Response to "Feremycorrhizal fungi: a confusing and erroneous term": feremycorrhiza means 'nearly mycorrhiza'; hence, it is a clear and correct term because the fungal partner has mycorrhizal traits and lineage

Article

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Letter to the Editor

Response to "Feremycorrhizal fungi: a confusing and erroneous term":

Feremycorrhiza means 'nearly mycorrhiza'; hence, it is a clear and correct term because the fungal partner has mycorrhizal traits and lineage

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Chanway et al. (2023) argue that 'feremycorrhiza' is an erroneous term. We contend otherwise, and explain why here. *Austroboletus occidentalis* Watling & N.M. Greg (Boletaceae, Boletales, Basidiomycota) establishes the feremycorrhizal (FM) symbiosis, conferring significant growth and nutritional benefits to diverse host plants, including woody species such as jarrah (*Eucalyptus marginata*) (Kariman et al., 2014), arbuscular mycorrhizal (AM) crops such as wheat and barley as well as the non-mycorrhizal canola (*Brassica napus*) growing in natural field soils containing indigenous microorganisms (Kariman et al., 2020, 2022). '*Fere*' means 'nearly' in Latin; hence, the term 'feremycorrhiza' means 'nearly mycorrhiza' (Kariman et al., 2018). This term is well suited for this symbiosis because of:

1) The Australian native fungus *A. occidentalis* phylogenetically belongs to an ectomycorrhizal (ECM) lineage
2) *A. occidentalis* possesses several established hallmarks of the ECM symbiosis
3) *A. occidentalis* does not enter roots and hence does not form any interface structures in the root. Therefore, it is not a fully developed mycorrhiza structurally; it is a 'nearly' mycorrhiza (with mycorrhiza-like effects on plant growth and nutrition)

In Latin, the first and best definition for 'fere' is 'nearly', and the 'companion' (Middle English) was added as an incidental additional term of note (Kariman et al., 2018); with the benefit of hindsight, it should not have been mentioned, and the Latin term should take precedence. We consider that Chanway et al. (2023) misinterpret the term ‘feremycorrhiza’ by ignoring the Latin meaning and inexplicably focusing on only the Middle English meaning of ‘fere’ (companion). However, we clearly state that we named the symbiosis ‘feremycorrhiza’ (nearly mycorrhiza) because of its mycorrhiza-like effects on plant growth and nutrition; i.e. we focused on the Latin meaning of ‘fere’ (nearly) when we explained the terminology (Kariman et al., 2018), not because feremycorrhiza is a companion of mycorrhiza as Chanway et al. (2023) incorrectly claim. Furthermore, Chanway et al. (2023) did not consider the clear and evident physiological and phylogenetic features that *A. occidentalis* shares with ECM fungi (see below), which substantially differ from those of saprotrophic fungi, reflecting a superficial assessment of mycorrhizal symbiosis and other plant-fungus relationships. We are happy to clarify this minor point of terminology; in addition, we refute the substantive point of their letter that the new term ‘feremycorrhiza’ is wrong or otherwise confusing. Here is our explanation of the terminology based on the similarities between feremycorrhiza and mycorrhiza:

***Austroboletus occidentalis* has ECM lineage and traits**: The Australian native fungus *A. occidentalis* phylogenetically belongs to an ECM lineage, the Austroboletoideae group, of the Boletaceae family (Boletales, Agaricomycetes, Basidiomycota) (Wu et al., 2014; Sato and Toju, 2019; Gelardi et al., 2020). In Boletales clade, the ECM lifestyle has evolved independently at least four times from brown-rot fungal lineages (Binder and Hibbett, 2006; Wu et al., 2022). The clade containing Boletaceae ECM lineages evolved at the second most recent event, with a sister clade in the family still containing brown-rot taxa (Sato and Toju, 2019). The relatively young Boletaceae represent the most species-rich family of Boletales, with the highest and most rapid transition from saprotrophic to ECM lifestyle complemented by a high evolutionary diversification (Sato and Toju, 2019). The ecological opportunities, which were largely created by coevolution with their angiosperm hosts and adaptation to a broad spectrum of hosts, resources, and habitats, resulted in a high genetic and functional variation among Boletaceae ECM fungi (Binder and Hibbett, 2006; Wu et al., 2022). Studies on other *Austroboletus* species revealed that they form ECM symbiosis with shrubs and trees; indeed, most genera from the fungal family of Boletaceae form ECM symbiosis (Halling, 1996; Orlovich and Cairney, 2004; Tedersoo et al., 2010; Smith et al., 2013; Tedersoo and Smith, 2013; Vasco Palacios et al, 2014). During the evolution of ECM lifestyle from diverse saprotrophic lineages, a massive loss of functional genes coding for plant cell wall-degrading enzymes (PCWDEs) has occurred (Borenstein et al., 2008; Martin et al., 2010; Nehls et al., 2010; Kohler et al., 2015; Murat et al., 2018; Miyauchi et al., 2020). However, ECM fungi retained the genes coding for a distinct set of PCWDEs essential in ECM formation, contributing to root penetration and apoplastic space colonization (Murat et al., 2018; Zhang et al., 2022). We have recently sequenced the genome of *A. occidentalis*, and compared this distinct set of PCWDEs genes (i.e. those involved in root colonization and ECM formation) with a broad range (19 species) of ECM fungi (*Astraeus odoratus, Boletus edulis, B. reticuloceps, Butyriboletus roseoflavus, Coniophora puteana, Chiua virens, Gyrodon lividus, Hydnomerulius pinastri, Laccaria bicolor, Melanogaster broomeanus, Paxillus involutus, Pisolithus tinctorius, Rhizopogon vinicolor, Scleroderma citrinum, Serpula lacrymans, Suillus brevipes, S. luteus, S. occidentalis,* and *Xerocomus badius*). The analysis provided preliminary evidence for the genetic basis behind the lack/loss of the root colonization feature in the feremycorrhizal fungus: a lower number of genes coding for PCWDEs in *A. occidentalis* (100 genes) compared to ECM fungi (111-209 genes) (unpublished data). *Austroboletus occidentalis* has the ECM lineage and several key ECM features (strong phosphorus solubilization activity, limited saprotrophic capacity, lack of invertases, utilization of hexoses but not sucrose, polyphosphate biosynthesis, etc.; see below), but it lacks the root colonization trait. Moreover, *A. occidentalis* effectively competes with other soil microbes, as growth/nutritional benefits were observed in inoculated plants grown in natural field soils containing indigenous microorganisms (Kariman et al., 2020, 2022). We accordingly hypothesize that *A. occidentalis* has developed stronger organic acid anion exudation and/or more effective capacity to capture organic molecules (e.g. hexoses) from the rhizosphere throughout the evolution; i.e. the feremycorrhizal fungus has evolved to function and being fed in the rhizosphere, as opposed to being fed directly within the root apoplast (which occurs in ECM symbiosis). Diverse host plants in feremycorrhizal symbiosis (woody/herbaceous species, mycorrhizal/non-mycorrhizal species) may have arisen from a lack of root colonization, which does require more complex compatibility (structural, metabolic, etc.) between host roots and hyphae. Feremycorrhiza is a remarkable phenomenon that occurred in the jarrah forests of Western Australia, where a broad range of trees, shrubs, and herbaceous plants co-exist.

***Austroboletus occidentalis* has limited saprotrophic capacity similar to ECM fungi**: The feremycorrhizal fungus does not grow on complex organic matter and lignocellulosic substrates and it only consumes hexoses such as glucose as a carbon source (Kariman et al., 2022), similar to ECM fungi (Shah et al., 2016; Zak et al., 2019). Therefore, it is not comparable to saprotrophs because saprotrophic fungi such as *Aspergillus* and *Penicillium* utilize organic matter, and may grow extensively in soil, leading to boosted mycelial biomass and competition with plants for nutrients; hence, they have limited use as bioinoculants and have not been successful biofertilisers in practice. However, ECM/feremycorrhizal fungi rely directly on the host plants as a carbon source and their growth is regulated by the host; therefore, they do not overgrow in soils and remain (primarily) beneficial to, rather than competing with, plants. In general, a strong saprotrophic capacity (i.e. the capability to utilize soil organic matter as a carbon source) could be an obstacle to mutual benefits in plant-fungus relationships, which is why the most ecologically successful/widespread root fungal symbionts are either obligate biotrophs (i.e. AM fungi) or have limited saprotrophic capacity and can only utilize hexose sugars provided by their host plant (i.e. ECM fungi).

***Austroboletus occidentalis* genome lacks invertase genes similar to ECM fungi**: our recent analysis of the *A. occidentalis* genome has revealed that this fungus does not have the invertase (sucrose-hydrolyzing enzyme) genes (thus, it is unable to use sucrose as a carbon source as we recently reported, Kariman et al., 2022), similar to AM and ECM fungi that generally lack invertases (with few exceptions), whereas invertase genes are common in saprotrophic, endophytic and phytopathogenic fungi (Parrent et al., 2009; Nehls et al., 2010; Tisserant et al., 2013; Miyauchi et al., 2020). The absence of invertase genes means fungal dependency on the host plant as a food source, i.e. a key trait behind the ecological success of AM and ECM fungi.

Austroboletus occidentalis has a strong phosphorus solubilization capacity that is mediated by the exudation of organic acid anions such as oxalate and citrate (Kariman et al., 2020), similar to ECM fungi (Landeweert et al., 2001; Courty et al., 2010). The feremycorrhizal fungus converts orthophosphate molecules to long-chain inorganic polyphosphate chains (Kariman et al., 2020), which are also regarded as a key phosphorus storage/transport strategy in AM and ECM fungi (Bücking and Heyser, 1999; Ashford and Allaway, 2002; Ezawa et al., 2002).

Taken together, we therefore contend that ‘feremycorrhiza’ is an appropriate term for the newly-discovered symbiosis between *A. occidentalis* and plants, as the fungal partner has ECM lineage and several typical features of ECM fungi. Furthermore, lack of root colonization in the feremycorrhizal symbiosis has presumably led to a broader range of host plants (woody/herbaceous plants; mycorrhizal/non-mycorrhizal plants), as compared to ECM fungi (e.g. ECM-forming *Austroboletus* species) that are, generally, limited to woody plants. *Austroboletus occidentalis* has ECM phylogeny and characteristics but does not form ECM structures in/on roots. Thus *A. occidentalis* is a feremycorrhizal fungus forming feremycorrhiza = ‘nearly’ mycorrhiza, a clear and correct term.

Declaration of competing interest

The authors declare that they do not have any conflicts of interest that could have influenced the works reported in this paper.

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