Questioning the Evidence for Host-Symbiont Codiversification in Mycorrhizal Symbioses

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Key words

Cophylogenetic signal, phylogenetic congruence, mycorrhizal symbioses, coevolution, codiversification, cospeciation.

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 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 and *vice-versa*), which may arise 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 evidence of codiversification in mycorrhizal symbioses actually detected cophylogenetic signal, indicating that there is no evidence of codiversification 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 (Chagnon et al., 2012; Jacquemyn et al., 2015; Perez-Lamarque et al., 2022; Petrolli et al., 2022; Rimington et al., 2019; 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 phylogenetic congruence can occur in mycorrhizal symbioses (Arifin et al., 2023; Merckx and Bidartondo, 2008; Van Galen et al., 2023). Whilst these studies discuss multiple processes by which these patterns could arise, one possibility is that plants and their associated mycorrhizal fungi could be undergoing simultaneous speciation events (*i.e.,* cospeciation), leading to codiversification (see Table 1 for definitions).

Testing for the occurrence of codiversification 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; Table 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 is a more stringent, specific case of cophylogenetic signal, occurring when the phylogenetic trees of plants and their associated mycorrhizal fungi closely mirror each other. Codiversification refers to this pattern of phylogenetic congruence combined with matching divergence times, reflecting parallel diversification histories in plants and their associated fungi (Suzuki et al., 2022). Distinguishing these two patterns of cophylogenetic signal and phylogenetic congruence is crucial because they may stem from different biological processes. A cophylogenetic signal may simply arise if present-day mycorrhizal interactions assemble according to the trait matching of evolutionarily conserved traits (Perez-Lamarque and Morlon, 2024). It can also be generated by past biogeographic events that have constrained the distributions of extant species. In contrast, phylogenetic

congruence arises from more stringent scenarios. For instance, codiversification can result from repeated cospeciation events driven by phylogenetic tracking, or from successive vicariance events that repeatedly affect both plants and their associated mycorrhizal fungi (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 fitting of model of phylogenetic tracking that allows for events of cospeciation, transfer, loss, and duplication. eMPRess 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 (Dorrell et al., 2021; Groussin et al., 2017; 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. Our results were robust despite uncertain deep phylogenetic resolution in some networks 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 using eMPRess. Therefore, given the high diversity of mycorrhizal networks analyzed, the consistency of our results indicates that they can be generalized beyond the specific sets of species and systems included in our analyses. Our findings suggest that previous studies that proposed codiversification in mycorrhizal symbioses based on visual inspections or global-fit methods detected cophylogenetic signal rather than phylogenetic congruence, and that there is in fact no evidence of codiversification 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 frequent cospeciation (Althoff et al. 2014; Poisot et al. 2015), although our results do not rule out the occurrence of rare cospeciation events that may sporadically happen in some mycorrhizal associations and whose signal may be blurred by other events. While our findings suggest that codiversification is uncommon in mycorrhizal networks, this result also 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 within individual communities (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, synthesizing our analyses with similar studies in other plant-associated interactions, such as pollination, seed dispersal, or parasitic interactions (Asar et al., 2022; Jackson, 2004; Russo et al., 2018; Taengon et al., 2025), would help clarifying whether codiversification has ever play a significant role in the evolutionary history of land plants.

Tables

Table 1: Cophylogenetic definitions in the context of mycorrhizal symbioses. These definitions are following the terminology of Perez-Lamarque and Morlon (2024).

Terminology	Process or pattern	Definition
Codiversification/ Co-cladogenesis:	Pattern	Parallel diversification of plant and mycorrhizal fungal lineages, in which the two clades exhibit phylogenetic congruence and matching divergence times. It can be generated through processes of phylogenetic tracking or successive vicariance events repeatedly affecting both clades.
Coevolution:	Process	Reciprocal evolutionary changes driven by selective pressures between two interacting lineages (pairwise coevolution). When more than two lineages are involved, the process is referred to as **diffuse coevolution**.
Cophylogenetic signal:	Pattern	Tendency of closely related plant species to interact with closely related mycorrhizal fungal partners (and *vice-versa*).
Cospeciation:	Process	Concomitant speciation event in a plant lineage and its associated mycorrhizal fungal lineage.
Phylogenetic congruence:	Pattern	Strong similarity between the phylogenetic trees of interacting plant and mycorrhizal fungal clades in terms of topology and relative branch lengths. When the divergence times of the two clades also coincide, this pattern corresponds to codiversification.
Phylogenetic tracking:	Process	Repeated plant speciation events leading to subsequent speciations in their associated mycorrhizal fungal lineages (**cospeciations**), for example through vertical transmission that isolates fungal populations between diverging plant lineages. Fungal lineages may also undergo host transfers, intra-host duplications, or losses at low frequency. Phylogenetic tracking typically results in a pattern of codiversification.
Trait matching	Process	Association of plants and fungi because of the complementarity of their mycorrhizal traits (for

		example plant root architecture, fungal nutrient exchange efficiency, or molecular signaling between plants and fungi). When these traits tend to be evolutionarily conserved, trait matching typically results in cophylogenetic signal.
Tree topology:	Pattern	Branching structure of the different nodes of a phylogenetic tree that does not consider absolute branch lengths (*i.e.* evolutionary time).

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; see Table 1 for a list of definitions of cophylogenetic patterns and processes).

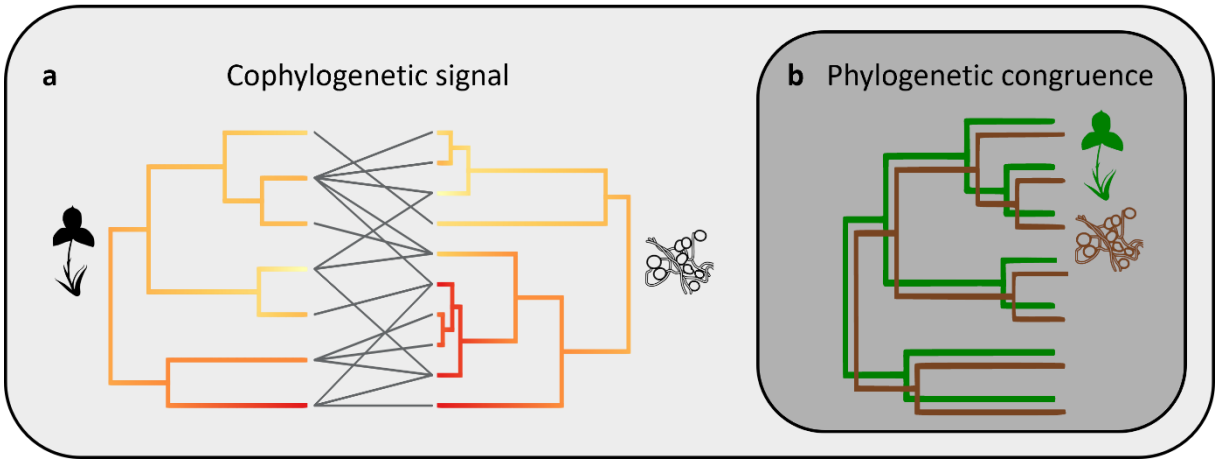
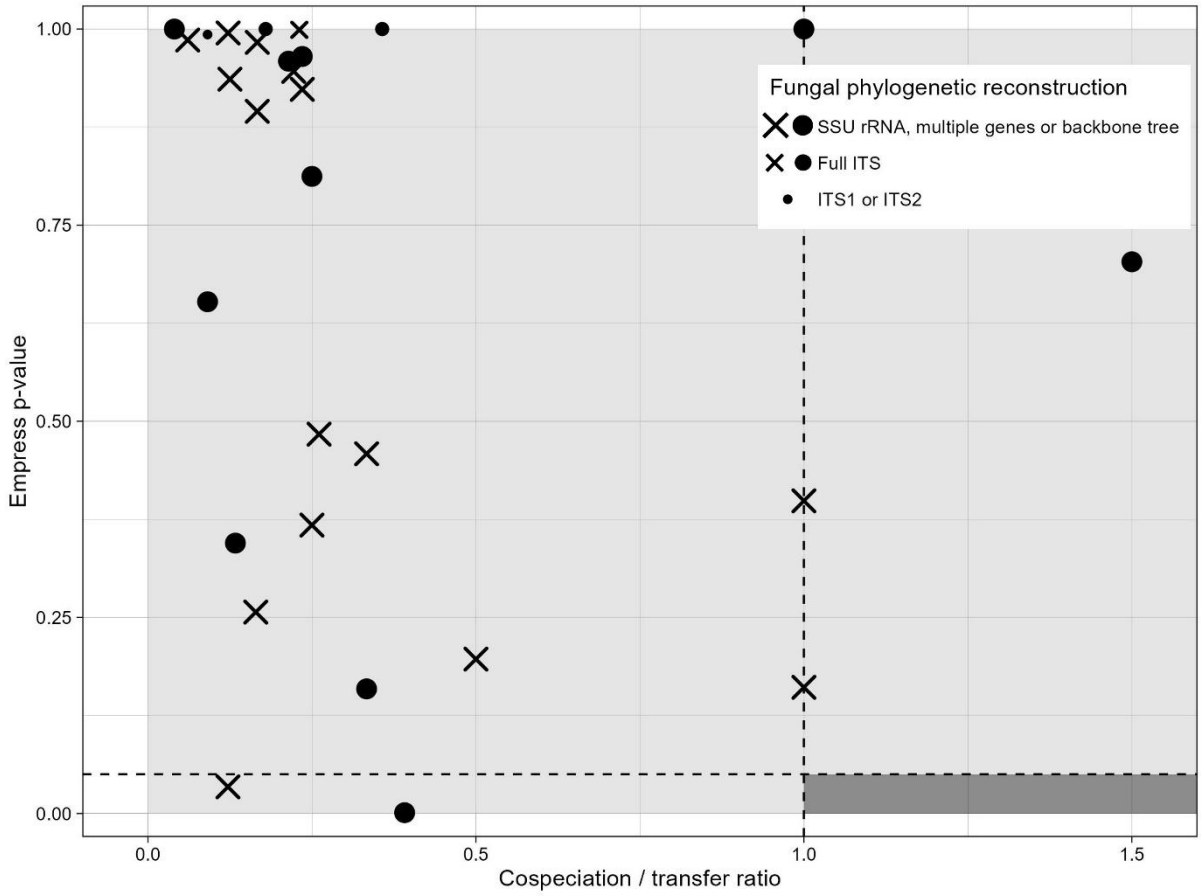


Figure 2: Cophylogenetic analysis of 29 mycorrhizal networks. For each mycorrhizal network, we report its eMPRess p-value as a function of the ratio of cospeciation to transfer events inferred by eMPRess. The grey regions indicate whether there is phylogenetic congruence: the dark grey area indicates 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.



Methods

Screening for available mycorrhizal networks

We manually screened the literature to compile as many mycorrhizal networks as possible (Supplementary Table 1). We targeted publications that provided freely accessible interaction networks, along with the phylogenetic trees or the DNA sequences of both plants and mycorrhizal fungi. We retained networks from natural habitats and with taxonomic delineations at the plant species level. Fungal operational taxonomic units (OTUs) were typically delineated using a 97% similarity threshold. We aimed to include all major types of mycorrhizal symbioses: arbuscular mycorrhizae (n=11), ectomycorrhizae (n=5), orchid mycorrhizae (n=7), and ericoid mycorrhizae (n=6). We also selected networks encompassing a broad range of spatial scales – from communities in specific habitats of La Réunion (Perez-Lamarque et al., 2022) to the global arbuscular mycorrhizal network between Glomeromycotina fungi and land plant species (Öpik et al., 2010; Perez-Lamarque et al., 2020) – and evolutionary scales – from a 14 million-year-old ectomycorrhizal association between Pisonieae and their thelephoroid fungal symbionts (Hayward and Horton, 2014) to more than 450 million years of diversification of all land plants forming arbuscular mycorrhizae. Our networks also encompass a diversity of sampling strategies: from sampling all the species present in a local community (for example Perez-Lamarque et al., 2022; Sepp et al., 2019), to intentionally targeting on some specific plant clades only (as in Arifin et al., 2023; Shefferson et al., 2010) or gathering all plant-fungal associations available at regional or global scale (for instance Öpik et al., 2009; Perez-Lamarque et al., 2020).

In total, we studied 29 networks from 13 different publications (Arifin et al., 2023; Hayward and Horton, 2014; Martos et al., 2012; Merckx and Bidartondo, 2008; Öpik et al., 2010, 2009; Otero et al., 2011; Perez-Lamarque et al., 2022; Perez-Lamarque et al., 2020; Sepp et al., 2019; Shefferson et al., 2010; Toju et al., 2016; Van Galen et al., 2023; Zhao et al., 2021).

Recovering phylogenetic trees

For some publications, robust phylogenetic trees were already publicly available (Supplementary Table 1). For the others, we retrieved DNA sequences from GenBank, aligned them with MAFFT (Katoh, 2002), trimmed them with trimAl (Capella-Gutiérrez et al., 2009), and then reconstructed the phylogenetic trees with IQ-TREE (Nguyen et al., 2015). When multiple sequences were available for a single species, we selected one representative (Supplementary Table 2). For the plants, we consistently used sequences from at least two genes, selected from trnL, ITS, matK, matR, 18S, and ATPa (Supplementary Table 1). When no genetic data were available in the original publications, we reconstructed the missing plant phylogenetic trees with V.PhyloMaker2 (Jin and Qian, 2022). For the fungal phylogenetic reconstructions, the available sequences primarily included the SSU rRNA gene and/or the ITS region (ITS1, ITS2, or the

full ITS1-5.8S-ITS2 fragment), sometimes in addition to other genes (28S, EF1 alpha, TOR2, ML 4-5). We considered robust the phylogenies obtained with multiple genes. While the SSU rRNA gene alone can support robust phylogenetic tree, the fungal ITS region is often too variable, especially ITS1 or ITS2 alone, to reliably resolve deeper evolutionary relationships (Perez-Lamarque et al., 2022; Schoch et al., 2012). Therefore, to obtain robust phylogenetic trees when only sequences of partial ITS1 or ITS2 were available, we informed the IQ-TREE reconstructions with a robust backbone tree built from multiple-gene datasets (following Talavera et al., 2022). Specifically, we used the backbone trees of Weiß et al. (2016) for Sebacinales, of Johnston et al. (2019) for Helotiales, and of Zhang et al. (2025) for Cantharellales (Supplementary Table 1). This backbone approach improves bootstrap branch support, raising values >70% for most nodes. In total, 15 fungal phylogenetic trees were reconstructed using either multi-gene datasets or a backbone-tree approach, 10 using the SSU rRNA gene, 3 using the full ITS region, and 1 using only ITS1, as no additional sequences or backbone trees were available for this mycorrhizal system (Supplementary Table 1).

Evaluating cophylogenetic signal

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

Evaluating phylogenetic congruence

We assessed phylogenetic congruence, *i.e.* whether the phylogenetic trees of plants and mycorrhizal fungi tend to mirror one another, with the event-based model eMPRess (Santichaivekin et al., 2021). eMPRess assumes a process of phylogenetic tracking and tries to reconcile the plant and fungal phylogenetic trees by fitting, using maximum parsimony, 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). Indeed, transfer events can obscure cospeciation signals and generate a symbiont phylogeny that no longer matches the host phylogeny.

Importantly, eMPRess, like most event-based methods, evaluates phylogenetic congruence by considering only the topologies of the plant and fungal phylogenetic trees. It does not take into account whether the divergence times of corresponding plant and fungal nodes are concomitant to fit a cospeciation event. Thus, to conclude that codiversification has occurred, both a significant phylogenetic congruence (as detected by eMPRess) and coincident divergence times between the two clades are needed. In this study, we did not evaluate the latter aspect, as most fungal phylogenies were not time-calibrated.

As phylogenetic congruence can only exist if one-to-one host-symbiont interactions are frequent, eMPRess cannot account for symbionts interacting with multiple hosts, like most event-based approach (De Vienne et al., 2013; Dismukes et al., 2022). Therefore, when a fungal species interacted with several plant species, we considered two approaches (Perez-Lamarque & Morlon, 2024): (1) we only picked the most abundant interaction (or one interaction at random when abundances were not available; Supplementary Table 1), or (2) we randomly simulated bifurcating sub-trees for fungal taxa with multiple plants, such that each tip in the fungal tree is associated with a single plant tip (Satler et al., 2019). As the second approach did not change our findings, we only reported the results of the first approach in the main text (Supplementary Table 1).

Finally, we verified that our results obtained with eMPRess were robust to the small size of some networks and the phylogenetic uncertainty in the fungal trees. Regarding the small sampling size, it has been demonstrated using simulations that eMPRess still performs well when the number of codiversifying species in low (Perez-Lamarque et al. 2024). For the phylogenetic uncertainty, we ran eMPRess with different fungal phylogenetic trees obtained with different reconstruction methods (ITS only or the backbone approach) and obtained consistent results in all cases.

Acknowledgments

The authors acknowledge members of the 'Modeling biodiversity' lab at IBENS and Laura Van Galen 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

References

Althoff, D.M., Segraves, K.A., Johnson, M.T.J., 2014. Testing for coevolutionary diversification: linking pattern with process. Trends Ecol. Evol. 29, 82–89. https://doi.org/10.1016/j.tree.2013.11.003

Arifin, A.R., Phillips, R.D., Linde, C.C., 2023. Strong phylogenetic congruence between *Tulasnella* fungi and their associated Drakaeinae orchids. J. Evol. Biol. 36, 221–237. https://doi.org/10.1111/jeb.14107

Asar, Y., Ho, S.Y.W., Sauquet, H., 2022. Early diversifications of angiosperms and their insect pollinators: were they unlinked? Trends Plant Sci. 27, 858–869. https://doi.org/10.1016/j.tplants.2022.04.004

Balbuena, J.A., Míguez-Lozano, R., Blasco-Costa, I., 2013. PACo: A novel Procrustes Application to Cophylogenetic analysis. PLoS ONE 8, e61048. https://doi.org/10.1371/journal.pone.0061048

Blasco-Costa, I., Hayward, A., Poulin, R., Balbuena, J.A., 2021. Next-generation cophylogeny: unravelling eco-evolutionary processes. Trends Ecol. Evol. 36, 907–918. https://doi.org/10.1016/j.tree.2021.06.006

Brundrett, M.C., Tedersoo, L., 2018. Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytol. 220, 1108–1115. https://doi.org/10.1111/nph.14976

Cairney, J.W.G., 2000. Evolution of mycorrhiza systems. Naturwissenschaften 87, 467–475. https://doi.org/10.1007/s001140050762

Capella-Gutiérrez, S., Silla-Martínez, J.M., Gabaldón, T., 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25, 1972–1973. https://doi.org/10.1093/bioinformatics/btp348

Castanedo, L., Bianconi, M., Pellen, T., Vernie, T., Lefort, C., Libourel, C., Le Ru, A., Rodde, N., Cauet, S., Callot, C., Forrest, L., Nishiyama, T., Wickell, D., Li, F.-W., Keller, J., Delaux, P.-M., 2025. Leafy liverwort genomes shed light on the evolution of ericoid symbiosis. https://doi.org/10.1101/2025.04.01.646537

Chagnon, P., Bradley, R.L., Klironomos, J.N., 2012. Using ecological network theory to evaluate the causes and consequences of arbuscular mycorrhizal community structure. New Phytol. 194, 307–312. https://doi.org/10.1111/j.1469-8137.2011.04044.x

Conow, C., Fielder, D., Ovadia, Y., Libeskind-Hadas, R., 2010. Jane: a new tool for the cophylogeny reconstruction problem. Algorithms Mol. Biol. 5. https://doi.org/10.1186/1748-7188-5-16

De Vienne, D.M., Refrégier, G., López-Villavicencio, M., Tellier, A., Hood, M.E., Giraud, T., 2013. Cospeciation vs host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. New Phytol. 198, 347–385. https://doi.org/10.1111/nph.12150

Delaux, P.-M., Schornack, S., 2021. Plant evolution driven by interactions with symbiotic and pathogenic microbes. Science 371, eaba6605. https://doi.org/10.1126/science.aba6605

Dismukes, W., Braga, M.P., Hembry, D.H., Heath, T.A., Landis, M.J., 2022. Cophylogenetic methods to untangle the evolutionary history of ecological interactions. Annu. Rev. Ecol. Evol. Syst. 53, 275–298. https://doi.org/10.1146/annurev-ecolsys-102320-112823

Dorrell, R.G., Villain, A., Perez-Lamarque, B., Audren De Kerdrel, G., McCallum, G., Watson, A.K., Ait-Mohamed, O., Alberti, A., Corre, E., Frischkorn, K.R., Pierella Karlusich, J.J., Pelletier, E., Morlon, H., Bowler, C., Blanc, G., 2021. Phylogenomic fingerprinting of tempo and functions of horizontal gene transfer within ochrophytes. Proc. Natl. Acad. Sci. 118, e2009974118. https://doi.org/10.1073/pnas.2009974118

Groussin, M., Mazel, F., Sanders, J.G., Smillie, C.S., Lavergne, S., Thuiller, W., Alm, E.J., 2017. Unraveling the processes shaping mammalian gut microbiomes over evolutionary time. Nat. Commun. 8, 14319. https://doi.org/10.1038/ncomms14319

Hayward, J., Horton, T.R., 2014. Phylogenetic trait conservation in the partner choice of a group of ectomycorrhizal trees. Mol. Ecol. 23, 4886–4898. https://doi.org/10.1111/mec.12903

Hutchinson, M.C., Cagua, E.F., Balbuena, J.A., Stouffer, D.B., Poisot, T., 2017. paco: implementing Procrustean Approach to Cophylogeny in R. Methods Ecol. Evol. 8, 932–940. https://doi.org/10.1111/2041-210X.12736

Jackson, A.P., 2004. A reconciliation analysis oh host switching in plant-fungal symbioses. Evolution 58, 1909–1923. https://doi.org/10.1111/j.0014-3820.2004.tb00479.x

Jacquemyn, H., Brys, R., Waud, M., Busschaert, P., Lievens, B., 2015. Mycorrhizal networks and coexistence in species-rich orchid communities. New Phytol. 206, 1127–1134. https://doi.org/10.1111/nph.13281

Jin, Y., Qian, H., 2022. V.PhyloMaker2: An updated and enlarged R package that can generate very large phylogenies for vascular plants. Plant Divers. 44, 335–339. https://doi.org/10.1016/j.pld.2022.05.005

Johnston, P.R., Quijada, L., Smith, C.A., Baral, H.-O., Hosoya, T., Baschien, C., Pärtel, K., Zhuang, W.-Y., Haelewaters, D., Park, D., Carl, S., López-Giráldez, F., Wang, Z., Townsend, J.P., 2019. A multigene phylogeny toward a new phylogenetic classification of Leotiomycetes. IMA Fungus 10. https://doi.org/10.1186/s43008-019-0002-x

Karandashov, V., Nagy, R., Wegmüller, S., Amrhein, N., Bucher, M., 2004. Evolutionary conservation of a phosphate transporter in the arbuscular mycorrhizal symbiosis. Proc. Natl. Acad. Sci. 101, 6285–6290. https://doi.org/10.1073/pnas.0306074101

Katoh, K., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30, 3059–3066. https://doi.org/10.1093/nar/gkf436

Legendre, P., Desdevises, Y., Bazin, E., 2002. A statistical test for host–parasite coevolution. Syst. Biol. 51, 217–234. https://doi.org/10.1080/10635150252899734

Martos, F., Munoz, F., Pailler, T., Kottke, I., Gonneau, C., Selosse, M., 2012. The role of epiphytism in architecture and evolutionary constraint within mycorrhizal networks of tropical orchids. Mol. Ecol. 21, 5098–5109. https://doi.org/10.1111/j.1365-294X.2012.05692.x

Merckx, V., Bidartondo, M.I., 2008. Breakdown and delayed cospeciation in the arbuscular mycorrhizal mutualism. Proc. R. Soc. B Biol. Sci. 275, 1029–1035. https://doi.org/10.1098/rspb.2007.1622

Nguyen, L.-T., Schmidt, H.A., Von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32, 268–274. https://doi.org/10.1093/molbev/msu300

Öpik, M., Metsis, M., Daniell, T.J., Zobel, M., Moora, M., 2009. Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. New Phytol. 184, 424–437. https://doi.org/10.1111/j.1469-8137.2009.02920.x

Öpik, M., Vanatoa, A., Vanatoa, E., Moora, M., Davison, J., Kalwij, J.M., Reier, Ü., Zobel, M., 2010. The online database Maarj *AM* reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol. 188, 223–241. https://doi.org/10.1111/j.1469-8137.2010.03334.x

Otero, J.T., Thrall, P.H., Clements, M., Burdon, J.J., Miller, J.T., 2011. Codiversification of orchids (Pterostylidinae) and their associated mycorrhizal fungi. Aust. J. Bot. 59, 480. https://doi.org/10.1071/BT11053

Page, R., 1994. Parallel phylogenies: reconstructing the history of host-parasite assemblages. Cladistics 10, 155–173. https://doi.org/10.1006/clad.1994.1010

Paradis, E., Claude, J., Strimmer, K., 2004. APE: Analyses of Phylogenetics and Evolution in R language. Bioinformatics 20, 289–290. https://doi.org/10.1093/bioinformatics/btg412

Perez-Lamarque, B., Morlon, H., 2024. Distinguishing Cophylogenetic Signal from Phylogenetic Congruence Clarifies the Interplay Between Evolutionary History and Species Interactions. Syst. Biol. syae013. https://doi.org/10.1093/sysbio/syae013

Perez-Lamarque, B., Öpik, M., Maliet, O., Afonso Silva, A.C., Selosse, M., Martos, F., Morlon, H., 2022. Analysing diversification dynamics using barcoding data: The case of an obligate mycorrhizal symbiont. Mol. Ecol. 31, 3496–3512. https://doi.org/10.1111/mec.16478

Perez-Lamarque, B., Petrolli, R., Strullu-Derrien, C., Strasberg, D., Morlon, H., Selosse, M.-A., Martos, F., 2022. Structure and specialization of mycorrhizal networks in phylogenetically diverse tropical communities. Environ. Microbiome 17, 38. https://doi.org/10.1186/s40793-022-00434-0

Perez-Lamarque, B., Selosse, M., Öpik, M., Morlon, H., Martos, F., 2020. Cheating in arbuscular mycorrhizal mutualism: a network and phylogenetic analysis of mycoheterotrophy. New Phytol. 226, 1822–1835. https://doi.org/10.1111/nph.16474

Petrolli, R., Zinger, L., Perez-Lamarque, B., Collobert, G., Griveau, C., Pailler, T., Selosse, M., Martos, F., 2022. Spatial turnover of fungi and partner choice shape mycorrhizal networks in epiphytic orchids. J. Ecol. 110, 2568–2584. https://doi.org/10.1111/1365-2745.13986

Remy, W., Taylor, T.N., Hass, H., Kerp, H., 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proc. Natl. Acad. Sci. 91, 11841–11843. https://doi.org/10.1073/pnas.91.25.11841

Rimington, W.R., Pressel, S., Duckett, J.G., Field, K.J., Bidartondo, M.I., 2019. Evolution and networks in ancient and widespread symbioses between Mucoromycotina and liverworts. Mycorrhiza 29, 551–565. https://doi.org/10.1007/s00572-019-00918-x

Ronquist, F., 2002. Parsimony analysis of coevolving species associations.

Russo, L., Miller, A.D., Tooker, J., Bjornstad, O.N., Shea, K., 2018. Quantitative evolutionary patterns in bipartite networks: Vicariance, phylogenetic tracking or diffuse co-evolution? Methods Ecol. Evol. 9, 761–772. https://doi.org/10.1111/2041-210X.12914

Santichaivekin, S., Yang, Q., Liu, J., Mawhorter, R., Jiang, J., Wesley, T., Wu, Y.-C., Libeskind-Hadas, R., 2021. eMPRess: a systematic cophylogeny reconciliation tool. Bioinformatics 37, 2481–2482. https://doi.org/10.1093/bioinformatics/btaa978

Satler, J.D., Herre, E.A., Jandér, K.C., Eaton, D.A.R., Machado, C.A., Heath, T.A., Nason, J.D., 2019. Inferring processes of coevolutionary diversification in a community of Panamanian strangler figs and associated pollinating wasps*. Evolution 73, 2295–2311. https://doi.org/10.1111/evo.13809

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., Fungal Barcoding Consortium, Fungal Barcoding Consortium Author List, Bolchacova, E., Voigt, K., Crous, P.W., Miller, A.N., Wingfield, M.J., Aime, M.C., An, K.-D., Bai, F.-Y., Barreto, R.W., Begerow, D., Bergeron, M.-J., Blackwell, M.,

Boekhout, T., Bogale, M., Boonyuen, N., Burgaz, A.R., Buyck, B., Cai, L., Cai, Q., Cardinali, G., Chaverri, P., Coppins, B.J., Crespo, A., Cubas, P., Cummings, C., Damm, U., De Beer, Z.W., De Hoog, G.S., Del-Prado, R., Dentinger, B., Diéguez-Uribeondo, J., Divakar, P.K., Douglas, B., Dueñas, M., Duong, T.A., Eberhardt, U., Edwards, J.E., Elshahed, M.S., Fliegerova, K., Furtado, M., García, M.A., Ge, Z.-W., Griffith, G.W., Griffiths, K., Groenewald, J.Z., Groenewald, M., Grube, M., Gryzenhout, M., Guo, L.-D., Hagen, F., Hambleton, S., Hamelin, R.C., Hansen, K., Harrold, P., Heller, G., Herrera, C., Hirayama, K., Hirooka, Y., Ho, H.-M., Hoffmann, K., Hofstetter, V., Högnabba, F., Hollingsworth, P.M., Hong, S.-B., Hosaka, K., Houbraken, J., Hughes, K., Huhtinen, S., Hyde, K.D., James, T., Johnson, E.M., Johnson, J.E., Johnston, P.R., Jones, E.B.G., Kelly, L.J., Kirk, P.M., Knapp, D.G., Kõljalg, U., Kovács, G.M., Kurtzman, C.P., Landvik, S., Leavitt, S.D., Liggenstoffer, A.S., Liimatainen, K., Lombard, L., Luangsa-ard, J.J., Lumbsch, H.T., Maganti, H., Maharachchikumbura, S.S.N., Martin, M.P., May, T.W., McTaggart, A.R., Methven, A.S., Meyer, W., Moncalvo, J.-M., Mongkolsamrit, S., Nagy, L.G., Nilsson, R.H., Niskanen, T., Nyilasi, I., Okada, G., Okane, I., Olariaga, I., Otte, J., Papp, T., Park, D., Petkovits, T., Pino-Bodas, R., Quaedvlieg, W., Raja, H.A., Redecker, D., Rintoul, T.L., Ruibal, C., Sarmiento-Ramírez, J.M., Schmitt, I., Schüßler, A., Shearer, C., Sotome, K., Stefani, F.O.P., Stenroos, S., Stielow, B., Stockinger, H., Suetrong, S., Suh, S.-O., Sung, G.-H., Suzuki, M., Tanaka, K., Tedersoo, L., Telleria, M.T., Tretter, E., Untereiner, W.A., Urbina, H., Vágvölgyi, C., Vialle, A., Vu, T.D., Walther, G., Wang, Q.-M., Wang, Y., Weir, B.S., Weiß, M., White, M.M., Xu, J., Yahr, R., Yang, Z.L., Yurkov, A., Zamora, J.-C., Zhang, N., Zhuang, W.-Y., Schindel, D., 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*. Proc. Natl. Acad. Sci. 109, 6241–6246. https://doi.org/10.1073/pnas.1117018109

Sepp, S., Davison, J., Jairus, T., Vasar, M., Moora, M., Zobel, M., Öpik, M., 2019. Non-random association patterns in a plant–mycorrhizal fungal network reveal host–symbiont specificity. Mol. Ecol. 28, 365–378. https://doi.org/10.1111/mec.14924

Shefferson, R.P., Cowden, C.C., Mccormick, M.K., Yukawa, T., Ogura-Tsujita, Y., Hashimoto, T., 2010. Evolution of host breadth in broad interactions: mycorrhizal specificity in East Asian and North American rattlesnake plantains (Goodyera spp.) and their fungal hosts: MYCORRHIZAL HOST BREADTH EVOLUTION. Mol. Ecol. 19, 3008–3017. https://doi.org/10.1111/j.1365-294X.2010.04693.x

Smith, S.E., Read, D.J., 2008. Mycorrhizal symbiosis, 3rd ed. ed. Academic Press, Amsterdam Boston.

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. https://doi.org/10.1111/nph.15076

Suzuki, T.A., Fitzstevens, J.L., Schmidt, V.T., Enav, H., Huus, K.E., Mbong Ngwese, M., Grießhammer, A., Pfleiderer, A., Adegbite, B.R., Zinsou, J.F., Esen, M., Velavan, T.P., Adegnika, A.A., Song, L.H., Spector, T.D., Muehlbauer, A.L., Marchi, N., Kang, H., Maier, L., Blekhman, R., Ségurel, L., Ko, G., Youngblut, N.D., Kremsner, P., Ley, R.E., 2022. Codiversification of gut microbiota with humans. Science 377, 1328–1332. https://doi.org/10.1126/science.abm7759

Taengon, C., Feng, Y., Zhang, Y., Aluthwattha, S.T., Chen, J., Wang, G., 2025. Cospeciation is not the dominant driver of plant–pollinator codiversification in

specialized pollination systems. Integr. Zool. 20, 437–442. https://doi.org/10.1111/1749-4877.12886

Talavera, G., Lukhtanov, V., Pierce, N.E., Vila, R., 2022. DNA Barcodes Combined with Multilocus Data of Representative Taxa Can Generate Reliable Higher-Level Phylogenies. Syst. Biol. 71, 382–395. https://doi.org/10.1093/sysbio/syab038

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Põldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Pärtel, K., Otsing, E., Nouhra, E., Njouonkou, A.L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.-H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T.W., Harend, H., Guo, L., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., De Kesel, A., Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S., Abarenkov, K., 2014. Global diversity and geography of soil fungi. Science 346, 1256688. https://doi.org/10.1126/science.1256688

Tedersoo, L., Bahram, M., Zobel, M., 2020. How mycorrhizal associations drive plant population and community biology. Science 367. https://doi.org/10.1126/science.aba1223

Toju, H., Tanabe, A.S., Ishii, H.S., 2016. Ericaceous plant–fungus network in a harsh alpine–subalpine environment. Mol. Ecol. 25, 3242–3257. https://doi.org/10.1111/mec.13680

van der Heijden, M.G.A., Martin, F.M., Selosse, M., Sanders, I.R., 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytol. 205, 1406–1423. https://doi.org/10.1111/nph.13288

Van Galen, L.G., Orlovich, D.A., Lord, J.M., Nilsen, A.R., Dutoit, L., Larcombe, M.J., 2023. Correlated evolution in an ectomycorrhizal host–symbiont system. New Phytol. 238, 1215–1229. https://doi.org/10.1111/nph.18802

Weiß, M., Waller, F., Zuccaro, A., Selosse, M., 2016. Sebacinales – one thousand and one interactions with land plants. New Phytol. 211, 20–40. https://doi.org/10.1111/nph.13977

Zhang, J., Gu, Z., Zhou, C., Zhou, H., 2025. Molecular phylogeny and morphology reveal four new species in Hymenochaetales and one new species in Cantharellales from Southwestern China. MycoKeys 115, 87–135. https://doi.org/10.3897/mycokeys.115.142433

Zhao, Z., Li, X., Liu, M.F., Merckx, V.S.F.T., Saunders, R.M.K., Zhang, D., 2021. Specificity of assemblage, not fungal partner species, explains mycorrhizal partnerships of mycoheterotrophic *Burmannia* plants. ISME J. 15, 1614–1627. https://doi.org/10.1038/s41396-020-00874-x

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.