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Article

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Still scratching the surface: how much of the "black box" of soil ectomycorrhizal communities remains in the dark?

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Article

Symbiotic soil organisms such as ectomycorrhizal fungi (EMF) were long thought of as an inscrutable “black-box”, yet the advent of molecular technologies has driven rapid advances in identification and enumeration of their diversity (Horton & Bruns, 2001; Buée *et al*., 2009). For instance, one 20 cm soil core can impressively yield 100’s of fungal OTUs (Taylor *et al.*, 2013). Importantly root symbionts play functional roles in sequestration or breakdown of soil carbon pools (Trumbore & Czimczik, 2008; Harrison *et al*., 2011a,b; Clemmensen *et al.*, 2013; Kramer *et al*., 2013), nutrient and water cycling (Virginia *et al*., 1986; Read & Perez-Moreno, 2003), alteration of soil porosity (Perry *et al.*, 1990), and provision of sustenance for different trophic levels (Coleman & Whitman, 2005).

Yet root symbionts occur and contribute to function far deeper in the soil than is usually sampled (Jenkins *et al*., 1988; Dalpé *et al*., 2000; Bornyasz *et al*., 2005). Soil properties vary considerably among ecosystems (Schenk, 2005; Dickie *et al.*, 2013), hence so too does rooting depth (see below) – even within a single species (Stone & Kalisz, 1991; Canadell *et al*., 1996). However, in practice, we are (understandably) encouraged to employ uniform sampling strategies, even if these are known to only scratch the surface of potential symbiont habitat in some ecosystems (see below). Although the issue of limited-depth sampling has been raised before (Taylor, 2002), we are unaware of any efforts to quantify how much of the "black box" typically remains out of reach of standard sampling techniques. Such information would be extremely timely, due to the growing interest in accurately characterizing global patterns of EMF diversity and distribution (Dickie & Moyerson 2008; Vellinga *et al*., 2009; Tedersoo *et al*., 2012).

To begin addressing this question, we gathered sampling depth data from recent field studies of EMF, and analysed these in relation to published data compiled by ecosystem ecologists regarding (i) maximum rooting depths of trees and shrubs, including 137 EMF host species distributed among 29 host genera, and (ii) estimates for 8 ecosystems of the mean depth above which 95% of all roots are located. Rooting depth data were derived from the following sources: (i) EMF host species/genera from Stone & Kalisz (1991) and Canadell *et al.* (1996), (ii) ecosystem data from Schenk & Jackson (2002). Sampling depth data were obtained from EMF studies published in the last 5 years of *New Phytologist* (Table S1). While the concepts discussed here are equally applicable to all soil-borne root symbionts, for the sake of brevity we focus our attention specifically on EMF and their hosts.

Based on 27 articles that reported sampling depth, the average was 13.4 cm (± 1.59 s.e.m.), with a median value of 10 cm. This sampling depth was approximately doubled in boreal and semi-arid ecosystems, and halved in semi-arid and tropical evergreen ecosystems. In comparison, none of the 29 ectomycorrhizal host genera for which data was available exhibited maximum rooting depths shallower than 50 cm (Fig. 1a), and on average maximum rooting depth among the 137 host species is 530 cm (± 44 cm s.e.m.) (Fig. 1b). Correspondingly, the average proportion of maximum rooting depth assessed is estimated to be 0.068 (± 0.0071 s.e.m.) across all host genera. If we consider maximum rooting depth as a proxy for the amount of habitat available to symbionts, then an enormous amount of potential habitat remains under-sampled, even within the Pinaceae (Fig. 1), which, according to a 2008 literature survey (Dickie & Moyerson, 2008), represented the focal family in 62% of all EMF studies.

Although maximum rooting depth is a crucial variable in research examining ecosystem function (Canadell *et al.*, 1996; Jackson *et al.*, 1996; Schenk, 2005), it could be argued that for our purposes it provides an overly pessimistic outlook on the completeness of current sampling efforts. We therefore also considered the EMF sampling depth data in relation to estimates of ecosystem-specific mean rooting depths calculated using 16 to 59 observations per ecosystem type, spanning all tree and shrub species for which rooting depth data existed (Schenk & Jackson, 2002). Using an average sampling depth of 13.4 cm, the proportion of the mean ecosystem rooting depth sampled varied from a high of 0.47 for tundra, to a low of 0.08 for

Mediterranean ecosystems (Fig. 2a), with a mean of 0.178 ($\pm$ 0.0442 s.e.m.). Thus, even in tundra ecosystems where rooting depths are comparatively shallow (Fig. 2b), typical sampling methods are likely to access less than 50% of the mean depth of host roots.

Although striking, our findings do not necessarily mean that standard sampling methods are always doomed to miss an important or sizeable component of the symbiont assemblage associated with any given host. Indeed, it is likely that some studies - especially those occurring in shallow rooting regions (e.g. tundra ecosystems) - could yield reasonable estimates of the actual number of symbiont species associated with the host (using appropriate analytical techniques; cf. Gotelli & Colwell, 2001). Nevertheless, it has long been acknowledged that important characteristics of EMF communities vary with depth, but only in recent years have studies begun to clarify these details. For example, fungal hyphae show vertical niche differentiation (Dickie *et al*., 2002), EMF community composition changes between soil horizons (Rosling *et al.*, 2003), ECM root tips and EMF extramatrical mycelium differ in their vertical structure (Genney *et al*., 2006), and other depth-associated patterns continue to emerge (e.g. Egerton-Warburton *et al.*, 2003; Landeweert *et al*., 2003; Baier *et al*., 2006; Lindahl *et al*., 2007; Courty *et al*., 2008; Scattolin *et al*., 2008; Beiler *et al*., 2010; Clemmensen *et al*., 2013; Taylor *et al*,. 2013). These observations, combined with our findings, substantiate earlier statements that current sampling methods provide a limited view of EMF assemblages (Taylor, 2002). Until more effort is spent sampling and characterising symbiont diversity and function at depth, we cannot know the true extent of these limitations.

Since deep roots are features of most ecosystems worldwide (Schenk & Jackson, 2005), the discoveries that could come with deeper sampling have the potential to profoundly change our outlook on patterns of EMF diversity and function. To illustrate, consider a recent and enlightening global-extent meta-analysis of local EMF diversity (Tedersoo *et al*., 2012). Based on data from 55 published studies, total species richness (representing site-level species richness) was significantly associated with a number of climate-based predictor variables (e.g.

mean annual temperature, mean annual precipitation), and not surprisingly, number of samples and total sample volume. However, the meta-analysis included data gathered from a variety of host genera and ecosystem types, meaning that the rooting depths of hosts also varied substantially (see above). It would be interesting to determine if and how their findings would change if sampling was deeper, or was adjusted to account for site-specific rooting depths. Because different communities arise with increasing depth, we predict that deeper sampling will increase estimates of total richness and reveal significant changes in community composition. Perhaps every additional 50 cm of depth explored could provide as much richness again as that found in the organic horizon (as per Rosling *et al.*, 2003 & Landeweert *et al.*, 2003)? Based upon our findings, we speculate that the magnitude of this total increase will vary significantly with ecosystem type due to the differences in host rooting depth and density.

Variation in the rooting depth of a given host species is related to multiple factors including age, depth to bedrock, mean annual precipitation, mean annual potential evapotranspiration, and depth to the water table (Schenk 2005), all co-varying with ecosystem type. Thus, a Douglas-fir growing in seasonally dry evergreen forest is more likely to develop deep roots than one growing in a cool-temperate to sub-boreal region (cf. Schenk & Jackson, 2002). This has implications for sampling strategies (see below), and suggests that host species distributed across multiple ecosystem types, like Douglas-fir, may be associated with a much more diverse pool of EMF symbionts than current estimates indicate. This combination of varied rooting depths and soil environments provides a greater diversity of habitat to symbionts than do hosts whose distributions are predominantly restricted to a single ecosystem type (e.g. black spruce).

Another important finding concerns the thoroughness with which sampling methods are described in published articles. Of the 30 EMF studies published in the past 5 years in *New Phytologist*, 3 (10%) failed to report details about sampling depth. More generally, whereas some authors give detailed descriptions of the soil environment in relation to sampling strategy (e.g. Smith *et al.*, 2005; Ryberg *et al.*, 2009), depth information occasionally has to be derived or is missing entirely. We suggest that where possible, details about sampling should be accompanied by estimates of average rooting depths at the site, for the host species of interest, even if these estimates are speculative. This would provide for better and more consistent estimates of realized sampling effort across studies.

Lastly, future research may not only require deeper sampling to minimise bias (depending upon the research objectives), but may also need to stratify sampling geographically according to potential rooting depth. Combining global estimates of soil depth (http://www.fao.org/nr/land/soils/harmonized-world-soil-database/en/) with global estimates of deep root distributions (Schenk & Jackson, 2005) and species' ranges (e.g. http://esp.cr.usgs.gov/data/little/) could help hone in on potential sampling regions, and ground penetrating radar technology (Sucre *et al*., 2011) could be used to identify final sample locations. The logistical impediments associated with deep soil sampling (including cost; Harrison *et al.*, 2011) are daunting, but other research areas point to possible solutions, such as using drilling equipment to acquire ice or sediment cores (Nogué *et al.*, 2013), or using excavation machinery such as a backhoe (Bornyasz *et al.*, 2005). These challenges are worth tackling given the potentially crucial roles that symbionts at depth may play in ecosystem function (e.g. Clemmensen *et al.* 2013; Kramer *et al.* 2013).

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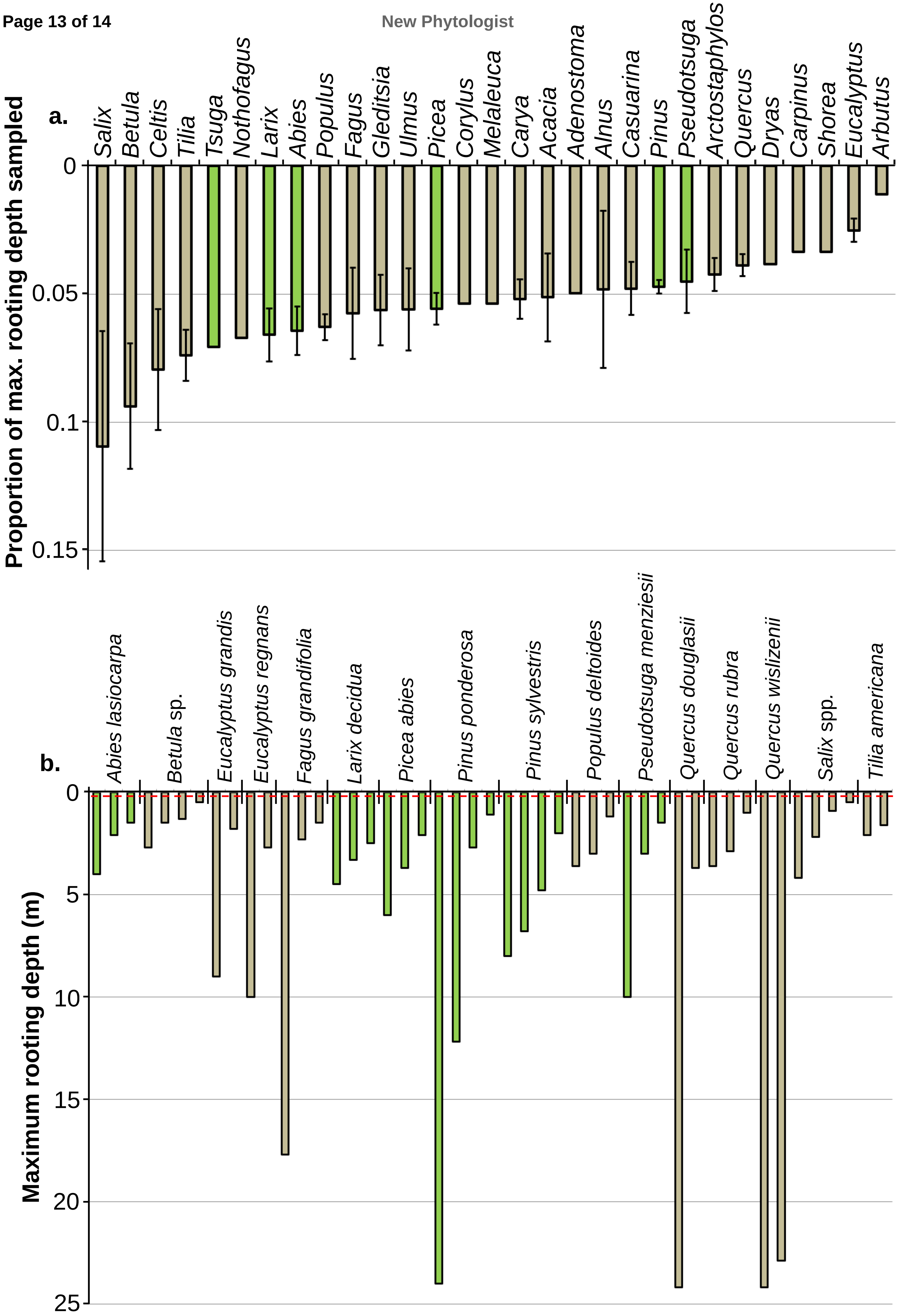
Figure legends

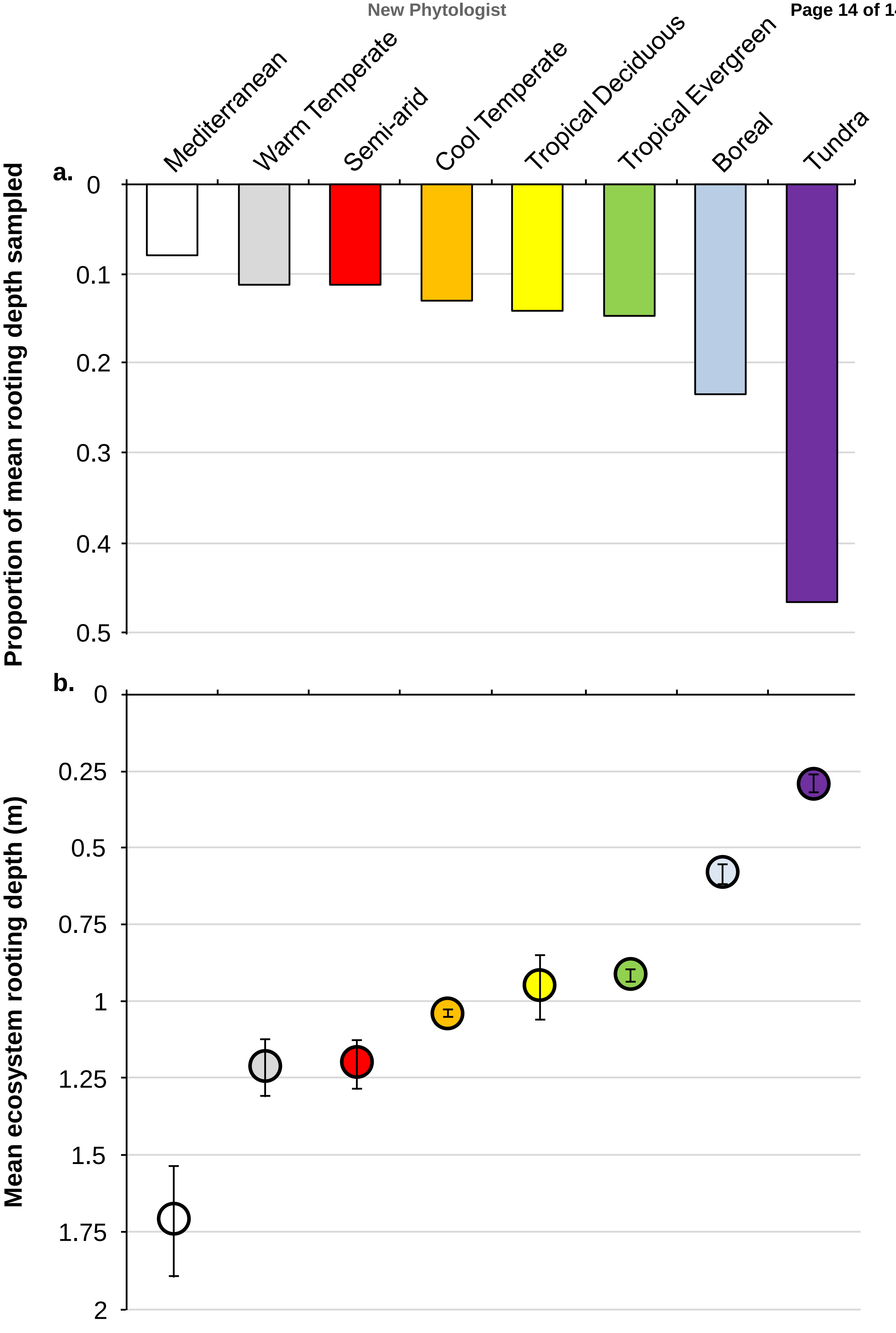
Figure 1. a. Proportion of maximum recorded rooting depth examined (± s.e.m.) across genera using mean sampling depth derived from values reported in the literature (Table S1). Note maximum y-axis value is a proportion of 0.15. b. Maximum rooting depths of selected host species, with multiple bars (records) per species. Dashed red line represents average sampling depth of EMF studies. In both panels, green bars indicate genera or species in the Pinaceae.

Figure 2. a. Sampled proportion of the mean depth at which 95% of ecosystem roots are located, calculated using mean of sampling values reported in literature (Table S1). b. Estimated mean depth (± s.e.m.) at which 95% of ecosystem roots are located using the interpolated values of Schenk & Jackson (2002).

Supporting Information

Table S1. Citation, sample depth and host species for all ectomycorrhizal articles from the last 5 years of *New Phytologist* in which sampling depth was provided.


a.
Proportion of max. rooting depth sampled
0
0.05
0.1
0.15
Salix
Betula
Celtis
Tilia
Tsuga
Nothofagus
Larix
Abies
Populus
Fagus
Gleditsia
Ulmus
Picea
Corylus
Melaleuca
Carya
Acacia
Adenostoma
Alnus
Casuarina
Pinus
Pseudotsuga
Arctostaphylos
Quercus
Dryas
Carpinus
Shorea
Eucalyptus
Arbutus
b.
Maximum rooting depth (m)
0
5
10
15
20
25
Abies lasiocarpa
Betula sp.
Eucalyptus grandis
Eucalyptus regnans
Fagus grandifolia
Larix decidua
Picea abies
Pinus ponderosa
Pinus sylvestris
Populus deltoides
Pseudotsuga menziesii
Quercus douglasii
Quercus rubra
Quercus wislizenii
Salix spp.
Tilia americana


Mediterranean
Warm Temperate
Semi-arid
Cool Temperate
Tropical Deciduous
Tropical Evergreen
Boreal
Tundra
a.
Proportion of mean rooting depth sampled
0
0.1
0.2
0.3
0.4
0.5
b.
Mean ecosystem rooting depth (m)
0
0.25
0.5
0.75
1
1.25
1.5
1.75
2