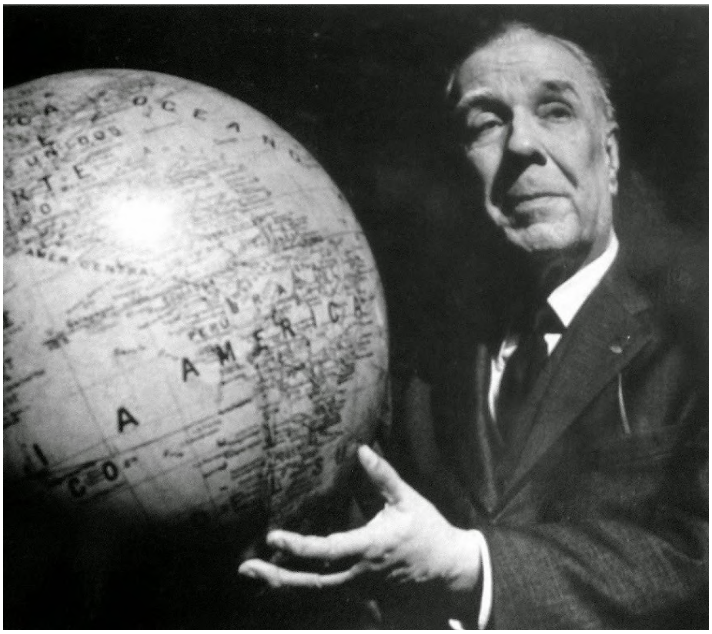
Research commentaries

Paradigm shifts and recent mycorrhizal 'controversies'

César Marín[1, 2*]

[1]Center for Research and Innovation for Climate Change, Santo Tomás University, Chile. [2]Amsterdam Institute for Life and Environment (A-LIFE), Section Systems Ecology, Vrije Universiteit Amsterdam, the Netherlands. *E-mail: cmarind@santotomas.cl

In his short story "*On Exactitude in Science*" (Borges 1998), Jorge Luis Borges compared the scientific endeavor to that of cartographers creating a map of an Empire. The cartographers created a map as big as the Empire itself, which ended up being useless. Models and scientific theories follow this logic and constitute approximations to reality – because an extremely detailed model ends up being useless, and as such, some degree of uncertainty is expected. Thus, as a map, scientific theories sometimes omit or summarize some information. The logical consequence of this is that paradigm shifts are expected (Kuhn 1962; Okasha 2016) – as each shift might represent an even closer approximation to 'reality'. Mycorrhizal research -particularly mycorrhizal ecology and evolution- is not exempt of such shifts, which cause significant controversy (Albornoz *et al*. 2021). In particular, in recent years, three important debates have developed in mycorrhizal research: 1. On misinformation regarding common mycorrhizal networks in forests . 2. On the definition of mycorrhizal types. 3. On the effectiveness of bio-fertilizers based on arbuscular mycorrhizal (AM) fungi.



Jorge Luis Borges. Photo from: Eterna Cadencia.

Misinformation on common mycorrhizal networks in forests

One of the most popularized mycorrhizal concepts that the general public is familiar with are the so called 'common mycorrhizal network' (CMN). A CMNs are "are physical, continuous linkages among the roots of at least two different individual plants, by the same genetic individual of mycorrhizal fungus" (Karst *et al*. 2023). A well known example of CMNs is given by mycoheterotrophic plants, which acquire carbon from mycorrhizal fungi that colonizes other plants (Merckx and Freudenstein 2010). But most of the focus on popular media regarding CMN has been in forests, particularly in the notion of 'Mother trees' able to communicate and share resources with seedlings through a CMN. Dozens of science outreach books, videos, movies, and other types of media have shared this notion, particularly since journalists at *Nature* deemed CMNs as the 'wood wide web' (and in their cover), based on a seminal paper by Simard *et al.* (1997) on the same journal issue.

Recently, though, Karst *et al.* (2023) have questioned three common claims regarding CMNs. First, that CMNs are present in all forests: after reviewing the literature, they found that just two tree (out of 73,300 global tree species; Cazzolla Gatti *et al.* 2022) and three ectomycorrhizal (ECM) fungal species have been genotipically mapped around the globe (in Canada and Japan). The hyphae of these shared fungal genets could easily break and does not prove nutrient transfer. A second common claim is that nutrients/resources are transferred through CMNs, which increases plant growth. After reviewing hundreds of papers, the authors got 18 highly cited studies and examined how those citations made supported or unsupported claims on the original research (Karst *et al.* 2023). These studies were directly performed in forests, *in situ* – the authors also comment about laboratory studies. Karst *et al.* (2023) identify alternative explanations for such positive effects: i. Part of the resource transfer pathway is discontinuous, involving the flow of solutes in the soil solution (and not necessarily in the CMN); ii. Fungal pathogen composition was altered due to experimental treatment (ie. when seedlings are grown in containers) which alters seedling performance; iii. Similarly, the mycorrhizal composition changed due to the treatments; iv. Access to surrounding roots positively influences seedling performance; and iv. The treatment reduced the soil volume for fungal foraging, affecting the non-CMN seedlings. Thus, it seems, the CMN experiments conducted so far do not exclude such alternative explanations which are consistent with the complexity of soil ecology. A third common claim is that mature trees communicate with offspring through CMNs, for which Karst *et al.* (2023) found no evidence at all.

Other recent articles have also questioned how common (or not) are CMNs (Figueiredo *et al.* 2021; Henriksson *et al.* 2023; Kuyper and Jansa 2023). Regarding this, Henriksson *et al.* (2023) inquired three aspects: ectomycorrhizal Carbon metabolism, patterns of (ectomycorrhizal) trees forest regeneration, and the CMN studies using isotopic methods. They conclude that there is evidence for C movement among plants but the role of CMNs is not clear, neither that CMNs provide growth benefits. Also, so far, we do not know any physiological mechanism allowing C fluxes from ECM fungi into the plant–fungal interface (Henriksson *et al.* 2023). Also, forest regeneration patterns in boreal forests are not congruent with what would be expected if they were connected through CMNs (Henriksson *et al.* 2023).

What this very relevant and on point criticism towards the CMN literature should cause is an improvement in experimental settings and controls *in vivo* and *in vitro*. Karst *et al.* (2023) propose some of these experimental design improvements. Some journals, like *Functional Ecology* have recently made an special issue call around mycorrhizal networks with this consideration. Still, some observations are thought-provoking. For example, it has been observed that seedlings growing together with larger, mycorrhized plants grew larger than seedlings growing with larger, non-mycorrhized plants (van der Heijden and Horton 2009) – in at least half of experiments. Processes other than CMNs could explain this, like the transfer of N and P, indirect effects (ie. being close to a fungus associated to a 'healthy' tree), or hyphospheres (Wang *et al.* 2023; Johnson and Marín 2023) might explain such patterns. Definitely more research is needed, considered all possible explanations. Also, anastomosis and hyphal healing mechanisms are quite common in arbuscular mycorrhizal fungi (de la Providencia et al. 2004; Purin and Morton 2013), which do not imply the functionality per sé of a CMN, but its existence is at least very interesting.

Die Sprache der Pilze (Biosemiotik I). By: Heiko Sievers. watercolor and pencil on paper, h 32 x w 24 cm, 2023 http://mushroom-of-the-day.blogspot.com/

Different approaches to define and diagnose mycorrhizal types

Symbiotic associations between plant and mycorrhizal fungi mediate plant populations and community diversity and ultimately impact global biogeochemical cycles and ecosystem services (Tedersoo et al. 2020). Different types of mycorrhizal associations exist including ecto-mycorrhizas (ECM), arbuscular mycorrhizas (AM), orchid mycorrhizas (OM) and ericoid mycorrhizas (ERM) (Moora 2014), which are broadly related to different nutrient economies (Phillips *et al.* 2013). It is important to identify and diagnose mycorrhizal types of vascular plants at all scales -from local to global, in order to identify effects of mycorrhizas in ecosystems. Furthermore, mapping global mycorrhizal types has been a priority in mycorrhizal ecology over the last three decades (Read 1991; Steidinger *et al.* 2019; Tedersoo *et al.* 2022).

In order to accomplish such global mapping efforts, it is necessary to know the mycorrhizal types of the global vascular flora. Bueno *et al.* (2019a) identified two approaches to assign mycorrhizal traits of plant species: the empirical approach and the taxonomic approach. The information source for the former is "published empirical studies describing plant root mycorrhizal associations", while "researcher expertise is used for extrapolating either mycorrhizal type or status to complete taxonomic groups, such as genus or family, based on background empirical information from one or a few species from the same group" for the latter (Bueno *et al.* 2019a). Both approaches have important assumptions: the empirical approach assumes that the accumulated empirical data are correct, while the taxonomic approach assumes that mycorrhizal traits are completely conserved at the chosen taxonomic level (Bueno *et al.* 2019a). A highly cited paper regarding the proportions of mycorrhizal types associated with global flora uses the taxonomic approach (Brundrett and Tedersoo 2018). Using such expertise-based generalizations has caused significant controversy (Bueno *et al.* 2019a; Sun *et al.* 2019).

Such debate and the above-mentioned assumptions have lead to some interesting developments. In particular, Brundrett and

Tedersoo (2019) have argued that a significant portion of the mycorrhizal literature contains 'mycorrhizal trait allocation errors' (Brundrett 2021) because root mycorrhizal structures have been incorrectly identified, and because such 'incorrect' diagnoses have been recycled many times in the citing literature.

Instead, they propose a specific set of criteria for diagnosing each mycorrhizal type and checking the mycorrhizal literature (and databases) against their curated list (Brundrett and Tedersoo 2018). As a response, Bueno *et al.* (2019b) ask the following questions: Should only the presence of arbuscules define AM plants? Can plant taxonomy be a reliable predictor of plant mycorrhizal traits? Should the results of published studies be considered 'incorrect' when they do not match any proposed 'standard reference'?

Ultimately, this debate raises important questions about what a mycorrhiza is. For example, Bueno *et al.* (2020) argues that in the *FungalRoot* database introduction (Soudzilovskaia *et al.* 2020), by proposing the only use of the 'arbuscule criterion' to assign a plant as AM, it is excluding the many (and highly understudied) non-nutritional functions that AM fungi perform (Delavaux *et al.* 2017), which could be expressed by the presence of arbuscular intraradical hyphae. This focus on nutritional exchange also seems to 'forget' that AM symbioses work in a mutualism-to-parasitism continuum (Johnson *et al.* 1997) and some AM fungi do not form arbuscules (e.g. ancient AMF families) (Redecker *et al.* 2013). Important issues regarding the definition of 'AM-facultivate' plants have also arise during this debate (Bueno *et al.* 2020; Soudzilovskaia *et al.* 2022) – recently, other authors have criticized the usefulness of this term at all (Kuyper and Jansa 2023).

I invite our readers to read all the cited literature here, to build their own opinions. Independently of the position taken, from the point of view of the development of mycorrhizal research, it is interesting to see how the need to establish global mapping efforts and global databases on mycorrhizal traits, has led to go back to the fundamental biology, ecology, functionality, and most importantly, definition of the mycorrhizal symbioses.

Effectiveness of bio-fertilizers based on AM fungi

Short articles by Matthias J. Salomon (Pag. 10) and by Vasilis Kokkoris and Nicolas Corradi (Pag. 13) in this issue discuss about the effectiveness of AM fungi as bio-fertilisers. In short, although over the last two or three decades there has been a lot of interest of using AM fungi as biofertilizers, just recently Salomon *et al.* (2022a) thoroughly evaluated 25 commercial inoculants containing AM fungi. They found that only 5 inoculants had viable propagules and a significant mycorrhizal root colonization (Salomon *et al.* 2022a). In addition, many AM-based biofertilizers do not contain the declared AM fungal species, as revealed by metabarcoding sequencing (Vahter *et al.* 2023). Given this, a general framework and criteria for AM fungal inoculants was established (Salomon *et al.* 2022b). Such criteria include: plant bio-assays to verify that the AM fungi have colonize plant roots, verify inoculum composition and its viability, avoid negative effects of the carrier material, and detailed labeling. Some companies do abide to such criteria in addition to use native consortia inoculum (4-5 species from different genera) and follow the inoculated crop soil ecology (Aguilera *et al.* 2023).

Acknowledgments

Many thanks to Marcel G.A. van der Heijden for helpful comments and suggestions to this article.

References

- Aguilera P, Borie F, Castillo C, Marín C. 2023. Native Chilean arbuscular mycorrhizal fungi consortia increase northern highbush blueberry growth, Chile. *Best Practices to Conserve Soil Biodiversity – FAO report*. Accepted.

- Albornoz FE, Dixon KW, Lambers H. 2021. Revisiting mycorrhizal dogmas: are mycorrhizas really functioning as they are widely believed to do?. Soil Ecol Lett 3:73-82. https://doi.org/10.1007/s42832-020-0070-2

- Borges JL. 1998. *Collected fictions*. London-New York, Penguin Press.

- Brundrett MC. 2021. Auditing data resolves systemic errors in databases and confirms mycorrhizal trait consistency for most genera and families of flowering plants. *Mycorrhiza* 31:671-683. https://doi.org/10.1007/s00572-021-01051-4

- Brundrett MC, 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

- Brundrett M, Tedersoo L. 2019. Misdiagnosis of mycorrhizas and inappropriate recycling of data can lead to false conclusions. *New Phytol* 221:18-24. https://doi.org/10.1111/nph.15440_

- Bueno CG, Gerz M, Zobel M, Moora M. 2019a. Conceptual differences lead to divergent trait estimates in empirical and taxonomic approaches to plant mycorrhizal trait assignment. *Mycorrhiza* 29:1-11. https://doi.org/10.1007/s00572-018-0869-1

- Bueno CG, Aldrich-Wolfe L, Chaudhary VB, *et al.* 2019b. Misdiagnosis and uncritical use of plant mycorrhizal data are not the only elephants in the room. *New Phytol* 224:1415-1418. https://doi.org/10.1111/nph.15976_

- Cazzolla Gatti R, Reich PB, Gamarra JG, *et al.* 2022. The number of tree species on Earth. Proc Natl Acad Sci USA 119:e2115329119. https://doi.org/10.1073/pnas.2115329119

- De la Providencia IE, de Souza FA, Fernández F, Delmas NS, Declerck S. 2005. Arbuscular mycorrhizal fungi reveal distinct pattNew Phytol erns of anastomosis formation and hyphal healing mechanisms between different phylogenic groups. *New Phytol* 165:261-271. https://doi.org/10.1111/j.1469-8137.2004.01236.x

- Delavaux CS, Smith-Ramesh LM, Kuebbing SE. 2017. Beyond nutrients: a meta-analysis of the diverse effects of arbuscular mycorrhizal fungi on plants and soils. *Ecology* 98:2111-2119. https://doi.org/10.1002/ecy.1892

- Figueiredo AF, Boy J, Guggenberger G. 2021. Common mycorrhizae network: a review of the theories and mechanisms behind underground interactions. *Front Fungal Biol* 2:735299. https://doi.org/10.3389/ffunb.2021.735299

- Henriksson N, Marshall J, Högberg MN, et al. 2023. Re-examining the evidence for the mother tree hypothesis–resource sharing among trees via ectomycorrhizal networks. *New Phytol* 239:19-28. https://doi.org/10.1111/nph.18935

- Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytol* 135:575-585. https://doi.org/10.1046/j.1469-8137.1997.00729.x

- Johnson NC, Marín C. 2023. Microbial villages in the geography of arbuscular mycorrhizal symbioses. *New Phytol* 238:461-463. https://doi.org/10.1111/nph.18803

- Karst J, Jones MD, Hoeksema JD. 2023. Positive citation bias and overinterpreted results lead to misinformation on common mycorrhizal networks in forests. *Nat Ecol Evol* 7:501-511. https://doi.org/10.1038/s41559-023-01986-1

- Kuhn T. 1962. *The structure of scientific revolutions*. Chicago, University of Chicago Press.

- Kuyper TW, Jansa J. 2023. Arbuscular mycorrhiza: advances and retreats in our understanding of the ecological functioning of the mother of all root symbioses. *Plant Soil. In press.* https://doi.org/10.1007/s11104-023-06045-z

- Merckx V, Freudenstein JV. 2010. Evolution of mycoheterotrophy in plants: a phylogenetic perspective. *New Phyto*l 185:605-609. https://doi.org/10.1111/j.1469-8137.2009.03155.x

- Moora M. 2014. Mycorrhizal traits and plant communities: perspectives for integration. *J Veg Sci* 25:1126-1132. https://doi.org/10.1111/jvs.12177

- Okasha S. 2016. *Philosophy of science: a very short introduction*. 2nd ed. Oxford, Oxford University Press.

- Phillips RP, Brzostek E, Midgley MG. 2013. The mycorrhizal-associated nutrient economy: a new framework for predicting carbon–nutrient couplings in temperate forests. *New Phytol* 199:41-51. https://doi.org/10.1111/nph.12221

- Purin S, Morton JB. 2013. Anastomosis behavior differs between asymbiotic and symbiotic hyphae of *Rhizophagus clarus*. *Mycologia* 105:589-602. https://doi.org/10.3852/12-135

- Read DJ. 1991. Mycorrhizas in ecosystems. *Experientia* 47:376-391. https://doi.org/10.1007/BF01972080

- Redecker D, Schüßler A, Stockinger H, *et al.* 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). *Mycorrhiza* 23:515-531. https://doi.org/10.1007/s00572-013-0486-y

- Simard SW, Perry DA, Jones MD, et al. 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* 388:579-582. https://doi.org/10.1038/41557

- Steidinger BS, Crowther TW, Liang J, *et al.* 2019. Climatic controls of decomposition drive the global biogeography of forest-tree symbioses. *Nature* 569:404-408. https://doi.org/10.1038/s41586-019-1128-0

- Sun T, Zhang H, Wang Z. 2019. Reply to Tedersoo *et al.*: Plant species within the same family or genus can have different mycorrhizal types?. *Proc Natl Acad Sci USA* 116:12141-12142. https://doi.org/10.1073/pnas.1903868116

- Tedersoo L, Bahram M, Zobel M. 2020. How mycorrhizal associations drive plant population and community biology. *Science* 367:eaba1223. https://doi.org/10.1126/science.aba1223

- Tedersoo L, Mikryukov V, Zizka A, *et al.* 2022. Global patterns in endemicity and vulnerability of soil fungi. *Glob. Change Biol* 28:6696-6710. https://doi.org/10.1111/gcb.16398

- Salomon MJ, Demarmels R, Watts-Williams SJ, *et al.* 2022a. Global evaluation of commercial arbuscular mycorrhizal inoculants under greenhouse and field conditions. *Appl Soil Ecol* 169:104225. https://doi.org/10.1016/j.apsoil.2021.104225

- Salomon MJ, Watts-Williams SJ, McLaughlin MJ, *et al.* 2022b. Establishing a quality management framework for commercial inoculants containing arbuscular mycorrhizal fungi. *iScience* 25:104636. https://doi.org/10.1016/j.isci.2022.104636

- Vahter T, Lillipuu EM, Oja J, Öpik M, Vasar M, Hiiesalu I. 2023. Do commercial arbuscular mycorrhizal inoculants contain the species that they claim?. *Mycorrhiza* 33:211-220. https://doi.org/10.1007/s00572-023-01105-9

- Van der Heijden MG, Horton TR. 2009. Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *J Ecol* 97:1139-1150. https://doi.org/10.1111/j.1365-2745.2009.01570.x

- Wang L, Zhang L, George TS, Feng G. 2023. A core microbiome in the hyphosphere of arbuscular mycorrhizal fungi has functional significance in organic phosphorus mineralization. *New Phytol* 238:859-873. https://doi.org/10.1111/nph.18642