Fine root endophytes under scrutiny: A review of the literature on arbuscule-producing fungi recently suggested to belong to the Mucoromycotina

Suzanne Orchard¹, Rachel J. Standish², Ian A. Dickie³, Michael Renton^{1,4}, Christopher Walker^{1,5}, Derrick Moot⁶, Megan H. Ryan¹

¹UWA School of Agriculture and Environment, and Institute of Agriculture, The University of Western Australia, 35 Stirling Hwy, Crawley (Perth), WA 6009, Australia

²School of Veterinary & Life Sciences, Murdoch University, 90 South Street, Murdoch, WA 6150, Australia

³Bio-Protection Research Centre, Lincoln University, Lincoln, 7647, New Zealand

⁴UWA School of Biological Sciences, The University of Western Australia, 35 Stirling Hwy, Crawley (Perth), WA 6009, Australia

⁵Royal Botanic Garden, 20A Inverleith Row, Edinburgh EH3 5LR, UK

⁶Department of Agricultural Sciences, Lincoln University, Lincoln, 7647, New Zealand

Author for correspondence

Ms Suzanne Orchard

School of Agriculture and Environment

The University of Western Australia, Crawley (Perth), WA 6009

Ph +61 8 6488 2208, Fax +61 8 6488 1108

Email: suzanne.orchard@research.uwa.edu.au

Acknowledgements

This research formed part of a project funded by an Australian Government Postgraduate Award, a Meat and Livestock Australia Postgraduate Scholarship and a Henry Schapper Postgraduate Research Scholarship to SO, and we gratefully acknowledge this funding. MHR was funded by ARC Future Fellowship FT140100103.

Abstract

Fine root endophytes (FRE) are arbuscule-forming fungi presently considered as a single species—*Glomus tenue* in the Glomeromycota (Glomeromycotina)—but probably belong within the Mucoromycotina. Thus, FRE are the only known arbuscule-forming fungi not within the arbuscular mycorrhizal fungi (AMF; Glomeromycotina) as currently understood. Phylogenetic differences between FRE and AMF could reflect ecological differences.

To synthesize current ecological knowledge, we reviewed the literature on FRE and identified 108 papers that noted the presence of FRE and, in some, the colonization levels for FRE or AMF (or both). We categorized these records by geographic region, host-plant family and environment (Agriculture, Moderate-natural, Low-temperature, High-altitude and Other) and determined their influence on the percentage of root length colonized by FRE in a meta-analysis.

We found FRE are globally distributed, with many observations from Poaceae, perhaps due to grasses being widely distributed. In agricultural environments, colonization by FRE often equalled or exceeded that of AMF, particularly in Australasia. In Moderate-natural and High-altitude environments, average colonization by FRE (~10%) was lower than that of AMF (~35%), whereas in Low-temperature environments colonization was similar (~20%). Several studies suggested that FRE can enhance host-plant phosphorus uptake and growth, and may be more resilient than AMF to environmental stress in some host plants.

Further research is required on the functioning of FRE in relation to the environment, host plant and co-occurring AMF and, in particular, to examine whether FRE are important for plant growth in stressful environments. Targeted molecular primers are urgently needed for further research on FRE.

Keywords: arbuscular mycorrhizal fungi; fine endophyte; *Glomus tenue*; *Glomus tenuis*; Mucoromycotina; *Rhizophagus tenuis*

Introduction

The root-colonizing fungi known as ‘fine root endophyte(s)’ (FRE) are presently placed, under the name *Glomus tenue*, within the phylum Glomeromycota *sensu* Walker & Schüßler, which has generally been considered to encompass all arbuscular mycorrhizal fungi (AMF; Schüßler et al. 2001). Recently, however, it has been proposed that the phylum name be replaced with Mucoromycota (Spatafora et al. 2016), with the inclusion of many former ‘zygomycetes’. Higher classifications are not subject to the same rules as those for family and below (McNeill et al. 2011), so it is up to individual researchers to decide if they wish to follow this classification. For the purpose of this review, the phylum-level classification can be ignored, but FRE and AMF are treated at the same subphylum level (Mucoromycotina and Glomeromycotina, respectively). This separation of FRE among the Mucoromycotina, rather than the Glomeromycotina, is consistent with the evidence of Orchard et al. (2017a) who used a combination of careful laboratory technique—including serial dilution and sieving, to enhance colonization by FRE relative to that of AMF—and molecular markers, to show that FRE are phylogenetically aligned with the subphylum Mucoromycotina, not the phylum Glomeromycota. This unexpected finding suggests that FRE are the only arbuscule-producing fungi outside of the Glomeromycota, as currently understood. If the ecology of FRE is shown to diverge from that of AMF, then there will be important implications for the mycorrhizal research community, as the two groups may routinely require separate and deliberate investigation.

To explore the current state of knowledge on FRE, we undertook a review of all published literature on FRE with the aim of identifying any key differences to AMF. We first describe the morphology of FRE and the history of research on FRE. This step was important given the influence of research methodology on findings (for both AMF and FRE). We then investigated the factors that influenced the level of colonization by FRE in isolation and relative to the level of colonization by AMF, using a quantitative meta-analysis of the published literature supported by case studies. The impact of FRE on the host plant is then discussed. We finish by highlighting research gaps and suggesting directions for future research.

1. Literature search

On 1 March 2016, searches were completed with the Web of Science

(www.webofknowledge.com) and Google Scholar (www.scholar.google.com) for papers on FRE published between 1963 and 2015 with the following search criteria: ‘fine endophyte’ OR ‘*Glomus tenuis*’ OR ‘*Glomus tenue*’ OR ‘*Rhizophagus tenuis*’. This search was then refined to include only papers in which the description of FRE fitted with the distinct morphological features described in Section 2. The search uncovered 116 papers, but only 108 papers, that we were confident that accurately identified FRE, were included for further analysis. While we tried to identify all papers containing observations of FRE, some may have been missed due to the ambiguous nature of the nomenclature and descriptions of FRE (see Section 3). A full list of the 108 papers is provided in Table 1.

2. Morphology of the colonization and spores of fine root endophytes

The morphology of FRE is distinctive when stained within plant roots, rendering it distinguishable from ‘coarse’ AMF (herein AMF; Fig. 1). While FRE may stain darker than many forms of AMF (Daft and Nicolson 1974; Rabatin 1979; Gianinazzi-Pearson et al. 1981), because of their fine nature, their features are best viewed at a magnification of $\geq 100\times$. In the epidermis and outer cortex of the root, FRE produce a fan-shaped or palmate entry point, followed by diverging fine hyphae ($< 2\ \mu\text{m}$ diameter) branching both intra- and inter-cellularly to spread within the root cortex (Fig. 1a) (Greenall 1963; Gianinazzi-Pearson et al. 1981; Abbott 1982). Multiple hyphae of FRE may group within inter- or intra-cellular spaces, sometimes forming ‘ropes’ of hyphae (Nicolson and Schenck 1979; Greenall 1963; Gianinazzi-Pearson et al. 1981). The aseptate hyphae produce terminal and intercalary swellings, approx. 5–10 μm in diameter, which have been referred to as vesicles (Fig. 1a, b) (Thippayarugs et al. 1999). Their function is unknown, and they are much smaller than the vesicles, or intraradical spores, of AMF. For instance, the intraradical spores (referred to as vesicles by Plenchette and Strullu (2003)) of the arbuscular mycorrhizal (AM) fungus *Rhizophagus intraradices* are $93\text{--}119 \times 112\text{--}131\ \mu\text{m}$ (Schenck and Smith 1982) (Fig. 1e). It should be noted that the literature on ‘vesicles’ of AMF is confusing, and makes it difficult to ascertain whether or not there is a significant difference between spores and vesicles of AMF. Mosse and Bowen (1968), following Schrader (1958), used the terms interchangeably. As yet, there seems to be no evidence that vesicles of FRE and AMF are either analogous to, or homologous with, each other.

Fine root endophytes form arbuscules in the mid to inner cortex of the root (Gianinazzi-Pearson et al. 1981) (Fig 1a, b). When colonies of FRE and AMF intermingle, which is often the case, their arbuscules are difficult to discriminate, although the arbuscule ‘trunk’ of the former may be less prominent (Fig. 1d, f) (Gianinazzi-Pearson et al. 1981). Spores of FRE (sensu Hall 1977) are reported to be $\leq 20\ \mu\text{m}$ diameter and colourless when young (Fig. 1c), turning dark brown with age (Hall 1977; Brundrett et al. 1996), attributes which perhaps have caused them to be rarely observed. Indeed, in some studies, FRE were reported to be non-sporulating (Baylis 1969). Hence, due to the fine morphology and often colourless spores, both requiring special, deliberate attention, FRE may have been overlooked, or not characterized separately from AMF, in many studies of mycorrhiza.

The species diversity of FRE is unknown. Close observation of the roots of *Trifolium subterraneum*, where AM fungal colonization was significantly reduced, Thippayarugs et al. (1999) identified five morphological types of FRE. It is, therefore, possible that FRE consist of several species (Thippayarugs et al. 1999; Orchard et al. 2017a); hence, FRE are referred to as a species complex in this review.

3. Evolution of the nomenclature of, and research on, fine root endophytes

The first published description of FRE, as *Rhizophagus tenuis*, was by Greenall (1963) from the roots of a woody plant native to New Zealand (Fig. 2). Since then, as long as visual observation was the key method to assess mycorrhizas and FRE were deliberately examined, their dark staining and distinct morphology made it relatively easy to compare colonization levels with ‘other’ or ‘coarse’ AMF (Crush 1975). Such comparison was the hallmark of plant growth experiments and studies investigating competitive interactions between FRE and AMF in the 1970s and 1980s (Crush 1973a; Abbott and Robson 1978, 1982). Over this period, descriptions of FRE in the literature were inconsistent, some authors included them as AMF, using the term ‘fine endophyte’ or the names *G. tenuis* or *G. tenue*, while others did not include them as AMF (Fig. 2). However, by the 1990s, research interest in FRE was clearly not keeping pace with that of AMF, with FRE only mentioned in 1.7% of the AM research literature in the 1990s and <0.9% since 2000, even when including papers which simply mentioned their presence (Fig. 2; Table 2).

This shift in research focus away from FRE was driven, in part, by the difficulty of producing root colonization by FRE without the presence of AMF (often referred to as a pot culture or, usually erroneously, an isolate). The tiny spores of FRE (Fig. 1c) cannot easily be separated from soils or roots; therefore, no spore-based pot cultures have ever been produced. In 1963, Greenall attempted to grow FRE on agar as a pure culture, but without success. Occasionally, FRE have survived soil sterilization or pasteurization processes and contaminated non-AM fungal control pots (e.g. Arines et al. 1988). However, several studies have used soil treatments, such as serial soil dilutions, to increase colonization by FRE and reduce colonization by AMF (e.g. Wilson and Trinick; 1983b; McGee 1989; Thippayarugs et al. 1999; Orchard et al. 2017a). For some time, a pot culture of FRE was held within the International Bank for the Glomeromycota (IBG), Dijon, France (IBG ref. LPA11 '*Glomus tenue*', source: New Zealand), however, it is unknown if this culture is still viable (D. Redecker, IBG collection curator, pers. comm. November 2016).

Of the 108 papers published on FRE between 1963 and 2015, only 17 used an established pot culture of FRE of identified origin (Table 1). As these cultures were often used for consecutive experiments and exchanged among research groups, it appears that FRE may have only been established in pot culture a few times in a few places, including New Zealand (Crush 1973a), Australia (Abbott 1982) and South Africa (Turnau et al. 2013). Experimentation with AMF generally followed similar protocols with regard to pot cultures—since AMF are easier to isolate and culture than FRE, this likely contributed to the greater number of papers on AMF (Table 2). From the 1990s onwards, AM fungal research shifted towards species reported to be more beneficial for plant growth, and for which spores and single species pot cultures were available (e.g. *Funneliformis mosseae* and *Rhizophagus irregularis*) (Table 2).

In addition to the challenge of producing a pot culture of FRE, there were further hindrances to research on FRE, such as i) the general taxonomic uncertainty of the identity of FRE (Fig. 2), ii) FRE appear to be more sensitive to host-plant changes and plant-sample storage methods than AMF, which may reduce the detection of FRE (Orchard et al. 2017b), and iii) the reduced reliance on light microscopy to assess AMF due to advances in molecular methods combined with the development of primers that target AMF but, in most cases, do not target FRE (Orchard et al. 2017a). This last point means that molecular studies of AM fungal diversity do not include FRE. Thus, the abundance and diversity of root colonizing, arbuscule-forming fungi may be

much higher than that suggested by molecular studies alone.

4. Meta-analysis methods

A meta-analysis was undertaken using data extracted from the papers identified in the literature review. In 69 of the 108 papers, colonization by FRE and AMF was not separately quantified, and many papers reported only the presence or absence of FRE rather than quantitative colonization data. Furthermore, the most commonly used method to quantify mycorrhizal colonization (the Magnified Gridline Intersect method) was not published until McGonigle et al. (1990). Before this time, the methods for quantifying colonization were inconsistent, and thus data are difficult to compare robustly among studies, and the data from some studies were not compatible for the meta-analysis (Table 1). Only 21 papers had FRE as the focus of the research.

We extracted two sets of data from the 108 papers. First, from all 108 publications, we extracted all observations of FRE both qualitative and quantitative; these data were expressed as presence or absence. Second, from the subset of papers where colonization was quantified, we extracted all data on colonization level by AMF and FRE. There were often multiple data points extracted from a paper due to the presence of more than one host-plant species. For each data point extracted, we noted the following (where information was available):

- 1) Geographic region (Australasia, USA-Canada (including the Canadian Arctic), Europe, South America, Asia, Africa, and sub-Antarctica, *i.e.* islands off the coast of Antarctica)
- 2) Host-plant family (Poaceae, Fabaceae, etc.)
- 3) Environmental category based on the dominant environment in the study:
 - Agriculture — plant or soil samples taken from agricultural fields, or focused on agricultural plant species, which had a high level of anthropogenic disturbance. These were often glasshouse experiments, and this has been included as a factor within the meta-analysis
 - Moderate-natural — plant samples collected from an environment with relatively low anthropogenic disturbance and not considered subject to extreme conditions
 - High-altitude (extreme) — plant samples collected from alpine regions ≥ 1000 m.

Samples collected from <1000 m were placed into the Moderate-natural category

- Low-temperature (extreme) — plant samples collected from the sub-Arctic or sub-Antarctica and, therefore, subject to extremely low temperatures for long durations
- Other — sampling or experimental conditions falling outside the criteria for the previous categories (for example, pH or CO₂ gradients).

The choice of these environment categories was informed by observations that colonization by FRE increased, compared to that of AMF, at higher elevations and lower temperatures (Crush 1973b; Read and Haselwandter 1981; Newsham et al. 2009), and reports of high colonization in agricultural environments and crops (Crush 1973a; Abbott and Robson 1978, 1982).

4) Glasshouse (pot) or field experiments

5) Whether or not an isolate of FRE was used; studies where inoculation with FRE occurred were excluded from the meta-analyses.

Table 1 lists all papers and how each was allocated to the above categories. The observation dataset (FRE presence/absence) was simply analyzed by summarizing the number of data points relative to the factors, or combinations of them, listed above, and the results are discussed throughout the review. The quantitative colonization level dataset was analyzed with a statistical meta-analysis following the methods used by Hoeksema et al. (2010). All statistical analyses for the meta-analysis of the quantitative colonization level dataset involved fitting mixed-effects models in R, using the base, nlme and multcomp packages (Hothorn et al. 2008; R Core Team 2014; Pinheiro et al. 2014). We considered two dependent variables: i) the percentage of root length colonized by FRE, and ii) the proportion of total colonization that consisted of FRE. The latter we termed the ‘FRE colonization ratio’. It was calculated using the formula:

$$[\text{Root length colonized by FRE (\%)} / (\text{Root length colonized by FRE (\%)} + \text{Root length colonized by AMF (\%)})] \times 100$$

We included region, plant family, environment and glasshouse (whether the data came from a glasshouse/pot study or not) as fixed effect explanatory variables. We also included a random effect for ‘study’ which was included to avoid pseudo-replication by accounting for possible within-study correlations. We weighted each data point by the number of replicates in each

study. Using log ratios for the statistical analysis ensured that the model assumptions regarding normality and homoscedasticity of residuals were met. The significance of model terms was tested using the ‘ANOVA’ function and confirmed using marginal likelihood ratio tests and AIC comparison, which all agreed in all cases. Where models indicated that global effects were significant, Tukey tests and subset ANOVA were used for pairwise comparisons. Statistical significance was determined at $P=0.05$.

5. Distribution of fine root endophytes

Observations of colonization by FRE come from studies undertaken on all continents, including islands off the coast of Antarctica (Fig. 3; Table 1). However, from 1973 to 1999, research on FRE from Australasia accounted for >50% of published papers despite, globally, an intense research focus on AMF (Table 1). More recently, *i.e.* 2012–2016, there has been an increase in the number of studies on FRE from USA-Canada and, in particular, Europe (Table 1; Fig. 3). Undoubtedly, the number of observations of FRE in a particular region is influenced by an interest in FRE by particular researchers or research groups (e.g. Abbott in Australia, Crush and Powell in New Zealand, and the Basinger-Kaminskyj research group in Canada). Thus, regions with no reports of FRE have likely never been deliberately sampled for FRE; it seems unlikely that FRE are absent.

In the subset of papers that quantified colonization, instances of high colonization by FRE were reported from all regions, although AMF were usually dominant. The meta-analysis showed that for field-grown plants, median colonization of FRE by region was around 20% of root length, and the median FRE colonization ratio was <20% for Australasia and Europe but was significantly higher for USA-Canada at ~50% (Figs. 4 and 5, $P<0.001$). For USA-Canada, much of the data were from an extreme environment (*i.e.* low temperature) and the reports of high colonization by FRE and high FRE colonization ratios were mostly from the Arctic (e.g. Olsson et al. 2004; Ormsby et al. 2007; Hodson et al. 2009; Walker et al. 2010). Even so, the limited data for less extreme environments from the same region showed that the level of colonization by FRE in Equisetaceae in the Canadian prairies did not differ from that of Equisetaceae in the Canadian Arctic (Hodson et al. 2009; Section 7.3). Further, there were instances from Australasia where colonization by FRE equalled or exceeded that of AMF, and colonization by FRE was >50% of root length; these included pastures in Western Australia (Abbott and Robson 1982; Orchard et al. 2016) and New Zealand (Crush 1973b, 1975), crops

in New South Wales (Ryan and Kirkegaard 2012), and native woodlands in New Zealand (Cooper 1976).

Overall, our review demonstrates that AMF generally dominate the community of arbuscule-forming root fungi in the field. However, it is also apparent that FRE, like AMF, are globally distributed with instances of high colonization reported in all regions. While the meta-analysis suggested that both the level of colonization by FRE and the FRE colonization ratio may be higher in USA-Canada, this could be due to bias towards extreme environments in the data from that region. This result is discussed further in Section 7.2.

6. Plant families that host fine root endophytes

Observations of colonization by FRE came from 53 plant families, with the highest number for any family being for the Poaceae ($n=155$ observations of 527). Almost 45% of Poaceae observations were from Australasia, and many of these were of New Zealand grasses (*e.g.* *Dactylis glomerata*, *Lolium perenne*, *Poa colensoi* and *Festuca novae-zelandiae*). The majority of observations in Poaceae were from Moderate-natural environments ($n=75$ observations of 155), followed by Agriculture ($n=40$). For instance, surveys from Moderate-natural environments, such as those by Read and Haselwandter (1981) and Schmidt et al. (2008), examined numerous plant species and included reports of colonization by FRE from many Poaceae species. To examine whether the prevalence of Poaceae represented a preference by FRE for this family or a sampling bias, we recorded the relative occurrence of Poaceae from the 13 studies that sampled >10 plant species. These studies included 333 sampled plants, of which 25% were Poaceae. As Poaceae is the fifth largest plant family in terms of species number, exceeded by both Fabaceae and Asteraceae, it appears that a disproportionately high number of samples did originate from Poaceae. This is perhaps due to i) Poaceae being relatively easy to sample (herbaceous and often commonly occurring), ii) grassland-type ecosystems being more often studied, as opposed to woodlands and other systems, iii) Poaceae species being used for agricultural crops, and/or iv) Poaceae being ubiquitous.

In the subset of papers that quantified colonization that was used in the meta-analysis, colonization ranges for FRE were mostly similar across the top eight observed plant families (Fig. 4), although, the observation number was low for most families other than Poaceae and

Fabaceae. The meta-analysis found no significant effect of family on the level of colonization by FRE, but there was a significant effect of family on the FRE colonization ratio, largely due to a high ratio for the Equisetaceae (Figs. 4 and 5, $P < 0.001$). This family is represented by a single genus, *Equisetum*, an ancient plant genus with ancestral links to Devonian plant groups (Hodson et al. 2009). Colonization of *Equisetum* by both FRE and AMF has been reported, but overall the fungal associations of these plants are little understood (Fernandez et al. 2008; Hodson et al. 2009). We also note that there are reports of colonization by FRE from families which are normally considered to be non- or rarely mycorrhizal (e.g. Caryophyllaceae, Juncaceae) (Brundrett 2009). However, these were few (≤ 7), with colonization of $\leq 10\%$.

In summary, FRE colonize a wide range of host plants and, therefore, appear similar to AMF in this regard (Brundrett 2009). There is some suggestion of host-plant preference, with high colonization by FRE relative to that of AMF in the Equisetaceae.

7. Effect of environment on colonization by fine root endophytes

The meta-analysis of the subset of papers that quantified colonization demonstrated that colonization by FRE varied among the five environmental categories we designated, with Agriculture, Low-temperature and Other being higher than High-altitude and Moderate-natural (Fig. 4, $P < 0.001$). However, the meta-analysis found no statistically significant impact of environment on the FRE colonization ratio (Fig. 5, $P = 0.12$). Below, we explore these findings in more detail with reference to three environments.

7.1 Agricultural environments

Observations of colonization by FRE for agricultural plants and in agricultural environments come from around the globe (Table 1). The colonization levels by FRE in agricultural environments ranged from 5–86% ($n = 68$). The majority of observations of colonization by FRE in agricultural environments originated from Australasia ($n = 75$ of 90). Therefore, we examined records for Australia and New Zealand in more detail.

Of the observations of colonization by FRE within Australian agricultural systems, most were from Western Australia (13 studies) for *Trifolium subterraneum*, the predominant (exotic) annual pasture legume in southern temperate Australia (Nichols et al. 2012). In these studies,

colonization by FRE ranged from 5–79% of root length ($n=36$). An excerpt from these data for 20 soils grown in a glasshouse pot experiment is shown in Fig. 6 and demonstrates that although AMF are generally dominant, FRE are a significant presence, being found in the roots of plants grown in every soil and with colonization levels often >20% of root length. Moreover, in 20% of the soils, FRE were dominant relative to AMF and, importantly, colonization levels by FRE and AMF did not vary in a similar manner among soils (Fig. 6a). In the one agronomic crop rotation experiment from Australia where FRE were distinguished from AMF, colonization by FRE was high for both *Pisum sativum* at up to ~40% of root length and *Triticum aestivum* at up to 60% of root length (Ryan and Kirkegaard 2012; Fig. 6b). Further, for *T. aestivum*, FRE and AMF responded differently to agronomic treatments with colonization by FRE less affected by the previous crop (perhaps due to higher inoculum levels in soil) but reduced to a greater extent by the addition of P fertilizer. Interestingly, a recent review failed to demonstrate an agronomically significant role for AMF in the growth and yield of field crops of southern Australia (Ryan and Kirkegaard 2012). Perhaps high levels of colonization by FRE, unrecognized because of being included mostly within the total AM fungal assessment data, influenced this outcome.

In New Zealand, colonization by FRE in agricultural environments ranged from 9–85% of root length ($n=22$) and these studies were dominated by grasses. However, recent sampling of *T. subterraneum* in New Zealand pastures (not included in our meta-analysis) found that FRE often colonized 40–50% of root length, a level of colonization generally similar to that of AMF (Fig. 7). Further, at ~10% of the sites, FRE were dominant relative to AMF (Fig. 7). Taken together, the data presented in Figs. 6 and 7 suggest that FRE and AMF are a substantial component of the arbuscule-forming fungal community in the agricultural environments of southern Australia and New Zealand.

The meta-analysis suggests that both total colonization by FRE and the abundance of FRE relative to that of AMF are strongly favored in glasshouse pot experiments compared with field conditions (Figs. 4 and 5, $P<0.001$ in both cases). The reasons why FRE may be favored in glasshouse pot conditions are unknown, but may include: more constant temperature and soil moisture regimes than in the field; a high degree of physical disturbance to substrate in the process of filling pots; and AMF being less tolerant of such conditions allowing FRE to out-compete them. The data presented in Figs. 6 and 7 show that colonization by FRE may be equal

to, or greater than, that of AMF in agricultural field conditions; this suggests that disturbance may be a factor which favors FRE.

Different colonization responses for FRE and AMF in agricultural systems have been reported in several other studies. For instance, colonization by FRE was increased, sometimes more so than that of AMF, in response to cooler, wetter conditions in Australia (McGee 1989; Braunberger et al. 1997), and at the beginning of the growing season (Thippayarugs et al. 1999). In a pasture in Western Australia, the colonization by FRE, relative to that of AMF, increased greatly in riparian zone soils compared with soils higher in the landscape due to both an increase in colonization by FRE and a decrease in colonization by AMF (Orchard et al. 2016). Thus, there is some evidence to suggest that FRE and AMF occupy different, albeit likely overlapping, ecological niches in agricultural environments.

7.2 Moderate-natural environments

Observations of colonization by FRE from within Moderate-natural environments were more evenly distributed across regions than those for Agricultural environments: Australasia $n=96$, USA-Canada $n=79$ and Europe $n=55$ (of 231 observations). Reported levels of colonization by FRE were 0–73% ($n=128$). From the Australasian region, the majority of observations for FRE were from New Zealand natural landscapes ($n=92$ from 17 plant families). Colonization ranged from 2–46%. Once again, observations were dominated by Poaceae ($n=34$), wherein colonization by FRE occasionally exceeded or equalled that of AMF, *e.g.* *Anthoxanthum odoratum* and *Poa colensoi* (Fig. 8). In addition, there were reports from a variety of species in the Fabaceae, wherein colonization by FRE occupied up to 30% of root length in *Trifolium* species (Fig. 8). Note that colonization by FRE was always much lower in New Zealand Moderate-natural environments than in Agricultural environments (Fig. 7), while colonization by AMF in Moderate-natural environments varied considerably among hosts, but still frequently reached the high levels found in pastures.

In Australia, there were four observations of FRE from natural landscapes (Fabaceae and Plantaginaceae species), but no accompanying colonization data (Abbott and Robson 1978; Hall and Abbott 1984). In USA-Canada, colonization by FRE in natural landscapes ranged from 2–73%, while in Europe the range was 1–43%. Interestingly, in Central Europe,

Smilauerova et al. (2012) found that FRE were abundant in *Plantago* seedlings, while AMF dominated in mature plants.

To summarize, colonization by FRE appears common in Moderate-natural environments from around the globe. In New Zealand, colonization by FRE appears to be substantially lower in Moderate-natural environments than in Agricultural environments. The reasons for this difference are unknown.

7.3 Extreme environments

There were 64 observations of colonization by FRE from Low-temperature environments. The meta-analysis suggested higher colonization by FRE in these environments ($P=0.004$), but no shift in the FRE colonization ratio (Figs. 4 and 5). However, a different picture emerges when these data are explored in more detail. Within the Canadian Arctic, relatively high colonization by FRE and clear dominance of FRE relative to AMF was reported in the Ranunculaceae and, to a lesser extent, the Equisetaceae and Poaceae (this trend was reversed in Natural-moderate environments — data not shown). For all three families, there was a much broader range of colonization levels for FRE than for AMF (Fig. 9; Olsson et al. 2004; Ormsby et al. 2007; Hodson et al. 2009; Walker et al. 2010). In contrast, colonization levels by FRE and AMF were similar for the Asteraceae. Hence, there appears to be a species-specific interaction among host plants and their fungal symbionts in response to Low-temperature environments, as has been observed under waterlogging (Orchard et al. 2016; see below). This interaction could reflect differences among plant species in the root physiological and morphological response to these environments. In addition, in specific Low-temperature plant communities, FRE may come to dominate AMF due to a competitive release from AMF in response to few plants being present that host AMF and, hence, low levels of AM fungal inoculum (Newsham et al. 2009). For instance, bryophytes such as liverworts, and lycopods (club mosses) tend to be persistent plant forms in these environments (Newsham et al. 2009), and mucoromycotinian fungi (including FRE) may be the dominant endophytes of these plants (Rabatin 1980; Turnau et al. 1999; Field et al. 2015; Kowal et al. 2016).

While some authors have observed that FRE may dominate at High-altitudes (Crush 1973b; Read and Haselwandter 1981), our meta-analysis does not support this (Fig. 5). Indeed, there

was weak evidence that the FRE colonization ratios were lower in High-altitude than Moderate-natural environments ($P=0.025$), where observations of FRE were dominated by Poaceae and Asteraceae ($n=18$ and 13 of 87 papers, respectively). In particular, within Europe, many of the observations were from plant surveys of alpine regions of altitudes >1000 m, and the FRE colonization ratio was lower in High-altitude environments compared with Moderate-natural environments (Fig. 5, $P=0.01$). However, several factors may have influenced this result, as the High-altitude category included a wide range of altitudes (1000 m to >4000 m) which may have introduced variability within the data. For example, some alpine slopes at the lower end of the range may receive fertilizer application or be grazed in the warmer months (*e.g.* Read and Haselwandter 1981), whereas slopes at the higher end of the range would be subject to highly extreme conditions such as low temperature, nutrient deficiency and erosion.

Additional studies of FRE from extreme environments were included within the Other category due to their unique nature. In these, several authors again reported that the response of FRE differed from that of AMF. For example, nitrogen eutrophication of soil, low pH, and primary successional, metal contaminated and waterlogged soils were all reported to be more favorable for FRE than AMF (Daft and Nicolson 1974; Arines et al. 1988; Sainz et al. 1990; Sigüenza et al. 2006a, b; Postma et al. 2007; Göransson et al. 2008; Orchard et al. 2016). However, colonization by FRE did not increase and may have decreased in response to increased soil CO₂ concentrations, or soil warming, while both were favorable for AMF (Rillig et al. 1999; Rillig and Field 2003; Staddon et al. 2004; Olsrud et al. 2010). Further, Turnau et al. (2013) hypothesized that FRE help to immobilize toxic metals in contaminated soils. Interestingly, for the waterlogged soils mentioned above, the favorable effect on FRE was far more prominent for *Lotus subbiflorus* than for *Lolium rigidum*, even though both are considered waterlogging tolerant (Orchard et al. 2016). This again suggests an interaction with host-plant physiology or, perhaps, root morphology.

In summary, colonization by FRE can be high in some extreme environments, particularly in low temperatures, and in these environments, FRE may dominate over AMF in some host plants. It is unknown whether high colonization by FRE results from the environmental extreme favoring their growth or a competitive release from AMF, or other edaphic factors such as nutrient availability. In some instances, competitive release could result from a lack of plant hosts for AMF. The failure of the meta-analysis to find an overall significant effect of

environment on the FRE colonization ratio is because colonization by FRE in extreme environments was variable among host plants.

8. Fine root endophytes: impact on host-plant growth

The high levels of colonization by FRE in Australian and New Zealand agricultural systems (Section 7.1) suggest that investigation of their role in pasture and crop growth and nutrition is merited. Few papers have investigated this question, partly due to the difficulties in producing isolates (Section 3). However, early papers have suggested that FRE can promote plant growth (*e.g.* Greenall 1963), with papers in the 1970–80s reporting that colonization by FRE enhanced plant growth (Johnson 1977; Powell and Daniel 1978; Powell 1979a, b; Williams 1985), notably for New Zealand grasses (Crush 1973a, b). For instance, Crush (1973b) found that colonization by FRE significantly improved growth of three grasses (*Dactylis glomerata*, *Anthoxanthum odoratum* and *Lolium perenne*) at extremely low P levels (Truog P $\leq 4 \text{ mg kg}^{-1}$), but that FRE may become mildly parasitic following an increase in soil fertility (Crush 1973b; Fig. 10).

Nevertheless, by the late 1980s, some studies reported that FRE were less competitive than many species of AMF and less beneficial to plant growth (*e.g.* Buwalda 1980; Lopez-Aguillon and Mosse 1987; Arines et al. 1988). More recently, few studies have examined the impact of FRE on the host plant through comparison of colonized and uncolonized plants in glasshouse experiments. However, the results of glasshouse experiments to assess invasive grass establishment indicated that FRE might give invasive grasses a competitive advantage in the USA in regenerating bushland (Hilbig and Allen 2015) and at a nitrogen deposition site (Siguenza et al. 2006a). This finding was also noted under field conditions (Siguenza et al. 2006b). In addition, some papers have hypothesized an impact on plant growth based on high colonization levels by FRE in field samples. For instance, it was hypothesized that FRE play an important role in Bromeliad epiphytes acquiring scarce nutrients (Rabatin et al. 1993), and in aiding plant nutrient uptake under low-temperature conditions (Newsham et al. 2009). Further, in the USA, Rabatin (1979) noted that FRE enhanced plant growth in low P conditions (Olsen P $< 1 \text{ } \mu\text{g g}^{-1}$) allowing meadow grasses to use brief influxes of available P when soil moisture was high. Indeed, following assessment of FRE among meadow sites at different times of the year, Rabatin (1979) concluded that FRE could respond quickly to change

indicating that they may be opportunists that can exploit favorable edaphic conditions.

In summary, it seems that FRE have the ability to enhance host phosphorus uptake like that of AMF, at least under extremely low P conditions. However, it is not known if FRE can provide the numerous other benefits ascribed to AMF, such as resistance to pathogens, drought tolerance, and uptake of other macro- and micro-nutrients (Smith and Read 2008). The benefits of a mixed community of AMF and FRE to plant hosts are unknown.

9. An anticipated resurgence in research of fine root endophytes

We have detailed the history of research on FRE during the period that they were included with AMF in the phylum Glomeromycota. Ahead is an exciting new era of research on FRE as their repositioning as members of the subphylum Mucoromycotina highlights their phylogenetic distinctiveness. Here we propose some possible research questions and directions.

As a priority, there is a need to understand if FRE consist of one or several species—the latter being highly likely (Thippayarugs et al. 1999; Orchard et al. 2017a). This work requires the establishment of a sound species concept for the group. Further, Mucoromycotina species with some morphological similarities to FRE reportedly colonize bryophytes (Field et al. 2015). Therefore, the pertinent question is, do the same species of FRE colonize both bryophytes and vascular plants, as is known to occur for some AMF?

Research on the function of FRE, both individually and in association with AMF, is needed, especially given their co-habitation of many plant roots. This call is especially critical given that much field-based research suggests the presence of a mixed community of AMF and FRE (*e.g.* Orchard et al. 2016). There may be merit, therefore, in designing more complex growth experiments where uncolonized control plants are compared with plants colonized by FRE only, AMF only and a mixture of AMF and FRE.

The evolutionary relationship between FRE (in the Mucoromycotina) and AMF (in the Glomeromycota) must be elucidated. For example, did the arbuscule evolve only once as implied by Morton (1990) or has it evolved independently in earlier common ancestors of these two groups? With current knowledge, it is not possible to come to a satisfactory conclusion on this critical aspect of scientific knowledge.

What is the role of FRE within extreme low-temperature environments? How do they persist within these, and other, extreme conditions? In particular, does this ability reflect an adaptation to survive these conditions *per se*, or does it represent adaptation in interaction with other factors, such as host-plant signals and competitive release from AMF?

Although the decline in research on FRE was partly due to the difficulty in isolating them, partial success towards their isolation has been achieved through soil sieving and dilution methods (Thippayarugs et al. 1999; Orchard et al. 2017a). Further refinements to this method may provide a way forward to create a reliable method for their isolation.

Molecular tools directed at sequencing AM fungal communities have not specifically targeted FRE. Even though this omission is understandable, given that FRE are phylogenetically separate from the AMF at the subphylum level, it means that a potentially large component of the arbuscule-forming, root symbiotic community has been excluded from recent molecular studies. Therefore, new targeted primers need to be developed to facilitate further research and to improve understanding of the phylogeny, diversity and ecology of FRE.

Techniques and technologies that have emerged during recent decades for AMF and other fungal endophytes have not been implemented for FRE. Thus, numerous possibilities exist for further research, such as advanced microscopy techniques (*e.g.* transmission and scanning electron microscopy) and, particularly, in areas such as molecular signalling between FRE and the host plant (*e.g.* effector molecules, manipulation of host-gene expression, and fungal exudates).

10. Conclusions

Our review of the literature suggests that there are influences on the level of colonization by FRE from the host-plant family, environment and geographic region. The high colonization levels in Agricultural environments and, for some host plants, in Low-temperature environments suggest that FRE have an important ecological role and that it may be distinct from, or perhaps complementary to, that of AMF. However, the current evidence provides only tantalizing glimpses into their role. Numerous research options offer exciting potential and opportunities to drive research on FRE and provide a better understanding of their function, particularly in agricultural and extreme environments, and so elucidate their role in both natural

and human-influenced ecosystems.

Reference list

- Abbott L (1982) Comparative anatomy of vesicular-arbuscular mycorrhizas formed on subterranean clover. *Aust J Bot* 30: 485–499
- Abbott L, Robson A (1978) Growth of subterranean clover in relation to the formation of endomycorrhizas by introduced and indigenous fungi in field soil. *New Phytol* 81: 575–585
- Abbott L, Robson A (1982) Infectivity of vesicular arbuscular mycorrhizal fungi in agricultural soils. *Aust J Agr Res* 33: 1049–1059
- Abbott L, Robson A, Hall I (1983) Introduction of vesicular arbuscular mycorrhizal fungi into agricultural soils. *Aust J Agr Res* 34: 741–749
- Allen N, Nordlander M, McGonigle T, Basinger J, Kaminskyj S (2006) Arbuscular mycorrhizae on Axel Heiberg Island (80 degrees N) and at Saskatoon (52 degrees N) Canada. *Can J Bot* 84: 1094–1100
- Anderson RC, Ebberts BC, Liberta AE (1986) Soil-moisture influences colonization of prairie cordgrass (*Spartina pectinata* Lind) by vesicular-arbuscular mycorrhizal fungi. *New Phytol* 102: 523–527
- Antoninka A, Reich PB, Johnson NC (2011) Seven years of carbon dioxide enrichment, nitrogen fertilization and plant diversity influence arbuscular mycorrhizal fungi in a grassland ecosystem. *New Phytol* 192: 200–214
- Arines J, Vilariño A, Sainz M (1988) ‘Fine’ and ‘coarse’ mycorrhizal fungi on red clover plants in acid soils: Root colonization and plant responses. *Plant Soil* 111: 135–145
- Baon JB, Smith SE, Alston AM, Wheeler RD (1992) Phosphorus efficiency of 3 cereals as related to indigenous mycorrhizal infection. *Aust J Agr Res* 43: 479–491
- Baylis GTS (1967) Experiments on the ecological significance of phycomycetous mycorrhizas. *New Phytol* 66: 231–243
- Baylis GTS (1969) Host treatment and spore production by *Endogone*. *New Zeal J Bot* 7: 173–174
- Beck A, Kottke I, Oberwinkler F (2005) Two members of the Glomeromycota form distinct ectendomycorrhizas with *Alzatea verticillata*, a prominent tree in the mountain rain forest of southern Ecuador. *Mycol Prog* 4: 11–22
- Binet M-N, van Tuinen D, Depretre N, Koszela N, Chambon C, Gianinazzi S (2011) Arbuscular mycorrhizal fungi associated with *Artemisia umbelliformis* Lam, an endangered aromatic species in Southern French Alps, influence plant P and essential oil contents. *Mycorrhiza* 21: 523–535
- Binet MN, Sage L, Malan C, Clement JC, Redecker D, Wipf D, Geremia RA, Lavorel S, Mouhamadou B (2013) Effects of mowing on fungal endophytes and arbuscular mycorrhizal fungi in subalpine grasslands. *Fungal Ecol* 6: 248–255
- Blaschke H (1991) Distribution, mycorrhizal infection, and structure of roots of calcicole floral elements at treeline, Bavarian Alps, Germany. *Arctic Alpine Res* 23: 444–450
- Blaszkowski J (1994) Arbuscular fungi and mycorrhizae (Glomales) of the Hel Peninsula, Poland. *Mycorrhiza* 5: 71–88
- Braunberger PG (1994) The effect of rain in the dry-season on the formation of vesicular-arbuscular mycorrhizas in the growing season of annual clover-based pastures. *New Phytol* 127: 107–114
- Braunberger PG, Abbott LK, Robson AD (1997) Early vesicular-arbuscular mycorrhizal colonisation in soil collected from an annual clover-based pasture in a Mediterranean environment: soil temperature and the timing of autumn rains. *Aust J Agr Res* 48: 103–

- Brundrett M (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant Soil* 320: 37–77
- Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N (1996) Working with mycorrhizas in forestry and agriculture. Australian Centre for International Agricultural Research, Canberra
- Buwalda JG (1980) Growth of a clover–ryegrass association with vesicular arbuscular mycorrhizas. *New Zeal J Agr Res* 23: 379–383
- Christie P, Nicolson TH (1983) Are mycorrhizas absent from the Antarctic. *Trans Br Mycol Soc* 80: 557–560
- Christie P, Kilpatrick DJ (1992) Vesicular-arbuscular mycorrhiza infection in cut grassland following long-term slurry application. *Soil Biol Biochem* 24: 325–330
- Cooper KM (1976) A field survey of mycorrhizas in New Zealand ferns. *New Zeal J Bot* 14: 169–181
- Crush JR (1973a) The effect of *Rhizophagus tenuis* mycorrhizas on ryegrass, cocksfoot and sweet vernal. *New Phytol* 72: 965–973
- Crush JR (1973b) Significance of endomycorrhizas in tussock grassland in Otago, New Zealand. *New Zeal J Bot* 11: 645–660
- Crush JR (1975) Occurrence of endomycorrhizas in soils of the McKenzie basin, Canterbury, New Zealand. *New Zeal J Agr Res* 18: 361–364
- Crush JR (1982) Effects of endomycorrhizas and phosphorus-fertilizer on nodulation and acetylene-reduction activity of white clover seedlings. *New Zeal J Exp Agr* 10: 297–299
- Daft MJ, Nicolson TH (1974) Arbuscular mycorrhizas in plants colonizing coal wastes in Scotland. *New Phytol* 73: 1129–1138
- Daft MJ, Chilvers MT, Nicolson TH (1980) Mycorrhizas of the Liliiflorae .1. Morphogenesis of *Endymion-non-scriptus* (L) Garcke and its mycorrhizas in nature. *New Phytol* 85: 181–189
- Dhillion SS, Vidiella PE, Aquilera LE, Friese CF, Deleon E, Armesto JJ, Zak JC (1995) Mycorrhizal plants and fungi in the fog-free Pacific coastal desert of Chile. *Mycorrhiza* 5: 381–386
- Druva-Lusite I, Ievinsh G (2010) Diversity of arbuscular mycorrhizal symbiosis in plants from coastal habitats. *Environ Exp Biol* 8: 17–34
- Ebbers BC, Anderson RC, Liberta AE (1987) Aspects of the mycorrhizal ecology of prairie dropseed, *Sporobolus heterolepis* (Poaceae). *Am J Bot* 74: 564–573
- Fernandez N, Messuti MI, Fontenla S (2008) Arbuscular mycorrhizas and dark septate fungi in *Lycopodium paniculatum* (Lycopodiaceae) and *Equisetum bogotense* (Equisetaceae) in a Valdivian temperate forest of Patagonia, Argentina. *Am Fern J* 98: 117–127
- Field KJ, Pressel S, Duckett JG, Rimington WR, Bidartondo MI (2015) Symbiotic options for the conquest of land. *Trends Ecol Evol* 30: 477–486
- Ganesan V, Ragupathy S, Parthipan B, Rani DBR, Mahadevan A (1991) Distribution of vesicular-arbuscular mycorrhizal fungi in coal, lignite, and calcite mine spoils of India. *Biol Fert Soils* 12: 131–136
- Gerdemann JW, Trappe JM (1974) The Endogonaceae in the Pacific Northwest. *Mycologia Memoir* (No. 5) 75 pp.
- Gianinazzi-Pearson V, Morandi D, Dexheimer J, Gianinazzi S (1981) Ultrastructural and ultracytochemical features of a *Glomus tenuis* mycorrhiza. *New Phytol* 88: 633–639

- Gnekow MA, Marschner H (1989) Influence of the fungicide pentachloronitrobenzene on VA-mycorrhizal and total root length and phosphorus uptake of oats (*Avena sativa*). Plant Soil 114: 91–98
- Göransson P, Olsson PA, Postma J, Falkengren-Grerup U (2008) Colonisation by arbuscular mycorrhizal and fine endophytic fungi in four woodland grasses — variation in relation to pH and aluminium. Soil Biol Biochem 40: 2260–2265
- Greenall JM (1963) The mycorrhizal endophytes of *Griselinia littoralis* (Cornaceae). New Zeal J Bot 1: 389–400
- Gucwa-Przepiora E, Blaszkowski J, Kurtyka R, Malkowski L, Malkowski E (2013) Arbuscular mycorrhiza of *Deschampsia cespitosa* (Poaceae) at different soil depths in highly metal-contaminated site in southern Poland. Acta Soc Bot Pol 82: 251–258
- Hall IR (1977) Species and mycorrhizal infections of New Zealand Endogonaceae. Trans Br Mycol Soc 68: 341–356
- Hall IR (1984) Field trials assessing the effect of inoculating agricultural soils with endomycorrhizal fungi. J Agr Sci 102: 725–731
- Hall IR, Abbott L (1984) Some Endogonaceae from south Western Australia. Trans Br Mycol Soc 83: 203–208
- Hilbig BE, Allen EB (2015) Plant–soil feedbacks and competitive interactions between invasive *Bromus diandrus* and native forb species. Plant Soil 392: 191–203
- Hodson E, Shahid F, Basinger J, Kaminskyj S (2009) Fungal endorhizal associates of *Equisetum* species from Western and Arctic Canada. Mycol Prog 8: 19–27
- Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, Koide RT, Pringle A, Zabinski C, Bever JD, Moore JC and others (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecol Lett 13: 394–407
- Hooker JE, Piatti P, Cheshire MV, Watson CA (2007) Polysaccharides and monosaccharides in the hyphosphere of the arbuscular mycorrhizal fungi *Glomus* E3 and *Glomus tenue*. Soil Biol Biochem 39: 680–683
- Hopkins NA (1987) Mycorrhizae in a California serpentine grassland community. Can J Bot 65: 484–487
- Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biometrical J 50: 346–363
- Jasper DA, Abbott LK, Robson AD (1989) Hyphae of a vesicular-arbuscular mycorrhizal fungus maintain infectivity in dry soil, except when the soil is disturbed. New Phytol 112: 101–107
- Jeffries P, Spyropoulos T, Vardavarkis E (1988) Vesicular-arbuscular mycorrhizal status of various crops in different agricultural soils of northern Greece. Biol Fert Soils 5: 333–337
- John J, Lundholm J, Kernaghan G (2014) Colonization of green roof plants by mycorrhizal and root endophytic fungi. Ecol Eng 71: 651–659
- Johnson PN (1977) Mycorrhizal Endogonaceae in a New Zealand forest. New Phytol 78: 161–170
- Knopf E, Blaschke H, Munch JC (2014) Facilitating AMF activities in *Moringa* species for reforestation purposes in Lake Victoria basin, Kenya. Agr Sci Eng Technol Res 2: 1–12
- Kowal J, Pressel S, Duckett JG, Bidartondo MI (2016) Liverworts to the rescue: an investigation of their efficacy as mycorrhizal inoculum for vascular plants. Funct Ecol 30: 1014–1023

- Lesica P, Antibus RK (1986) Mycorrhizae of alpine fell-field communities on soils derived from crystalline and calcareous parent materials. *Can J Bot* 64: 1691–1697
- Lopez-Aguillon R, Mosse B (1987) Experiments on competitiveness of three endomycorrhizal fungi. *Plant Soil* 97: 155–170
- Majewska ML, Blaszkowski J, Nobis M, Rola K, Nobis A, Lakomiec D, Czachura P, Zubek S (2015) Root-inhabiting fungi in alien plant species in relation to invasion status and soil chemical properties. *Symbiosis* 65: 101–115
- McGee PA (1989) Variation in propagule numbers of vesicular-arbuscular mycorrhizal fungi in a semi-arid soil. *Mycol Res* 92: 28–33
- McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol* 115: 495–501
- McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J, Prud'Homme vanReine WF, Smith GF, Wieersma JH, Turland NJ (2011) International Code of Nomenclature for algae, fungi and plants (Melbourne Code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. Publ. 2012. (Regnum Vegetabile, 154); 240 p. gr8vo. ISBN 978-3-87429-425-6
- Merryweather J, Fitter AH (1998) The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta*. I. Diversity of fungal taxa. *New Phytol* 138: 117–129
- Morton JB (1990) Evolutionary relationships among arbuscular mycorrhizal fungi in the Endogonaceae. *Mycologia* 82: 192–207
- Mosse B, Bowen GD (1968) A key to the recognition of some *Endogone* spore types. *Trans Br Mycol Soc* 51: 469–483
- Mullen RB, Schmidt SK (1993) Mycorrhizal infection, phosphorus uptake, and phenology in *Ranunculus adoneus*: implications for the functioning of mycorrhizae in alpine systems. *Oecologia* 94: 229–234
- Newsham KK, Upson R, Read DJ (2009) Mycorrhizas and dark septate root endophytes in polar regions. *Fungal Ecol* 2: 10–20
- Nichols PGH, Revell CK, Humphries AW, Howie JH, Hall EJ, Sandral GA, Ghamkhar K, Harris CA (2012) Temperate pasture legumes in Australia — their history, current use, and future prospects. *Crop Pasture Sci* 63: 691–725
- Nicolson TH, Schenck NC (1979) Endogonaceous mycorrhizal endophytes in Florida. *Mycologia* 71: 178–198
- Olsrud M, Carlsson BA, Svensson BM, Michelsen A, Melillo JM (2010) Responses of fungal root colonization, plant cover and leaf nutrients to long-term exposure to elevated atmospheric CO₂ and warming in a subarctic birch forest understory. *Glob Change Biol* 16: 1820–1829
- Olsson PA, Eriksen BE, Dahlberg A (2004) Colonization by arbuscular mycorrhizal and fine endophytic fungi in herbaceous vegetation in the Canadian High Arctic. *Can J Bot* 82: 1547–1556
- Orchard S, Standish RJ, Nicol D, Gupta VVSR, Ryan MH (2016) The response of fine root endophyte (*Glomus tenue*) to waterlogging is dependent on host plant species and soil type. *Plant Soil* 403: 305–315
- Orchard S, Hilton S, Bending GD, Dickie IA, Standish, RJ, Gleeson D,Ryan MH (2017a) Fine endophytes (*Glomus tenue*) are related to Mucoromycotina not Glomeromycota. *New Phytol* 213: 481–486
- Orchard S, Standish RJ, Nicol D, Dickie IA, Ryan MH (2017b) Sample storage conditions alter

- colonisation structures of arbuscular mycorrhizal fungi and, particularly, fine root endophyte. *Plant Soil* 412: 35–42
- Ormsby A, Hodson E, Li Y, Basinger J, Kaminskyj S (2007) Quantitation of endorhizal fungi in high Arctic tundra ecosystems through space and time: The value of herbarium archives. *Can J Bot* 85: 599–606
- Parfitt RL (1979) Availability of P from phosphate-goethite bridging complexes — desorption and uptake by ryegrass. *Plant Soil* 53: 55–65
- Parfitt RL, Hart PBS, Meyrick KF, Russell M (1982) Response of ryegrass and white clover to phosphorus on an allophanic soil, egmont black loam. *New Zeal J Agr Res* 25: 549–555
- Parke JL, Linderman RG, Trappe JM (1983) Effect of root zone temperature on ectomycorrhiza and vesicular arbuscular mycorrhiza formation in disturbed and undisturbed forest soils of southwest Oregon. *Can J Forest Res* 13: 657–665
- Parmar A, Mall TP, Singh RB (2013) Natural population dynamics and morphological characters of mycorrhizal fungi in rhizosphere of wheat (*Triticum aestivum* L.). *Res Environ Life Sci* 6: 65–68
- Pinheiro J, Bates D, DebRoy S, Sarkar D and R Core Team (2014) nlme: linear and nonlinear Mixed Effects Models. R package version 3.1-117. URL <http://CRAN.R-project.org/package=nlme>
- Plenchette C, Strullu DG (2003) Long-term viability and infectivity of intraradical forms of *Glomus intraradices* vesicles encapsulated in alginate beads. *Mycol Res*, 107, 614–616
- Postma JWM, Olsson PA, Falkengren-Grerup U (2007) Root colonisation by arbuscular mycorrhizal, fine endophytic and dark septate fungi across a pH gradient in acid beech forests. *Soil Biol Biochem* 39: 400–408
- Powell C (1976) Mycorrhizal fungi stimulate clover growth in New Zealand hill country soils. *Nature* 264: 436–438
- Powell C (1979a) Inoculation of white clover and ryegrass seed with mycorrhizal fungi. *New Phytol* 83: 81–85
- Powell CL (1979b) Spread of mycorrhizal fungi through soil. *New Zeal J Agr Res* 22: 335–339
- Powell CL (1980) Phosphate response curves of mycorrhizal and non-mycorrhizal plants .1. Responses to super-phosphate. *New Zeal J Agr Res* 23: 225–231
- Powell CL, Daniel J (1978) Mycorrhizal fungi stimulate uptake of soluble and insoluble phosphate fertilizer from a phosphate-deficient soil. *New Phytol* 80: 351–358
- Powell CL, Groters M, Metcalfe D (1980) Mycorrhizal inoculation of a barley crop in the field. *New Zeal J Agr Res* 23: 107–109
- R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>
- Rabatin SC (1979) Seasonal and edaphic variation in vesicular-arbuscular mycorrhizal infection of grasses by *Glomus tenuis*. *New Phytol* 83: 95–102
- Rabatin SC (1980) The occurrence of the vesicular-arbuscular mycorrhizal fungus *Glomus tenuis* with moss. *Mycologia* 72: 191–195
- Rabatin SC, Stinner BR, Paoletti MG (1993) Vesicular-arbuscular mycorrhizal fungi, particularly *Glomus tenue*, in Venezuelan bromeliad epiphytes. *Mycorrhiza* 4: 17–20
- Read DJ, Haselwandter K (1981) Observations of the mycorrhizal status of some alpine plant communities. *New Phytol* 88: 341–352
- Rillig MC, Field CB (2003) Arbuscular mycorrhizae respond to plants exposed to elevated atmospheric CO₂ as a function of soil depth. *Plant Soil* 254: 383–391

- Rillig MC, Field CB, Allen MF (1999) Fungal root colonization responses in natural grasslands after long-term exposure to elevated atmospheric CO₂. *Glob Change Biol* 5: 577–585
- Rosendahl S, Rosendahl CN, Sochting U (1990) Distribution of VA mycorrhizal endophytes amongst plants from a Danish grassland community. *Agr Ecosyst Environ* 29: 329–335
- Ruotsalainen AL, Vare H, Oksanen J, Tuomi J (2004) Root fungus colonization along an altitudinal gradient in North Norway. *Arct Antarct Alp Res* 36: 239–243
- Ryan MH, Kirkegaard JA (2012) The agronomic relevance of arbuscular mycorrhizas in the fertility of Australian extensive cropping systems. *Agr Ecosyst Environ* 163: 37–53
- Ryszka P, Blaszkowski J, Jurkiewicz A, Turnau K (2010) Arbuscular mycorrhiza of *Arnica montana* under field conditions—conventional and molecular studies. *Mycorrhiza* 20: 551–557
- Sainz MJ, Vilarino A, Arines J (1990) Competition between *Glomus tenue* and some coarse fungi for colonizing red clover roots in acid soils. *Agr Ecosyst Environ* 29: 337–340
- Sanginga N, Carsky RJ, Dashiell K (1999) Arbuscular mycorrhizal fungi respond to rhizobial inoculation and cropping systems in farmers' fields in the Guinea savanna. *Biol Fert Soils* 30: 179–186
- Sannazzaro AI, Ruiz OA, Alberto E, Menendez AB (2004) Presence of different arbuscular mycorrhizal infection patterns in roots of *Lotus glaber* plants growing in the Salado River basin. *Mycorrhiza* 14: 139–142
- Schenck NC, Smith GS (1982) Additional new and unreported species of mycorrhizal fungus (Endogonaceae) from Florida. *Mycologia* 74: 77–92
- Schmidt SK, Sobieniak-Wiseman LC, Kageyama SA, Halloy SRP, Schadt CW (2008) Mycorrhizal and dark-septate fungi in plant roots above 4270 meters elevation in the Andes and Rocky Mountains. *Arct Antarct Alp Res* 40: 576–583
- Schrader, R (1958) Untersuchungen zur Biologie der Getreide-Thysanopteren. *Arch Mikrobiol* 32: 81–114.
- Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycol Res* 105: 1413–1421
- Sigüenza C, Crowley DE, Allen EB (2006b) Soil microorganisms of a native shrub and exotic grasses along a nitrogen deposition gradient in southern California. *Appl Soil Ecol* 32: 13–26
- Sigüenza C, Corkidi L, Allen E (2006a) Feedbacks of soil inoculum of mycorrhizal fungi altered by N deposition on the growth of a native shrub and an invasive annual grass. *Plant Soil* 286: 153–165
- Smilauerova M, Lokvencova M, Smilauer P (2012) Fertilization and forb:graminoid ratio affect arbuscular mycorrhiza in seedlings but not adult plants of *Plantago lanceolata*. *Plant Soil* 351: 309–324
- Smith SE, Manjarrez M, Stonor R, McNeill A, Smith FA (2015) Indigenous arbuscular mycorrhizal (AM) fungi contribute to wheat phosphate uptake in a semi-arid field environment, shown by tracking with radioactive phosphorus. *Appl Soil Ecol* 96: 68–74
- Smith SE, Read DJ (2008) *Mycorrhizal symbiosis*, 3rd ed. Elsevier, Burlington
- Sparling GP, Tinker PB (1978) Mycorrhizal infection in Pennine grassland. I. Levels of infection in the field. *J Appl Ecol* 15: 943–950
- Spatafora JW, Chang Y, Benny GL, Lazarus K, Smith ME, Berbee ML, ..., Stajich JE (2016) A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia* 108: 1028–1046. doi:10.3852/16-042
- Staddon PL, Gregersen R, Jakobsen I (2004) The response of two *Glomus* mycorrhizal fungi

- and a fine endophyte to elevated atmospheric CO₂, soil warming and drought. *Glob Change Biol* 10: 1909–1921
- Tanner CC, Clayton JS (1985) Effects of vesicular arbuscular mycorrhizas on growth and nutrition of a submerged aquatic plant. *Aquat Bot* 22: 377–386
- Thippayarugs S, Bansal M, Abbott LK (1999) Morphology and infectivity of fine endophyte in a Mediterranean environment. *Mycol Res* 103: 1369–1379
- Thomson BD, Robson A, Abbott L (1992) The effect of long-term applications of phosphorus fertilizer on populations of vesicular-arbuscular mycorrhizal fungi in pastures. *Aust J Agr Res* 43: 1131–42
- Trappe JM (1982) Synoptic keys to the genera and species of zygomycetous mycorrhizal fungi. *Phytopathology* 72: 1102–1108
- Turnau K, Mesjasz-Przybylowicz J (2003) Arbuscular mycorrhiza of *Berkheya coddii* and other Ni-hyperaccumulating members of Asteraceae from ultramafic soils in South Africa. *Mycorrhiza* 13: 185–190
- Turnau K, Ronikier M, Unrug J (1999) Role of mycorrhizal links between plants in establishment of liverworts thalli in natural habitats. *Acta Soc Bot Pol* 68: 63–68
- Turnau K, Anielska T, Jurkiewicz A (2005) Mycothallic/mycorrhizal symbiosis of chlorophyllous gametophytes and sporophytes of a fern, *Pellaea viridis* (Forsk.) Prantl (Pellaeaceae, Pteridales). *Mycorrhiza* 15: 121–128
- Turnau K, Przybylowicz WJ, Ryszka P, Orlowska E, Anielska T, Mesjasz-Przybylowicz J (2013) Mycorrhizal fungi modify element distribution in gametophytes and sporophytes of a fern *Pellaea viridis* from metaliferous soils. *Chemosphere* 92: 1267–1273
- Upton R, Newsham KK, Read DJ (2008) Root-fungal associations of *Colobanthus quitensis* and *Deschampsia antarctica* in the maritime and subantarctic. *Arct Antarct Alp Res* 40: 592–599
- Visser S, Maynard D, Danielson RM (1998) Response of ecto- and arbuscular mycorrhizal fungi to clear-cutting and the application of chipped aspen wood in a mixed wood site in Alberta, Canada. *Appl Soil Ecol* 7: 257–269
- Walker X, Basinger J, Kaminskyj S (2010) Endorhizal fungi in *Ranunculus* from Western and Arctic Canada: Predominance of fine endophytes at high latitudes. *Open Mycol* 4: 1–9
- Wang GM, Stribley DP, Tinker PB, Walker C (1993) Effects of pH on arbuscular mycorrhiza. I. Field observations on the long-term liming experiments at Rothamsted and Woburn. *New Phytol* 124: 465–472
- Williams PG (1985) Orchidaceous rhizoctonias in pot cultures of vesicular arbuscular mycorrhizal fungi. *Can J Bot* 63: 1329–1333
- Wilson JM (1984) Comparative development of infection by three vesicular-arbuscular mycorrhizal fungi. *New Phytol* 97: 413–426
- Wilson JM, Trinick MJ (1983a) Infection development and interactions between vesicular-arbuscular mycorrhizal fungi. *New Phytol* 93: 543–553
- Wilson JM, Trinick MJ (1983b) Factors affecting the estimation of numbers of infective propagules of vesicular arbuscular mycorrhizal fungi by the most probable number method. *Aust J Soil Res* 21: 73–81
- Zubek S, Blaszkowski J, Buchwald W (2012) Fungal root endophyte associations of medicinal plants. *Nova Hedwigia* 94: 525–540
- Zubek S, Blaszkowski J, Delimat A, Turnau K (2009) Arbuscular mycorrhizal and dark septate endophyte colonization along altitudinal gradients in the Tatra Mountains. *Arct Antarct Alp Res* 41: 272–279

Zubek S, Nobis M, Blaszkowski J, Mleczko P, Nowak A (2011) Fungal root endophyte associations of plants endemic to the Pamir Alay Mountains of Central Asia. *Symbiosis* 54: 139–149

Figures

Fig. 1 Images of root colonization structures by fine root endophytes (FRE) and, in contrast, arbuscular mycorrhizal fungi (AMF): (a) FRE within the roots of *Trifolium subterraneum* (reproduced from Orchard et al. (2016)); (b) morphological features of FRE, an arbuscule (a), a swollen hypha (sw) and a (terminal) vesicle (tv) (reproduced from Thippayarugs et al. (1999)); (c) a spore of FRE (white arrow) attached to fine hyphae on the root surface; (d) stained hyphae (black arrows) and arbuscules (A) of FRE in *Plantago lanceolata*; (e) an AM fungal spore (*Diversispora epigaea*); and (f) stained hyphae and arbuscules of AMF (*Acaulospora* sp.) in *Mirbelia dilatata*. Scale bars are all 50 μ m.

<i>Rhizophagus tenuis</i> Phylum: Zygomycota	1963	The first description and diagram of "fine endophyte" within the roots of the native New Zealand shrub <i>Griselinia littoralis</i> (Raoul). Greenall (1963) was the first to distinguish FRE from other AMF and named it <i>Rhizophagus tenuis</i> (family Endogonaceae) in line with the nomenclature of AMF at that time; she conceded it may be several species rather than one.
<i>Glomus tenuis</i> Phylum: Zygomycota	1974	A taxonomic revision of the Endogonaceae reclassified several <i>Rhizophagus</i> species to <i>Glomus</i> : FRE became <i>Glomus tenuis</i> (Gerdemann & Trappe 1974).
	1977	An assessment of New Zealand Endogonaceae provided a detailed description of <i>G. tenuis</i> . The placement of FRE within the <i>Glomus</i> genus was questioned, but insufficient knowledge hindered an alternative classification (Hall 1977).
<i>Glomus tenue</i> Phylum: Zygomycota	1982	Neuter gender assigned to <i>Glomus</i> (Trappe 1982).
	1970s to late 1990s	During this period the distinct morphology of FRE became useful to distinguish its colonisation from that of AMF (often described as coarse AMF). Spores of FRE were rarely observed (Crush 1975; Hall 1977). FRE were often grouped within the "indigenous AMF" in field soils and compared with specific/introduced AM fungal species (e.g. Abbott & Robson 1978; Powell & Daniel 1978).
<i>Glomus tenue</i>; fine endophyte Phylum: Glomeromycota	2001	A review based on molecular evidence reassigned all AMF to the phylum Glomeromycota and <i>Glomus</i> species to within the family Glomeraceae (Schussler <i>et al.</i> , 2001). Once again, FRE classification as <i>Glomus</i> was questioned, but knowledge was still lacking to provide an alternative.
	late 1990s To 2015	During this period, authors often referred to FRE as 'fine endophyte' rather than <i>G. tenue</i> . Some authors no longer included FRE within the AMF. As identification and inoculum of coarse AM fungal species became readily available, research focus shifted away from FRE. In molecular studies, the primers for Glomeromycota mostly did not target FRE.
fine root endophyte Sub-phylum: Mucoromycotina	2016	The phylogenetic identity of FRE was proposed to be within the sub-phylum Mucoromycotina (Orchard <i>et al.</i> , 2017a).

Fig. 2 Timeline of the nomenclature and descriptions of fine root endophytes (FRE) from 1963 to date, describing the changing classifications and research focus relative to that of arbuscular mycorrhizal fungi (AMF).

Fig. 3 The approximate geographical origin of each paper that reported the presence of fine root endophytes from 1963 to 2015. Increasing size of the black dots indicates a larger number of publications. Two observations from islands off the coast of Antarctica are not shown on the map. Refer to Table 1 and the Reference List for the countries and full publication details.

Fig. 4 The reported FRE colonization level by those factors found significant in the meta-analysis: region ($P<0.0001$), environment ($P<0.0001$) and whether the study was a glasshouse/pot experiment (white) or field-based (grey) ($P<0.0001$); there was no effect of plant family ($P>0.05$). Boxes show median and upper and lower quartiles, and whiskers indicate the full range. The width of boxes is proportional to the square root of the number of data values. Visual differences here may not match statistical modelling and testing results because weighting by sample size is not included here. Untransformed data are plotted to ensure a meaningful scale.

Fig. 5 The FRE colonization ratio by those factors found significant in the meta-analysis: region ($P < 0.0001$), family ($P < 0.0001$) and whether study was a glasshouse/pot experiment (white) or field-based (grey) ($P < 0.0001$); there was no effect of environment ($P > 0.05$). Boxes show median and upper and lower quartiles, and whiskers indicate the full range. The width of boxes is proportional to the square root of the number of data values. Visual differences here may not match statistical modelling and testing results because weighting by sample size is not included here. Untransformed data are plotted to ensure a meaningful scale.

Fig. 6 The percentage of the total root length that was colonized (mean + standard error) by fine root endophytes (green) and arbuscular mycorrhizal fungi (grey) reported for field soils and field experiments of winter-grown annual agricultural pastures and crops in southern Australia. (a) *Trifolium subterraneum* grown in a range of agricultural field soils (A to T) from Western Australia within a glasshouse pot experiment (adapted from Abbott and Robson (1982)); (b) *Triticum aestivum* grown in an agronomic field experiment in southern New South Wales with either no added phosphorus (No P) or added phosphorus at 20 kg ha⁻¹ (Plus P), and following five crop rotations or treatments: pasture (Past), linseed (Lin; *Linum usitatissimum*), plant-free long fallow maintained with chemicals (CF) or tillage (TF), or canola (Can; *Brassica napus*) (adapted from Ryan and Kirkegaard (2012)).

Fig: 7: The percentage root length colonized (mean + standard error) by fine root endophytes (green) and arbuscular mycorrhizal fungi (AMF) (grey) for *Trifolium subterraneum* sampled from 30 pastures in New Zealand in October 2014 (Suzanne Orchard, unpublished data).

Fig. 8: The percentage root length colonized (mean + standard error) by fine root endophytes (green) and arbuscular mycorrhizal fungi (AMF) (grey) for various plants sampled in New Zealand from a natural environment (data reproduced from Crush 1973b and 1975).

Fig 9: Colonization by fine root endophytes (FRE) and arbuscular mycorrhizal fungi (AMF) from Low-temperature environments in the Canadian Arctic (Olsson et al. 2004; Ormsby et al. 2007; Hodson et al. 2009; Walker et al. 2010) from Asteraceae, Equisetaceae, Poaceae, Ranunculaceae and Rosaceae ($n=24, 14, 4, 14$ and 2 , respectively). The boxes show the median, 90th percentiles and outliers (black dots) of the reported data.

Fig. 10: The shoot dry weight of *Lolium perenne* grown for 76 days in a New Zealand glasshouse experiment using grassland soil, either inoculated with a culture of fine root endophytes (FRE; green) or without FRE (sterile soil; white), and with treatments: no fertilizer (nil), complete fertilizer minus phosphorus (C-P) or phosphorus supplied (+P). Numbers above the bars indicates the percentage of root length colonized by FRE in the inoculated treatments (reproduced from Crush 1973a).