Commentary

A step forward in tree physiological research on soil copper contamination

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In this issue of *Tree Physiology*, Almeida-Rodríguez et al. (2016) present an interesting and innovative study that evaluates the role of arbuscular mycorrhizal fungi (AMF) in plant tolerance to soil copper (Cu) contamination. The study has important implications for phytoremediation of this inorganic soil pollutant. Industrial and mining activities, since ancient times, have heavily polluted the environment. However, a very promising green technology, namely 'phytoremediation', has emerged in the last decade (Ali et al. 2013, Lee 2013). This biotechnology, in many cases, involves the combined use of fast biomass producing forest trees (e.g., willows and poplars) and AMF. Thanks to the extension of the root apparatus with the formation of the so-called 'Wood Wide Web' (Giovannetti et al. 2006), the plant is equipped with a more efficient system for the absorption of nutrients, and even better, it is protected against excessive concentrations of metals in the soil in general, and of Cu in particular.

Copper is an essential micronutrient. In plants, Cu is required for photosynthesis, cell metabolism and wall synthesis. Moreover, it plays an essential role as a reducing or oxidizing cofactor in several biochemical reactions (Chen et al. 2015). However, in excessive doses, Cu proves to be extremely toxic to cells of all organisms due to the production of free radicals. Copper detoxification and homeostasis, in plant cells, are maintained by efficient systems of chelation and compartmentalization. After its uptake, intracellular detoxification occurs via chelation by scavenging polypeptides (e.g., metallothioneins), or by capturing with small binding proteins. The majority of plant responses to Cu stress are related to plant growth, cell differentiation and physiological processes. Almeida-Rodríguez et al. (2016) investigate the symbiosis between *Salix purpurea* L. and *Rhizophagus irregularis* (Blaszk., Wubet, Renker & Buscot) C.Walker &

A.Schüßler (formerly *Glomus intraradices* Błaszk., Wubet, Renker & Buscot) and its effect on xylem morphology, metabolic and physiological parameters, and expression of aquaporin (AQP) genes related to Cu stress response and/or tolerance.

In agreement with a previous study (Chen et al. 2012), Almeida-Rodríguez et al. show that *S. purpurea* plants had normal growth after Cu applications and no visible symptoms of toxicity. However, a reduced growth (height) was observed when plants were induced to form mycorrhizal root tips with *R. irregularis* and when Cu was applied, as already shown for other plant species (Dai et al. 2014). Growth reduction in mycorrhizal plants is commonly attributable to a reduction in carbon available for growth in host plants, due to carbon partitioned to AMF (Citterio et al. 2005). Almeida-Rodríguez et al. (2016) also observe a reduced plant root mycorrhization when Cu was added to the soil. These observations are in line with other studies, and might help elucidate why Cu has an effect on reducing the mycorrhization in *S. purpurea*. It is well known that differences in AMF root colonization arise from differential host susceptibility, mycorrhizal dependency, environmental conditions, soil pollution and plant nutritional status (Dai et al. 2014). Although the Glomeraceae family can adjust the pattern of sporulation under stress conditions, thus having greater adaptability compared with other AMF families, even this family shows different mycorrhizal root colonization and sporulation in metal-contaminated soils (Lingua et al. 2008, Wei et al. 2014). Indeed, studies have reported the inhibition of mycorrhizal colonization in metal-polluted soils, reducing, delaying or even eliminating AMF and/or spores (Lingua et al. 2008).

Another interesting aspect of the study conducted by Almeida-Rodríguez et al. (2016) relates to Cu accumulation in willows. They found that in *S. purpurea*, Cu accumulated exclusively

in the roots and rhizosphere; the presence of AMF did not significantly affect the absorption and/or the accumulation of Cu in other plant organs. Enhanced metal accumulation in the presence of AMF has been reported for other plant species, while, in the Almeida-Rodríguez et al. (2016) study, the initial amount of Cu in the soil was reduced, yet plant uptake was not improved. Evidently, *S. purpurea* actively immobilizes Cu ions in the root apparatus by preventing their transport to the aerial parts, regardless of the AMF root colonization, confirming the natural high capacity of this species to phytostabilize metal. Copper retention by roots, even in the case of mycorrhizal plants, is probably due to increased accumulation within root/mycorrhizal structures, including fungal and plant cell walls, where polysaccharides efficiently bind Cu (Zhang et al. 2009). In fact, the cell wall is a known subcellular compartment involved in determining plant tolerance and adaptation to Cu. Immobilization of Cu in roots is an evolutionary adaptation of plants that protects the photosynthetic apparatus against metal toxicity, especially when high amounts of metals are absorbed. In addition, this defense system can alter the lipid composition of the cellular membranes both in root and leaf. In plants, the regulation of lipid composition and the adjustment of levels of fatty acid unsaturation are essential for restoring the optimal chemicophysical properties of cell membranes in response to deleterious environmental factors (e.g., metal toxicity), and for survival (Firmin et al. 2015). In *S. purpurea* leaves, a low level of peroxidation was observed in Cu-treated plants compared with the controls (Almeida-Rodríguez et al. 2016). This protection system is probably due to more efficient reactive oxygen species scavenging processes, or to a relatively low level of free radicals inducing lipid peroxidation. This was also linked to an overproduction of antioxidant enzymes, as superoxide dismutase and ascorbate peroxidase in *S. purpurea* leaves, where metal concentration was lower compared with that observed in the roots.

Although AMF colonization is restricted to the root system, its effect is detectable, even macroscopically, in the aboveground plant parts. In fact, evidence is emerging on the capacity of AMF to regulate gene expression and metabolic processes in shoots and leaves (Cicatelli et al. 2010, Zouari et al. 2014). The study of Almeida-Rodríguez et al. (2016) reports that AMF did not diminish the ability of *S. purpurea* to accumulate or stabilize Cu in the roots. On the other hand, AMF modulated the sequestration of this metal in the cell wall and also influenced plant–water balance, in particular leaf-specific conductivity and root hydraulic conductance of plants grown on Cu-polluted soil.

Generally, exposure to toxic metals negatively affects physiological responses related to plant–water balance (e.g., root permeability, stomatal opening; Han et al. 2013). The dynamic regulation of water uptake, which allows plants to inhabit environments where water levels fluctuate throughout the season, is controlled by two coordinated systems: stomatal conductance and hydraulic conductivity. Stomatal conductance controls the rate at which water vapor is lost from leaves during transpiration, regulating the exchange of CO_2 for H_2O as the gases diffuse 'in' or 'out' of leaves. Hydraulic conductivity controls the rate at which water and solutes enters the roots, the radial and axial transport of water within root system and the radial water outflow through the leaf toward the evaporation sites on the mesophyll cell walls. In this context, the structure of the xylem tissue is the corner stone of plant hydraulic architecture. In some plants, in addition to reducing net photosynthesis, metals can also induce other biochemical and even anatomical alterations, such as the reduction of the number and diameter of xylem vessels. Susceptibility of xylem vessels to embolisms and wilting are strongly influenced by the number and size of the conduits (Tyree and Ewers 1991). The documented effects of toxic metals on xylem structure, such as smaller vessel size (Kasim 2007), lower vessel density (Barceló et al. 1988) or lower hydraulic conductivity, perturb water flow to shoots. Previous studies reported that exposure to metals may decrease plant–water potential (Barceló et al. 1988), or increase it (Disante et al. 2011). Reduced hydraulic conductance in response to metals has been observed in other species (Kasim 2007, de Silva et al. 2012). The reduction was attributed to the limited absorption and retention of water due to inhibition of root elongation and/or to changes in xylem structure, observable as fewer functioning vessels not clogged or cavitated, and/or altered vessel diameter. In the study of Almeida-Rodríguez et al. (2016), the wood anatomy of *S. purpurea* was altered by AMF under Cu exposure. Specifically, the authors found that Cu-induced changes in xylem structure influence hydraulic conductance in roots and leaves, and the effect was different in mycorrhizal plants.

In non-mycorrhizal plants, exposure to low Cu soil contamination increased wall thickness, while exposure to high Cu contamination increased vessel lumen diameter and area. Nevertheless, non-mycorrhizal *S. purpurea* plants did not exhibit an altered transpiration rate, suggesting that they were able to compensate for Cu stress and maintain their water status. Although root hydraulic conductance decreased with increasing Cu content, stomatal conductance was unaffected and leaf hydraulic conductivity remained high. Such imbalance between processes controlling water uptake and loss may have consequences whenever water availability is greatly limited. Thus, metals can aggravate water stress in an additive manner by making trees more vulnerable to drought due to a reduced water uptake capacity of roots, as previously reported by de Silva et al. (2012). In contrast, in mycorrhizal willow exposed to Cu, Almeida-Rodríguez et al. (2016) show that vessels were smaller with thicker cell walls (AMF effect). Similar changes to the vessels were observed in other studies on the effect of metal ions, for example, Cu or Cd, on vascular systems (Bouazizi et al. 2010, Akhter et al. 2014). In the roots of bean plants stressed

with Cu, cell wall thickenings were not only confined to the xylem but also extended to the endodermis and the phloem, and the degree of thickening increased with metal concentration. Finally, in the presence of AMF, Almeida-Rodríguez et al. (2016) show that roots had a low hydraulic conductance that was unaffected by Cu, but stomatal conductance and leaf conductivity were reduced, as well as chlorophyll content. The authors conclude that the decrease of stomatal conductance and leaf conductivity were involved in protecting *S. purpurea* by shielding the hydraulic system from failure, further facilitated by the increased thickening of the vessel walls.

Almeida-Rodríguez et al. (2016) also investigated the role of AQPs genes in regulating water flow in *S. purpurea* grown on Cu-polluted soils, in the presence or absence of AMF. Aquaporins are membrane channels, localized to plasmalemma and many cellular endomembranes (e.g., tonoplast), that facilitate the transport of water and small molecule and uncharged solutes. In *S. purpurea*, the authors identified multiple isoforms of AQPs, reflecting a high diversity of cellular localizations, transport selectivity, regulation properties and functions. Putative roles for tonoplast intrinsic proteins and/or plasma membrane intrinsic proteins have been reported recently (Moshelion et al. 2015). They are involved in the dynamics of leaf hydraulic properties and stomatal conductance, and in stress tolerance. In the Almeida-Rodríguez et al. (2016) study, a modulation of *TIP2;2* and *PIP1;2* genes, in response to Cu contamination in the presence or absence of AMF, was observed. In AMF-inoculated *S. purpurea* plants, gene expression levels were higher, showing a modulation of AQP expression exerted by the AMF symbiosis under different stress conditions. In non-mycorrhizal plants, Almeida-Rodríguez and colleagues report the overexpression of the tonoplast AQP *TIP2;2* gene with increasing Cu content. This observation confirms the capacity of AQP *TIP2;2* to maintain high water permeability of the tonoplast, to extend the capacity of the vacuole in osmotic buffering of the cytoplasm under stress conditions and to allow the cells to stabilize the water potential of cytoplasm (Sade et al. 2009). In the *S. purpurea* mycorrhizal plants, the AQP *PIP1;2* gene was upregulated in the presence of low Cu content. According to the composition of AQPs selection ability, the effect of the *R. irregularis* on AQP *PIP1;2* gene expression might be related to a role of this AQP channel in the root water uptake and in the maintenance of root hydraulic conductivity for plants grown on Cu-polluted soils. This would be in agreement with a recent study (Armada et al. 2015) that reports the upregulation of several PIP genes in roots of microbial-inoculated maize plants under drought-stress conditions, even correlated with an improved root hydraulic conductivity.

The work of Almeida-Rodríguez et al. (2016) has demonstrated in mycorrhizal *S. purpurea* trees the relationship between soil Cu contamination, morphological variations of the hydraulic apparatus, stomatal conductance and AQP genes modulation, a novel finding in the field of phytoremediation. The data obtained would aid in the more widespread use of native willows and/or specific AMF for cheaper and more sustainable phytoremediation processes.

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