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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'.

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Manuscripts

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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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.

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.

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194   **Author contributions**

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

196

197   **References**

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