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# 1 **Micronutrient transport in mycorrhizal symbiosis; zinc steals the show**

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21 **Abstract**

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

38

39 **Keywords**

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

## 42 **Introduction**

43 The conquest of land by plants, around 450 million years ago, was facilitated by the recruitment of  
44 fungal root symbionts, forming the premise of mycorrhizal symbiosis (Field *et al.*, 2015; Strullu-  
45 Derrien *et al.*, 2018). Today, the vast majority of land plants still associate with mycorrhizal fungi  
46 and rely on them for their nutrient supply (Brundrett and Tedersoo, 2018). Different types of  
47 mycorrhiza can be distinguished. The most widespread and studied types are arbuscular  
48 mycorrhizal (AM) and ectomycorrhizal (EcM) symbioses. Less common types are restricted to  
49 particular plant hosts and include ericoid mycorrhizal (ErM) symbiosis. Regardless of their striking  
50 morphological divergence and evolutionary independence, all types ensure reciprocal exchanges  
51 between host plant and fungus. Plants allocate photosynthetic carbon (C) to their fungal symbionts  
52 in exchange for water and essential nutrients (Garcia *et al.*, 2016; Martin *et al.*, 2016; Rich *et al.*,  
53 2017). Regulation of these exchanges and the mechanisms by which nutrients impact the  
54 establishment and maintenance of mycorrhiza remain largely unclear (Carbonnel and Gutjahr,  
55 2014; Garcia *et al.*, 2015).

56         Recent evidence indicates that reciprocity in C/phosphorus (P) or C/nitrogen (N) exchanges  
57 determines sustainability in the symbiosis (Hammer *et al.*, 2011; Kiers *et al.*, 2011; Fellbaum *et*  
58 *al.*, 2012; Bogar *et al.*, 2019; Kafle *et al.*, 2019). In mycorrhizal fungi, glucose availability triggers  
59 the degradation of soil organic N sources, induces N assimilation pathways, and alters the  
60 transcription of various N transporters (Fellbaum *et al.*, 2012; Rineau *et al.*, 2013). However, N  
61 provision towards the host plant does not always explain the amount of C a particular fungal partner  
62 receives (Corrêa *et al.*, 2015), and reduction of N supply by EcM fungi results in host defense  
63 response rather than in restricted C allocation (Hortal *et al.*, 2017). This suggests that the regulation  
64 of nutrient exchange and its impact on maintenance of the symbiosis must be more complex than  
65 originally thought and might involve multiple elements. Experiments with *Oryza sativa* colonized

66 by the AM fungus *Rhizophagus irregularis*, pointed to changes in C/N exchange ratio depending  
67 on Zn conditions (Corrêa *et al.*, 2014). This indicates the synergy of N and Zn to be important for  
68 mycorrhizal plant responses. Therefore, the bioavailability and plant needs in micronutrients,  
69 particularly in Zn, seem to play a more important role in the maintenance of the AM symbiosis and  
70 C allocation than previously thought.

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

84

### 85 **The dual effect of mycorrhizal fungi on plant micronutrient status**

86 To balance micronutrient concentration in their tissues, plants develop various strategies, including  
87 modification of root architecture, change in the chemistry of root exudates, or interaction with soil  
88 microbes like AM and EcM fungi (Rasouli-Sadaghiani *et al.*, 2011; Ferrol *et al.*, 2016; Nanda and  
89 Wissuwa, 2016). These fungi play a dual role on plant micronutrient status, either by improving

90 their acquisition under limiting conditions, or by preventing their accumulation in plant tissues in  
91 contaminated soils. Besides, mycorrhizal symbiosis may result in alleviation of plant stress,  
92 induced by environmental micronutrient excess, through activation of detoxification mechanisms  
93 (Bui and Franken, 2018; Cicatelli *et al.*, 2010; Merlos *et al.*, 2016). This results in considerable  
94 plant growth, fitness, and crop quality improvement (Watts-Williams *et al.*, 2013), making possible  
95 the use of these fungi in phytoremediation/mycoremediation, as well as biofortification practices  
96 (*e.g.* Abu-Elsaoud *et al.*, 2017).

97         Examples of mycorrhizal fungi contributing to Zn, Cu, Fe and Mn acquisition under  
98 deficient conditions and survival under potentially toxic conditions include both EcM and AM  
99 symbioses (*e.g.* Adriaensen *et al.*, 2005; Canton *et al.*, 2016; Liu *et al.*, 2000, 2018). However, the  
100 involvement of mycorrhizal fungi in these responses greatly depends on the plant species or even  
101 cultivars. *R. irregularis* AM colonization increases Cu tolerance of particular maize cultivars  
102 whereas it has no effect on growth in other cultivars (Merlos *et al.*, 2016). Similar results were  
103 obtained when comparing plants growing on soils from Mexican Mn-mining sites, with AM fungi  
104 decreasing Mn uptake in native vegetation (*Ambrosia psilostachya*) but not in maize (Rivera-  
105 Becerril *et al.*, 2013). This differential Mn response might ultimately have supported environmental  
106 adaptation of the particular native plant species. Facilitation of adaptation towards high  
107 environmental Cu by AM symbiosis was also described for *Elsholzia splendens* (Li *et al.*, 2017), a  
108 Cu hyperaccumulating plant and indicator species of Chinese Cu mining sites (Lou *et al.*, 2004).  
109 Many crops colonized by AM fungi, including barley (Watts-Williams and Cavagnaro, 2018),  
110 tomato (Cavagnaro *et al.*, 2006; Watts-Williams *et al.*, 2015), maize (Jansa *et al.*, 2003; Ortas,  
111 2012), pepper (Ortas, 2012), or soybean (Ibiang *et al.*, 2017) display higher Zn concentrations than  
112 non-mycorrhizal plants in control or Zn-limiting conditions. Interestingly, plant Zn acquisition via  
113 these symbiotic fungi depends on soil P concentrations (Watts-Williams and Cavagnaro, 2012;

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

124

### 125 **Micronutrient transport and homeostasis in mycorrhizal symbiosis**

126 Knowledge on transport of nutrients towards and throughout the fungal symbiont on the molecular  
127 level is key for a good understanding of mycorrhizal symbiosis. Undoubtedly, the most complete  
128 view on micronutrient acquisition, transport and translocation within mycorrhizal symbiosis is  
129 available from the AM fungus *R. irregularis* and its plant partners (Casieri *et al.*, 2013). Different  
130 compounds of the high affinity reductive Fe uptake system, including a ferric reductase RiFRE1  
131 and two Fe permeases RiFTR1-2, were identified and functionally characterized by heterologous  
132 expression in yeast (Tamayo *et al.*, 2018). Glutaredoxins, RiGRX4 and RiGRX5, are highly  
133 regulated by the presence of excess Fe. These oxidoreductases most likely impact on Fe  
134 homeostasis by influencing cellular redox state (Tamayo *et al.*, 2016). Cu uptake in *R. irregularis*  
135 is mediated by RiCTR1, a plasma membrane localized CTR-family transporter, and regulated by  
136 environmental Cu availability. RiCTR2 is involved in the mobilization of vacuolar Cu-stores in  
137 severe Cu deficient conditions. Interestingly, a particular splicing variant RiCTR3A of a CTR-like

138 protein (RiCTR3) might function as a Cu receptor in sensing external Cu availability (Gómez-  
139 Gallego *et al.*, 2019). A metallothionein, RiMT1 (originally GintMT1), complexes excess cytosolic  
140 Cu (González-Guerrero *et al.*, 2007). Moreover, particular fungal transporters supposed to be  
141 involved in micronutrient uptake are highly regulated at the fungal-plant interface despite growth  
142 in control conditions (Tamayo *et al.*, 2018; Gomez-Gallego *et al.*, 2019) and stress the barely  
143 understood importance of micronutrients in mycorrhizal symbiosis.

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

#### 154 ***Cellular zinc homeostasis in the mycelium***

155 To ensure growth and overcome toxicity, mycorrhizal fungi need to control cytoplasmic Zn  
156 concentrations tightly by transport and compartmentalization into organelles (Fig. 1). Membrane  
157 transporters contribute to cellular Zn homeostasis by regulating Zn uptake, efflux, and  
158 redistribution. Vacuoles and ER-derived vesicles (so-called zincosomes; Blaudez and Chalot,  
159 2011) are cellular Zn accumulation sites (Fig. 1a,g). Different metal transporter families, mediating  
160 Zn uptake or redistribution, have been found in all organisms and are particularly well described  
161 in yeast (Gaither and Eide, 2001; Eide, 2006). The main transporter family for Zn release into the



162 cytosol is the ZIP family (zinc-iron permease or ZRT-IRT-like Protein). Redistribution of Zn out  
163 of the cytosol towards intracellular organelles or the environment is mediated by members of the  
164 CDF family (Cation Diffusion Facilitator). However, ZIP and CDF families are not restricted to  
165 Zn transport and can also transport other metals, including Fe, Mn and Cu (Guerinot, 2000;  
166 Montanini *et al.*, 2007). Members of both families harbor histidine-rich regions which are assumed  
167 to mediate metal selectivity. Yet, in certain conditions most members have the potential to transport  
168 multiple metals to a lower extent.

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

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

189 In addition to tightly controlled membrane transport, Zn sequestration by bio-chelators  
190 contributes to the cellular Zn homeostasis. In particular, cysteine-rich metallothionein-like proteins  
191 (so-called zinc binding proteins) were identified in the Zn-accumulating EcM fungus *R.*  
192 *atropurpurea* (RaZBP1 and 2). They are described to bind a major part of the cellular Zn pool (Fig.  
193 1f; Leonhardt *et al.*, 2014). In vacuoles and vesicles, Zn is bound to polyphosphate granules  
194 (Bücking and Heyser, 1999; Olsson *et al.*, 2011).

#### 195 ***Zinc allocation into mycorrhizal roots***

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

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

### 216 ***Regulation of zinc transport in mycorrhizal fungi***

217 Sensing the environment is required to support adaptive growth in suboptimal conditions. In  
218 response to external Zn availability, mycorrhizal fungi regulate the expression of some Zn  
219 transporters along with genes involved in primary metabolism (Fig. 1c, e) (González-Guerrero *et*  
220 *al.*, 2005; Muller *et al.*, 2007; Coninx *et al.*, 2017). Zn sensor(s) and eventual molecules involved  
221 in cellular signal transduction are unknown. Current knowledge of the regulation of homeostasis  
222 in response to Zn availability in fungi mainly relies on extensive experimentation in yeasts. Both  
223 *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* sense Zn availability intracellularly  
224 by a transcription factor (Zhao and Eide, 1997; Corkins *et al.*, 2013). Zn stabilizes binding of the  
225 particular transcription factor to promoter elements and alters directly the transcription of target  
226 genes (Wilson and Bird, 2016; Fig. 1a). Orthologs of the *S. cerevisiae* transcription factor are  
227 widely dispersed in fungi, including those with a mycorrhizal lifestyle (Tisserant *et al.*, 2012;  
228 Kohler *et al.*, 2015). Consequently, they might orchestrate Zn responses in these important root  
229 symbionts (Fig. 1a). Alternatively, Zn is sensed by yeast at the plasma membrane. The ZIP family  
230 Zn transceptor ScZRT1 has a dual function as Zn transporter and receptor. Changes in Zn  
231 availability trigger ScZRT1-dependent activation (cAMP-independent) of the protein kinase A  
232 (PKA) pathway (Schothorst *et al.*, 2017). Zn-dependent activation of a phosphorylation pathway

233 results in a swift adaptation of growth in changing environments (Fig. 1e). In plants, Zn is sensed  
234 by a ZIP transporter and was shown to induce phosphorylation pathways to result in local responses  
235 to micronutrient availability (Cointry and Vert, 2019). Zn availability alters root morphology and  
236 architecture through interaction with auxin metabolism (Sofa *et al.*, 2017) but a direct interaction  
237 of ZIP transporter-activated phosphorylation pathways and auxin homeostasis is not yet  
238 investigated. Up to now, only two ScZRT1 orthologs in mycorrhizal fungi were functionally  
239 characterized (Coninx *et al.*, 2017; Leonhardt *et al.*, 2018). Their potential as activators of  
240 phosphorylation pathways is not yet evidenced. However, it might be worth to test this and eventual  
241 associated implications in mycorrhizal symbiosis. A function as nutrient transceptor is proven for  
242 the phosphate transporter GigmPT of AM fungus *Gigaspora margarita* and suggested for the CTR-  
243 like protein RiCTR3A of *R. irregularis*.

244         Several putative Zn transporters of the CDF and ZIP family are regulated in mycorrhizal  
245 fungi as a consequence of their symbiotic status (Table 1; Tisserant *et al.*, 2012; Kohler *et al.*,  
246 2015). In the AM fungus *R. irregularis*, ZIP transporters localized at the plasma membrane and  
247 tonoplast show a 3 to 8-fold change in gene expression when comparing intra- and extra-radicular  
248 mycelium (Tisserant *et al.*, 2012; Tamayo *et al.*, 2014). Although it differs among species (Table  
249 1), a stronger alteration of the expression of Zn transporters can be observed in EcM fungi upon  
250 symbiosis (*e.g.* 233-fold for a *Piloderma croceum* CDF transporter). It is still unclear whether Zn  
251 at the symbiotic interface is only restricted to a trophic resource that both symbiotic partners may  
252 compete for, as suggested in AM symbiosis (Tamayo *et al.*, 2014). Indeed, Zn has the potential to  
253 act as a signaling molecule. In animals, Zn release from intracellular compartments or into the  
254 apoplastic space results in Zn waves that facilitates the transduction of many signaling cascades in  
255 response to external stimuli (Yamasaki *et al.*, 2007). Although it needs to be experimentally  
256 demonstrated, we hypothesize that Zn waves might also exist in fungi (Fig. 1d). Indeed, in the

257 fungus *Candida albicans*, glucose is able to induce mobilization of intracellular Zn through the  
258 activation of the cyclic AMP pathway (Kjellerup *et al.*, 2018). It will be worth exploring the  
259 influence of glucose, or other plant-derived molecules, on Zn metabolism in mycorrhizal fungi to  
260 elucidate the regulation of symbiotic Zn transporters, and the possible regulatory role of this  
261 micronutrient at the plant-fungus interface.

262

### 263 **Conclusion**

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

278

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286

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567

568 **Figure 1**

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

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

581

## 582 **Table 1**

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