Tansley review

Dual-mycorrhizal plants: their ecology and relevance

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Contents

Summary

Dual-mycorrhizal plants are capable of associating with fungi that form characteristic arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) structures. Here, we address the following questions: (1) How many dual-mycorrhizal plant species are there? (2) What are the advantages for a plant to host two, rather than one, mycorrhizal types? (3) Which factors can provoke shifts in mycorrhizal dominance (i.e. mycorrhizal switching)? We identify a large number (89 genera within 32 families) of confirmed dual-mycorrhizal plants based on observing arbuscules or coils for AM status and Hartig net or similar structures for EM status within the same plant species. We then review the possible nutritional benefits and discuss the possible mechanisms leading to net costs and benefits. Cost and benefits of dual-mycorrhizal status appear to be context dependent, particularly with respect to the life stage of the host plant. Mycorrhizal switching occurs under a wide range of abiotic and biotic factors, including soil moisture and nutrient status. The relevance of dual-mycorrhizal plants in the ecological restoration of adverse sites where plants are not carbon limited is discussed. We conclude that dual-mycorrhizal plants are underutilized in ecophysiological-based experiments, yet are powerful model plant–fungal systems to better understand mycorrhizal symbioses without confounding host effects.

I. Introduction

With the emergence of the first terrestrial plants *c.* 400 Ma, soil fungi of the Glomeromycotina and Mucoromycotina began to form structures in the roots of early Devonian plants. One of these structures resembled arbuscules (Taylor *et al.*, 1995), forming what is now commonly called arbuscular mycorrhizas. As the land masses evolved and ecosystems developed along with pedogenesis, so did other fungi. At *c.* 190 Ma, multiple groups of saprotrophic fungi, such as brown- and white-rot fungi (Skrede *et al.*, 2011; Floudas

et al., 2012) from the Basidiomycota, Ascomycota, and Endogonales from the Mucoromycotina (Desirò *et al.*, 2017) began to form a new type of association, primarily with gymnosperm trees species (e.g. *Gnetum* spp.). These were the first ectomycorrhizas, although key fungal structures such as Hartig nets, commonly characterizing the ectomycorrhizal (EM) type today, were only first seen in fossil records of Pinaceae roots some 50 Ma (Lepage *et al.*, 1997; Strullu-Derrien *et al.*, 2018). Other mycorrhizal types also evolved later than arbuscular mycorrhizas within specific lineages of plants, including the orchid and ericoid mycorrhizas.

Today, most terrestrial plants require an association with at least one type of mycorrhiza to adequately grow and complete their life cycle in natural ecosystems, with arbuscular mycorrhizal (AM) plants being the most common (Smith & Read, 2008). There remain considerable gaps on the role of the mycorrhizal symbiosis in improving plant fitness given the difficulties involved in maintaining nonmycorrhizal controls (Jones & Smith, 2004). Still, it is well recognized that mycorrhizal fungi are in large part responsible for improving the mineral nutrition of host plants that need to cope with low nitrogen (N) and phosphorus (P) concentrations in soil. Mycorrhizas can also benefit plants by helping them tolerate drought stress, heavy metals, and pathogens, via both nutritional and direct effects (Smith & Read, 2008).

Plants are generally considered to form a single mycorrhizal type. However, there are plants that can form both arbuscular mycorrhizas and ectomycorrhizas, either simultaneously within the same root system (Fig. 1) or at different life stages or in different environments; we call these 'dual-mycorrhizal plant species'. Dual-mycorrhizal plants have traditionally been considered uncommon and unusual (Lodge, 2000). We review the literature supporting dual-mycorrhizal status of a wide range of plant species, starting with plant genera where dual-mycorrhizal status is well established and then plant genera not generally considered dual mycorrhizal. We evaluated evidence for structures (i.e. arbuscules, vesicles, and coils for arbuscular mycorrhiza; Hartig net and mantle for ectomycorrhiza), evidence of nutrient transfer or growth enhancement, and whether fungal partner identity has been shown to be consistent with mycorrhizal status. The minimum requirement to be considered dual mycorrhizal was the observation of arbuscules or coils and Hartig net or similar EM structures (e.g. transfer cells) within the roots of the same plant species (Fig. 2).

Dual-mycorrhizal plants are more than curiosities; they offer great potential in determining which mycorrhizal type provides the greatest benefits or costs to their host plants and the benefits or costs of specialization on one type. They also offer insights into the abiotic factors that 'drive' AM and EM root colonization levels within the same host plant, thus providing evidence of how the two main mycorrhizal types partition both fundamental niches, the root system and soil nutrients. Finally, they also highlight the important functions mycorrhizas can play in ecosystems, in particular during rapid abiotic changes and ecological restoration.

Here, we first highlight the challenges in defining mycorrhizal status and propose more inclusive and functional definitions of AM and EM types to define dual-mycorrhizal plants. We then focus on
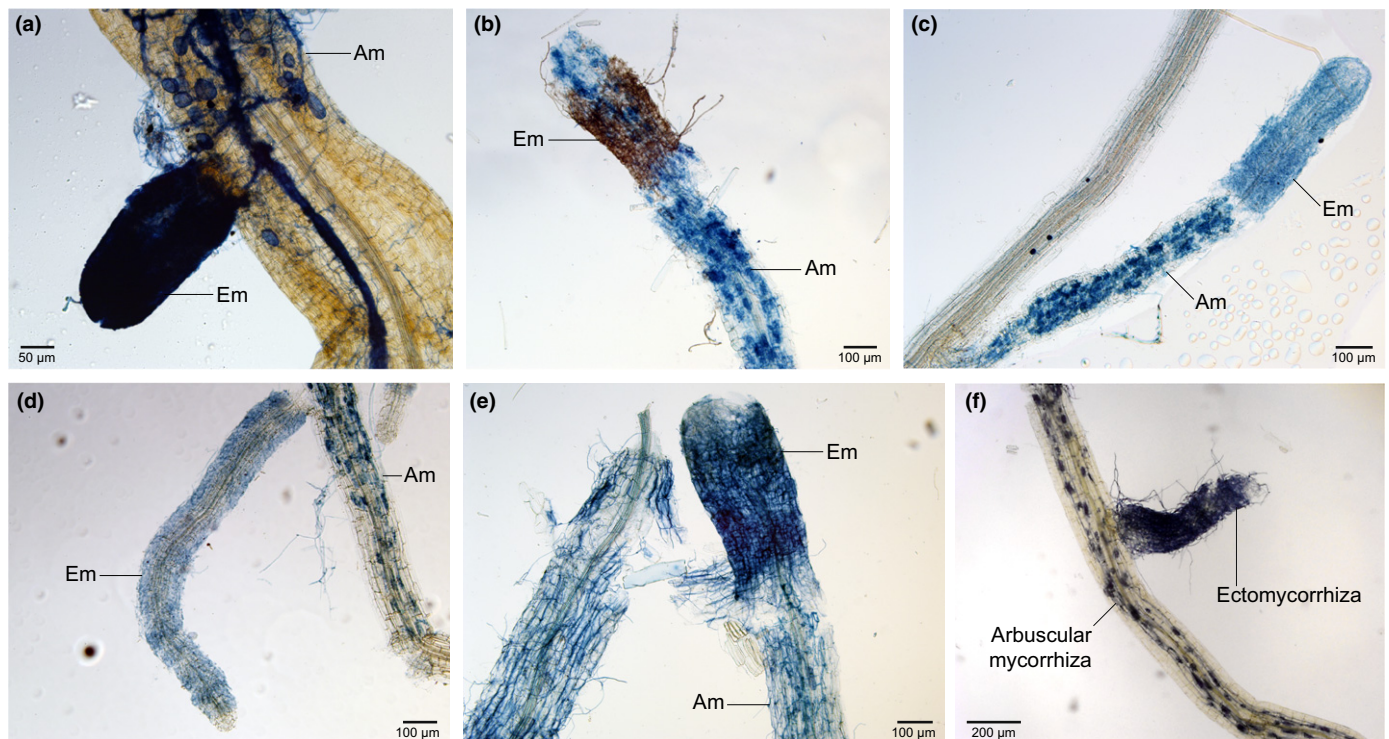
Fig. 1 Dual-mycorrhizal symbioses on the same root fragment of Australian plants. These are examples of 'simultaneous' dual-mycorrhizal plants or 'context-free dual-mycorrhizal plants' (Fig. 2). Root fragments host both arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) key structures (arbuscules and Hartig net as indicated) in (a, b) *Calothamnus sanguineus*, (c) *Eremaea asterocarpa*, (d) *Eucalyptus todtiana*, (e) *Gastrolobium capitatum*, and (f) *Melaleuca systena*.

Fig. 2 Flowchart summarizing how to identify dual-mycorrhizal plant species. Marked in bold are the different subtypes and benefit types of dual-mycorrhizal plants that we propose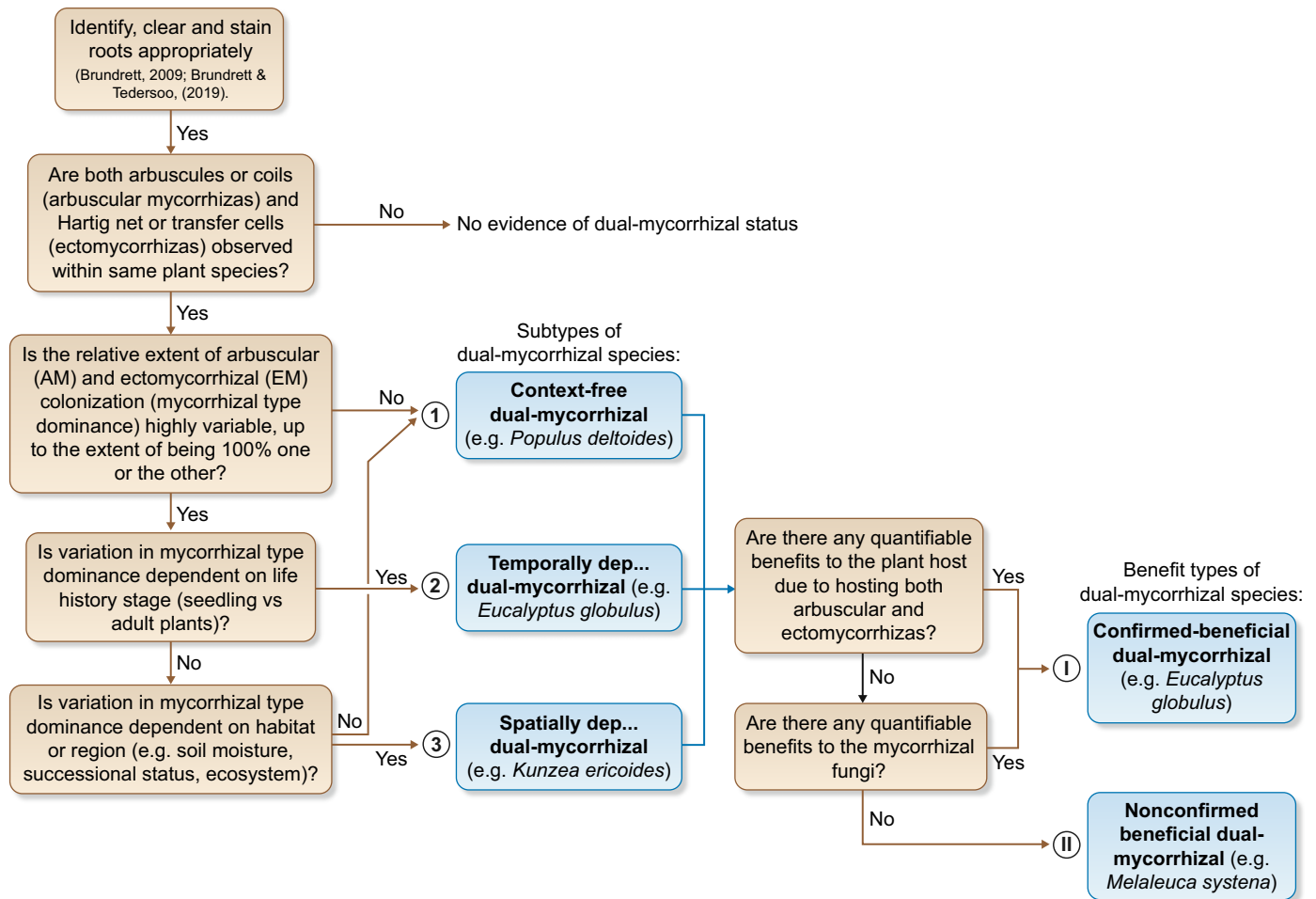 based on the spatiotemporal occurrences of the both arbuscular mycorrhizal and ectomycorrhizal root colonization on the same or different plant individuals.

identifying all possible dual-mycorrhizal plant taxa by re-examining numerous reports from the literature. We searched as far back as the classic work of McDougall (1914), who was the first to describe a 'heterotrophic' mycorrhiza (i.e. showing both EM and AM structures) in *Tilia americana*, although this observation has not been subsequently robustly supported. Based on this review, we showcase three subtypes and two benefit types of dual-mycorrhizal species based on their context of occurrence and quantified benefits (Fig. 2). We then discuss possible costs and benefits that can result from hosting both arbuscular mycorrhizas and ectomycorrhizas, discuss whether they are really independent traits, and reanalyze data on how abiotic and biotic factors can shift the dominance of arbuscular mycorrhizas or ectomycorrhizas on dual-mycorrhizal plants.

Misdiagnosis of mycorrhizas and erroneously assigning mycorrhizal types to plant species has become a major concern recently since ecologists are now conducting more trait-base and meta-studies (Box 1; Brundrett & Tedersoo, 2019). To minimize errors, we relied heavily on studies providing photographs of the characteristic mycorrhizal structures or clear methodology that would have avoided misdiagnoses. For example, *Fraxinus* is a tree genus that has traditionally been considered AM (Harley

& Harley, 1987), but recent studies clearly show EM structures in some species, such as *Fraxinus uhdei* (Ambriz *et al.*, 2010). Another example is the EM status of the perennial herb *Pulsatilla patens* that grows in *Pinus sylvestris* forests (Hoeksema *et al.*, 2018). The roots of this herb had Hartig net and mantle from genuine ectomycorrhizas as a result of associations with *Cenococcum geophilum* and *Piloderma olivaceum* amongst other EM fungi (Hoeksema *et al.*, 2018). However, given the relatively small body of literature on dual-mycorrhizal status, we considered all types of studies ranging from lab experiments using pure AM or EM inoculum to sampling of roots from the field. We do not consider plant species hosting only AM in one region (i.e. in a given study) to be in conflict with the same plant species reported as being EM in a different region (i.e. another study). Rather, these types of reports were considered indications of the temporal- and spatial-context dependency of mycorrhizal status.

II. Challenges in defining mycorrhizal types

There is no one strict definition of what constitutes a dual-mycorrhizal plant, in part because of a lack of clear definitions of

Box 1 Challenges of mycorrhizal status databases and analyses

Mycorrhizal status is increasingly incorporated into broad-scale studies of plant physiology and ecosystem function (Koele *et al.*, 2012; Hempel *et al.*, 2013; Phillips *et al.*, 2013). These databases rely heavily on literature that used different definitions of mycorrhizas (Koide & Mosse, 2004). Uncertainty and limitations around initial observations are frequently lost when these data are integrated into databases (Brundrett & Tedersoo, 2019). This presents a challenge with dual-mycorrhizal status, where a single observation can change a plant from being considered single status to a dual status. Hence, the more observations that are made, the greater chance a species will be listed as dual status on the basis of a single error.

Analysis of dual status also presents challenges. Different statistical approaches have been used, including treating mycorrhizal status as a multiple-level factor (e.g. arbuscular mycorrhizal (AM), ectomycorrhizal (EM), dual mycorrhizal; Cornelissen *et al.*, 2001), considering only one type of mycorrhiza as a binary trait (e.g. EM, non-EM in Koele *et al.* (2012)), or assigning arbitrary units along a continuum from AM to EM (Comas *et al.*, 2014). Considering the ability to form arbuscular mycorrhizas and ectomycorrhizas as alternative states of the same trait, with dual-mycorrhizal plants considered a third possible state, does not make ecological sense. EM status has evolved in multiple lineages from nonmycorrhizal ancestors (Tedersoo & Brundrett, 2017), suggesting the ability to evolve ectomycorrhizas is independent of AM status. We suggest treating the ability to form AM and EM symbioses as independent traits. Whether these are considered binary or continuous traits (reflecting facultative status) may depend on the goals of the analysis. In either case, a significant interaction term between AM and EM treatments can be used to test statistically whether simultaneous dual colonization has costs or benefits above and beyond the two mycorrhizal types independently.

what constitutes an AM or EM plant. There is current debate over whether functional or morphological traits are more diagnostic (Brundrett & Tedersoo, 2019; Bueno *et al.*, 2019). Mycorrhizal symbiosis has traditionally been defined as mostly involving the mutualistic transfer of carbon (C) from plant to fungus and mineral nutrients from fungus to plant, yet some associations have neutral to negative effects on plant growth in spite of nutrient exchange, especially in higher fertility soil (Smith *et al.*, 2003; Jones & Smith, 2004; Hoeksema *et al.*, 2010). Furthermore, it is not practical to test for nutrient exchange in the field, and fitness effects can never be evaluated on long-lived hosts. Morphologically, arbuscular mycorrhizas are typically defined by the formation of arbuscules (either *Paris* or *Arum* type) and vesicles, but arbuscules are ephemeral and some AM fungi form neither structure (Smith & Read, 2008). Creating further confusion, typically non-AM plants can sometimes be infected by AM fungi (Giovannetti & Sbrana, 1998). In *Salsola*, for example, root cell penetration and short-lived arbuscule formation occurs, but the plant is nonetheless considered nonmycotrophic (Allen *et al.*, 1989). Ectomycorrhizas were first defined by Frank in 1885 on the basis of an ensheathing mantle (Trappe, 2005), and the presence of a Hartig net is commonly considered a defining characteristic. Nonetheless, some authors have considered plants to be EM on the basis of a fungal mantle covering as little as a

single epidermal cell (Warcup, 1980). As in arbuscular mycorrhizas, atypical infection of plant species is not uncommon; for example, despite colonization of *Carex* by *Cortinarius*, *Carex* is not generally considered to be EM due to the lack of a Hartig net and lack of evidence of mutualism (Harrington & Mitchell, 2002; Brundrett, 2009; Tedersoo & Brundrett, 2017). Because the typical AM and EM symbioses are fairly clear morphologically, we used morphological characteristics in our compilation of dual-mycorrhizal plants; however, we acknowledge that there are many plant–fungal symbioses that do not fit rigid morphological definitions.

Given the problems in fitting strict definitions of mycorrhizal types to plants, it is not surprising that defining dual-mycorrhizal plants is equally or even more problematic. There are a number of plants that are widely considered to be dual mycorrhizal, with both AM and EM types frequently reported, along with positive growth responses from both, including *Acacia*, *Alnus*, *Eucalyptus*, *Fraxinus*, *Populus*, *Salix*, *Shorea* and *Uapaca*. At the extreme, Molina *et al.* (1992) lists *c.* 110 genera as hosting both AM and EM types. They do, however, mention in a footnote that some of the genera listed are poorly documented or that their ecological significance is slight or unknown. Hempel *et al.* (2013) compiled mycorrhizal status data from multiple databases and report 66 plant species listed as having both AM and EM associations. Several of these appear to be erroneous. *Campanula scheuchzeri* and *Saxifraga paniculata*, for example, are listed as having ectomycorrhizas on the sole basis of an association of 'Cenococcum-type' hyphae with roots, with no evidence of any mycorrhizal structures (Read & Haselwandter, 1981). A number of plants are often considered as being exclusively EM, despite periodic records of arbuscular mycorrhizas, including species in the Pinaceae and Fagaceae. *Festuca rubra* is also claimed to be EM based on a citation chain from Harley & Harley (1987) back to (Read & Haselwandter, 1981), but the original citation does not include *Festuca rubra*, and none of the *Festuca* species that are cited in that publication are claimed to have ectomycorrhizas. Other database entries – for example, the association of *Acer campestre* with EM fungi – are based at least in part on the observation of fruiting bodies in proximity to trees; that is, Trappe (1962) as cited in Harley & Harley (1987) as cited in Wang & Qiu (2006) as cited in Hempel *et al.* (2013). Simply observing EM fungi growing near a tree does not provide clear evidence that the fungus is associating with that tree. The mycorrhizal status of several other plant species, such as *Ilex aquifolium*, also trace back to Harley & Harley (1987), but the original sources cited therein are difficult to recover (in that case a 1935 publication written in Czech). Hence, errors in designating plants as dual mycorrhizal have been propagated through the literature, and some designations are very difficult to confirm.

We acknowledge that defining a mycorrhiza is still debated (e.g. International Conference on Mycorrhiza 10, Mérida, Mexico, 2019), yet the definition we propose here is clear and in line with recent commentaries (Bueno *et al.*, 2019) that also propose a more inclusive definition. As such, we included plant species that tend to be dominated by AM as seedlings only,

given that this life stage is so critically important in determining plant lifetime fitness.

III. Dual-mycorrhizal plant taxonomy and distribution

Central to determining dual-mycorrhizal status is the observation of arbuscules and Hartig nets within roots of the same plant species. We suggest that roots should represent different life stages (i.e. seedling, sapling, to mature trees) in the case of tree species. This differs from Brundrett & Tedersoo (2019) in not requiring dual plants to have both EM and AM structures in mature plant roots, which we believe is justified given that seedling establishment and early growth is a critical plant life history stage. As others pointed out, hyphal coils from the *Paris* colonization type can effectively function like arbuscules (Bueno *et al.*, 2019). As such, we consider any internal root structures of AM fungi that are directly linked to conducting nutrient exchange at the interfaces of the symbioses to be valid. Similarly, with ectomycorrhizas, there are reports of so-called 'unusual' versions of the Hartig net. For example, *Pisonia grandis* has transfer cells found in the epidermis and cortex cells of its roots (Ashford & Allaway, 1982). These structures may simply be poorly developed forms of a Hartig net and likely still function as nutrient-exchange structures (Ashford & Allaway, 1982); thus, we included these cases.

We conducted an extensive search of the literature, based, wherever possible, on primary data rather than databases. We identified 211 plant genera within 67 families that have previously been considered to have a dual-mycorrhizal status (Supporting Information Table S1). Notably, many of these are not well documented, and we have labelled these as possibly erroneous. To create a list of species we designate as 'confirmed dual-mycorrhizal plants' we removed studies that did not explicitly mention or show arbuscules/coils for arbuscular mycorrhizas and Hartig net/transfer cells for ectomycorrhizas, retaining 89 plant genera within 32 families (Table S2). These genera contain *c.* 7355 species in total (84% woody taxa), although only a small proportion of these have confirmed dual-mycorrhizal status (238 plant species; Table S2). The remainder should be examined more intensively to determine within-genus variability in dual-mycorrhizal status, because observation of only mycorrhiza type does not mean that the other type does not occur. We mapped the global distribution of these confirmed dual-mycorrhizal species and found that they are widespread globally (Fig. S1), generally covering most areas where EM plants occur (Steidinger *et al.*, 2019). The major exception would be Nothofagaceae-dominated forests of southern South America, where dual status has been suggested for *Nothofagus* spp. (Smith & Read, 2008) but not confirmed (Table S1). Our analysis of the global distribution of confirmed dual-mycorrhizal plants highlights Australia as a potential 'hotspot' for dual status (Fig. S1).

IV. Net costs and benefits of dual-mycorrhizal status

There are few studies on dual-mycorrhizal plants aimed at quantifying the benefits or costs of hosting both AM and EM fungi. We found dual-inoculation studies spanning only 10 dual-mycorrhizal genera (Table S3). As such, most (> 90%) dual-mycorrhizal plant genera listed in Table S2 are nonconfirmed

beneficial duals (Fig. 2). It is not unusual to observe earlier colonization by AM fungi than EM fungi in lab experiments, with positive growth response and P uptake occurring around the same time as colonization (Lapeyrie & Chilvers, 1985; van der Heijden & Kuyper, 2001). Nevertheless, dual colonization is sometimes inhibitory or has no effect on plant growth relative to a single type of mycorrhiza formation. For example, survival, biomass and nutrient content of *Quercus agrifolia* were lower in dual-colonized plants than plants colonized with a mixture of AM fungi or a single species of EM fungi (Egerton-Warburton & Allen, 2001). Seedlings had higher total P content in foliage if EM only and higher foliar N content if AM. Similarly, EM *Eucalyptus marginata* seedlings were larger than nonmycorrhizal controls or AM plants, but dual-inoculated plants were no larger than controls and significantly smaller than AM or EM plants (Kariman *et al.*, 2012). However, a Hartig net was not observed in *Eucalyptus marginata* seedlings inoculated with EM fungi; thus, results from that study should be interpreted cautiously. In *Eucalyptus grandis*, dual inoculation stimulated belowground growth, but inhibited aboveground growth, with no effect on nutrient content relative to controls (Holste *et al.*, 2017). EM *Eucalyptus urophylla* accumulated *c.* 50% more N and P than AM plants, with dual-colonized plants showing intermediate levels of nutrient accumulation (Gange *et al.*, 2005). In *Populus fremontii*, inoculation with a mixture of AM fungi appeared to stimulate total plant biomass compared with nonmycorrhizal controls, whereas a mixture of EM or EM + AM fungi reduced root growth so much that it was not offset by a stimulation in shoot growth (Meinhardt & Gehring, 2012). These results highlight that there can be disadvantages associated with hosting both AM and EM fungi simultaneously.

Despite some cases of negative responses to dual colonization, a compilation of dual-inoculation studies using dual-mycorrhizal plant species shows that, overall, there are more frequent positive and neutral effects than negative ones (Fig. 3; Table S3). More frequently reported are positive effects of dual inoculations compared with controls (Fig. 3), which was also the case for single-mycorrhizal-type inoculations (Fig. 3). When compared with each other, dual inoculations vs either type, AM or EM, produced more neutral results (Fig. 3). From this analysis, we propose that dual-mycorrhizal status is frequently a positive or neutral trait, despite the examples of growth reduction described in the previous paragraph. For example, Chatarpaul *et al.* (1989) found that *Alnus incana* produced more biomass when inoculated with a combination of one AM fungus, one EM fungus, and *Frankia*, rather than *Frankia* alone or *Frankia* with either mycorrhizal type separately. When two *Eucalyptus* species were inoculated with individual AM fungi alone or in combination with one EM fungus, plant height was generally greater with the combination of dual mycorrhizas than arbuscular mycorrhizas alone. For one species, EM plants were larger than AM plants, but with no further increase in size with dual colonization (Chen *et al.*, 2000). Nutrient content was not measured in either case, so it is difficult to state whether N or P was more important, but more likely P given that *Alnus* plants were actinorhizal and, for the *Eucalyptus*, the response to mycorrhizal colonization was much larger at low P (< 5 mg kg^{-1} soil).
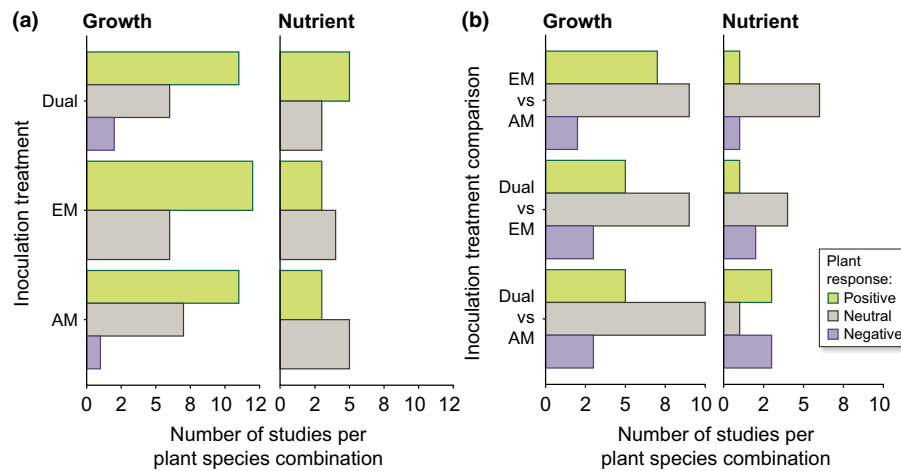
Fig. 3 Number of studies that showed a positive, neutral, or negative plant host response to inoculations with arbuscular mycorrhizal (AM) fungi, ectomycorrhizal (EM) fungi, or both (Dual; dual inoculation). Inoculated plant responses compared with (a) a control (i.e. noninoculated or grown in sterile soil) and (b) each other (i.e. Dual vs AM or EM). Plant response types were separated as Growth (i.e. plant-growth based, such as survival or biomass increase) or Nutrient (i.e. plant-nutrient uptake based, such as foliar P content increase). When multiple plant responses were reported in the same study, we used total plant DW as our 'gold standards'. Shoot DW was favored over root DW when total values were not analyzed. Other plant responses, such as plant survival and final height, were also considered when no biomass data were presented. Please refer to Supporting Information Table S3 for more information about the studies that were used to generate these results; those studies are as follows: Chatarpaul *et al.* (1989), Chen *et al.* (2000), Egerton-Warburton & Allen (2001), Founoune *et al.* (2002), Duponnois *et al.* (2003), Diouf *et al.* (2005), Gange *et al.* (2005), Misbahuzzaman & Newton (2006), Ramanankierana *et al.* (2007), Ambriz *et al.* (2010), Kariman *et al.* (2012), Meinhardt & Gehring (2012), Báez-Pérez *et al.* (2015, 2017), Tapwal *et al.* (2015), and Cortese & Bunn (2017). These results are based on inoculation trials of seedlings and thus may not be representative of plant responses when individuals are mature.

Given the paucity of direct studies of dual inoculation, we attempted to make some predictions about the growth benefits of hosting both arbuscular mycorrhizas and ectomycorrhizas in dual-mycorrhizal plant genera that have not been tested. As such, we analyzed the log response ratio (log of plant growth with/without mycorrhizal inoculum) of our confirmed dual-mycorrhizal plant genera (Table S2) included in the MycoDB (Chaudhary *et al.*, 2016). Whether plants were inoculated with AM or EM fungi, we found a more frequent positive response than neutral or negative response in dual-mycorrhizal plant genera (Fig. S2). From this preliminary analysis, we hypothesize that *Acacia*, *Eucalyptus*, *Fraxinus* and *Pinus* will typically respond positively to inoculations by both AM and EM fungi (Fig. S2; effect sizes per plant species are shown in Figs S3, S4), suggesting these genera may contain plant species that benefit from dual inoculations.

V. Nutritional advantages of being dual

Each type of mycorrhiza has well-documented benefits to plants in terms of growth, nutrient acquisition, and protection from pathogens (Smith & Read, 2008). Therefore, the obvious question concerns why a plant would form associations with both AM and EM fungi simultaneously, consecutively, or in different environments. In certain plant species, a gradual shift from AM- to EM-type dominance occurs over time or along abiotic gradients, yet both mycorrhizal types persist. Why? We consider first the potential nutritional advantages and then a series of hypotheses around non-nutritional benefits. We also consider fungal-based explanations in Box 2.

The best documented benefits to plants from either type of mycorrhiza are nutritional, with most research focused on N and P.

Box 2 Myco-centric explanations for dual-mycorrhizal status

Though dual-mycorrhizal status is often considered from a plant perspective, it is possible that dual-mycorrhizal status is not driven by plant benefit, but rather by fungal interactions. Ectomycorrhizal (EM) colonization in predominately arbuscular mycorrhizal (AM) plants may reflect hyperpromiscuity by fungi. One fungus, *Cenococcum geophilum*, is particularly common among EM fungi reported on otherwise AM plants and has also been reported to form ectomycorrhizas on ericoid mycorrhizal plants (Stevens *et al.*, 1996; Vohník *et al.*, 2007). Tedersoo & Brundrett (2017) have argued that these reports are due to either misidentification of the fungus or the plant root, but it is also possible that this fungus is simply highly promiscuous.

It is possible that AM colonization in typically EM hosts is not necessarily beneficial, but rather represents a relict of the evolutionary past. The EM status has evolved in *c.* 30 independent lineages of plants, with all but five being from predominately AM ancestors (Tedersoo & Brundrett, 2017). If the costs to plants of AM colonization in otherwise EM hosts are low in ecosystems, there may be limited evolutionary pressure to exclude AM colonization following evolution of EM status in plants, whereas the AM fungus may still benefit. Exclusion of AM colonization in pure EM plants may reflect fungal competition rather than plant control, in which case a lack of EM inoculum may drive temporary AM presence. EM fungi may outcompete AM fungi due to some of the mechanisms shown in interspecific EM fungal competition studies (Kennedy, 2010), including mycelial overgrowth, greater scavenging of nutrients in return for plant carbon, and colonizing roots first leading to 'priority effects' (Kennedy, 2010). Further investigation of EM–AM fungal interactions in dual-mycorrhizal plants is needed.

Increased nutrient uptake is driven by different mechanisms, which vary with mycorrhizal type. Uptake of mineral nutrients from soil by AM hyphae has been characterized as 'scavenging', which was defined by Lambers *et al.* (2008) as physical exploration and uptake of nutrients without changing their chemical form. By contrast, EM fungi are generally considered capable of also 'mining' nutrients, defined as releasing otherwise unavailable nutrients by excreting enzymes or low molecular weight organic acids (LMWOAs; Plassard & Dell, 2010). This raises the possibility that AM and EM colonization result in complementarity in nutrient acquisition.

Scavenging involves fungal hyphae extending many centimeters beyond the colonized root to expand the volume of soil from which nutrients can be absorbed (Smith & Read, 2008). This mechanism is important in both mycorrhizal types and is considered to be most important for nutrients such as orthophosphate, ammonium, copper and zinc, where low diffusion coefficients limit mobility in soil solutions (Tinker & Nye, 2000). Both types of hyphae can transport P through the soil at rates faster than would occur by diffusion alone (Cox *et al.*, 1980; Timonen *et al.*, 1996). The relative effectiveness of AM and EM hyphae in facilitating nutrient uptake via direct scavenging will depend on proliferation of hyphae beyond the depletion zones that form around roots. In the field, EM hyphae appear better able than AM hyphae to proliferate in nutrient-rich patches, although this was observed on different plant species for each mycorrhizal type (Chen *et al.*, 2016). In one of the few comparisons of hyphal production by EM and AM fungal species on the same host, Jones *et al.* (1998) found three to seven times greater hyphal production by two EM fungi than three AM fungi, and this was correlated with shoot growth and P uptake of *Eucalyptus coccifera*. P inflow rates (i.e. uptake per unit root length) and total P accumulation were 1.5–3.5 times as great in EM plants as in AM plants, depending on the fungal species, whereas percentage P was not affected (Jones *et al.*, 1998). Therefore, a difference in the propensity to produce exploratory hyphae may be an advantage of EM fungi, even though it comes with increased absolute C partitioning belowground (Jones *et al.*, 1998). By contrast, retention of nutrients by the fungus to meet its own needs has been demonstrated for both EM and AM symbioses; therefore, the larger proportion of fungal tissue in EM than AM roots may be detrimental to plants in low-nutrient soils (Hasselquist *et al.*, 2016; Püschel *et al.*, 2016; Teste *et al.*, 2016).

In many soils, the majority of N and P is found in organic forms (Cosgrove, 1967), with the ratio of organic to inorganic P increasing with time (Walker & Syers, 1976; Turner & Condron, 2013). EM fungi utilize a range of oxidative and hydrolytic enzymes to break down soil organic matter and release N and P in absorbable forms (Antibus *et al.*, 1997; Plassard & Dell, 2010; Nicolás *et al.*, 2019), albeit with lower capability than saprotrophic fungi (Kohler *et al.*, 2015). By contrast, whereas AM fungi can take up and transfer N from organic matter to their host plant (Hodge *et al.*, 2001; Fellbaum *et al.*, 2012; Thirkell *et al.*, 2016), the weight of evidence is that AM fungi take up N or P primarily after mineralization by other soil microbes (Joner & Jakobsen, 1995; Leigh *et al.*, 2009; Whiteside *et al.*, 2012; Wang *et al.*, 2017). Indeed, there is increasing evidence that AM hyphae can stimulate

mineralization of organic matter by influencing the metabolism of soil bacteria (Zhang *et al.*, 2018) and compete effectively with soil microbes for those nutrients (Bukovská *et al.*, 2018).

Consistent with the generally lower contribution of AM fungi than EM fungi to soil extracellular enzymes (Joner & Johansen, 2000; Phillips *et al.*, 2014), higher soil phosphomonoesterase and β-glucosidase occurred in treatments where *Salix* clones had higher EM colonization relative to AM colonization (Baum *et al.*, 2018), and soils from around EM trees generally have higher enzyme activities than AM fungi-colonized soils (Phillips *et al.*, 2013). A comparison of N uptake by four AM and four EM tree species under controlled conditions confirmed that the ratio of organic (supplied as an amino acid) to inorganic (nitrate + ammonium) taken up per unit root surface area was higher in EM species than in AM species (Liese *et al.*, 2017). AM trees accumulated six times more N from inorganic forms than EM trees did, independent of tree size, with no difference in uptake of N from amino acids. In the field, root exudation in AM trees appears to result in increased inorganic N in the rhizosphere, whereas the extracellular enzymes stimulated by root exudates in EM root systems resulted in increased availability of amino acids (Brzostek *et al.*, 2013). Hence, the traditional view is that an AM or AM-dominated dual-mycorrhizal plant may be able to gain access to additional organic N and P by allowing colonization by EM fungi, but access to organic nutrients by AM fungi may have been underestimated (Jansa *et al.*, 2019). This is a topic ripe to be examined using dual-mycorrhizal hosts.

A third mycorrhizal mechanism that aids plants with nutrient uptake is the solubilization of nutrients from primary and secondary minerals. This is especially important for P, which occurs primarily as apatite (calcium phosphate) in young soils, and strongly complexed with iron and aluminium oxides or as secondary calcium phosphates in older or highly weathered soils (Walker & Syers, 1976). Roots and microbes, including mycorrhizal hyphae, LMWOAs (Griffiths *et al.*, 1994; Rineau *et al.*, 2008), which facilitate release of orthophosphate from phosphate minerals through complex and poorly understood mechanisms (Zhu *et al.*, 2018). Although both EM and AM hyphae release LMWOAs (Plassard & Dell, 2010), Allen *et al.* (1996) found oxalate crystals on only EM hyphae in southern California. In a field study, where most of the P was present as calcium phosphate, dual-mycorrhizal *Salix sitchensis* had lower N : P ratios than AM clones did, indicating that EM fungi had been more effective at alleviating P stress in this substrate (Cortese & Bunn, 2017). In direct measurement of rock surface weathering, Quirk *et al.* (2012) found that trees with EM associations caused higher weathering than trees with AM associations did, but Dickie *et al.* (2014) noted that these differences could be explained by soil pH. Although these studies suggest that release of LMWOAs is a more common mechanism for P acquisition by EM hyphae than AM hyphae, further research comparing the release of LMWOAs by AM and EM fungi/roots and its effect on the liberation of P from soil minerals is needed.

Taken as a whole, there is evidence that AM and EM fungi differ in nutrient acquisition strategies, with EM fungi generally having greater capability. This only partially supports the

complementarity in nutrient uptake hypothesis, as no clear advantage of AM status has been shown for any nutrient. Some key knowledge gaps remain, however, such as how the interaction of the two types of mycorrhizal hyphae with soil bacteria influences nutrient availability. There remains the possibility that AM status may be more efficient (lower C cost per nutrient gain) for uptake of available nutrients than EM status; but except for Jones *et al.* (1998), who found no difference in efficiency, this remains unexplored.

VI. Non-nutritional benefits of dual-mycorrhizal status

Nutritional complementarity is only one possible cause of dual-mycorrhizal status. We consider the following additional mechanisms:
1 Lowering costs of seedling establishment;
2 Insurance strategy;
3 Ability to cope with flooding and/or drought;
4 Greater ability to exploit whole soil depth profile;
5 Greater flexibility with soil nutrient availability through ecosystem development;
6 Greater flexibility for other relevant soil properties: temperature, salinity, litter compounds;
7 Greater pathogen and pest protection.

1. Lowering costs of seedling establishment

In *Eucalyptus* seedlings, it is common to observe rapid colonization by AM fungi, which is then replaced by EM fungi when inocula of both fungal groups is available (Chen *et al.*, 2000; Gange *et al.*, 2005). In extreme cases, seedlings are completely AM early in life (Bellei *et al.*, 1992; Chen *et al.*, 2000) and then simply lose AM associations and become almost completely EM after 1 yr, particularly when growing after severe site disturbance or as exotics in plantations. These shifts from AM- to EM-dominated seedlings in *Eucalyptus* (Lapeyrie & Chilvers, 1985) have also been reported in alien ranges such as Algeria and Brazil (dos Santos *et al.*, 2001; Adjoud-Sadadou & Halli-Hargas, 2017). Here, we show this clear switching with seedling age in seven *Eucalyptus* species from two studies that provided sufficient data to calculate a mycorrhizal-type dominance ratio (Fig. 4).

Earlier colonization by AM fungi may be advantageous to seedlings if the C costs of an AM root system are lower than those of an EM root system. When nutrient fluxes occur early in the spring when plants are small, this could allow plants to form a P-acquiring symbiosis when needed, but with lower C costs (van der Heijden, 2001). In a range of field and laboratory studies, C allocated to EM fungal tissue alone reached up to 22% of total allocation (Hobbie (2006), whereas belowground C allocation to the entire mycorrhizal root system can increase by 4–36% (Reid *et al.*, 1983; Durall *et al.*, 1994). Such values for AM root systems are somewhat lower on average (4–13%; Lambers, 1987; Lendenmann *et al.*, 2011), although Jones *et al.* (1998) found no difference in percentage of fixed C allocated to AM and EM root systems of 3-month-old *E. coccifera*.

In cases where a dual-mycorrhizal seedling is promptly colonized by EM fungi instead of AM fungi, the C costs of ectomycorrhizas

may be offset by subsidies provided by mycorrhizal networks (Simard *et al.*, 2012), which have been shown to be more positive for EM plants than AM plants (van der Heijden & Horton, 2009). However, these network benefits are only available when EM trees are already established, and seedlings growing into AM vegetation or in early succession may benefit from forming lower C cost AM associations.

Interestingly, a reduction of C fixation by clipping 50% of the shoots did not result in a change in formation rate of either type of mycorrhiza (Saravesi *et al.*, 2011), suggesting that relative C cost did not influence colonization in *Salix repens*. This may be because C is usually not a limiting resource for plants (Millard *et al.*, 2007), and plants can compensate for higher C sink strength of mycorrhizal root systems with higher photosynthetic rates per unit leaf area (Reid *et al.*, 1983; Ingestad *et al.*, 1986; Lendenmann *et al.*, 2011). Consequently, a thorough analysis by Correa *et al.* (2012) concluded that, for EM plants, C costs were not a factor in whether a symbiosis was established. Therefore, although it is intuitive that AM associations would require lower C inputs from the plant than EM associations would, this idea is not supported by studies that have compared AM and EM root systems in the same plant species, even on young seedlings. More studies using dual-mycorrhizal hosts are required to determine whether plants can always compensate to the same extent for the C demands of either type of mycorrhiza, even in high-stress environments.

2. Insurance strategy

An alternative hypothesis is that dual-mycorrhizal status is an insurance strategy to secure benefits from the mycorrhizal symbiosis regardless of the type. This benefit would be relevant in ecosystems where the inoculum of one or both types of mycorrhizal fungi is sometimes absent or insufficient. Some site disturbances can greatly reduce EM fungal inoculum (e.g. severe forest fires) with lesser impact to AM fungal communities (Lapeyrie & Chilvers, 1985; Horton *et al.*, 1998). In such cases, arbuscular mycorrhizas could help dual-mycorrhizal plant species to establish and regenerate the sites more quickly than EM-type plant species could. Many dual-mycorrhizal species can occur in early succession, and Read (1991) suggested that the dual status might allow plants to establish with AM colonization and later switch to EM colonization. Dickie *et al.* (2014) suggest that this is most likely to occur in secondary, rather than primary succession, on the basis that AM inoculum is frequently more limiting than EM inoculum in primary succession. For example, in the very early stages (first few years) of primary succession, dual-mycorrhizal species such as *Salix* get heavily colonized by EM fungi and only several years later are arbuscular mycorrhizas detected (Allen *et al.*, 2005). A strong switch to AM dominance occurs in some stands, and such shifts in colonization patterns appear to be driven by the buildup of soil organic matter (Allen *et al.*, 2018).

Some evidence supports the insurance strategy hypothesis. For example, Dickie *et al.* (2001) found that *Quercus rubra* seedlings planted in a highly disturbed site had high AM colonization when growing away from EM trees, while *Quercus* seedlings growing near EM *Quercus* trees had consistently high EM colonization and low

AM colonization (Fig. 4). In another study, Horton *et al.* (1998) looked at the post-fire fungal colonization frequency of dual-mycorrhizal *Pinus muricata*. They found strong evidence for AM fungal colonization early after fire, and particularly high levels on seedlings growing on sites that only had AM plant species pre-fire. With time they noted a gradual dominance of EM fungal colonization. This differs from the lower establishment cost hypothesis, in that AM colonization dominates only when EM fungi are absent.

3. Ability to cope with flooding and/or drought

Flooding typically produces large variations in soil texture and nutrient patches, resulting in a soil with a heterogeneous moisture-holding capacity. As such, floodplains tend to be hostile grounds for plants not adapted to these conditions and the rapid changes in soil conditions that typically occur. Interestingly, flooding is an important disturbance in many ecosystems dominated by dual-mycorrhizal trees such as *Populus*, *Salix* and *Alnus*, but also some species of *Quercus* (Watson *et al.*, 1990). For example, colonization by AM fungi was strongly favored in moist soil conditions for *Populus deltoides* and *Salix nigra* when grown as seedlings in a controlled experiment, but the pattern reversed (Fig. 4) based on assessments of field roots (Lodge, 1989). Moyersoen & Fitter

(1999) also suggest that waterlogged soils favor AM fungi over EM fungi in *Uapaca staudtii* (Fig. 4), and Watson *et al.* (1990) found a greater abundance of arbuscular mycorrhizas in lowland, poorly draining and periodically flooded sites on *Quercus rubra* (Fig. 4). For *Quercus palustris*, during very wet years, resulting in poorly aerated conditions, on floodplain sites, AM roots predominated with a considerable reduction of EM colonization (Watson *et al.*, 1990), which supports the hypothesis that flooding favors dual status. Further, *Leptospermum scoparium* was reported as being entirely AM in four out of 10 samples from wet coastal sites in New Zealand (Moyersoen & Fitter, 1999) and nearly entirely EM in dry montane sites (Weijtmans *et al.*, 2007). Similar results for *Kunzea ericoides* support the importance of moisture in determining the mycorrhizal-type dominance of Myrtaceae in these systems (Olsen, 2015).

Soil drought is a very frequent environmental stressor for plants. In *A. incana*, drought significantly decreased EM colonization levels, while increasing the formation of arbuscules from AM fungi (Kilpelainen *et al.*, 2017). Specificity of *Alnus* in forming EM symbioses may have partly confounded these results by limiting the number of EM fungal symbionts tolerant to drought stress. Still, others also found that dry soil favored dominance by AM fungi on dual-mycorrhizal *Populus angustifolia* and *Q. agrifolia* (Gehring *et al.*, 2006; Querejeta *et al.*, 2009).
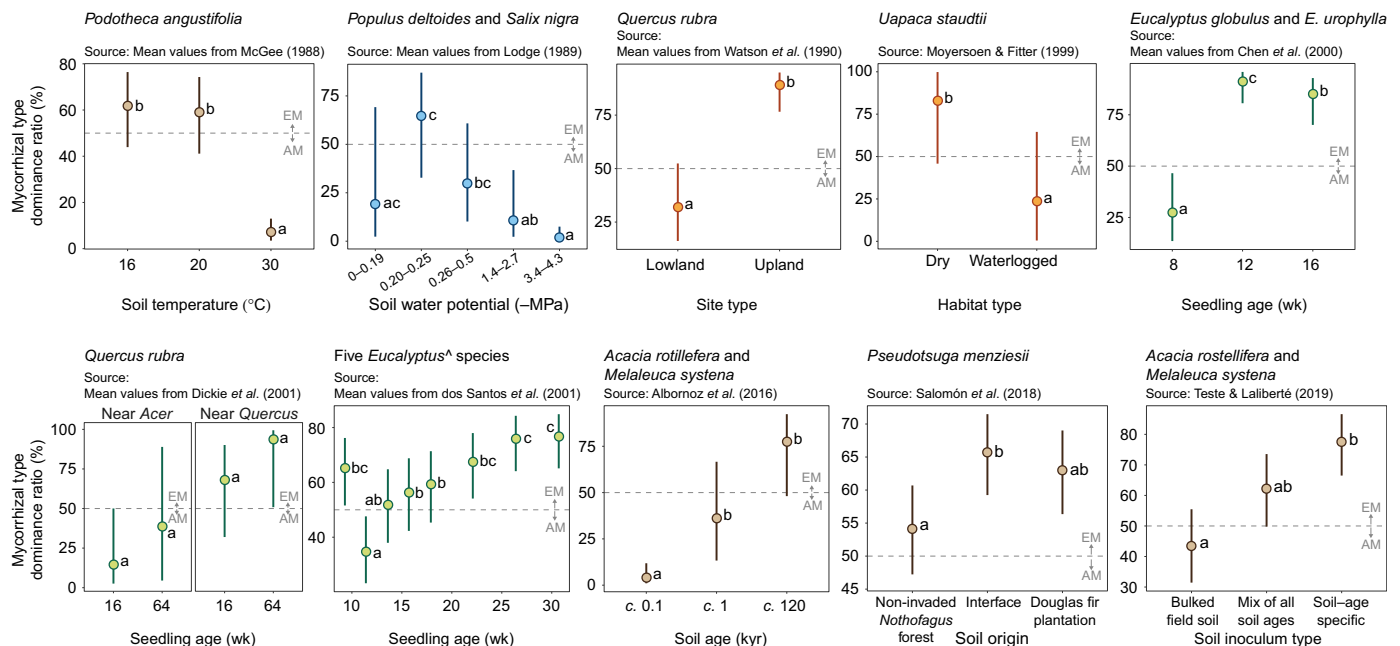

Fig. 4 Abiotic and biotic factors regulating shifts in the dominance of arbuscular mycorrhizas or ectomycorrhizas on roots of dual-mycorrhizal plants. These shifts (i.e. 'mycorrhizal switching') were estimated with a mycorrhizal-type dominance ratio, which is the ratio of ectomycorrhizal (EM) to arbuscular mycorrhizal (AM) fungal colonization levels: %EM/(%EM + %AM). We used a logistic transformation (Warton & Hui, 2011) in all cases except for the data in Moyersoen & Fitter (1999) and Teste & Laliberté (2019), which required an arcsine square-root transformation to avoid infinity values. Linear mixed effects or standard linear models were used to fit the data, and 95% confidence intervals were generated from model fitted values. Dots are means with 95% confidence intervals based on published means when raw data were not available. Different letters indicate statistically significant differences ($P < 0.05$) according to Tukey honest significant difference tests. We used data from the following studies: McGee (1988), Lodge (1989), Watson *et al.* (1990), Moyersoen & Fitter (1999), Chen *et al.* (2000), Dickie *et al.* (2001), dos Santos *et al.* (2001), Albornoz *et al.* (2016), Salomón *et al.* (2018), and Teste & Laliberté (2019). ^*Eucalyptus camaldulensis*, *Eucalyptus citriodora*, *Eucalyptus cloeziana*, *Eucalyptus grandis*, *Eucalyptus urophylla*. Soil inoculum origin from Teste & Laliberté (2019) refers to treatments where plants were grown directly in unaltered field soil, or an average mix of all soil-age-specific inoculum, or a soil-age-specific soil inoculum (see section VI.5 and Teste & Laliberté (2019) for more detail). Colonization values in most studies presented here were based on a per root length basis. In some cases, data points were extracted from graphs with DATATHIEF III (Tummers, 2006).

We propose the following mechanisms responsible for higher AM than EM colonization in very dry and very wet soils: (1) poor oxygen (O_2) availability in soil reduces EM dominance on roots since EM fungi do not develop properly in poorly aerated soil (Read & Armstrong, 1972); (2) EM fungi are more competitive and can displace AM fungi on roots when grown in well-drained (but not dry) soil; (3) AM fungal propagules have superior drought tolerance compared with EM fungal propagules, resulting in dual-mycorrhizal plants that are more AM dominated in dry soil (Kilpelainen *et al.*, 2017); (4) AM fungi can increase the hydraulic conductivity of soil (Bitterlich *et al.*, 2018); and (5) aquatic plants, which tend to be AM, can transport gases, including O_2, within roots, and AM fungi may be capable of surviving by residing in cells of these roots. From the plant perspective, being able to form different types of mycorrhizas along moisture gradients may increase habitat breadth and resilience to flooding or drought.

4. Greater ability to exploit whole soil depth profile

Belowground ecology and our current understanding of ecosystem functioning remain based on only the surficial sampling of roots (Binkley, 2015). Whereas soil nutrient levels can be highly variable within the uppermost soil layers, there are major changes in soil properties, particularly nutrient availability and uptake by roots, in deeper soil (McCulley *et al.*, 2004). As a result, ecologists have hypothesized that the coexistence of both arbuscular mycorrhizas and ectomycorrhizas could involve the partitioning of soil nutrients horizontally within surficial layers (Nilsson *et al.*, 2005) or vertically with depth (> 10 cm; Moyersoen *et al.*, 1998). The study of Neville *et al.* (2002) supports this hypothesis, since they found a negative correlation between EM and AM fungal colonization with *Populus tremuloides* over three soil depths (0–5, 5–10 and > 10 cm). Similarly, vertical segregation between AM and EM roots was found down to 35 cm soil depth in a tropical forest (Moyersoen *et al.*, 1998). They also found a negative relationship between AM and EM root colonization in the two top soil layers. The ability of *P. tremuloides* to form dual-mycorrhizal symbioses that occupy different soil depths may contribute to its wide geographic distribution, since a wider range of habitats, including primary successional sites or deep well-developed soils, could be used (Neville *et al.*, 2002). We suggest the mechanisms underlying a resulting switch in mycorrhizal status with soil depth could include, first, vertical distribution of soil niches promoting vertical segregation of mycorrhizal fungi and, second, EM fungi outcompeting AM fungi at the top soil layers since organic matter is abundant and P is not as limiting as in the deeper layers (Read, 1991).

5. Greater flexibility with soil nutrient availability through ecosystem development

There are few studies directly testing the effect of soil nutrient availability on root colonization patterns in dual-mycorrhizal plants. Here, we briefly discuss two recent studies conducted with soil from a well-established soil chronosequence in Western Australia that has a strong soil P availability gradient (Turner & Laliberté, 2015). First, the study of Albornoz *et al.* (2016) on a part of the gradient found a distinct decrease in AM root colonization in conjunction with a clear increase in EM root colonization with increasing soil age using two phylogenetically distant dual-mycorrhizal plants: *Acacia rostillefera* and *Melaleuca systena* (Fig. 4). The study of Teste & Laliberté (2019), which used the full range of soil ages, including the oldest most impoverished sands, did not find such strong evidence for mycorrhizal switching. Indeed, *M. systena* simply remained mostly EM along the strong nutrient-availability gradient, whereas *A. rostillefera* showed higher variation but a tendency to form mostly arbuscular mycorrhizas when natural levels of AM and EM inoculum were used. The novel finding from Teste & Laliberté (2019) was that, overall, switching to EM dominance was found only when plants were inoculated with soil-age-specific inoculum soil (Fig. 4). Specifically, *Acacia* had a strong switch to EM in the least impoverished soil, in terms of both N and P (i.e. c. 1000-yr-old soil), along this soil chronosequence (Teste & Laliberté, 2019). The switch to EM dominance in *Acacia* when grown in soil age-specific inoculum demonstrates how other factors are at play; in this case, EM propagule density and locally adapted mycorrhizal fungi can interact and influence whether arbuscular mycorrhizas or ectomycorrhizas are formed.

We suggest that the mechanisms underlying mycorrhizal switching due to soil nutrient availability involve the following: first, the ability of EM fungi to access organic P, which accumulates in older soil, via excretion of phosphatases; second, the ability of EM fungi to scavenge more effectively and at further distances from the host roots compared with AM fungi; and third, the host plant's ability to control the level of AM fungal colonization and/or arbuscule development depending on nutrient requirements (Wipf *et al.*, 2019).

6. Greater flexibility for other relevant soil properties: temperature, salinity, litter compounds

There are other important physicochemical soil properties that could promote mycorrhizal switching, but very few studies using dual-mycorrhizal plants exist. The few studies published highlight the interesting complexity that is present in ecosystems where dual-mycorrhizal plants occur (Fig. 5). In one example, McGee (1988) found a reduction of ectomycorrhizas at high soil temperatures, which resulted in a shift to AM dominance (Fig. 4). In another study, the development and functioning of AM fungi in *A. incana* were reduced at very low soil temperatures, whereas EM fungi were not affected (Kilpelainen *et al.*, 2016). The key result of Kilpelainen *et al.* (2016) shows greater ability of EM fungi, compared with AM fungi, to colonize roots via propagules after soil freezing. The authors concluded that this partly explains the predominance of EM plants in cold climates (Read, 1991).

As a second example of context dependence, Piotrowski *et al.* (2008) tested the effects of *Populus* litter and its components on the levels of fungal colonization of *Populus trichocarpa* roots. All compounds tested significantly reduced AM fungal colonization but had little effect on non-AM fungi (i.e. mostly EM fungi but
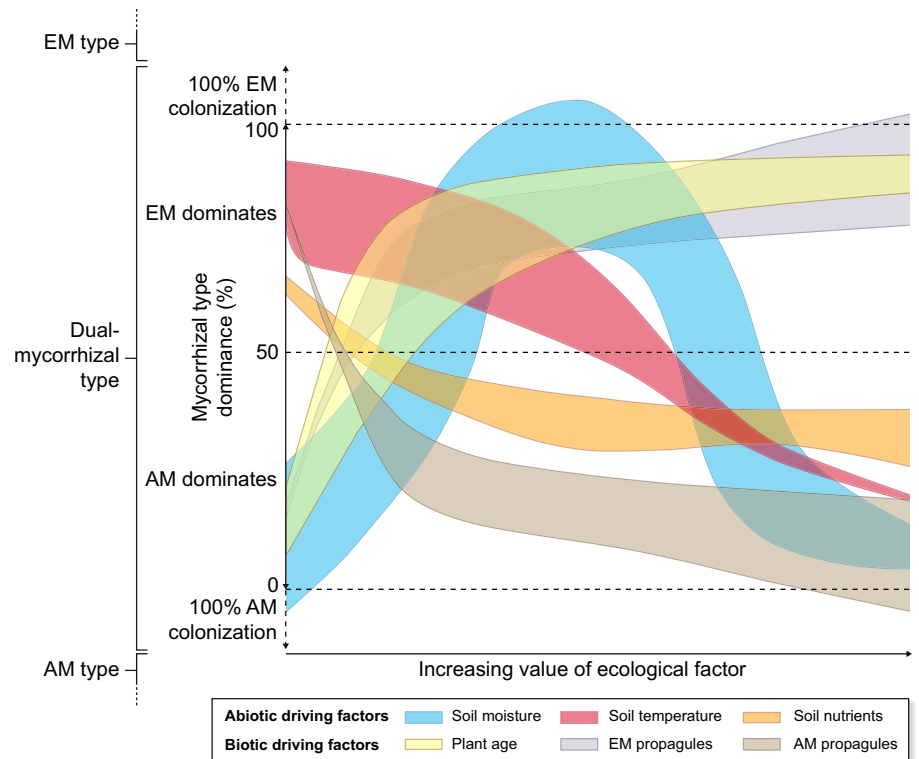
Fig. 5 Synthesis of factors that drive the dominance of one mycorrhizal type over another in dual-mycorrhizal plant species. The plot shows expected confidence bands that were derived from the mycorrhizal-type dominance response data presented in Fig. 4. Multiple factors are likely to interact. For example, dual-mycorrhizal *Eucalyptus* seedlings are not dominated by arbuscular mycorrhizas early in life when growing in severely impoverished and/or cold soil.

included other non-AM groups). They concluded that secondary compounds found in *Populus* litter would effectively give EM fungi another advantage at dominating this tree's roots in natural ecosystems.

Finally, experimental work with *S. repens* also points to strong context dependency in the levels of AM and EM colonization (van der Heijden & Vosatka, 1999; van der Heijden *et al.*, 1999). Under a soil pH of 4 and a low soil N : P ratio of 5.4, as well as at neutral soil pH and a high soil N : P ratio of 48.6, *S. repens* was completely dominated by ectomycorrhizas (van der Heijden & Kuyper, 2001). The other seven experimental growing conditions (e.g. soil pH of 5.5, soil N : P ratio of 16.2 and all other interactions) showed a more even proportion of AM and EM root colonization, although *S. repens* is overall considered more EM than AM (van der Heijden, 2001; van der Heijden & Kuyper, 2001). These three examples not only support the idea that dual-mycorrhizal status is highly context dependent, but also suggests that environmental factors are major drivers in shifts in the type of mycorrhiza formed by plant species capable of forming either AM or EM associations (Fig. 5).

7. Greater pathogen and pest protection

Both AM and EM symbioses can influence the interactions of plants with pathogens, herbivores and competitors (Meinhardt & Gehring, 2012; Cameron *et al.*, 2013; Gonthier *et al.*, 2019), so another hypothesis is that being capable of forming either type of mycorrhiza provides greater protection from pests and pathogens. A recent review by Laliberté *et al.* (2015) concluded that EM fungi were more effective than AM fungi in ameliorating pathogen damage to woody plants, particularly under P-limiting conditions. Further, AM seedlings planted under conspecific adults had more

root lesions than EM seedlings planted under conspecifics (Bennett *et al.*, 2017).

We could not find studies directly testing dual-mycorrhizal plant responses to root pathogens; however, the study of Teste *et al.* (2017), which had eight dual-mycorrhizal plant species in a plant–soil feedback experiment, supports the hypothesis that hosting AM and EM fungi simultaneously may bolster protection against soil pathogens. Poor growth in conspecific soil, compared with nonconspecific soil, is often associated with species-specific root pathogens and renders negative plant–soil feedback (Brinkman *et al.*, 2010). Yet, this scenario was uncommon among dual-mycorrhizal plant species grown in nutrient-impoverished soil, since there was only one of the eight dual-mycorrhizal species that showed negative feedback (Teste *et al.*, 2017). Finally, when examining the effects of damage from three different insect herbivores, either separate AM or EM colonization of *E. urophylla* increased initial damage caused by geometrid larvae, whereas only EM colonization reduced damage by *Anomala cupripes* (Coleoptera) adults and leaf folding *Strepsicrates* spp. (Lepidoptera) larvae (Gange *et al.*, 2005). Clearly, more research is needed on the relative effects of AM and EM associations, and of dual colonization, on multitrophic interactions.

VII. A proposed classification of dual-mycorrhizal subtypes

To aid diagnosing dual-mycorrhizal status and context-dependent subtypes, we developed a simple decision tree (Fig. 2). The classification of dual-mycorrhizal plants into subtypes and benefit types is useful to contextualize the ecological mechanisms leading to dual-mycorrhizal status. Obviously, some plant species will possess

more than one subtype; as such, our subtypes are not mutually exclusive, and in many cases are mutually necessary. Whereas some plants are consistently dual mycorrhizal, others can have entirely AM or EM colonization. These 'switch-hitters' can be divided into spatially context dependent and temporally context dependent.

1. Temporally dependent duals

Temporally dependent dual-mycorrhizal species form one type of mycorrhiza when young and then become dominated by the other type of mycorrhiza as the seedling matures. Plant species that are dual-mycorrhizal in order to facilitate seedling establishment or as an insurance policy (Sections VI.1 and 2) are likely to have temporally dependent dual mycorrhizal status. Australian *Eucalyptus* is the archetypal genus that represent temporally dependent dual-mycorrhizal plant species. They typically get colonized rapidly by AM fungi in the first few weeks of root development and generally transition into an EM plant (Lapeyrie & Chilvers, 1985; Chen *et al.*, 2000; dos Santos *et al.*, 2001). This temporal replacement or succession from AM status to EM status with plant age is characteristic, and when *Eucalyptus* individuals are over a year old they are often observed to be completely EM (Bellei *et al.*, 1992). However, recent studies show that even mature *Eucalyptus* can still retain a considerable level of AM colonization (Adjoud-Sadadou & Halli-Hargas, 2000; Adams *et al.*, 2006). Comprehensive mycorrhizal colonization surveys in *Eucalyptus* plantations also show that dual status vs complete EM status in this genus is context dependent (Adams *et al.*, 2006; Chen *et al.*, 2007). As such, mature *Eucalyptus* trees can also be considered spatially dependent duals (see following subsection). In other plant genera, such as *Populus*, there may also be mycorrhizal switching driven by ontogenetic development; for example, the development of sufficient root-storage mass to provide local reserves of C (starch and sugars) to EM fungi and fine roots (Dickmann *et al.*, 2001).

2. Spatially dependent duals

Species that are dual mycorrhizal in order to increase niche breadth are likely to have spatially dependent dual mycorrhizal status. As described in Sections VI.3–5, mycorrhizal type can vary in the same species depending on factors such as flooding or drought, or soil depth, nutrient status, or development. *Populus* and *Salix* serve as good model genera of spatially dependent duals since they respond strongly to differences in soil moisture levels. In extreme cases, spatially dual plant species can have populations that are entirely AM or EM, because either the biotic or abiotic environments of the populations differ substantially, or the populations differ in some heritable characteristics favoring colonization by AM or EM fungi.

3. Benefit types

Confirmed beneficial dual-mycorrhizal species (Fig. 2) show positive responses to hosting both AM and EM fungi. Greater survival, growth, or nutrient uptake, compared with the single-type states (i.e. AM only and EM only), provides sufficient evidence to designate a species as confirmed beneficial dual-mycorrhizal. We

acknowledge, however, that fully functioning AM or EM symbioses can exist without enhancement of plant growth or nutrient status. Conversely, arbuscular mycorrhizas, ectomycorrhizas, or dual mycorrhizas may be beneficial to plant fitness without faster growth or high nutrient contents. Therefore, nonconfirmed beneficial dual-mycorrhizal plant species (Fig. 2) may still derive a benefit from a dual association; we may just not be able to measure it with current methodology.

Although we consider dual-mycorrhizal status a plant trait, we envision other subtypes based on a myco-centric viewpoint (Box 2) if we quantify growth or fitness responses of the mycorrhizal fungi. For instance, splitting the confirmed beneficial dual-mycorrhizal subtype into 'confirmed beneficial to the host plant' and 'confirmed beneficial to the mycorrhizal fungus' will be ecologically informative. For example, we hypothesize that sustained intraradical hyphal growth by AM fungi may be supported or perhaps even facilitated by neighboring EM fungi in dual-mycorrhizal *Eucalyptus* trees, as a result of long-distance N scavenging and subsequent indirect sharing by EM fungi. Obviously, an individual AM or EM fungus cannot form both types of mycorrhizas, yet the effect of living alongside another mycorrhizal type can involve antagonism (Chilvers *et al.*, 1987; Lodge & Wentworth, 1990; Moyersoen *et al.*, 1998) or coexistence regardless of carbohydrate availability (Saravesi *et al.*, 2011), or niche partitioning of the soil with depth (Neville *et al.*, 2002) and/or between nutrient patches (van der Heijden & Kuyper, 2001).

VIII. Future directions

Our current knowledge of dual-mycorrhizal status and its ecological relevance, as demonstrated by this first complete synthesis, remains in its infancy. We summarize key research questions that surfaced from our synthesis (Table 1). Dual-mycorrhizal plant species have unique potential to serve as model plant systems to test hypotheses about the role of abiotic and biotic factors on dual-colonization levels by AM and EM fungi without confounding host species effects (Table 1). Regardless of the research question, we emphasize the need to minimize errors through careful host-plant identification and avoiding dying and dead roots, while also remaining open to 'atypical' colonization events and promiscuous fungi (Box 2). For example, most researchers of EM plants do not routinely stain roots and test for the presence of AM colonization. We suggest that checking for AM colonization in 'typical' EM hosts should be considered, particularly for studies considering early stages of seedling establishment, extreme habitats' or plant succession. A further benefit of clearing and staining EM roots is that dark-septate endophytes and sometimes oomycete colonization can be simultaneously quantified. However, accurately quantifying dual colonization requires approaches that standardize infection rates per root length or per root segment available for colonization. Therefore, the widely used gridline intersect method (Giovannetti & Mosse, 1980) adjusted for EM root segments, or adjusting commonly used EM methodology for AM root segments, should be used with dual-mycorrhizal plant species.

Identifying mycorrhizal fungi associated with roots of plants using high-throughput DNA sequencing can certainly have

Table 1 Future research questions involving the use of dual-mycorrhizal species as model plant–fungal systems to accelerate our understanding of the role of mycorrhizal symbioses in ecosystems undergoing rapid change.

Trait ecology	(1) Are all ectomycorrhizal (EM) plant species colonized by arbuscular mycorrhizal (AM) fungi during early-life stages (i.e. seedling stage)? (2) Can *Cenococcum* spp. form root symbioses with all AM plants? (3) Are there other categories of dual-mycorrhizal plants based on the constant dominance of one mycorrhiza type over the other for noncontext-dependent duals?
Ecophysiology of root symbioses	(1) Does the amount of carbon (C) partitioned to a mycorrhizal root system, accounting for both respiration and tissue costs, differ between AM or EM symbioses? If so, do differences in sink strength between EM and AM symbioses drive differences in compensatory photosynthetic rates? This should be evaluated under different environmental stresses. (2) When associated with the same plant species, are nutrient-acquisition strategies considered typical for each mycorrhizal type retained? For example, how do activities of extracellular enzymes or release of organic acids differ between AM and EM roots? What differences exist, if any, in the ability of EM and AM hyphae to withdraw nutrients from organic matter or secondary minerals? (3) Considering any extra C partitioned to the mycorrhizal root system, how does the efficiency of nitrogen and phosphorus uptake compare between the two types of mycorrhizas? (4) Does drought tolerance differ between AM and EM plants of the same species? (5) Do EM hyphae proliferate more than AM hyphae in nutrient-rich hotspots, when associated with the same plant species? (6) When associated with the same plant species, how do EM and AM hyphae interact with other soil microbes?
Evolution of ectomycorrhizas	(1) Can dual-mycorrhizal plants better explain the evolutionary mechanisms behind the global rise and dominance of EM vegetation (Dickie *et al.*, 2014) without confounding effects of plant host species? (2) Is the evolution of EM status gradual, with dual-mycorrhizal status being an intermediate state (Brundrett, 2002)?
Fungal ecology	(1) Do EM fungi always outcompete AM fungi when inoculum is not limiting? Such EM–AM competition experiments with dual-mycorrhizal plants could be addressed with the use of stable isotope probing, real-time PCR, or high-throughput-based randomization analyses (Yamamoto *et al.*, 2014), and split-root or hyphal exclusion experiments. (2) Are there any quantifiable benefits to the mycorrhizal fungi of associating with a simultaneously dual-mycorrhizal plant that would lead to a stable symbiosis?
Plant ecology	Are dual-mycorrhizal plant species less susceptible to root pathogen damage or mortality compared with their single-type counterparts (i.e. AM only or EM only)?
Ecological restoration	(1) Is plant establishment on adverse sites more successful when dual-mycorrhizal inoculations of dual-mycorrhizal species used? (2) Can dual inoculation improve seedling survival of a typical EM plant (e.g. Pinaceae) compared with single type inoculation?

advantages, yet these newer techniques are not currently robust enough to be used on their own to determine dual-mycorrhizal status. These molecular techniques cannot distinguish between superficial colonization of roots and genuine mycorrhizal colonization with key structures. As such, we advocate that dual-mycorrhizal status, and single mycorrhizal status for that matter, should be based on the observations of the key structures (arbuscules or coils for AM status; and a Hartig net or similar structures for EM status) using direct viewing methods (e.g. microscopes, high-resolution digital cameras) or indirectly (e.g. X-ray micro-computed tomography). However, high-throughput sequencing could be used as an early detection technique to screen for possible candidates with genuine dual colonization by both AM and EM before applying any viewing methodology.

IX. Conclusions

Dual-mycorrhizal plants are more common than previously thought. Although most are represented by woody plant taxa (i.e. shrubs and trees), 16% are herbaceous species. In this review we aimed to demonstrate that dual-mycorrhizal plants can serve as powerful plant–fungal model systems to experimentally distinguish the roles and net benefits of arbuscular mycorrhizas and ectomycorrhizas. We also aimed to showcase the strong 'mycorrhizal switching' that occurs in dual-mycorrhizal plants and which abiotic and biotic factors are known to drive such shifts in the dominance of arbuscular mycorrhizas or ectomycorrhizas. C cost-to-benefit thresholds of hosting both AM and EM fungi are central

to adequately determining whether dual-mycorrhizal status is a stable state throughout the life of plant species. Though fitness comparisons cannot be made for long-lived plants such as trees, the short-term benefits to young plants of hosting both arbuscular mycorrhizas and ectomycorrhizas remain relevant for efforts to restore harsh sites, where seedling establishment represents the most important step.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Global map of the occurrences of confirmed dual-mycorrhizal plant species listed in Table S2.

Fig. S2 Effects of arbuscular (AM) and ectomycorrhizal (EM) fungal inoculations on the growth of plants in genera containing confirmed dual-mycorrhizal species listed in Table S2 and also found in the MycoDB.

Fig. S3 Effects of arbuscular (AM) and ectomycorrhizal (EM) fungal inoculations on the growth of plant species from plant genera containing confirmed dual-mycorrhizal plant species (Table S2) found in the MycoDB.

Fig. S4 Effects of arbuscular (AM) and ectomycorrhizal (EM) fungal inoculations on the growth of confirmed dual-mycorrhizal species listed in Table S2 that are also found in the MycoDB.

Notes S1 Reference list for Table S1.

Table S1 Lists of all plant families and genera with records indicating both arbuscular and ectomycorrhizal fungal colonization of root systems.

Table S2 Dual-mycorrhizal plants with confirmed occurrence of key mycorrhizal structures.

Table S3 Summary of studies quantifying the responses of confirmed dual-mycorrhizal plants to controlled dual- and single-inoculations.

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