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Bueno, C. Guillermo, Aldrich-Wolfe, Laura, Chaudhary, V. Bala et al. (10 more authors) (2019) Misdiagnosis and uncritical use of plant mycorrhizal data are not the only elephants in the room. *New Phytologist*. pp. 1-4. ISSN 1469-8137

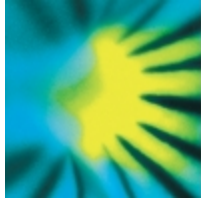
<https://doi.org/10.1111/nph.15976>

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New Phytologist

Misdiagnosis and uncritical use of plant mycorrhizal data are not the only elephants in the room: A response to Brundrett & Tedersoo (2018) 'Misdiagnosis of mycorrhizas and inappropriate recycling of data can lead to false conclusions'.

Journal:	<i>New Phytologist</i>
Manuscript ID	NPH-L-2019-29346.R1
Manuscript Type:	L - Letter
Date Submitted by the Author:	n/a
Complete List of Authors:	Bueno, Guillermo; University of Tartu, Department of Botany Aldrich-Wolfe, Laura; North Dakota State University, Biological Sciences Chaudhary, Bala; DePaul University, Department of Environmental Science and Studies Gerz, Maret; University of Tartu, Department of Botany, Institute of Ecology and Earth Sciences. Lai 40. EE 51005 Helgason, Thorunn; University of York, Department of Biology; Hoeksema, Jason; University of Mississippi, Department of Biology Klironomos, John; University of British Columbia, Department of Biology Lekberg, Ylva; MPG Ranch, Soil Ecology; University of Montana, Ecosystem and Conservation Sciences Leon, Daniela; University of Tartu, Department of Botany Maherali, Hafiz; University of Guelph, Integrative Biology; Öpik, Maarja; University of Tartu, Department of Botany Zobel, Martin; University of Tartu, Department of Botany Moora, Mari; University of Tartu, Department of Botany, Institute of Ecology and Earth Sciences;
Key Words:	plant mycorrhizal type, plant mycorrhizal status, facultative mycorrhiza, obligate mycorrhiza, non-mycorrhizal plants, databases, diagnosis error, arbuscular mycorrhiza

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1 **Misdiagnosis and uncritical use of plant mycorrhizal data are not the only elephants in the**
2 **room: A response to Brundrett & Tedersoo (2018) ‘Misdiagnosis of mycorrhizas and**
3 **inappropriate recycling of data can lead to false conclusions’.**

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24 Word count: 2160

25 There is increasing interest in using plant mycorrhizal traits – characteristics related to a plant’s
26 ability to form mycorrhizal symbiosis – to understand the role of mycorrhizas within and across
27 communities, ecosystems and biogeographical regions (Moora, 2014; Tedersoo, 2017). Recent
28 studies incorporating plant mycorrhizal traits, mainly mycorrhizal type (e.g. ecto-, ericoid-,
29 arbuscular-, and non-mycorrhizal; ECM, ERM, AM, NM) and mycorrhizal status (obligately and
30 facultatively mycorrhizal; OM, FM; e.g. Correia et al. 2018; Gerz et al. 2018), have assigned trait
31 values from published empirical data for plant species of interest. Dedicated efforts to build
32 and improve databases of empirical plant mycorrhizal traits (e.g. Chaudhary et al. 2016; Bueno
33 et al. 2017) are making these resources increasingly comprehensive, transparent and
34 accessible.

35 A recent viewpoint paper by Brundrett and Tedersoo (2019) highlights several challenges
36 connected with assigning mycorrhizal traits to plant species and criticizes the use of mycorrhizal
37 trait databases. The authors argue that the use of such trait databases (which they refer to as
38 “recycled data”) is ‘inappropriate’ due to two underappreciated problems: the databases (i)
39 may include ‘mycorrhizal trait allocation errors’ due to misidentification of root mycorrhizal
40 structures; or (ii) may contain data derived using diagnostic criteria that are flawed. They
41 propose instead (1) diagnostic criteria for defining mycorrhizas that make specific assumptions
42 about the biology of the interaction, and (2) checking databases against their curated list,
43 derived from prior experience of assigning mycorrhizal traits to plant species (hereafter
44 referred to as a 'standard reference', Brundrett, 2009; Tedersoo, 2017). Although we fully agree
45 that standard protocols are required, there are several questions raised by the Brundrett and
46 Tedersoo (2019) viewpoint that deserve critical consideration if consensus is to be reached
47 among researchers in the field: 1) should the presence of minimum number of arbuscules
48 define the AM symbiosis; 2) does plant taxonomy accurately predict plant mycorrhizal traits
49 (i.e. high phylogenetic trait conservatism); 3) should the results of published studies be
50 considered ‘incorrect’ when they do not match this, or any proposed, ‘standard reference’?
51 Below, we discuss these three questions and address an additional topic that arises from
52 consideration of Brundrett and Tedersoo’s criticisms of prior research: 4) the level of

53 transparency involved in building plant mycorrhizal trait databases and directions for future
54 research.

55 **Point 1. Should the presence of arbuscules define AM plants?**

56 Symbiosis describes any intimate association of two organisms, while mutualism is an
57 association that provides benefits for both. Mycorrhizal symbiosis is often interpreted as a
58 mutualism, but depending on the environmental conditions in nature, the association varies
59 along a continuum from mutualism to parasitism (Johnson *et al.*, 1997; Johnson & Graham,
60 2013). The nutritional benefit of mycorrhizal interactions to plants has received most attention,
61 but non-nutritional benefits, such as improved biotic and abiotic stress tolerance for host plants
62 (Delavaux *et al.*, 2017) and habitat for the fungal partner (Brundrett, 2002), can occur and are
63 also potential drivers of the symbiosis. Thus, in our opinion, the criterion proposed by Brundrett
64 and Tedersoo, which focuses solely on the nutritional exchange, is too limited. Furthermore,
65 Brundrett and Tedersoo argue that AM plant species should be defined by mycorrhizal
66 structures devoted to nutrient exchange: the presence of arbuscules. While the presence of
67 arbuscules could indicate P-transfer (if arbuscules are functional), the absence of arbuscules
68 does not necessarily mean that nutrients are not transferred. For example, nutrient exchange in
69 AM also occurs through hyphal coils (Paris colonization type) with no arbuscules involved
70 (Dickson, 2004), or even in the absence of coils and arbuscules (Manjarrez *et al.*, 2010). Besides,
71 the lifetime of arbuscules is short, and their presence depends on plant developmental stage
72 (Montero & Paszkowski, 2018), meaning that detection of arbuscules is context dependent and
73 practically challenging (Vierheilig *et al.*, 2005). Therefore, the distinction between AM plants
74 (with arbuscules) and non-mycorrhizal (NM) plants with 'Glomeromycotan Fungal Colonization'
75 (with no arbuscules or non-functional ones) based on potential nutritional function or lack
76 thereof (Brundrett and Tedersoo 2019), is inconsistent with published observations. Also, any
77 criterion that is based on terms such as "few", "many" and "low" (Table 1, Brundrett and
78 Tedersoo 2019) is subjective. Based on this, we think it is premature to use the definition of AM
79 proposed by Brundrett and Tedersoo 2019 and advocate for the more inclusive morphological
80 criterion previously proposed by Smith & Read (2008), which is based only on root intracellular
81 colonization by glomeromycotinan fungi. This definition is widely applicable to the different

82 contexts that occur in nature (e.g., during development of mycorrhizal fungal colonization,
83 plant-specific developmental stages, seasonal and soil fertility variation) and avoids any criteria
84 linked to a function (i.e., nutritional, non-nutritional) of the symbiosis, where the degree of
85 mutualism may fluctuate independently from morphological structures. Moreover, in ECM
86 plants, N-transfer does not necessarily require a Hartig net (Sa *et al.*, 2019). This demonstrates
87 that for ECM symbiosis there are potential new discoveries that may change our understanding
88 about the functioning of mycorrhizal symbiosis. It is our hope, however, that a definition
89 encompassing all the complexities of the mycorrhizal symbiosis will evolve as our knowledge
90 advances.

91 **Point 2. Can plant taxonomy be a reliable predictor of plant mycorrhizal traits?**

92 Brundrett and Tedersoo proposed a 'standard reference' for checking whether any new list of
93 plant mycorrhizal traits contains potential errors. This 'standard reference' approach is based,
94 in part, on the assumption that mycorrhizal traits can be assigned to species by extrapolating
95 from higher taxonomic units, such as families or genera. The approach relies on the expectation
96 that plant mycorrhizal traits are phylogenetically conserved within plant families or genera to a
97 degree that allows predictions to be made. However, this assumption is not always justified.
98 Taxonomy based extrapolations at lower taxonomic levels (e.g. within families and genera) may
99 be more suitable for some mycorrhizal types (ECM, ERM, ORM), where well-studied plant
100 groups are expected to exhibit highly conserved mycorrhizal traits. However, the approach
101 could even yield errors in these groups because assumptions about the uniformity of
102 mycorrhizal traits within well-studied plant groups are regularly disproved. For example, the
103 ECM association was unexpectedly documented (based on morphology) in *Pulsatilla patens*
104 (Hoeksema *et al.*, 2018), a species belonging to a family (Ranunculaceae) that contains a
105 number of species, including *P. patens*, that have been shown experimentally to be highly
106 dependent on AM symbiosis (Moora *et al.*, 2004).

107 A recent comparison of the Brundrett and Tedersoo's 'standard reference' and literature-
108 derived databases of European plant species indicated frequently diverging mycorrhizal trait
109 assignment. Based on the literature databases, only 19% and 6% of plant families (out of 75

110 European plant families with more than five studied species) comprised a single plant
111 mycorrhizal type or status, respectively (Bueno *et al.*, 2019). One cause for low phylogenetic
112 conservatism in mycorrhizal traits is that some plant species have adapted to different
113 environmental conditions in a way that elicits changes in mycorrhizal traits (Gerz *et al.*, 2018).
114 For instance, Osborne *et al.* (2018) recently described how adaptation to different soil
115 conditions was accompanied by a divergence of mycorrhizal traits in sister palm species. This
116 type of adaptation has not been systematically explored, but it may explain why phylogeny is
117 not a consistently reliable tool for determining plant mycorrhizal traits within plant families or
118 genera.

119 Given the discrepancies noted by Bueno *et al.* (2019) and the limited volume of existing plant
120 mycorrhizal trait data (fewer than 5% of all plant species; Brundrett 2009) we argue that strong
121 generalizations about the predictability of plant mycorrhizal traits from plant taxonomy are
122 unwarranted. In fact, rigid adherence to this assumption could lead to misdiagnosis when newly
123 explored plant species diverge from expectations or a new type of mycorrhizal association is
124 discovered for a plant species. Therefore, care needs to be taken in making predictions based
125 on plant's taxonomic placement, particularly for AM and NM types or FM status, which are less
126 phylogenetically conserved than ECM, ERM and ORM types (Maherali *et al.*, 2016; Bueno *et al.*,
127 2019). The amount of putative misclassification errors seems directly related to the level of
128 taxonomy extrapolated, being higher within plant families than within genera (Bueno *et al.*,
129 2019). Still, in contexts where the available evidence is absent for a high number of species,
130 plant phylogenetic relationships, which are not yet fully resolved (APG, 2016), could be used as
131 a reasonable starting point for formulating hypotheses regarding missing plant mycorrhizal
132 traits, but those hypotheses should be tested and not be assumed to be correct in advance. We
133 advocate for a more flexible view of mycorrhizal symbioses, where plant and fungal partners, as
134 well as our knowledge about them, are constantly evolving (Selosse *et al.*, 2018). Overall, more
135 empirical research, observing and analyzing mycorrhizal fungal colonization of plant species in
136 natural systems is needed to overcome limitations, improve our extrapolations and hypotheses,
137 and ultimately build our knowledge of mycorrhiza on more solid ground.

138 **Point 3. Constructing a 'standard reference'**

139 We agree with Brundrett and Tedersoo that detailed comparison with a critical review of
140 knowledge accumulated in the field is necessary to contextualize new findings. However, there
141 are unstated assumptions in the 'standard reference' proposed by Brundrett and Tedersoo that
142 should be addressed in order for the field to advance. First, the binary evaluation ('correct' or
143 'incorrect') of earlier studies disregards differences in the conceptual frameworks or definitions
144 used by those studies. We are not suggesting that existing databases are error free, but genuine
145 errors in plant mycorrhizal traits need to be distinguished from variation that is generated by
146 using alternate, but still valid definitions of the mycorrhizal symbiosis (see point 1). Given
147 differences in the definitions used by researchers to assign mycorrhizal traits, opinions about
148 the conclusions of prior studies without appropriate analytical and/or empirical support should
149 not be treated as evidence that prior conclusions were incorrect (Table 4, Brundrett & Tedersoo
150 2019). In light of this, we do not think that researchers should be dissuaded from using curated
151 databases, nor inherently disregard the output of analyses stemming from them without first
152 examining the assumptions, objectives and definitions used by the authors. Second, it should
153 be noted that the 'standard reference' cited in Brundrett and Tedersoo's viewpoint did not
154 appear to be presented with detailed references or supporting information that could justify
155 decisions about each family assignment to a mycorrhizal type or status (See point 4).

156 **Point 4. Towards a transparent database of mycorrhizal traits and future research**

157 Any methodological approach is expected to be independently reproducible. Reproducibility is
158 enabled by a clear methodological description that includes the criteria, background
159 information and evidence used to justify conceptual or analytical decisions ('Towards
160 transparency', 2014; Powers & Hampton, 2018). This is crucial if methodologies are to be
161 assessed, discussed and improved in light of both conceptual developments and the availability
162 of new information. To achieve this, we suggest that the research community strives to build a
163 standard reference that represents a review of empirical evidence for all examined species,
164 providing references and evidence to support mycorrhizal trait assignment (by morphological
165 and ideally by functional features), and highlighting known uncertainties and knowledge gaps
166 for which more empirical research is needed.

167 We hope that the discussion prompted by Brundrett and Tedersoo's viewpoint draws attention
168 to a lack of consensus about core concepts in mycorrhizal ecology and provides incentive for
169 researchers to openly discuss the various challenges, and to agree on core concepts. Moving
170 forward, we advocate that researchers are transparent about the different conceptual
171 frameworks used to make plant mycorrhizal trait assignments in their studies, and that readers
172 consider such frameworks when drawing inferences from those studies. To spur expansion of
173 the empirical knowledge base, we advocate the development of more inclusive definitions of
174 mycorrhizal symbiosis and suggest that researchers strive to reach consensus on suitable
175 definitions that allow us to capture features of the symbiosis that we are just beginning to
176 discover. In this respect, meetings and forums such as ICOM (International Conference on
177 Mycorrhiza) are ideal. A concerted effort to account for this complexity in detailed data
178 collation will pave the way for future multidisciplinary work and enable new discoveries, such
179 as unearthing possibly novel roles of the symbiotic partners. This approach could encompass
180 experimental and observational studies at molecular to global scales, and could lead us towards
181 a more comprehensive understanding of the functional roles of mycorrhizal symbioses in
182 ecosystems.

183 **Acknowledgements**

184 This research has been supported by the Estonian Ministry of Education and Research (IUT20-
185 28), and by the European Union through the European Regional Development Fund (Centre of
186 Excellence EcolChange) and ERA-NET Cofund BiodivERsA3 (Project SoilMan). YL is grateful to
187 MPG Ranch for funding. TH is supported by NERC grant NE/M004864/1. LAW was supported by
188 NSF EPSCoR Track-1 Cooperative Agreement OIA-1355466. HM is supported by the NSERC
189 Discovery grant program (RGPIN-2018-04620). Any opinions, findings, and conclusions or
190 recommendations expressed in this material are those of the author(s) and do not necessarily
191 reflect the views of the National Science Foundation. We thank John Davison, Roger Koide, Brad
192 Oberle, Marc-André Selosse and three anonymous reviewers for suggestions on an earlier
193 version on this manuscript.

194 **Author contributions**

195 CGB and MM wrote the first draft, and all authors significantly contributed to revisions.

196

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