

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

Article

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1 **Still scratching the surface: how much of the “black box” of soil**  
2 **ectomycorrhizal communities remains in the dark?**

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16

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20 **Article**

21 Symbiotic soil organisms such as ectomycorrhizal fungi (EMF) were long thought of as an  
22 inscrutable “black-box”, yet the advent of molecular technologies has driven rapid advances in  
23 identification and enumeration of their diversity (Horton & Bruns, 2001; Buée *et al.*, 2009).  
24 For instance, one 20 cm soil core can impressively yield 100’s of fungal OTUs (Taylor *et al.*,  
25 2013). Importantly root symbionts play functional roles in sequestration or breakdown of soil  
26 carbon pools (Trumbore & Czimczik, 2008; Harrison *et al.*, 2011a,b; Clemmensen *et al.*, 2013;  
27 Kramer *et al.*, 2013), nutrient and water cycling (Virginia *et al.*, 1986; Read & Perez-Moreno,  
28 2003), alteration of soil porosity (Perry *et al.*, 1990), and provision of sustenance for different  
29 trophic levels (Coleman & Whitman, 2005).

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

42 To begin addressing this question, we gathered sampling depth data from recent field studies  
43 of EMF, and analysed these in relation to published data compiled by ecosystem ecologists  
44 regarding (i) maximum rooting depths of trees and shrubs, including 137 EMF host species  
45 distributed among 29 host genera, and (ii) estimates for 8 ecosystems of the mean depth above

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46 which 95% of all roots are located. Rooting depth data were derived from the following  
47 sources: (i) EMF host species/genera from Stone & Kalisz (1991) and Canadell *et al.* (1996),  
48 (ii) ecosystem data from Schenk & Jackson (2002). Sampling depth data were obtained from  
49 EMF studies published in the last 5 years of *New Phytologist* (Table S1). While the concepts  
50 discussed here are equally applicable to all soil-borne root symbionts, for the sake of brevity  
51 we focus our attention specifically on EMF and their hosts.

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

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

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

75 Although striking, our findings do not necessarily mean that standard sampling methods are  
76 always doomed to miss an important or sizeable component of the symbiont assemblage  
77 associated with any given host. Indeed, it is likely that some studies - especially those  
78 occurring in shallow rooting regions (e.g. tundra ecosystems) - could yield reasonable  
79 estimates of the actual number of symbiont species associated with the host (using appropriate  
80 analytical techniques; cf. Gotelli & Colwell, 2001). Nevertheless, it has long been  
81 acknowledged that important characteristics of EMF communities vary with depth, but only in  
82 recent years have studies begun to clarify these details. For example, fungal hyphae show  
83 vertical niche differentiation (Dickie *et al.*, 2002), EMF community composition changes  
84 between soil horizons (Rosling *et al.*, 2003), ECM root tips and EMF extramatrical mycelium  
85 differ in their vertical structure (Genney *et al.*, 2006), and other depth-associated patterns  
86 continue to emerge (e.g. Egerton-Warburton *et al.*, 2003; Landeweert *et al.*, 2003; Baier *et al.*,  
87 2006; Lindahl *et al.*, 2007; Courty *et al.*, 2008; Scattolin *et al.*, 2008; Beiler *et al.*, 2010;  
88 Clemmensen *et al.*, 2013; Taylor *et al.*, 2013). These observations, combined with our  
89 findings, substantiate earlier statements that current sampling methods provide a limited view  
90 of EMF assemblages (Taylor, 2002). Until more effort is spent sampling and characterising  
91 symbiont diversity and function at depth, we cannot know the true extent of these limitations.

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

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

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

119 Another important finding concerns the thoroughness with which sampling methods are  
120 described in published articles. Of the 30 EMF studies published in the past 5 years in *New*  
121 *Phytologist*, 3 (10%) failed to report details about sampling depth. More generally, whereas  
122 some authors give detailed descriptions of the soil environment in relation to sampling strategy  
123 (e.g. Smith *et al.*, 2005; Ryberg *et al.*, 2009), depth information occasionally has to be derived



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124 or is missing entirely. We suggest that where possible, details about sampling should be  
125 accompanied by estimates of average rooting depths at the site, for the host species of interest,  
126 even if these estimates are speculative. This would provide for better and more consistent  
127 estimates of realized sampling effort across studies.

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

141

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151 **References**

- 152 Baier R, Ingenhaag J, Blaschke H, Gottlein A, Agerer R. 2006. Vertical distribution of an  
153 ectomycorrhizal community in upper soil horizons of a young Norway spruce (*Picea abies* [L.]  
154 Karst.) stand of the Bavarian Limestone Alps. *Mycorrhiza* **16**: 197-206.
- 155 Beiler KJ, Durall DM, Simard SW, Maxwell SA, Kretzer AM. 2010. Architecture of the  
156 wood-wide web: *Rhizopogon* spp. genets link multiple Douglas-fir cohorts. *New Phytologist*  
157 **185**: 543-553.
- 158 Bornyasz MA, Graham RC, Allen MF. 2005. Ectomycorrhizae in a soil-weathered granitic  
159 bedrock regolith: Linking matrix resources to plants. *Geoderma* **126**: 141-160.
- 160 Buée M, Reich M, Murat C, Morin E, Nilsson RH, Uroz S, Martin F. 2009. 454  
161 pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New*  
162 *Phytologist* **184**: 449-456.
- 163 Canadell J, Jackson RB, Ehleringer JR, Mooney HA, Sala OE, Schulze E-D. 1996. Maximum  
164 rooting depth of vegetation types at the global scale. *Oecologia* **108**: 583-595.
- 165 Clemmensen KE, Bahr A, Ovaskainen O, Dahlberg A, Ekblad A, Wallander H, Stenlid J,  
166 Finlay RD, Wardle DA, Lindahl BD. 2013. Roots and associated fungi drive long-term carbon  
167 sequestration in Boreal forest. *Science* **339**: 1615-1618.
- 168 Coleman DC, Whitman WB. 2005. Linking species richness, biodiversity and ecosystem  
169 function in soil systems. *Pedobiologia* **49**: 479-497.
- 170 Courty P-E, Franc A, Pierrat J-C, Garbaye J. 2008. Temporal changes in the ectomycorrhizal  
171 community in two soil horizons of a temperate oak forest. *Applied and Environmental*  
172 *Microbiology* **79**: 5792-5801.
- 173 Dalpé Y, Diop TA, Plenchette C, Gueye M. 2000. Glomales species associated with surface  
174 and deep rhizosphere of *Faidherbia albida* in Senegal. *Mycorrhiza* **10**: 125-129.

Pickles & Pither 2013

- 175 Dickie IA, Moyerson B. 2008. Towards a global view of ectomycorrhizas. *New Phytologist*  
176 **180**: 263-265.
- 177 Dickie IA, Xu B, Koide RT. 2002. Vertical niche differentiation of ectomycorrhizal hyphae in  
178 soil as shown by T-RFLP analysis. *New Phytologist* **156**: 527-535.
- 179 Dickie IA, Martinez-Garcia LB, Koele N, Grelet G-A, Tylianakis JM, Peltzer DA, Richardson  
180 SJ. 2013. Mycorrhizas and mycorrhizal fungal communities throughout ecosystem  
181 development. *Plant & Soil* **367**: 11-39.
- 182 Egerton-Warburton LM, Graham RC, Hubbert KR. 2003. Spatial variability in mycorrhizal  
183 hyphae and nutrient and water availability in a soil-weathered bedrock profile. *Plant and Soil*  
184 **249**: 331-342.
- 185 Genney DR, Anderson IC, Alexander IA. 2006. Fine-scale distribution of pine  
186 ectomycorrhizas and their extramatrical mycelium. *New Phytologist* **170**: 381-390.
- 187 Gotelli NJ, Coldwell RK. 2001. Quantifying biodiversity: procedures and pitfalls in the  
188 measurement and comparison of species richness. *Ecology Letters* **4**: 379-391.
- 189 Harrison RB, Footen PW, Strahm BD. 2011a. Deep soil horizons: contribution and importance  
190 to soil carbon pools and in assessing whole-ecosystem response to management and global  
191 change. *Forest Science* **57**: 67-76.
- 192 Harrison RB, Richter D, Fox T. 2011b. Deep soils. *Forest Science* **57**: 1-2.
- 193 Horton TR, Bruns TD. 2001. The molecular revolution in ectomycorrhizal ecology: peeking  
194 into the black-box. *Molecular Ecology* **10**: 1855-1871.
- 195 Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED. 1996. A global  
196 analysis of root distributions for terrestrial biomes. *Oecologia* **108**: 389-411.
- 197 Jenkins MB, Virginia RA, Jarrell WM. 1988. Depth distribution and seasonal populations of  
198 Mesquite-nodulating *Rhizobia* in warm desert ecosystems. *Soil Science Society of America*  
199 *Journal* **52**: 1644-1650.

Pickles & Pither 2013

- 200 Kraft NJB, Comita LS, Chase JM, Sanders NJ, Swenson NG, Crist TO, Stegan JC, Vellend M,  
201 Boyle B, Anderson MJ *et al.* 2011. Disentangling the drivers of  $\beta$  diversity along latitudinal  
202 and elevational gradients. *Science* **333**: 1755-1758.
- 203 Kramer S, Marhan S, Haslwimmer H, Ruess L, Kandeler E. 2013. Temporal variation in  
204 surface and subsoil abundance and function of the soil microbial community in an arable soil.  
205 *Soil Biology and Biochemistry* **61**: 76-85.
- 206 Landeweert R, Leeftang P, Kuyper TW, Hoffland E, Rosling A, Wernars K, Smit E. 2003.  
207 Molecular identification of ectomycorrhizal mycelium in soil horizons. *Applied Environmental*  
208 *Microbiology* **69**: 327-333.
- 209 Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Högberg P, Stenlid J, Finlay RD. 2007.  
210 Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest.  
211 *New Phytologist* **173**: 611-620.
- 212 Nogué S, de Nascimento L, Fernández-Palacios JM, Whittaker RJ, Willis KJ. 2013. The  
213 ancient forests of La Gomera, Canary Islands, and their sensitivity to environmental change.  
214 *Journal of Ecology* **101**: 368-377.
- 215 Perry DA, Borchers JG, Borchers SL, Amaranthus MP. 1990. Species migrations and  
216 ecosystem stability during climate change: the belowground connection. *Conservation Biology*  
217 **4**: 266-274.
- 218 Read DJ, Perez-Moreno J. 2003. Mycorrhizas and nutrient cycling in ecosystems – a journey  
219 towards relevance? *New Phytologist* **157**: 475-492.
- 220 Rosling A, Landeweert R, Lindahl BD, Larsson K-H, Kuyper TW, Taylor AFS Findlay RD.  
221 2003. Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. *New*  
222 *Phytologist* **159**: 775-783.
- 223 Ryberg M, Andreassen M, Björk RG. 2010. Weak habitat specificity in ectomycorrhizal  
224 communities associated with *Salix herbacea* and *Salix polaris* in alpine tundra. *Mycorrhiza* **21**:  
225 289-296.

Pickles & Pither 2013

- 226 Scattolin L, Montecchio L, Mosca E, Agerer R. 2008. Vertical distribution of the  
227 ectomycorrhizal community in the top soil of Norway spruce stands. *European Journal of*  
228 *Forest Research* **127**: 347-357.
- 229 Schenk HJ. 2005. Vertical vegetation structure below ground: Scaling from root to globe.  
230 *Progress in Botany* **66**: 341-373.
- 231 Schenk HJ, Jackson RB. 2002. The global biogeography of roots. *Ecological Monographs*  
232 **72**: 311-328.
- 233 Schenk HJ, Jackson RB. 2005 Mapping the global distribution of deep roots in relation to  
234 climate and soil characteristics. *Geoderma* **126**: 129-140.
- 235 Smith JE, McKay D, Brenner G, McIver J, Spatafora JW. 2005. Early impacts of forest  
236 restoration treatments on the ectomycorrhizal fungal community and fine root biomass in a  
237 mixed conifer forest. *Journal of Applied Ecology* **42**: 526-535.
- 238 Stone EL, Kalisz PJ. 1991. On the maximum extent of tree roots. *Forest Ecology &*  
239 *Management* **46**: 59 –102.
- 240 Sucre EB, Tuttle JW, Fox TR. 2011. The use of ground-penetrating radar to accurately  
241 estimate soil depth in rocky forest soils. *Forest Science* **57**: 59-66.
- 242 Taylor AFS. 2002. Fungal diversity in ectomycorrhizal communities: sampling effort and  
243 species detection. *Plant & Soil* **244**: 19-28.
- 244 Taylor DL, Hollingsworth TN, McFarland, Lennon NJ, Nusbaum C, Ruess RW. 2013. A first  
245 comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche  
246 partitioning. *Ecology*, in press: <http://dx.doi.org/10.1890/12-1693.1>
- 247 Trumbore SE, Czimczik CI. 2008. An uncertain future for soil carbon. *Science* **321**: 1455-1456.
- 248 Vellinga EC, Wolfe BE, Pringle A. 2009. Global patterns of ectomycorrhizal introductions.  
249 *New Phytologist* **181**: 960-973.
- 250 Virginia RA, Jenkins MB, Jarell WM. 1986. Depth of root symbiont occurrence in soil. *Biology*  
251 *and Fertility of Soils* **2**: 127-130.

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252 **Figure legends**

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

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

262

263 **Supporting Information**

264 **Table S1.** Citation, sample depth and host species for all ectomycorrhizal articles from the last  
265 5 years of *New Phytologist* in which sampling depth was provided.



