The paleosymbiosis hypothesis: host plants can be colonized by root symbionts that have been inactive for centuries to millenia

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Jason Pither[1] and Brian J. Pickles[2]*

[1]Okanagan Institute for Biodiversity, Resilience, and Ecosystem Services, University of British Columbia, Okanagan campus, ASC 367, 3187 University Way, Kelowna, BC, V1V 1V7, Canada.

[2]School of Biological Sciences, University of Reading, Harborne Building, Whiteknights, Reading, RG6 8AS, UK.

Tel: +44 118 378 7955

**Email: b.j.pickles@reading.ac.uk*

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Abstract

Paleoecologists have speculated that post-glacial migration of tree species could have been facilitated by mycorrhizal symbionts surviving glaciation as resistant propagules belowground. The general premise of this idea, which we call the "paleosymbiosis hypothesis", is that host plants can access and be colonized by fungal root symbionts that have been inactive for millennia. Here, we explore the plausibility of this hypothesis by synthesizing relevant findings from a diverse literature. For example, the paleoecology literature provided evidence of modern roots penetrating paleosols containing ancient (> 6000 yr) fungal propagules, though these were of unknown condition. With respect to propagule longevity, the available evidence is of mixed quality, but includes convincing examples consistent with the paleosymbiosis hypothesis (i.e. > 1000 yr viable propagules). We describe symbiont traits and environmental conditions that should favour the development and preservation of an ancient propagule bank, and discuss the implications for our understanding of soil symbiont diversity and ecosystem functioning. We conclude that the paleosymbiosis hypothesis is plausible in locations where propagule deposition and preservation conditions are favourable (e.g. permafrost regions). We encourage future below-ground research to consider and explore the potential temporal origins of root symbioses.

One-sentence summary

The authors assess evidence that certain mycorrhizal fungi may produce resistant propagules that remain viable for hundreds to thousands of years before colonizing host plants, a process they call "paleosymbiosis".

Introduction

In his review of post-glacial conifer species migration in the Pacific Northwest, Elias (2013) speculated about the role naturally resurrected mycorrhizal fungi might have played: "Did mycorrhizal fungi survive the last glaciation in soils buried by glacial ice?" (p. 67). Much earlier, Wilkinson (1998) penned similar thoughts, wondering whether fungal spores could have remained viable in permafrost / sediment for thousands of years, and subsequently played a role in plant migration. We suspect that many mycorrhizal researchers would, at first glance, consider these ideas rather fanciful, and would instead favour co-migration (e.g. Murat et al. 2004; Kennedy et al. 2011) or long-range dispersal of fungal propagules (e.g. Moyersoen, Beever and Martin 2003; Geml *et al.* 2012) as processes by which mycorrhizal fungi assist host migration. However, despite a long-standing interest in fungal persistence and viability (e.g. Willetts 1971; Miller et al. 1994), and despite the crucial roles that "resistant propagule banks" (RPBs) can play in ecosystems for plants (Venable and Brown 1988) and microbes (Jones and Lennon 2010; Lennon and Jones 2011), our knowledge about the longevity and infectivity of fungal spores and sclerotia remains extremely limited. Here, we further develop the initial ideas of Wilkinson (1998) and Elias (2013), and propose what we call the "paleosymbiosis hypothesis": host plants can access and be colonized by fungal root symbionts that have been inactive for centuries to millenia. We summarize what is known and assumed about the key requirements of this hypothesis, namely the availability of ancient and viable inoculum, and the likelihood that said inoculum would colonize the roots of contemporary hosts. Next we provide a brief overview of the evidence in support of long-term viability of fungal inoculum. We then discuss favourable conditions for the development and preservation of ancient RPBs, suggest promising locations for future study, and explore the implications of paleosymbiosis. We

conclude by proposing a strategy for future research aimed at rigorously testing our hypothesis, especially as a complement and / or alternative to dispersal-related hypotheses.

Facts and assumptions about the RPB and its relevance to hosts

1. *General characteristics of the RPB.* A vast reservoir of dormant but potentially viable bacterial and fungal inoculum exists belowground (Jones & Lennon, 2010; Lennon & Jones, 2011). Soil microbial biomass, of which the RPB is a component, generally decreases in size with increasing sampling depth (Xu, Thornton and Post 2013). One exception may include fire-prone ecosystems, where deeper mineral soils that escape lethal heat can host greater abundance of viable ectomycorrhizal fungi (EMF) propagules (e.g. Baar *et al.* 1999). In the largest RPB study to date, and based on soil samples 14 cm deep, Glassman *et al.* (2015) (see also Talbot et al. 2014) found the RPBs of *Pinus*-dominated ecosystems in North America to be highly variable in taxonomic composition, hosting some common genera that are often conspicuous components of the RPB in similar ecosystems (e.g. *Cenococcum, Laccaria, Rhizopogon, Suillus, Tuber, Wilcoxina*) alongside numerous rarer taxa, and often differing significantly in composition from paired soil fungal sequences (as has been observed previously; e.g. Taylor & Bruns 1999). As most ectomycorrhizal RPB research has concerned *Pinus*-dominated forests (e.g. Baar et al. 1999; Bruns et al. 2009; Glassman et al. 2015), we can only assume that similar dominance-rarity patterns would be found in other ecosystems.

2. *Age distribution within the RPB.* Based on available evidence and sound reasoning, the majority of viable inoculum most likely spans a range of ages from a few years or less (e.g. recently released spores of fruiting bodies; Nara 2009) to multiple decades (e.g. the resistant spores and sclerotia of species that associate with seedlings but are found in mature pine forests; Glassman et al., 2015). The precise form of the age distribution likely varies amongst

ecosystems (via taxonomic and environmental variation), though the historical focus on temperate forest ecosystems (particularly *Pinus* forests) precludes more refined statements on this. The extent to which plant root growth, animal activity, and percolation contribute to temporal mixing of inoculum has not been explored, and we know very little about the absolute abundances (effective inoculum load) that comprise the age distribution (Bruns *et al.* 2009; Nguyen, Hynson and Bruns 2012). Thus the relevance of the inoculum within the tail of the age distribution remains unknown.

3. *Roots interact with ancient inoculum*. In a study undertaken to inform Holocene paleoclimate reconstructions in Lapland, northern Sweden, Hormes et al. (2004) dated sclerotia of the ubiquitous EMF species *Cenococcum geophilum* within paleosols. They found increasingly ancient sclerotia with increasing depth: between ca. 4ky and 6ky and 20 to 30cm, respectively), and plant roots of modern origin penetrating the paleosol layer. Although viability was not tested, this study illustrates that (i) modern hosts can access symbiont propagules of ancient origin, (ii) ancient propagules need not be deep belowground, and (iii) deeper roots likely interact with mycorrhizal inoculum of varied age. Coupled with Thorn et al.'s (2009) evidence of an association between depth and spore age for arbuscular mycorrhizal fungi (AMF), these studies highlight the need to critically evaluate the present working assumption that all root associations – even those at depth – originate with modern inoculum. It is typically assumed that colonizing mycelium follows the downward growth of roots, however this has not been experimentally addressed. Even if this is generally true, wherever deep roots are colonized by spores this may have significant consequences for the population genetics of the EMF species involved. Mechanisms for vertical migration of recent propagules into mineral soil horizons

remain mostly unexplored and, more generally, mycorrhizal communities at depth are rarely studied (Dickie *et al.* 2013; Pickles and Pither 2014).

4. *Colonization by ancient inoculum*. The initiation of symbioses from contemporary mycelium or propagules is well studied, particularly in regards to interspecific competition (e.g. Kennedy, 2010). Where roots do access ancient RPBs, it is assumed that the mechanisms by which ancient inoculum could successfully break dormancy and colonize modern hosts are similar to those described for modern inoculum (e.g. stimulation by root exudates). Much more research on this is warranted (Juge *et al.* 2002; Nara 2009).

5. *Ancient RPBs constitute a potential local source of propagules*. In un-vegetated areas, including recently deglaciated landscapes, established plant communities at the regional scale (e.g. within tens of km) are assumed to be the source of viable mycorrhizal propagules, via long-distance dispersal (e.g. Jumpponen *et al.* 2012; Dickie *et al.* 2013). However, this assumption has rarely, if ever, been directly tested. Spore dispersal by animal vectors (e.g. Colgan and Claridge 2002; Lilleskov and Bruns 2005) is generally biased towards short distances, though exceptions have been noted (e.g. Elk dispersal of AMF spores; Allen 1987). Aerial spore trapping studies have yielded mixed results (propagules rare or common) depending in part upon the mycorrhizal type (AMF versus EMF) (Allen 1987; Allen, Klironomos and Harney 1997; Peay *et al.* 2012; Egan, Li and Klironomos 2014; Kivlin *et al.* 2014), and variation in sampling scale and methodology precludes unequivocal conclusions. Aerial dispersal may be an important process over landscape scales for some taxa (e.g. Kivlin *et al.* 2014), but rather restricted over broad spatial scales (Talbot *et al.* 2014). It is significant that, in their work on aerial spore dispersal by EMF, Peay et al. (2012) and Peay and Bruns (2014) found strong evidence that the dispersal of aerial spores was limited to distances below a few km, and that only approx. 50% of

seedlings ≥ 1 km from a forest edge were colonized by aerially-dispersing EMF. Additionally the diversity of EMF species represented in the aerial spore community was only a small proportion of the EMF community fruiting in these forests. Hence long distance dispersal of host seeds, e.g. by wind or animal vectors, likely increases the chances of colonization of resulting seedlings from the RPB. Thus, any contributions to EMF communities from long distance or landscape-level dispersal do not preclude a role for local RPBs (e.g. see Baar *et al.* 1999), and ancient RPBs could account for the observation by Jumpponen (2003) that *Laccaria* DNA was detected in recently exposed soil on the forefront of the Lyman Glacier.

Evidence of ancient and viable fungal symbiont propagules

The strength of evidence for propagule viability varies depending upon the methods used to test it. Inoculating host seedlings with spores of known age is ideal, though some symbionts appear to favour mature hosts over seedlings (e.g. AMF: Hart *et al.* 2014; EMF: Twieg et al. 2007). Seedling inoculation approaches have frequently been used to test EMF spore viability. In EMF bioassay trials with *Eucalyptus* hosts, Chen *et al.* (2006) found that infectivity of air-dried *Scleroderma* spores was high following storage at 4˚C for 5 years, with significant variation in the response to storage treatments among the three species examined. Five years into a 99-year experiment, Bruns et al. (2009) reported that spores of four *Rhizopogon* species were still viable, and displayed increasing infectivity. As part of the same long-term experiment, Nguyen et al. (2012) reported that spores of *Laccaria proxima*, *Suillus brevipes*, and *Wilcoxina mikolae* were viable after at least 6 years of inactivity. Thus available evidence shows that the spores of some EMF taxa remain viable for several years, but to our knowledge the viability of ancient spores has never been tested. Culture-based methods in which spores are germinated on a growth medium provide the next strongest evidence, but many mycorrhizal species are not

culturable (Smith & Read, 2008; but see Richter, 2008). Furthermore, culturing fungi on agar does not inform about their inoculation potential on hosts. In the absence of inoculation or culture, RNA-based detection of transcribed fungal genes can prove that cellular processes are taking place, i.e. that the spore remains alive (Blazewicz *et al.* 2013), at least in the subset of spores transitioning from dormancy to viability. Cellular activity can also be examined using various viability 'stains' (Miller, Torres and McClean 1993; Torres and Honrubia 1994), although some commonly applied stains cannot detect spores that are viable but currently dormant (Bassani et al. 2013). DNA-based detection methods have been used to detect mycorrhizal spores in soil (Guidot *et al.* 2004) but may be the least diagnostic technique for viability assessment, since, unlike RNA, DNA can persist in the soil environment after cell death (Willerslev and Cooper 2005; Anderson and Cairney 2007). However, DNA from dead material may degrade quickly due to microbial DNase production, resulting in shorter fragments than are attainable from living tissue (see reviews of soil DNA cycling; Levy-Booth et al. 2007; Pietramellara et al. 2008). Therefore DNA evidence might be diagnostic for fungi when restricted to read lengths that are typically obtained from living fungal tissue (e.g. > 400bp), but this has not been clearly established and may vary with the target region.

We searched a broad literature for evidence consistent with long-term viability of fungal symbiont propagules (using a conservative 400bp threshold for DNA evidence based on the activity of restriction endonuclease I), and summarize our findings in Table 1. Although the strength of evidence varies, propagules of fungal genera containing mycorrhizal species (AMF, EMF, and ericoid mycorrhizas), and putatively mutualistic endophytes (Newsham 2011), retained their viability after tens to thousands of years in an inactive state (Table 1). In one case (Kochkina et al. 2012) the preservation of readily culturable fungal spores coincided with

molecular detection of EMF species that resisted growth in culture. These propagules may also have been viable, given that the shared storage environment had successfully preserved the viability of other fungi.

Notwithstanding the evidence we have compiled and presented in Table 1 there are more general, evolutionary reasons to suspect that ancient and viable RPBs exist in nature. The primary determinants of seed longevity are water, temperature, and oxygen, with decreasing temperature and relative humidity tending to favour seed survival (Roberts and Ellis 1989). Bruns et al. (2009), Nara (2009), and Glassman et al. (2015) briefly drew the analogy between resistant plant seeds and resistant fungal propagules (including spores), with Nara (2009) pointing out the likelihood that EMF spores exhibit as much physiological variation in germination/dormancy mechanisms as plant seeds. This appears to be a useful and reasonable analogy, and expanding on it bolsters support for the existence of ancient and viable RPBs. For example, just as some plant species' seeds exhibit traits such as hard coats that limit the detrimental effects of high ambient humidity (e.g. Daws et al. 2007), the spores and sclerotia of many fungal taxa exhibit a variety of traits that confer longevity, including melanin (to guard against UV) and cryoprotectants (e.g. Duman & Olsen, 1993; Robinson, 2001; Tibbett et al. 2002; Tibbett & Cairney, 2007; Maggi et al. 2013). Sclerotia in particular are recognized for their resilience, and are currently known to be produced by EMF taxa from 12 genera (Smith, Henkel and Rollins 2015), including several that are geographically widespread (e.g. *Cenococcum*, *Cortinarius*, *Hebeloma*, *Scleroderma*). Furthermore, to the extent that seed longevity serves as a reasonable guide (e.g. Walters et al. 2005; Daws et al. 2007), timescales of centuries (e.g. *Acacia* spp.; Leino and Edqvist (2010) and millennia (e.g. *Phoenix dactylifera*; Sallon *et al.* 2008) may be reasonable estimates for spores and sclerotia of certain taxa under

specific conditions. For example, germination has been induced in seeds extracted from immature fruits frozen in permafrost ~ 30 Kya (Yashina *et al.* 2012). Given that the germination requirements of fungal spores are likely to vary (Nara 2009), and that spore longevity appears to show high inter- and intra-specific variability (e.g. in insect and plant pathogens; Hong, Ellis and Moore 1997), it would be challenging to disprove viability in the absence of trials that include varied growth conditions. A case in point is provided by spores of the EMF basidiomycete *Pisolithus microcarpus*, which, despite their extreme recalcitrance, clearly possess cellular constituents indicative of provisioning for future germination (Campos & Costa, 2010). For these reasons, we suspect that the list in Table 1 could expand considerably given increased research effort.

Potential reservoirs of ancient, viable propagules

Numerous recent studies discuss methods for long-term preservation of mycorrhizal fungal inoculum (Stielow *et al.* 2011; Homolka 2014; Lalaymia, Cranenbrouck and Declerck 2014), building on older work showing that viable spores of AMF and other fungi can be preserved for extended periods (Douds and Schenck 1990; Chandler 1994; Declerck and Angelo-Van Coppenolle 2000). This research shows that both cryopreservation (e.g. temperatures in the range of -130^{o}C) and refrigeration (e.g. ~ 4^{o}C) can effectively preserve the inoculum of some mycorrhizal fungi for years with no loss in viability (Crahay *et al.* 2013a, 2013b), though the limits of cryopreservation remain to be discovered. Additionally, storage of basidiomycete tissue cultures on Agar in cold water for 20 years was effective in preserving 27 out of 35 EMF isolates (Richter 2008). Thus, maintenance of low temperatures for long periods of time appears conducive to long-term storage of viable inoculum (spores or tissue), perhaps by minimizing susceptibility to degradation by parasitic microbes (e.g. Obase et al. 2014).

When considering specific locations to search for long-term propagule viability *in situ*, studies that successfully isolated and identified ancient fungal DNA, but did not assess viability, provide a useful starting point. Some of the more impressive examples in Table 1 concern propagules collected from permafrost, where subsurface environments may best approximate the lab conditions used in cryopreservation research. Covering approximately 26% of the exposed land area on earth, permafrost represents a vast potential reservoir of viable inoculum. Importantly, suitable mycorrhizal host species (especially generalist taxa such as *Dryas* and *Salix*) have been present intermittently in various Arctic regions throughout the Quaternary (Delcourt and Delcourt 1993; Bartlein *et al.* 1998; Parducci *et al.* 2012; Willerslev *et al.* 2014), serving as a source of potentially diverse inoculum. For example, Bellemain et al. (2013) found ancient fungal communities in permafrost samples from Beringia, which included DNA from putative EMF/endophyte species (approximately 5 – 10 % of the total). The samples were dated to 32 –16.5 Kya. Repeating such a study with the addition of inoculation, culturing, RNA, and/or staining techniques would enable comment on the potential viability of recovered spores. Cryoturbation, in which freeze-thaw cycles led to the burial of topsoil in permafrost, may be a common mechanism that alters the age structure in RPBs of permafrost soils (see Gittel *et al.* 2014), though freeze-thaw cycles may also reduce the viability of inoculum. Glaciers may also serve as a source of viable, ancient inoculum, as previously predicted (Wilkinson, 1998; Ma et al. 2000), both in soil trapped beneath and in ice and cold water inside. Buried forests such as those found in Michigan's Upper Peninsula (Pregitzer & Reed, 2000) and more recently on Kruzof Island, Alaska (http://blogs.usda.gov/2014/03/07/the-buried-forest-of-alaskas-kruzof-island-a-window-into-the-past/; last accessed December 12, 2016) may be especially profitable to explore,

as the preserved hosts remain on site, minimizing uncertainties regarding potential sources of any ancient symbiont inoculum.

In contrast to the above examples, hot-dry environments may also promote longevity of ancient spores, e.g. the aridity of the Great Basin may favour preservation of fungal tissue (Homolka 2014; Lalaymia, Cranenbrouck and Declerck 2014). Fungal communities associated with long-lived bristlecone pine (*Pinus longaeva*) individuals within the Great Basin (USA) may provide promising sampling opportunities, as their associated fungal symbionts could have deposited spores centuries or thousands of years ago. Interestingly, Bidartondo et al. (2001) explored the inoculation potential of soils in this very region, but used only the top 20 cm of soil which is likely to be comparatively recent in origin (Holliday, Meltzer and Mandel 2011).

Stratigraphic cores collected as part of paleoecological and paleoclimatological studies could provide a means to test the more general hypothesis that ancient propagules can be viable. For example, Anderson et al. (1984) and van Geel et al. (2011) found *Glomus* spores in their respective lake sediment cores (Maine, USA and Lake Challa, Kenya), which they used to interpret the vegetation history of the region over the last 12.5 Kya or 25 Kya, respectively. Despite the preponderance of paleoecological studies relying on fungal material (see Porter et al. 2013), we are unaware of any studies documenting attempts to revive said material. The sample preparation process for palynology destroys most basidiomycota and renders ascomycota non-viable (van Geel *et al.* 2011). Nonetheless, many paleoecologists may be in the possession of soil, sediment, or ice cores that could harbour ancient, viable inoculum. While contamination may be a problem in older cores (Willerslev, Hansen and Poinar 2004) those extracted using newer, cleaner methods (e.g. Rogers et al. 2004) should prove suitable targets for revival attempts.

Implications of ancient, viable RPBs and the paleosymbiosis hypothesis

If RPBs are found to include ancient and viable inoculum, the implications are potentially far-reaching, particularly for our understanding of diversity patterns (Locey 2010) and ecosystem functioning (Lennon and Jones 2011). With respect to biogeographical patterns, gene flow among fungal populations via RPBs could provide an additional and/or alternative explanation to long-distance dispersal (spatial gene flow) for the lack of spatial genetic structuring among arctic fungi (Geml 2011; Geml *et al.* 2012; Timling *et al.* 2014). Likewise, ancient RPBs could explain the presence of EMF and AMF propagules in recently exposed substrate at glacial forefronts (Jumpponen 2003; Jumpponen *et al.* 2012; Rime *et al.* 2015; Rime, Hartmann and Frey 2016). With respect to diversity patterns, RPBs can be expected to reduce rates of local extinction, thereby maintaining higher levels of α-diversity. However, all of these potential mechanistic effects of RPBs remain largely untested. Thus, increased focus on the RPB, particularly at depths beyond those typically surveyed (Pickles and Pither 2014), is likely to modify the recently documented diversity patterns among EMF (Tedersoo *et al.* 2012, 2014) and AMF (Davison *et al.* 2015) taxa. However, the degree to which overall diversity patterns are altered by accounting for the RPB will depend upon the proportion of taxa that contribute to it – about which little is known (see Schadt & Rosling's (2015) discussion of hidden fungal diversity). One of the most extensive studies of EMF RPBs concluded that spore bank α-diversity is low across the pine forests of North America (Glassman *et al*. 2015). However, this study examined seedling root tip EMF communities that formed on pine seedlings placed in dried soil as a proxy for the RPB spore community, and seedlings are typically observed to associate with a small subset of the total EMF community (see for e.g. Massicotte *et al*. 1999; Twieg *et al*. 2007; Pickles *et al*. 2015). We also note that in their study of glacial forefront bacterial and fungal communities, at different

depths and stages of soil development, Rime *et al.* (2015) found that the relative abundance of Glomeromycotan sequences (AMF) increased with increasing soil development, but was greatest at the lowest sampled depths (18-20 cm) of the least vegetated soils. The same study detected the presence of several EMF species at 4-6 cm and/or 18-20 cm depth, but not at 0-1 cm, in barren and sparsely vegetated soils. These observations suggest that the mycorrhizal fungi encountered at depth were old spores rather than recently dispersed.

In addition to containing reservoirs of genetic diversity, ancient RPBs could also be important reservoirs of functional diversity (Kerfoot, Robbins and Weider 1999; Lennon and Jones 2011). Frisch et al. (2014) showed this was the case in populations of *Daphnia*: lineages derived from centuries-old cysts displayed significantly higher growth efficiency in low phosphorus conditions compared to recently-derived lineages exposed to eutrophic conditions. The mixing of functional capabilities among lineages of varied temporal origin (e.g. Kerfoot & Weider, 2004) may be an important aspect of host-symbiont ecology (as is intra-specific functional diversity; Johnson et al. 2012), but to our knowledge has not been explored. For instance, successful colonists from the RPB could include symbiont lineages that bear the unique phylogenetic and functional legacies of associations from the distant past. Such details could be especially relevant to better understanding host responses to global change (Lennon and Jones 2011; Jumpponen and Brown 2014).

Lastly, ancient RPBs have the potential to dramatically extend the scope of so-called "legacy effects" in soils (i.e. the influence of historical soil communities on contemporary ecosystem processes), which have generally been applied to short term effects (e.g. months to years; Van de Voorde et al. 2011; Van der Putten & Bardgett, 2013; Meisner et al. 2013). In

short, a form of ‘deep time legacy effect’ could emerge, and be especially pronounced if the functionality of the RPB differs from that of contemporary symbionts.

Strategies for future research

Key evidence in support of the paleosymbiosis hypothesis would be successful inoculation of present-day plant roots with ancient fungal inoculum. To this end, we suggest the following research strategy: Identify locations with favourable conditions for long-term preservation of a RPB. Extract soil cores for propagule isolation and depth profile dating (e.g. carbon dating via accelerator mass spectrometry). Ideally, dating would be conducted on subsamples of propagule material throughout the soil profile, though collecting sufficient material will be challenging. Collect and surface sterilize seeds of naturally occurring host taxa that would encounter the propagules during their lifecycle (e.g. through glacial melt or defrosting permafrost). Perform growth experiments (with controls) in which seeds are sown in soil inoculated with collected propagules. Inventory fungal communities isolated from roots and soil using DNA/RNA approaches. Communicate experimental outcomes, whether germination was successful or not. A tendency to publish only “successful” germination trials will yield a biased view of the relevance of the ancient RPB. It is also important to utilize multiple potential host species, to expand the potential diversity of mycorrhizal fungi exhibiting inoculation success, and to attempt to extract spores from the soil to determine the total potential diversity of the RPB rather than purely estimating its diversity from the subset of species that associate with bait seedlings.

Conclusion

Although the extent of its role in ecosystem processes is currently unknown, the key components of the paleosymbiosis hypothesis appear plausible and worthy of further

examination. We therefore encourage research that tests this hypothesis, especially as an alternative for, or compliment to, hypotheses invoking rapid and/or long-distance dispersal of viable fungal propagules. Paleosymbioses are most likely to be found among the subset of symbiont taxa that possess the traits required for long-term persistence, and in locations where all the necessary conditions exist, including (i) a large source of symbiont propagules through time, (ii) preservation conditions that are favourable, (iii) RPBs that are accessible to contemporary hosts, and (iv) a contemporary vegetation that includes compatible hosts. To our knowledge, these characteristics have not previously been considered in research concerning RPBs, hence our optimism and excitement for what the future holds.

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Table 1. Examples of studies from the literature in which evidence consistent with long-term viability of fungal propagules was provided.

Viability assessment	**Organism**	**Ecology[1]**	**Storage Material**	**Estimated age (ya)**	**Reference**
Inoculation	*Acaulospora* spp.	AM	Arctic tundra soil	$\geq$ 10	(Varga *et al.* 2015)
	Ambispora sp.	AM	frozen at -20^{o}C	$\geq$ 10	
	Archaeospora sp.	AM		$\geq$ 1	
	Funneliformis sp.	AM		$\geq$ 8	
	Glomus spp.	AM		$\geq$ 10	
	Paraglomus sp.	AM		$\geq$ 10	
	Rhizophagus sp.	AM		$\geq$ 8	
	Sclerocystis sp.	AM		$\geq$ 8	
	Scutellospora sp.	AM		$\geq$ 8	
	Septoglomus sp.	AM		$\geq$ 10	

Inoculation	*Laccaria* sp. *Rhizopogon* sp. *Suillus* sp. *Wilcoxina* sp.	ECM ECM ECM ECM	Forest soil	≥ 6	(Nguyen, Hynson and Bruns 2012)
Inoculation	*Scleroderma* spp.	ECM	Sealed container, 4°C	5	(Chen, Dell and Malajczuk 2006)
Inoculation	*Rhizopogon* spp.	ECM	Refrigerated tap water	3	(Castellano and Molina 1989)
Cultured	*Oidiodendron* *Trichoderma*	ErM Multi	Antarctic permafrost Arctic permafrost	0.17 M 0.1 M	(Kochkina *et al.* 2001)
Cultured	*Pleurotus* sp.	Sapro	Greenland ice core	0.14 M	(Ma *et al.* 2000)
Culture failed; DNA obtained	*Cortinarius* sp. *Imaia* sp. [2]	ECM ECM	Antarctic ice core	>7.5 K	(Kochkina *et al.* 2012)
rRNA and DNA obtained[2]	*Phialocephala* sp. Agaricomycotina spp. Pezizomycotina spp.	DSE Multi Multi	Alaskan permafrost	≤4.1 K	(Coolen *et al.* 2011)
rRNA obtained	*Diversispora* sp. *Glomus* sp. Agaricales Dothidiomycetes *Entoloma* sp. Pyronemataceae Hypocreales *Rhizoctonia* sp. *Mycena* spp.	AM AM ECM+ ECM+ ECM+ ECM+ ErM+ Patho Sapro	Deep sea sediment	0.03 M 0.03 M 0.1–2.6 M 2.77 M 0.1 M 2.6 M 0.1–2 M 2.77 M 0.03-2.77 M	(Orsi, Biddle and Edgcomb 2013)
DNA obtained	*Cadophora* sp. *Cortinarius* sp. *Entoloma* sp. *Hydnum* sp. *Suillus* sp.	ECM+ ECM ECM+ ECM ECM	Western Beringian Permafrost	0.3 – 0.4 M >1 K 0 – 0.4 M >1 K >1 K	(Lydolph *et al.* 2005)

[1] AM = Arbuscular mycorrhizal; DSE = Dark Septate Endophyte; ECM = Ectomycorrhizal; ECM+ = Phylogenetic group includes ECM species; ErM = Ericoid mycorrhizal; ErM+ = Phylogenetic group includes ErM species; Multi = Multiple ecological types; Patho = Pathogenic species; Sapro = Saprotrophic species.

[2] DNA sequence length unknown.