many crops colonized by AM fungi, including barley (Watts-Williams and Cavagnaro, 2018), tomato (Cavagnaro *et al*., 2006; Watts-Williams *et al*., 2015), maize (Jansa *et al*., 2003; Ortas, 2012), pepper (Ortas, 2012), or soybean (Ibiang *et al*., 2017) display higher Zn concentrations than non-mycorrhizal plants in control or Zn-limiting conditions. Interestingly, plant Zn acquisition via these symbiotic fungi depends on soil P concentrations (Watts-Williams and Cavagnaro, 2012;

Watts-Williams *et al*., 2014; 2019), highlighting the synergy between nutrients in mycorrhizal associations. In northern hemisphere forests, tree Zn nutrition is also greatly modulated by the presence of root colonizing EcM fungi (Adriaensen *et al*., 2004), as described for example for *Pinus radiata* and *Araucaria cunninghamii* (Bowen *et al*., 1974). The efficiency of mycorrhizal fungi to regulate plant Zn supply is not well understood, but we can reasonably assume that this might be related to their ability to tolerate a wide range of external Zn concentrations. Indeed, Zn tolerant *Suillus* isolates (EcM) protect their pine host more effectively from Zn toxicity compared to their non-tolerant relatives by reducing Zn translocation (Adriaensen *et al*., 2004). However, further studies are needed to characterize mechanisms of Zn homeostasis and transport and to investigate their natural diversity among mycorrhizal fungi.

Micronutrient transport and homeostasis in mycorrhizal symbiosis

Knowledge on transport of nutrients towards and throughout the fungal symbiont on the molecular level is key for a good understanding of mycorrhizal symbiosis. Undoubtedly, the most complete view on micronutrient acquisition, transport and translocation within mycorrhizal symbiosis is available from the AM fungus *R. irregularis* and its plant partners (Casieri *et al*., 2013). Different compounds of the high affinity reductive Fe uptake system, including a ferric reductase RiFRE1 and two Fe permeases RiFTR1-2, were identified and functionally characterized by heterologous expression in yeast (Tamayo *et al*., 2018). Glutaredoxins, RiGRX4 and RiGRX5, are highly regulated by the presence of excess Fe. These oxidoreductases most likely impact on Fe homeostasis by influencing cellular redox state (Tamayo et al., 2016). Cu uptake in *R. irregularis* is mediated by RiCTR1, a plasma membrane localized CTR-family transporter, and regulated by environmental Cu availability. RiCTR2 is involved in the mobilization of vacuolar Cu-stores in severe Cu deficient conditions. Interestingly, a particular splicing variant RiCTR3A of a CTR-like protein (RiCTR3) might function as a Cu receptor in sensing external Cu availability (Gómez-Gallego *et al.*, 2019). A metallothionein, RiMT1 (originally GintMT1), complexes excess cytosolic Cu (González-Guerrero *et al.*, 2007). Moreover, particular fungal transporters supposed to be involved in micronutrient uptake are highly regulated at the fungal-plant interface despite growth in control conditions (Tamayo *et al.*, 2018; Gomez-Gallego *et al.*, 2019) and stress the barely understood importance of micronutrients in mycorrhizal symbiosis.

In other AM fungal species and EcM fungi, knowledge on the molecular mechanisms involved in micronutrient transport and homeostasis is rather scarce and limited to the characterization of several metallothioneins (Bellion *et al.*, 2007; Lanfranco *et al.*, 2002; Nguyen *et al.*, 2017; Reddy *et al.*, 2016) and a couple of Cu transporters in *Amanita strobiliformis* (Beneš *et al.*, 2018, 2016). Concerning Zn however, physiology and molecular mechanisms of transport and homeostasis are well explored in AM and EcM fungi and some data are also available for ErM fungi. Extending current knowledge on Zn transport, homeostasis and their regulation in different environmental and developmental conditions for several species could set light on the role of this particular element in mycorrhizal symbiosis and serve as a model to uncover the role of micronutrients in general.

Cellular zinc homeostasis in the mycelium

To ensure growth and overcome toxicity, mycorrhizal fungi need to control cytoplasmic Zn concentrations tightly by transport and compartmentalization into organelles (Fig. 1). Membrane transporters contribute to cellular Zn homeostasis by regulating Zn uptake, efflux, and redistribution. Vacuoles and ER-derived vesicles (so-called zincosomes; Blaudez and Chalot, 2011) are cellular Zn accumulation sites (Fig. 1a,g). Different metal transporter families, mediating Zn uptake or redistribution, have been found in all organisms and are particularly well described in yeast (Gaither and Eide, 2001; Eide, 2006). The main transporter family for Zn release into the cytosol is the ZIP family (zinc-iron permease or ZRT-IRT-like Protein). Redistribution of Zn out of the cytosol towards intracellular organelles or the environment is mediated by members of the CDF family (Cation Diffusion Facilitator). However, ZIP and CDF families are not restricted to Zn transport and can also transport other metals, including Fe, Mn and Cu (Guerinot, 2000; Montanini *et al*., 2007). Members of both families harbor histidine-rich regions which are assumed to mediate metal selectivity. Yet, in certain conditions most members have the potential to transport multiple metals to a lower extent.

Members of the CDF and ZIP families are present in mycorrhizal fungi (Table 1; Tisserant *et al*., 2012; Kohler *et al*., 2015) but only few have been functionally characterized. As predicted, CDF transporters from mycorrhizal fungi are involved in Zn storage and exclusion (Fig. 1a,b,g). Zn homeostasis was modified in yeast by expression of the CDF member from the AM fungus *R. irregularis* GintZnT1 (ID: 70407; González-Guerrero *et al*., 2005). The CDF-type transporter HcZnT1 from the EcM fungus *Hebeloma cylindrosporum* is localized at the ER membrane and mediates specifically Zn tolerance upon functional expression in yeast, presumably by vesicle mediated efflux (Blaudez and Chalot, 2011). The homologue OmZnT1 has been identified in the ErM fungus *Oidiodendron maius* and attributed the same function based on heterologous expression experiments in yeast (Khouja *et al.*, 2013). Two members of the CDF family were identified from the EcM fungus *Suillus luteus,* SlZnT1 (ID: 807028) and SlZnT2 (ID: 814105), and for SlZnT1 its contribution to transport Zn in excess towards the vacuole was suggested (Ruytinx *et al*., 2017). In *Russula atropurpurea*, RaCDF1 and RaCDF2 were identified. They both rescue the Zn sensitive phenotype of yeast mutants and localize to the tonoplast and plasma membrane, respectively (Sácký *et al*., 2016). ZIP family transporters were characterized in the EcM fungi *S. luteus* and *R. atropurpurea*. Respectively, SlZRT1 (ID: 22926) and RaZIP1 were predicted as plasma membrane transporters and were shown to mediate Zn uptake upon expression in yeast

(Coninx *et al*., 2017; Leonhardt *et al*., 2018). In the ErM fungus *O. maius,* the Fe permease OmFET4 is suggested to import Zn and Fe in deficient conditions whereas it is counteracting Zn toxicity by importing Mg in environmental Zn excess (Khouja *et al*., 2013).

In addition to tightly controlled membrane transport, Zn sequestration by bio-chelators contributes to the cellular Zn homeostasis. In particular, cysteine-rich metallothionein-like proteins (so-called zinc binding proteins) were identified in the Zn-accumulating EcM fungus *R. atropurpurea* (RaZBP1 and 2). They are described to bind a major part of the cellular Zn pool (Fig. 1f; Leonhardt *et al*., 2014). In vacuoles and vesicles, Zn is bound to polyphosphate granules (Bücking and Heyser, 1999; Olsson *et al*., 2011).

Zinc allocation into mycorrhizal roots

Extraradical hyphae of mycorrhizal fungi extend the rhizosphere, allowing the host plant to explore a larger volume of soil. As mentioned above, Zn is acquired by fungi through ZIP transporters (Fig. 1), stored into vacuoles through CDF transporters (Fig. 1g), translocated towards intraradical hyphae along with polyphosphates (Fig. 1h), released to the plant-fungus interface, and taken up by plant cortical cells (reviewed in Becquer *et al*., 2019 for EcM). The black-box in this cascade of events touches on the plant-fungus interface. Indeed, information about the molecular mechanisms driving Zn delivery from fungal hyphae to the symbiotic interface is sparse. For instance, no transport system, passive diffusion, or vesicle-dependent mechanism, has been identified so far. We can hypothesize that characterized fungal ZIP or CDF transporters might facilitate Zn unload at the interface. It was shown recently in EcM symbiosis that the same fungal transporter is involved in the uptake of P from the soil and its release to plant cells, following an unknown regulatory mechanism (Becquer *et al*., 2018). On the plant side, genes encoding for ZIP transporters were found up- or down-regulated in AM roots, compared to non-mycorrhizal ones.

More specifically, *HvZIP13* and *MtZIP6* transcripts were up-regulated in barley and *Medicago truncatula* AM roots, respectively, at low Zn (Watts-Williams *et al*., 2017; Watts-Williams and Cavagnaro, 2018). On the other hand, *MtZIP2* was found down-regulated in *M. truncatula* AM roots upon Zn fertilization, presumably preventing the plant from over-accumulating Zn (Burleigh *et al*., 2003). In the fungus, Zn excess results in either its immobilization by compartmentalization in vacuoles or its release outside the hyphae, preventing its transfer to the host plant (González-Guerrero *et al*., 2008; Ruytinx *et al*., 2013; Leonhardt *et al*., 2014).

Regulation of zinc transport in mycorrhizal fungi

Sensing the environment is required to support adaptive growth in suboptimal conditions. In response to external Zn availability, mycorrhizal fungi regulate the expression of some Zn transporters along with genes involved in primary metabolism (Fig. 1c, e) (González-Guerrero *et al*., 2005; Muller *et al*., 2007; Coninx *et al*., 2017). Zn sensor(s) and eventual molecules involved in cellular signal transduction are unknown. Current knowledge of the regulation of homeostasis in response to Zn availability in fungi mainly relies on extensive experimentation in yeasts. Both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* sense Zn availability intracellularly by a transcription factor (Zhao and Eide, 1997; Corkins *et al*., 2013). Zn stabilizes binding of the particular transcription factor to promoter elements and alters directly the transcription of target genes (Wilson and Bird, 2016; Fig. 1a). Orthologs of the *S. cerevisiae* transcription factor are widely dispersed in fungi, including those with a mycorrhizal lifestyle (Tisserant *et al*., 2012; Kohler *et al*., 2015). Consequently, they might orchestrate Zn responses in these important root symbionts (Fig. 1a). Alternatively, Zn is sensed by yeast at the plasma membrane. The ZIP family Zn transceptor ScZRT1 has a dual function as Zn transporter and receptor. Changes in Zn availability trigger ScZRT1-dependent activation (cAMP-independent) of the protein kinase A (PKA) pathway (Schothorst *et al*., 2017). Zn-dependent activation of a phosphorylation pathway results in a swift adaptation of growth in changing environments (Fig. 1e). In plants, Zn is sensed by a ZIP transporter and was shown to induce phosphorylation pathways to result in local responses to micronutrient availability (Cointry and Vert, 2019). Zn availability alters root morphology and architecture through interaction with auxin metabolism (Sofo *et al.*, 2017) but a direct interaction of ZIP transporter-activated phosphorylation pathways and auxin homeostasis is not yet investigated. Up to now, only two ScZRT1 orthologs in mycorrhizal fungi were functionally characterized (Coninx *et al*., 2017; Leonhardt *et al*., 2018). Their potential as activators of phosphorylation pathways is not yet evidenced. However, it might be worth to test this and eventual associated implications in mycorrhizal symbiosis. A function as nutrient transceptor is proven for the phosphate transporter GigmPT of AM fungus *Gigaspora margarita* and suggested for the CTR-like protein RiCTR3A of *R. irregularis*.

Several putative Zn transporters of the CDF and ZIP family are regulated in mycorrhizal fungi as a consequence of their symbiotic status (Table 1; Tisserant *et al*., 2012; Kohler *et al*., 2015). In the AM fungus *R. irregularis*, ZIP transporters localized at the plasma membrane and tonoplast show a 3 to 8-fold change in gene expression when comparing intra- and extra-radicular mycelium (Tisserant *et al*., 2012; Tamayo *et al*., 2014). Although it differs among species (Table 1), a stronger alteration of the expression of Zn transporters can be observed in EcM fungi upon symbiosis (*e.g.* 233-fold for a *Piloderma croceum* CDF transporter). It is still unclear whether Zn at the symbiotic interface is only restricted to a trophic resource that both symbiotic partners may compete for, as suggested in AM symbiosis (Tamayo *et al*., 2014). Indeed, Zn has the potential to act as a signaling molecule. In animals, Zn release from intracellular compartments or into the apoplastic space results in Zn waves that facilitates the transduction of many signaling cascades in response to external stimuli (Yamasaki *et al*., 2007). Although it needs to be experimentally demonstrated, we hypothesize that Zn waves might also exist in fungi (Fig. 1d). Indeed, in the fungus *Candida albicans*, glucose is able to induce mobilization of intracellular Zn through the activation of the cyclic AMP pathway (Kjellerup *et al*., 2018). It will be worth exploring the influence of glucose, or other plant-derived molecules, on Zn metabolism in mycorrhizal fungi to elucidate the regulation of symbiotic Zn transporters, and the possible regulatory role of this micronutrient at the plant-fungus interface.

Conclusion

Despite many efforts in the characterization of transporters (González-Guerrero *et al*., 2005; Blaudez and Chalot, 2011; Sácký *et al*., 2016; Coninx *et al*., 2017; Ruytinx *et al*., 2017; Leonhardt *et al*., 2018), our knowledge on cellular Zn and other micronutrient transport in mycorrhizal fungi remains fragmented. There is no single mycorrhizal fungus for which the complete Zn transportome, including all identified ZIP and CDF encoding genes, is functionally characterized. Observations only point to differences in transporter regulation due to external Zn availability and symbiotic status (Table 1). With around 9% of the proteome interacting with Zn for catalytic or structural traits (Andreini *et al*., 2006), it is clear that alterations in cytoplasmic Zn availability, even temporary, have far-reaching consequences and result in adaptive growth responses. A role for Zn as secondary messenger in symbiosis establishment and maintenance besides its trophic role deserves to be considered. Therefore, incorporating Zn and other micronutrients in the current models of symbiotic transport is urgently needed to understand better the links between nutrient availability, symbiosis development and functioning, and to provide valuable insights in how mycorrhizal fungi balance the nutritional status of plants.

Acknowledgments

JR acknowledges Research Foundation Flanders for financial support (grant n° G079213). MU is supported by a PhD fellowship from the Pakistan Higher Education Commission. LC holds a Flanders Innovation & Entrepreneurships PhD fellowship (IWT project 141461). KG acknowledges support of the North Carolina Agriculture Research Service (NCARS) and the North Carolina Soybean Producers Association (2019-1656). SZ and JR are supported by the French ANR project "MYCOTRANS".

References

Abu-Elsaoud, A.M., Nafady, N.A., Abdel-Azeem, A.M., 2017. Arbuscular mycorrhizal strategy for zinc mycoremediation and diminished translocation to shoots and grains in wheat. PLoS One 12, e0188220.

Adriaensen, K., Van Der Lelie, D., Van Laere, A., Vangronsveld, J., Colpaert, J. V, 2004. A zinc-adapted fungus protects pines from zinc stress. New Phytol. 161, 549–555.

Adriaensen, K., Vrålstad, T., Noben, J.-P., Vangronsveld, J., Colpaert, J. V, 2005. Copper-adapted *Suillus luteus*, a symbiotic solution for pines colonizing Cu mine spoils. Appl. Environ. Microbiol. 71, 7279–7284.

Alloway, B.J., 2008. Zinc in soils and crop nutrition. International Zinc Association, International Fertilizer Industry Association. Second edition. Brussels, Belgium and Paris, France.

Andreini, C., Banci, L., Bertini, I., Rosato, A., 2006. Zinc through the three domains of life. J. Proteome Res. 5, 3173–3178.

Becquer, A., Garcia, K., Amenc, L., Rivard, C., Doré, J., Trives-Segura, C., Szponarski, W., Russet, S., Baeza, Y., Lassalle-Kaiser, B., Gay, G., Zimmermann, S.D., Plassard, C., 2018. The *Hebeloma cylindrosporum* HcPT2 Pi transporter plays a key role in ectomycorrhizal symbiosis. New Phytol. 220, 1185–1199.

Becquer, A., Guerrero-Galán, C., Eibensteiner, J.L., Houdinet, G., Bücking, H., Zimmermann, S.D., Garcia, K., 2019. The ectomycorrhizal contribution to tree nutrition. Adv. Bot. Res. 89, 77–126.

Bellion, M., Courbot, M., Jacob, C., Guinet, F., Blaudez, D., Chalot, M., 2007. Metal induction of a *Paxillus involutus* metallothionein and its heterologous expression in *Hebeloma cylindrosporum*. New Phytol. 174, 151–158.

Beneš, V., Hložková, K., Matěnová, M., Borovička, J., Kotrba, P., 2016. Accumulation of Ag and Cu in *Amanita strobiliformis* and characterization of its Cu and Ag uptake transporter genes AsCTR2 and AsCTR3. BioMetals 29, 249–264.

Beneš, V., Leonhardt, T., Sácký, J., Kotrba, P., 2018. Two P1B-1-ATPases of *Amanita strobiliformis* with distinct properties in Cu/Ag transport. Front. Microbiol. 9, 747.

Blaudez, D., Chalot, M., 2011. Characterization of the ER-located zinc transporter ZnT1 and identification of a vesicular zinc storage compartment in *Hebeloma cylindrosporum*. Fungal Genet. Biol. 48, 496–503.

Bogar, L., Peay, K., Kornfeld, A., Huggins, J., Hortal, S., Anderson, I., Kennedy, P., 2019. Plant-mediated partner discrimination in ectomycorrhizal mutualisms. Mycorrhiza 29, 97–111.

Bowen, G.D., Skinner, M.F., Bevege, D.I., 1974. Zinc uptake by mycorrhizal and uninfected roots of *Pinus radiata* and *Araucaria cunninghamii*. Soil Biol. Biochem. 6, 141–144.

Brundrett, M.C., Tedersoo, L., 2018. Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytol. 220, 1108–1115.

Bücking, H., Heyser, W., 1999. Elemental composition and function of polyphosphates in ectomycorrhizal fungi — an X-ray microanalytical study. Mycol. Res. 103, 31–39.

Bui, V.C., Franken, P., 2018. Acclimatization of *Rhizophagus irregularis* enhances Zn tolerance of the fungus and the mycorrhizal plant partner. Front. Microbiol. 9, 3156.

Burleigh, S.H., Kristensen, B.K., Bechmann, I.E., 2003. A plasma membrane zinc transporter from *Medicago truncatula* is up-regulated in roots by Zn fertilization, yet down-regulated by arbuscular mycorrhizal colonization. Plant Mol. Biol. 52, 1077–1088.

Canton, G.C., Bertolazi, A.A., Cogo, A.J.D., Eutrópio, F.J., Melo, J., de Souza, S.B., A. Krohling, C., Campostrini, E., da Silva, A.G., Façanha, A.R., Sepúlveda, N., Cruz, C., Ramos, A.C., 2016. Biochemical and ecophysiological responses to manganese stress by ectomycorrhizal fungus *Pisolithus tinctorius* and in association with *Eucalyptus grandis*. Mycorrhiza 26, 475–487.

Carbonnel, S., Gutjahr, C., 2014. Control of arbuscular mycorrhiza development by nutrient signals. Front. Plant Sci. 5, 462.

Casieri, L., Ait Lahmidi, N., Doidy, J., Fourrey, C., Migeon, A., Bonneau, L., Courty, P.E., Garcia, K., Charbonnier, M., Delteil, A., Brun, A., Zimmermann, S., Plassard, C., Wipf, D. 2013. Biotrophic transportome in mutualistic plant-fungal interactions. Mycorrhiza. 23, 597–625.

Cavagnaro, T.R., Jackson, L.E., Six, J., Ferris, H., Goyal, S., Asami, D., Scow, K.M., 2006. Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. Plant Soil 282, 209–225.

Chowdhary, P., Yadav, A., Singh, R., Chandra, R., Singh, D.P., Raj, A., Bharagava, R.N., 2018. Stress response of *Triticum aestivum* L. and *Brassica juncea* L. against heavy metals growing at distillery and tannery wastewater contaminated site. Chemosphere 206, 122–131.

Cicatelli, A., Lingua, G., Todeschini, V., Biondi, S., Torrigiani, P., Castiglione, S., 2010. Arbuscular mycorrhizal fungi restore normal growth in a white poplar clone grown on heavy metal-contaminated soil, and this is associated with upregulation of foliar metallothionein and polyamine biosynthetic gene expression. Ann. Bot. 106, 791–802.

Cointry, V., Vert, G., 2019. The bifunctional transporter-receptor IRT1 at the heart of metal sensing and signalling. New Phytol. 223, 1173–1178.

Coninx, L., Thoonen, A., Slenders, E., Morin, E., Arnauts, N., Op De Beeck, M., Kohler, A., Ruytinx, J., Colpaert, J.V., 2017. The SlZRT1 gene encodes a plasma membrane-located ZIP (Zrt-, Irt-Like Protein) transporter in the ectomycorrhizal fungus *Suillus luteus*. Front. Microbiol. 8, 2320.

Connorton, J.M., Balk, J., Rodríguez-Celma, J., 2017. Iron homeostasis in plants - a brief overview. Metallomics 9, 813–823.

Corkins, M.E., May, M., Ehrensberger, K.M., Hu, Y.-M., Liu, Y.-H., Bloor, S.D., Jenkins, B., Runge, K.W., Bird, A.J., 2013. Zinc finger protein Loz1 is required for zinc-responsive regulation of gene expression in fission yeast. Proc. Natl. Acad. Sci. U. S. A. 110, 15371–15376.

Corrêa, A., Cruz, C., Pérez-Tienda, J., Ferrol, N., 2014. Shedding light onto nutrient responses of arbuscular mycorrhizal plants: Nutrient interactions may lead to unpredicted outcomes of the symbiosis. Plant Sci. 221–222, 29–41.

Corrêa, A., Cruz, C., Ferrol, N., 2015. Nitrogen and carbon/nitrogen dynamics in arbuscular mycorrhiza: the great unknown. Mycorrhiza 25, 499–515.

Eide, D.J., 2006. Zinc transporters and the cellular trafficking of zinc. Biochim. Biophys. Acta - Mol. Cell Res. 1763, 711–722.

Fellbaum, C.R., Gachomo, E.W., Beesetty, Y., Choudhari, S., Strahan, G.D., Pfeffer, P.E., Kiers, E.T., Bücking, H., 2012. Carbon availability triggers fungal nitrogen uptake and transport in arbuscular mycorrhizal symbiosis. Proc. Natl. Acad. Sci. U. S. A. 109, 2666–2671.

Ferrol, N., Tamayo, E., Vargas, P., 2016. The heavy metal paradox in arbuscular mycorrhizas: from mechanisms to biotechnological applications. J. Exp. Bot. 67, 6253–6265.

Field, K.J., Pressel, S., Duckett, J.G., Rimington, W.R., Bidartondo, M.I., 2015. Symbiotic options for the conquest of land. Trends Ecol. Evol. 30, 477–486.

Gaither, L.A., Eide, D.J., 2001. Eukaryotic zinc transporters and their regulation. Biometals 14, 251–270.

Garcia, K., Delaux, P.-M., Cope, K.R., Ané, J.-M., 2015. Molecular signals required for the establishment and maintenance of ectomycorrhizal symbioses. New Phytol. 208, 79–87.

Garcia, K., Doidy, J., Zimmermann, S.D., Wipf, D., Courty, P.-E., 2016. Take a trip through the plant and fungal transportome of mycorrhiza. Trends Plant Sci. 21, 937–950.

Gitan, R.S., Lou, H., Rodgers, J., Broderius, M., Eide, D., 1998. Zinc induced inactivation of the yeast ZRT1 zinc transporter occurs through endocytosis and vacuolar degradation. J. Biol. Chem. 273, 28617-28624.

Gómez-Gallego, T., Benabdellah, K., Merlos, M.A., Jiménez-Jiménez, A.M., Alcon, C., Berthomieu, P., Ferrol, N., 2019. The *Rhizophagus irregularis* genome encodes two CTR copper transporters that mediate Cu import into the cytosol and a CTR-Like protein likely involved in copper tolerance. Front. Plant Sci. doi.org/10.3389/fpls.2019.00604

González-Guerrero, M., Azcón-Aguilar, C., Mooney, M., Valderas, A., MacDiarmid, C.W., Eide, D.J., Ferrol, N., 2005. Characterization of a *Glomus intraradices* gene encoding a putative Zn transporter of the cation diffusion facilitator family. Fungal Genet. Biol. 42, 130–140.

González-Guerrero, M., Cano, C., Azcón-Aguilar, C., Ferrol, N., 2007. GintMT1 encodes a functional metallothionein in *Glomus intraradices* that responds to oxidative stress. Mycorrhiza 17, 327–335.

González-Guerrero, M., Melville, L.H., Ferrol, N., Lott, J.N.A., Azcón-Aguilar, C., Peterson, R.L., 2008. Ultrastructural localization of heavy metals in the extraradical mycelium and spores of the arbuscular mycorrhizal fungus *Glomus intraradices*. Can. J. Microbiol. 54, 103–110.

Guerinot, M. Lou, 2000. The ZIP family of metal transporters. Biochim. Biophys. Acta - Biomembr. 1465, 190–198.

Hammer, E.C., Pallon, J., Wallander, H., Olsson, P.A., 2011. Tit for tat? A mycorrhizal fungus accumulates phosphorus under low plant carbon availability. FEMS Microbiol. Ecol. 76, 236–244.

He, M., Shen, H., Li, Z., Wang, L., Wang, F., Zhao, K., Liu, X., Wendroth, O., Xu, J., 2019. Tenyear regional monitoring of soil-rice grain contamination by heavy metals with implications for target remediation and food safety. Environ. Pollut. 244, 431–439.

Hortal, S., Plett, K.L., Plett, J.M., Cresswell, T., Johansen, M., Pendall, E., Anderson, I.C., 2017. Role of plant–fungal nutrient trading and host control in determining the competitive success of ectomycorrhizal fungi. ISME J. 11, 2666–2676.

Ibiang, Y.B., Mitsumoto, H., Sakamoto, K., 2017. Bradyrhizobia and arbuscular mycorrhizal fungi modulate manganese, iron, phosphorus, and polyphenols in soybean (*Glycine max* (L.) Merr.) under excess zinc. Environ. Exp. Bot. 137, 1–13.

Jansa, J., Mozafar, A., Frossard, E., 2003. Long-distance transport of P and Zn through the hyphae of an arbuscular mycorrhizal fungus in symbiosis with maize. Agronomie 23, 481–488.

Johnson-Beebout, S.E., Goloran, J.B., Rubianes, F.H.C., Jacob, J.D.C., Castillo, O.B., 2016. Zn uptake behavior of rice genotypes and its implication on grain Zn biofortification. Sci. Rep. 6, 38301.

Kafle, A., Garcia, K., Wang, X., Pfeffer, P.E., Strahan, G.D., Bücking, H., 2019. Nutrient demand and fungal access to resources control the carbon allocation to the symbiotic partners in tripartite interactions of *Medicago truncatula*. Plant. Cell Environ. 42, 270–284.

Khouja, H.R., Abbà, S., Lacercat-Didier, L., Daghino, S., Doillon, D., Richaud, P., Martino, E., Vallino, M., Perotto, S., Chalot, M., Blaudez, D., 2013. OmZnT1 and OmFET, two metal transporters from the metal-tolerant strain Zn of the ericoid mycorrhizal fungus *Oidiodendron maius*, confer zinc tolerance in yeast. Fungal Genet. Biol. 52, 53–64.

Kiers, E.T., Duhamel, M., Beesetty, Y., Mensah, J. a, Franken, O., Verbruggen, E., Fellbaum, C.R., Kowalchuk, G. a, Hart, M.M., Bago, A., Palmer, T.M., West, S.A., Vandenkoornhuyse, P., Jansa, J., Bücking, H., 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333, 880–882.

Kjellerup, L., Winther, A.-M.L., Wilson, D., Fuglsang, A.T., 2018. Cyclic AMP pathway activation and extracellular zinc induce rapid intracellular zinc mobilization in *Candida albicans*. Front. Microbiol. 9, 502.

Kohler, A., Kuo, A., Nagy, L.G., Morin, E., Barry, K.W., Buscot, F., Canback, B., Choi, C., Cichocki, N., Clum, A., Colpaert, J., Copeland, A., Costa, M.D., Dore, J., Floudas, D., Gay, G., Girlanda, M., Henrissat, B., Herrmann, S., Hess, J., Hogberg, N., Johansson, T., Khouja, H.-R., LaButti, K., Lahrmann, U., Levasseur, A., Lindquist, E.A., Lipzen, A., Marmeisse, R., Martino, E., Murat, C., Ngan, C.Y., Nehls, U., Plett, J.M., Pringle, A., Ohm, R.A., Perotto, S., Peter, M., Riley, R., Rineau, F., Ruytinx, J., Salamov, A., Shah, F., Sun, H., Tarkka, M., Tritt, A., Veneault-Fourrey, C., Zuccaro, A., Consortium, M.G.I., Tunlid, A., Grigoriev, I. V, Hibbett, D.S., Martin, F., 2015. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. Nat. Genet. 47, 410–415.

Lanfranco, L., Bolchi, A., Ros, E.C., Ottonello, S., Bonfante, P., 2002. Differential expression of a metallothionein gene during the presymbiotic versus the symbiotic phase of an arbuscular mycorrhizal fungus. Plant Physiol. 130, 58–67.

Leonhardt, T., Sácký, J., Šimek, P., Šantrůček, J., Kotrba, P., 2014. Metallothionein-like peptides involved in sequestration of Zn in the Zn-accumulating ectomycorrhizal fungus *Russula atropurpurea*. Metallomics 6, 1693–1701.

Leonhardt, T., Sácký, J., Kotrba, P., 2018. Functional analysis RaZIP1 transporter of the ZIP family from the ectomycorrhizal Zn-accumulating *Russula atropurpurea*. BioMetals 31, 255–266.

Li, J., Liang, H., Yan, M., Chen, L., Zhang, H., Liu, J., Wang, S., Jin, Z., 2017. Arbuscular mycorrhiza fungi facilitate rapid adaptation of *Elsholtzia splendens* to copper. Sci. Total Environ. 599–600, 1462–1468.

Liu, A., Hamel, C., Hamilton, R.I., Ma, B.L., Smith, D.L., 2000. Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. Mycorrhiza 9, 331–336.

Liu, L., Li, J., Yue, F., Yan, X., Wang, F., Bloszies, S., Wang, Y., 2018. Effects of arbuscular mycorrhizal inoculation and biochar amendment on maize growth, cadmium uptake and soil cadmium speciation in Cd-contaminated soil. Chemosphere 194, 495–503.

Lou, L., Shen, Z., Li, X., 2004. The copper tolerance mechanisms of *Elsholtzia haichowensis*, a plant from copper-enriched soils. Environ. Exp. Bot. 51, 111–120.

Martin, F., Kohler, A., Murat, C., Veneault-Fourrey, C., Hibbett, D.S., 2016. Unearthing the roots of ectomycorrhizal symbioses. Nat. Rev. Microbiol. 14, 760–773.

Merlos, M.A., Zitka, O., Vojtech, A., Azcón-Aguilar, C., Ferrol, N., 2016. The arbuscular mycorrhizal fungus *Rhizophagus irregularis* differentially regulates the copper response of two maize cultivars differing in copper tolerance. Plant Sci. 253, 68–76.

Montanini, B., Blaudez, D., Jeandroz, S., Sanders, D., Chalot, M., 2007. Phylogenetic and functional analysis of the Cation Diffusion Facilitator (CDF) family: improved signature and prediction of substrate specificity. BMC Genomics 8, 107.

Muller, L.A.H., Craciun, A.R., Ruytinx, J., Lambaerts, M., Verbruggen, N., Vangronsveld, J., Colpaert, J.V., 2007. Gene expression profiling of a Zn-tolerant and a Zn-sensitive *Suillus luteus* isolate exposed to increased external zinc concentrations. Mycorrhiza 17, 571–580.

Nanda, A.K., Wissuwa, M., 2016. Rapid crown root development confers tolerance to zinc deficiency in rice. Front. Plant Sci. 7, 428.

Nguyen, H., Rineau, F., Vangronsveld, J., Cuypers, A., Colpaert, J. V, Ruytinx, J., 2017. A novel, highly conserved metallothionein family in basidiomycete fungi and characterization of two representative SlMTa and SlMTb genes in the ectomycorrhizal fungus *Suillus luteus*. Environ. Microbiol. 19, 2577–2587.

Olsson, P.A., Hammer, E.C., Pallon, J., van Aarle, I.M., Wallander, H., 2011. Elemental composition in vesicles of an arbuscular mycorrhizal fungus, as revealed by PIXE analysis. Fungal Biol. 115, 643–648.

Ortas, I., 2012. The effect of mycorrhizal fungal inoculation on plant yield, nutrient uptake and inoculation effectiveness under long-term field conditions. F. Crop. Res. 125, 35–48.

Pilon, M., Cohu, C.M., Ravet, K., Abdel-Ghany, S.E., Gaymard, F., 2009. Essential transition metal homeostasis in plants. Curr. Opin. Plant Biol. 12, 347–357.

Plett, J.M., Tisserant, E., Brun, A., Morin, E., Grigoriev, I.V., Kuo, A., Martin, F., Kohler, A., 2015. The mutualist *Laccaria bicolor* expresses a core gene regulon during the colonization of diverse host plants and a variable regulon to counteract host-specific defenses. Mol. Plant Microbe Interact. 28, 261–273.

Rasouli-Sadaghiani, M., Sadeghzadeh, B., Sepehr, E., Rengel, Z., 2011. Root exudation and zinc uptake by barley genotypes differing in Zn efficiency. J. Plant Nutr. 34, 1120–1132.

Reddy, M.S., Kour, M., Aggarwal, S., Ahuja, S., Marmeisse, R., Fraissinet-Tachet, L., 2016. Metal induction of a *Pisolithus albus* metallothionein and its potential involvement in heavy metal tolerance during mycorrhizal symbiosis. Environ. Microbiol. 18, 2446–2454.

Rich, M.K., Nouri, E., Courty, P.-E., Reinhardt, D., 2017. Diet of arbuscular mycorrhizal fungi: Bread and butter? Trends Plant Sci. 22, 652–660.

Rineau, F., Shah, F., Smits, M.M., Persson, P., Johansson, T., Carleer, R., Troein, C., Tunlid, A., 2013. Carbon availability triggers the decomposition of plant litter and assimilation of nitrogen by an ectomycorrhizal fungus. ISME J. 7, 2010–2022.

Rivera-Becerril, F., Juárez-Vázquez, L. V, Hernández-Cervantes, S.C., Acevedo-Sandoval, O.A., Vela-Correa, G., Cruz-Chávez, E., Moreno-Espíndola, I.P., Esquivel-Herrera, A., de León-González, F., 2013. Impacts of manganese mining activity on the environment: Interactions among soil, plants, and arbuscular mycorrhiza. Arch. Environ. Contam. Toxicol. 64, 219–227.

Ruytinx, J., Nguyen, H., Van Hees, M., Op De Beeck, M., Vangronsveld, J., Carleer, R., Colpaert, J.V., Adriaensen, K., 2013. Zinc export results in adaptive zinc tolerance in the ectomycorrhizal basidiomycete *Suillus bovinus*. Metallomics 5, 1225–1233.

Ruytinx, J., Coninx, L., Nguyen, H., Smisdom, N., Morin, E., Kohler, A., Cuypers, A., Colpaert, J.V., 2017. Identification, evolution and functional characterization of two Zn CDF-family transporters of the ectomycorrhizal fungus *Suillus luteus*. Environ. Microbiol. Rep. 9, 419–427.

Sácký, J., Leonhardt, T., Kotrba, P., 2016. Functional analysis of two genes coding for distinct cation diffusion facilitators of the ectomycorrhizal Zn-accumulating fungus *Russula* *atropurpurea*. BioMetals 29, 349–363.

Schothorst, J., Zeebroeck, G. V, Thevelein, J.M., 2017. Identification of Ftr1 and Zrt1 as iron and zinc micronutrient transceptors for activation of the PKA pathway in *Saccharomyces cerevisiae*. Microb. Cell 4, 74–89.

Sinclair, S.A., Krämer, U., 2012. The zinc homeostasis network of land plants. Biochim. Biophys. Acta - Mol. Cell Res. 1823, 1553–1567.

Sofo, A., Bochicchio, R., Amato, M., Rendina, N., Vitti, A., Nuzzaci, M., Altamura, M.M., Falasca, G., Rovere, F. Della, Scopa, A., 2017. Plant architecture, auxin homeostasis and phenol content in *Arabidopsis thaliana* grown in cadmium- and zinc-enriched media. J. Plant Physiol. 216, 174–180.

Strullu-Derrien, C., Selosse, M., Kenrick, P., Martin, F.M., 2018. The origin and evolution of mycorrhizal symbioses: from palaeomycology to phylogenomics. New Phytol. 220, 1012–1030.

Tamayo, E., Gómez-Gallego, T., Azcón-Aguilar, C., Ferrol, N., 2014. Genome-wide analysis of copper, iron and zinc transporters in the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. Front. Plant Sci. 5, 547.

Tamayo, E., Benabdellah, K., Ferrol, N., 2016. Characterization of three new glutaredoxin genes in the arbuscular mycorrhizal fungus *Rhizophagus irregularis*: Putative role of RiGRX4 and RiGRX5 in iron homeostasis. PLoS One 11, e0149606.

Tamayo, E., Knight, S.A.B., Valderas, A., Dancis, A., Ferrol, N., 2018. The arbuscular mycorrhizal fungus *Rhizophagus irregularis* uses a reductive iron assimilation pathway for high-affinity iron uptake. Environ. Microbiol. 20, 1857–1872.

Tisserant, E., Kohler, A., Dozolme-Seddas, P., Balestrini, R., Benabdellah, K., Colard, A., Croll, D., Da Silva, C., Gomez, S.K., Koul, R., Ferrol, N., Fiorilli, V., Formey, D., Franken, P., Helber, N., Hijri, M., Lanfranco, L., Lindquist, E., Liu, Y., Malbreil, M., Morin, E., Poulain, J., Shapiro, H., van Tuinen, D., Waschke, A., Azcón-Aguilar, C., Bécard, G., Bonfante, P., Harrison, M.J., Küster, H., Lammers, P., Paszkowski, U., Requena, N., Rensing, S.A., Roux, C., Sanders, I.R., Shachar-Hill, Y., Tuskan, G., Young, J.P.W., Gianinazzi-Pearson, V., Martin, F., 2012. The transcriptome of the arbuscular mycorrhizal fungus *Glomus intraradices* (DAOM 197198) reveals functional tradeoffs in an obligate symbiont. New Phytol. 193, 755–769.

Watts-Williams, S.J., Cavagnaro, T.R., 2012. Arbuscular mycorrhizas modify tomato responses to soil zinc and phosphorus addition. Biol. Fertil. Soils 48, 285–294.

Watts-Williams, S.J., Patti, A.F., Cavagnaro, T.R., 2013. Arbuscular mycorrhizas are beneficial under both deficient and toxic soil zinc conditions. Plant Soil 371, 299–312.

Watts-Williams, S.J., Turney, T.W., Patti, A.F., Cavagnaro, T.R., 2014. Uptake of zinc and phosphorus by plants is affected by zinc fertiliser material and arbuscular mycorrhizas. Plant Soil 376, 165–175.

Watts-Williams, S.J., Smith, F.A., McLaughlin, M.J., Patti, A.F., Cavagnaro, T.R., 2015. How important is the mycorrhizal pathway for plant Zn uptake? Plant Soil 390, 157–166.

Watts-Williams, S.J., Tyerman, S.D., Cavagnaro, T.R., 2017. The dual benefit of arbuscular mycorrhizal fungi under soil zinc deficiency and toxicity: linking plant physiology and gene expression. Plant Soil 420, 375–388.

Watts-Williams, S.J., Cavagnaro, T.R., 2018. Arbuscular mycorrhizal fungi increase grain zinc concentration and modify the expression of root ZIP transporter genes in a modern barley (*Hordeum vulgare*) cultivar. Plant Sci. 274, 163–170.

Watts-Williams, S.J., Jewell, N., Brien, C., Berger, B., Garnett, T., Cavagnaro, T.R., 2019. Using high-throughput phenotyping to explore growth responses to mycorrhizal fungi and zinc in three plant species. Plant Phenomics 2019, 5893953.

Wilson, S., Bird, A.J., 2016. Zinc sensing and regulation in yeast model systems. Arch. Biochem. Biophys. 611, 30–36.

Yamasaki, S., Sakata-Sogawa, K., Hasegawa, A., Suzuki, T., Kabu, K., Sato, E., Kurosaki, T., Yamashita, S., Tokunaga, M., Nishida, K., Hirano, T., 2007. Zinc is a novel intracellular second messenger. J. Cell Biol. 177, 637–645.

Zhao, H., Eide, D.J., 1997. Zap1p, a metalloregulatory protein involved in zinc-responsive transcriptional regulation in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 17, 5044–5052.

Figure 1

Figure 1. Working model of Zn homeostasis in mycorrhizal fungi, including potential cross-links with growth and development. Zn enters the cytosol mainly via ZIP transporters, and excess Zn is redistributed by CDF transporters towards cellular compartments or the environment. Thus, it may be imported into endoplasmic reticulum (ER) derived zincosomes (a) or directly into the ER (b) eventually followed by vesicle-mediated efflux. The cytosolic Zn pool supplies proteins,

including transcription factors (c) and metallothionein-like zinc binding proteins (f), to support their structure and function. Although it should be demonstrated for mycorrhizal fungi, Zn is released in the cytoplasm from zincosomes and can lead to temporary concentration changes (Zn waves) (d). Alterations in cytoplasmic Zn concentrations result in an adaptive growth response mediated via an unknown regulation mechanism (e). Finally, Zn can also be stored in vacuoles (g) and may be allocated to intraradical hyphae to supply colonized plant roots through unknown delivery mechanisms (h).

Table 1

Table 1. Putative Zn ZIP and CDF transporters identified in selected AM and EcM fungi and the symbiotic regulation of their gene expression. Putative Zn transporters are organized following protein similarity and clusters are named according to their yeast ortholog. Ratio of expression levels (fold change) in symbiotic (ECM) to free-living mycelium (FLM), or intraradical (IRM) to extraradical mycelium (ERM), was calculated from published micro-array (*L. bicolor*; Plett *et al.*, 2015) and RNAseq data (*R. irregularis*, Tisserant *et al.*, 2012; all other species, Kohler *et al.*, 2015). Genes more than two times up- or downregulated upon symbiosis are highlighted in red and blue, respectively. "-", non-detected transcript; blank, missing gene copy.