# Questioning the Evidence for Host-Symbiont Codiversification in Mycorrhizal Symbioses

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## Key words

Mycorrhizal symbioses, coevolution, codiversification, cospeciation, cophylogenetic signal, phylogenetic congruence

## Abstract

A large majority of plants form mycorrhizal symbioses. The ecological importance of these mutualistic interactions has sparked interest in their long evolutionary history. By examining interaction networks and phylogenetic trees, several studies have suggested that plants codiversify or cospeciate with their associated mycorrhizal fungi. However, recent research has demonstrated that phylogenetic congruence (often interpreted as codiversification) has often been conflated with another pattern called cophylogenetic signal (i.e., closely related plants interacting with closely related fungi), which arises from different biological processes. We performed cophylogenetic analyses on 29 diverse mycorrhizal networks to reevaluate the evidence for codiversification in mycorrhizal symbioses. We found significant cophylogenetic signal but no phylogenetic congruence: closely related plants interact with closely related fungi, but their phylogenies do not match. Instead of codiversification, this finding suggests that plants and fungi undergo diffuse coevolution, likely driven by trait-matching, where compatible, evolutionarily conserved plant and fungal traits govern their interactions. Our work highlights the importance of appropriately interpreting the cophylogenetic methods used to study the macroevolution of plants and their mycorrhizal fungi. It suggests that previous studies proposing codiversification in mycorrhizal symbioses actually detected cophylogenetic signal, indicating that there is no evidence of codiversification or cospeciation occurring during the evolution of mycorrhizal symbioses.

## Main text

More than 90% of vascular plants form mycorrhizal symbioses (Brundrett and Tedersoo, 2018). These mutualistic associations are crucial for nutrition of most plants and their associated fungi and thus for the functioning of whole ecosystems (Smith and Read, 2008; Tedersoo et al., 2020; van der Heijden et al., 2015). Mycorrhizal symbioses originated more than 400 million years ago, likely facilitating the transition of plants from water to land (Remy et al., 1994; Strullu-Derrien et al., 2018). Over time, these associations evolved and diversified, leading to the recognition of several types of mycorrhizal symbioses (van der Heijden et al., 2015). The ancient origin and the ecological importance of these interactions have sparked interest in their assembly and evolution. In natural communities, mycorrhizal fungi typically interact with multiple plant species, rather than forming exclusive one-to-one associations (van der Heijden et al., 2015) . Therefore, they shape complex interaction networks (Perez-Lamarque et al., 2022; Sepp et al., 2019). By considering the structure of mycorrhizal networks and the phylogenetic trees of plants and their associated mycorrhizal fungi, it has been suggested that codiversification occurs in mycorrhizal symbioses (Arifin et al., 2023; Merckx and Bidartondo, 2008; Van Galen et al., 2023), meaning that plants and their associated mycorrhizal fungi may undergo simultaneous speciation events (cospeciations).

Testing for the occurrence of codiversification events in host-symbiont associations is notoriously challenging, and has spurred the development of several cophylogenetic methods (Blasco-Costa et al., 2021; De Vienne et al., 2013; Dismukes et al., 2022; Page, 1994; Perez-Lamarque and Morlon, 2024; Ronquist, 2002). However, two distinct patterns – cophylogenetic signal and phylogenetic congruence – have often been conflated (Perez-Lamarque and Morlon, 2024) (Figure 1). In the context of mycorrhizal symbioses, cophylogenetic signal indicates that closely related plants tend to interact with closely related mycorrhizal fungi and *vice-versa*. In contrast, phylogenetic congruence occurs when the phylogenetic trees of plants and their associated fungi mirror one another. Distinguishing these two patterns is crucial because they stem from different biological processes. A cophylogenetic signal may simply arise from trait matching of evolutionarily conserved traits or from past biogeographic events (Perez-Lamarque and Morlon, 2024). In contrast, phylogenetic congruence results from more constrained scenarios, for

instance codiversification through successive cospeciation events (Althoff et al., 2014; De Vienne et al., 2013; Perez-Lamarque and Morlon, 2024). Global-fit methods such as ParaFit or PACo (Balbuena et al., 2013; Hutchinson et al., 2017; Legendre et al., 2002) can assess cophylogenetic signal, but only event-based methods such as Jane or eMPRess (Conow et al., 2010; Santichaivekin et al., 2021) can evaluate phylogenetic congruence.

Here, we reevaluate the evidence for codiversification in mycorrhizal symbioses in light of recent cophylogenetic developments (Perez-Lamarque and Morlon, 2024), using an extensive collection of mycorrhizal networks. We manually screened the literature and selected 29 networks from 13 different publications, ensuring coverage of various spatial and evolutionary scales, as well as all four main types of mycorrhizal symbioses (arbuscular mycorrhizae, ectomycorrhizae, orchid and ericoid mycorrhizae). For each mycorrhizal network, if the phylogenetic trees were not already available, we retrieved representative DNA sequences for both plant and mycorrhizal fungi, using multiple genes when possible (Supplementary Table 1). Sequences were aligned using MAFFT (Katoh, 2002) and refined using trimAl (Capella-Gutiérrez et al., 2009). Robust phylogenetic trees were then reconstructed using IQ-TREE (Nguyen et al., 2015), constraining deep nodes by a robust backbone tree when necessary (Supplementary Table 1). To assess cophylogenetic signal, we applied the global-fit method PACo (Balbuena et al., 2013; Hutchinson et al., 2017) to each mycorrhizal network. We then evaluated phylogenetic congruence using the event-based model eMPRess (Santichaivekin et al., 2021). This tool reconciles phylogenetic trees by accounting for events of cospeciation, transfer, loss, and duplication, returns the number of each event, and assesses the significance of the reconciled scenario through permutations. We considered phylogenetic congruence to be supported when the reconciled scenario had a significantly low cost ($p$-value $< 0.05$) and included more cospeciation events than transfer events (Perez-Lamarque and Morlon, 2024).

We detected a significant cophylogenetic signal in 13 out of 29 mycorrhizal networks (Figure 2). This pattern was consistently observed across the different types of mycorrhizal symbioses, regardless of the spatial and evolutionary scales (Supplementary Table 1). This confirms that there is evidence of frequent cophylogenetic signal in mycorrhizal associations. In contrast, phylogenetic congruence was absent in all 29

networks (Figure 2). Our results were consistent across phylogenetic reconstruction methods (Supplementary Table 1), meaning that uncertainty in fungal phylogenies does not explain the observed patterns. The fungal phylogenies of 4 of the 29 networks were built with only the ITS region, which may poorly resolve deep evolutionary relationships, but generally supports recent splits. We are confident in our results on these networks despite uncertain deep phylogenetic resolution, because recent splits play the major role in the detection of phylogenetic congruence, while incongruence at deeper nodes can often be reconciled by a few host-transfer events. Therefore, our findings suggest that previous studies that proposed codiversification in mycorrhizal symbioses based on visual inspections or global-fit methods (Arifin et al., 2023; Merckx and Bidartondo, 2008; Van Galen et al., 2023) detected cophylogenetic signal rather than phylogenetic congruence, and that there is in fact no evidence of codiversification or cospeciation between plants and their associated mycorrhizal fungi.

The absence of codiversification in mycorrhizal symbioses may stem from the fact that most mycorrhizal partners do not engage in highly specialized or one-to-one associations, but instead participate in complex interaction networks characterized by high partner sharing (Arifin et al., 2023; Hayward and Horton, 2014; Öpik et al., 2009; Perez-Lamarque et al., 2022; Sepp et al., 2019; Toju et al., 2016; Van Galen et al., 2023; Zhao et al., 2021). Such generalized interactions reduce the likelihood of cospeciation (Althoff et al. 2014; Poisot et al. 2015). While our findings suggest that codiversification is uncommon in mycorrhizal networks, this result does not rule out the role of coevolution (Brundrett 2002, van der Heijden 2015). The widespread presence of cophylogenetic signal suggests that closely related plants tend to interact with closely related fungi. At regional and global scales, this signal could be at least partially explained by the biogeography of plants and/or mycorrhizal fungi. Vicariance events and dispersal limitations may lead to allopatric speciations and geographically independent radiations in both plants and fungi. As a result, closely related plants and closely related fungi tend to be present in similar places (Tedersoo et al., 2014), generating cophylogenetic signal (Perez-Lamarque and Morlon, 2024). However, we also observed cophylogenetic signal in mycorrhizal networks at local scale (Supplementary Table 1), which cannot be due to biogeography. Therefore, the cophylogenetic signal we observed is likely explained by the

fact that mycorrhizal interactions require a minimal level of trait matching, and that the involved mycorrhizal traits are evolutionary conserved. In this scenario, diffuse coevolution between interacting plants and fungi drives the evolutionary conservatism of key mycorrhizal traits (Cairney, 2000), such as plant root architecture, fungal nutrient exchange efficiency, or molecular signaling between plants and fungi (Castanedo et al., 2025; Delaux and Schornack, 2021; Karandashov et al., 2004; van der Heijden et al., 2015).

Our study highlights the importance of careful interpretation of cophylogenetic methods, and suggests that mycorrhizal symbionts did not codiversify with their host plants. Evolutionary history nevertheless plays an important role in shaping plant-mycorrhizal networks, as evidenced by the phylogenetic conservatism of interactions. Trait matching and trait conservatism likely mediates this phylogenetical signal, suggesting that a deeper understanding of the functional traits underlying mycorrhizal compatibility is key to understand the evolution of mycorrhizal symbioses. Finally, similar analyses in other plant-associated interactions, such as pollination, seed dispersal, or parasitic interactions, would clarify whether codiversification has played any role in the evolutionary history of land plants.

## Figures

**Figure 1: Cophylogenetic signal *versus* phylogenetic congruence. a**. Cophylogenetic signal: closely related plants tend to interact with closely related mycorrhizal fungi and *vice-versa* (this pattern can be generated by trait matching of evolutionarily conserved traits, as represented here with colors representing trait values, or by past biogeographic events leading to closely related species being present in similar places). **b.** Phylogenetic congruence: the phylogenetic trees of plants and their associated fungi mirror one another (this pattern can be generated by codiversification, *i.e.* concomitant diversification events happening in both plant and fungal interacting lineages).

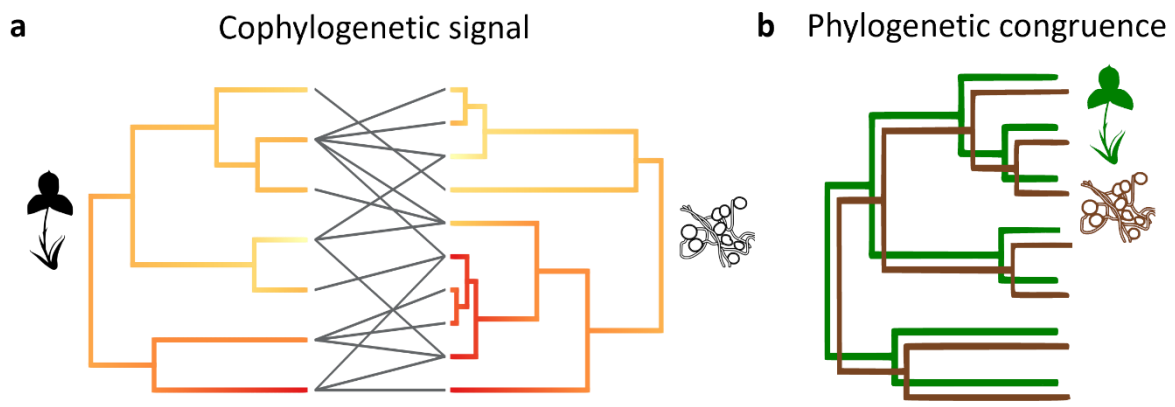
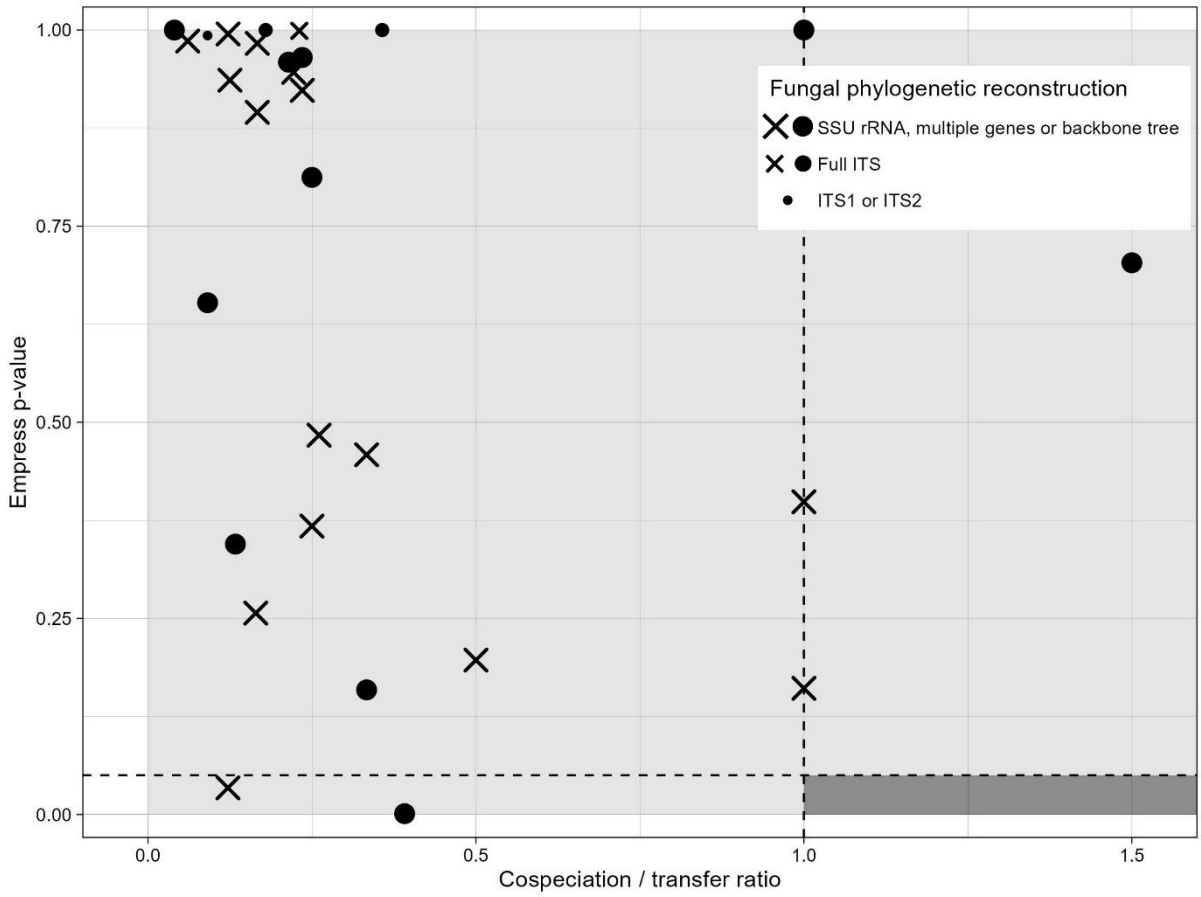


**Figure 2: Cophylogenetic analysis of 29 mycorrhizal networks.** For each mycorrhizal network, we report its eMPRess p-value as a function of the ratio of cospeciation to transfer events inferred by eMPRess. The grey regions indicate whether there is phylogenetic congruence: the dark grey area indicates significant phylogenetic congruence (eMPRess p-value < 0.05 and cospeciation / transfer ratio > 1), whereas the light grey areas indicate no significant phylogenetic congruence (eMPRess p-value > 0.05 and/or cospeciation / transfer ratio < 1). In addition, the shape of each symbol reflects the significance of the cophylogenetic signal inferred using PACo: a dot represents a network with significant cophylogenetic signal (PACo p-value < 0.05), whereas a cross represents a network without cophylogenetic signal (PACo p-value > 0.05). The size of each shape indicates the type of genetic data available for reconstructing the fungal phylogenetic trees. Detailed information on each network can be obtained in Supplementary Table 1.



175 # Methods

176 **Screening for available mycorrhizal networks**

177 We manually screened the literature to compile as many mycorrhizal networks as
178 possible (Supplementary Table 1). We targeted publications that provided freely
179 accessible interaction networks, along with the phylogenetic trees or the DNA sequences
180 of both plants and mycorrhizal fungi. We retained networks from natural habitats and with
181 taxonomic delineations at the plant species level. Fungal operational taxonomic units
182 (OTUs) were typically delineated using a 97% similarity threshold. We aimed to include all
183 major types of mycorrhizal symbioses: arbuscular mycorrhizae (n=11), ectomycorrhizae
184 (n=5), orchid mycorrhizae (n=7), and ericoid mycorrhizae (n=6). We also selected
185 networks encompassing a broad range of spatial scales – from communities in specific
186 habitats of La Réunion (Perez-Lamarque et al., 2022) to the global arbuscular mycorrhizal
187 network between Glomeromycotina fungi and land plant species (Öpik et al., 2010; Perez-
188 Lamarque et al., 2020) – and evolutionary scales – from a 14 million-year-old
189 ectomycorrhizal association between Pisonieae and their thelephoroid fungal symbionts
190 (Hayward and Horton, 2014) to more than 450 million years of diversification of all land
191 plants forming arbuscular mycorrhizae. In total, we studied 29 networks from 13 different
192 publications (Arifin et al., 2023; Hayward and Horton, 2014; Martos et al., 2012; Merckx
193 and Bidartondo, 2008; Öpik et al., 2010, 2009; Otero et al., 2011; Perez-Lamarque et al.,
194 2022; Perez-Lamarque et al., 2020; Sepp et al., 2019; Shefferson et al., 2010; Toju et al.,
195 2016; Van Galen et al., 2023; Zhao et al., 2021).

196 **Recovering phylogenetic trees**

197 For some publications, robust phylogenetic trees were already publicly available
198 (Supplementary Table 1). For the others, we retrieved DNA sequences from GenBank,
199 aligned them with MAFFT (Katoh, 2002), trimmed them with trimAl (Capella-Gutiérrez et
200 al., 2009), and then reconstructed the phylogenetic trees with IQ-TREE (Nguyen et al.,
201 2015). When multiple sequences were available for a single species, we selected one
202 representative (Supplementary Table 2). For the plants, we consistently used sequences
203 from at least two genes, selected from trnL, ITS, matK, matR, 18S, and ATPa
204 (Supplementary Table 1). When no genetic data were available in the original

205 publications, we reconstructed the missing plant phylogenetic trees with V.PhyloMaker2
206 (Jin and Qian, 2022). For the fungal phylogenetic reconstructions, the available
207 sequences primarily included the SSU rRNA gene and/or the ITS region (ITS1, ITS2, or the
208 full ITS1-5.8S-ITS2 fragment), sometimes in addition to other genes (28S, EF1 alpha,
209 TOR2, ML 4-5). We considered robust the phylogenies obtained with multiple genes.
210 While the SSU rRNA gene alone can support robust phylogenetic tree, the fungal ITS
211 region is often too variable, especially ITS1 or ITS2 alone, to provide robust phylogenetic
212 information to reliably resolve deeper evolutionary relationships (Perez-Lamarque et al.,
213 2022; Schoch et al., 2012). Therefore, to obtain robust phylogenetic trees when only
214 sequences of partial ITS1 or ITS2 were available, we informed the IQ-TREE
215 reconstructions with a robust backbone tree built from multiple-gene datasets (following
216 Talavera et al., 2022). Specifically, we used the backbone trees of Weiß et al. (2016) for
217 Sebacinales, of Johnston et al. (2019) for Helotiales, and of Zhang et al. (2025) for
218 Cantharellales (Supplementary Table 1). In total, 15 fungal phylogenetic trees were
219 reconstructed using either multi-gene datasets or a backbone-tree approach, 10 using
220 the SSU rRNA gene, 3 using the full ITS region, and 1 using only ITS1, as no additional
221 sequences or backbone trees were available for this mycorrhizal system (Supplementary
222 Table 1).

223 **Evaluating cophylogenetic signal**

224 We assessed cophylogenetic signal, *i.e.* whether closely related plants tend to interact
225 with closely related fungi, using the global-fit method PACo (Balbuena et al., 2013;
226 Hutchinson et al., 2017). This method returns a $R^2$ and a p-value obtained by comparison
227 with permutations. We ran 10,000 permutations for each mycorrhizal network.
228 Additionally, we checked the results with the global-fit method parafit (Legendre et al.,
229 2002; Paradis et al., 2004). Both methods yielded similar results (Supplementary Table 1).

230 **Evaluating phylogenetic congruence**

231 We assessed phylogenetic congruence, *i.e.* whether the phylogenetic trees of plants and
232 mycorrhizal fungi tend to mirror one another, with the event-based model eMPRess
233 (Santichaivekin et al., 2021). eMPRess reconciles the plant and fungal phylogenetic trees
234 using maximum parsimony to model events of cospeciation, host transfer, duplication,

and loss, with a given cost associated to each event. We ran eMPRess with the following costs: 0 for a cospeciation event, 4 for a duplication event, 1 for a loss event, and 1 for a transfer event. We obtained similar results using different cost values (Supplementary Table 1). To assess the significance of each eMPRess reconciliation, we performed 1,000 permutations of the mycorrhizal associations. We considered a reconciliation as significant when the total cost was lower than 95% of the costs obtained from the permuted data (eMPRess p-value < 0.05) and when the number of cospeciation events was larger than the number of transfer events (cospeciation / transfer ratio > 1; Perez-Lamarque & Morlon, 2024).

As eMPRess cannot account for symbionts interacting with multiple hosts, when a fungal species interacted with several plant species, we only picked the most abundant interaction (Supplementary Table 1).

## Acknowledgments

The authors acknowledge members of the 'Modeling biodiversity' lab at IBENS for helpful discussions, as well as Alice Garcia for initiating the project with preliminary analyses.

The authors also acknowledge Arild Arifin and Zhongtao Zhao for sharing the interaction networks and phylogenetic trees of plants and mycorrhizal fungi they studied, as well as all other authors whose data were directly available in their publications.

## Competing interests

The authors declare no conflict of interests.

## Author contributions

FB, HM, and BPL designed the study, FB performed the analyses, FB and BPL wrote a first version of the manuscript and all authors contributed to the revisions.

## Code availability

All the scripts for performing the analyses are available through the following link: https://github.com/fantine-bodin/Cophylogenetics_mycorrhizal-symbioses.git

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## Supporting information

**Supplementary Table 1: Summary table with characteristics of each mycorrhizal network and the corresponding results of the cophylogenetic analyses.** The table reports, for each network: the spatial, evolutionary and taxonomic scales, the network size, the type of mycorrhizal associations, how we recovered phylogenetic trees of both plants and fungi, the results of the evaluation of cophylogenetic signal (with 2 methods: PACo and ParaFit), and the results of the evaluation of phylogenetic congruence (with the event-based method eMPRess with different cost values for each reconciliation event).

**Supplementary Table 2: Data selection for the mycorrhizal networks from Arifin et al. (Arifin et al., 2023).** Modification of Table S1 from Arifin et al. (2023). As different sequences of the same OTU were available, the representative sequence of each fungal OTU chosen to build the phylogenetic tree are highlighted in green.