

Commentary

Interactions of saprotrophic fungi with tree roots: can we observe the emergence of novel ectomycorrhizal fungi?

In this issue of *New Phytologist*, Smith *et al.* (pp. 747–755) re-address the question about the mode of interaction between tree roots and saprotrophic fungi. Roots of ectomycorrhizal (EcM) coniferous trees are known to interact with a large suite of different fungi. These interactions are typified by distinct anatomical and morphological characteristics and are specific for each type of interaction. The presence of root pathogens is characterized by various morphological changes to infected roots and leads to the rapid dieback of small roots. Root interior colonization by dark septate endophytes (that are most likely commensalists utilizing host resources) is rather limited in size so that they can live asymptotically with their hosts. The EcM symbiosis is characterized by a distinct root shape, fungal penetration between cortical cells (the so called Hartig net), formation of a hyphal mantle encasing root tips and mycelia extending into the surrounding soil (van der Heijden *et al.*, 2015). Anatomico-morphological features of the EcM symbiosis reflect its role in functioning as the plant root protection against pathogens, as well as the exchange zone for carbohydrates and mineral nutrients between the host plant and the mycobiont. If similar structures are observed in healthy roots, it is improbable that they are formed by fungi that have endophytic or pathogenic lifestyles.

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Previously, determination of fungi associated with EcM tree roots relied either on root tip morphotyping or on Sanger sequencing of fungal rDNA from a limited number of root tips. Although these studies provided substantial insights into EcM fungal diversity and ecology, they were biased towards capturing dominant or morphologically distinct fungal species. Similarly, fungal sequences obtained with affinity to non-EcM fungal lineages

might often have been discarded from the studies as putative contaminants. The implementation of high throughput sequencing (HTS) methods has significantly increased the possibility of detecting less abundant root-associated mycobionts, including such species present in EcM-like structures. Studies based on HTS have revealed greater taxonomical, as well as ecological, diversity of the tree root mycobiome, than previously expected (e.g. Tedersoo *et al.*, 2010; Menkis *et al.*, 2016).

Earlier works recorded the presence of saprotrophic fungi in living tree roots (Menkis *et al.*, 2012; Tedersoo & Smith, 2013), as well as the ability of some saprotrophic fungal taxa to form EcM-like structures in tree roots under *in vitro* conditions (Vasiliauskas *et al.*, 2007). The extent of this phenomenon is still elusive since earlier morphotyping might have missed the opportunity to identify the fungal symbiont, and the current preference for molecular tools leads to a lesser appreciation of the morphological phenomena of root–fungal structures.

In the paper by Smith *et al.* a comprehensive screening shows that a relatively high proportion of wood-decay fungi, 34 out of 201 tested taxa, are occasionally able to associate *in vitro* with otherwise intact roots of *Pinus sylvestris* or *Picea abies*. Some of the fungal genera (e.g. *Gymnopus*, *Bjerkandera*, *Mycena*, *Pleurotus*), which showed the ability to colonize roots of tested trees, were previously also found as root-associated fungi in EcM plants under natural conditions (Tedersoo & Smith, 2013). Together, these results strongly suggest that the association of wood-decay fungi with roots of coniferous trees is not an exception and might indicate the optional endophytic lifestyle of some fungi. Endophytism can be seen as a newly acquired lifestyle option of these typical saprotrophs, which might serve as an intermediary step between saprotrophic and mycorrhizal lifestyles (Selosse *et al.*, 2009). It should be noted that the endophytic lifestyle of fungal lineages which are sister lineages to EcM fungi is currently known from a few fungal groups only (e.g. Helotiales, Cantharellales, Sebaciales; Tedersoo & Smith, 2013).

Why are the saprotrophic fungi potentially interested in creating associations with roots? The fact that both saprotrophic and EcM fungi proliferate in summer, in the period of high photosynthetic activity (Voříšková *et al.*, 2014), indicates that roots might be considered as a rich source of easily available carbon (C) compounds and it is only tree defences that keep potentially dangerous saprotrophs away from this resource. For the fungi, the benefit from being able to overcome the root defence is obvious. More frequent association of *Pinus sylvestris* than *Picea abies* with saprotrophic fungi reported by Smith *et al.* is worthwhile considering, because the former tree species is typically found in more nutrient-limited soils (Sterkenburg *et al.*, 2015), where the energetic costs of root defence may be limiting and the benefit from establishing a mycorrhizal association would be greater.

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Smith *et al.* showed the ability of three *Phellinus* spp. to asymptotically colonize roots of both tested trees, as well as the ability of the fourth one – *Phellinus igniarius* – to form anatomomorphological structures resembling EcM on *Picea abies*. Species from the genus *Phellinus* represent typical wood-decaying saprotrophs with a few also possessing some parasitic abilities. If forthcoming studies identify features characteristic of EcM in this association, the *Phellinus* genus will represent an interesting model for studying the evolutionary shifts from free-living wood-decay saprotrophs to root-associated endophytes and subsequently to EcM fungi.

During the course of evolution, EcM symbiosis was established at least 70 times in multiple lineages by conversion of a saprotrophic fungus (Tedersoo & Smith, 2013; Kohler *et al.*, 2015); this indicates that the transition to biotrophy is relatively ‘easy’ and/or that it is very positively selected. If this is the case, the emergence of novel symbioses cannot be ruled out.

The transition from saprotrophy to an EcM lifestyle was typically associated with the reduction of the saprotrophic suite of glycoside hydrolases and peroxidases that were no longer needed by fungi receiving C compounds from their host (Kohler *et al.*, 2015). Although the saprotrophic potential of EcM fungi was often questioned (Talbot *et al.*, 2008; Baldrian, 2009), it seems that C acquisition via biotrophy and saprotrophy is not present in the same taxon and the potential for organic matter decomposition in EcM fungi, if present, serves rather for the acquisition of nitrogen (N) from organic resources (Lindahl & Tunlid, 2015). The newly developing EcM fungi thus seem to be forced to make a choice between using complex C compounds from soil organic matter or C from their host.

While the loss of saprotrophic capability is easy to imagine, it is more difficult to propose the evolutionary path to the bilateral trade of C for mineral nutrients that needs to be established between the tree host and its fungal partner and that is demonstrated by the increase of genes coding for N and phosphorus transport, common to EcM fungi (Martin *et al.*, 2010). Among others, it includes the development of a complex recognition and signalling apparatus between the partners. The observation that a large proportion of genes involved in EcM symbiosis are fungal species-specific (Kohler *et al.*, 2015), supports the idea that the development of biotrophic mechanisms is indeed able to occur *de novo* in various genetic backgrounds. Due to the complexity of the symbiosis functioning, it is likely that the formation of fungal root-associated structures, often fails to be successfully completed by symbiosis development and if so, the observation of anatomo-morphological structures resembling EcM is likely to be much more frequent than the full development of novel mutualistic symbioses.

In order to recognize whether the observed EcM-like structures formed by saprotrophs may be of mycorrhizal nature, it will be necessary to analyse the interaction in more detail, for example using nutrient tracing or transcriptomics. This should be technically feasible, as demonstrated for other *Pinus*-associated fungi (Liao *et al.*, 2014). To prove the symbiotic status of the observed tree–fungus associations, some aspects of the symbiosis need to be confirmed, such as the impact on plant growth and fitness, the expression of specific transporters, differentiation of expression between root-associated and free living mycelia, or the production

of mycorrhiza-induced small secreted proteins by the tree host (van der Heijden *et al.*, 2015). Furthermore, observations of the occurrence of such interactions *in situ* should answer the question as to whether or not they are artefacts of the culturing conditions. Therefore, studies addressing tree root-associated fungal communities should not present the EcM fungal occurrences only, but rather a more complex picture of all detected fungi. It is tempting to imagine that we may have the chance to observe the development of a novel EcM symbiosis in reality at the stage where one fungal species can operate two contrasting lifestyles.

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Petr Baldrian^{1*} and Petr Kohout^{1,2}

¹Laboratory of Environmental Microbiology of the CAS, Vídeňská 1083, 14220, Prague 4, Czech Republic;

²Department of Experimental Plant Biology, Faculty of Science, Charles University, 12844, Prague 2, Czech Republic
(*Author for correspondence: tel +420 723 770 570; email baldrian@biomed.cas.cz)

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