
Article:
Brearley, F.Q., Saner, P., Uchida, A. et al. (5 more authors) (2017) Testing the importance of a common ectomycorrhizal network for dipterocarp seedling growth and survival in tropical forests of Borneo. Plant Ecology & Diversity, 9 (5-6). pp. 563-576. ISSN 1755-0874

https://doi.org/10.1080/17550874.2017.1283649

Testing the importance of a common ectomycorrhizal network for dipterocarp seedling growth and survival in tropical forests of Borneo

Francis Q. Brearley[1,2,+*], Philippe Saner[3,+], Ayuho Uchida[4], David F. R. P. Burslem[4], Andy Hector[3,5], Reuben Nilus[6], Julie D. Scholes[1] and Simon Egli[7]

[1] Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK; [2] School of Science and the Environment, Manchester Metropolitan University, Chester Street, UK; [3] Department of Evolutionary Biology and Environmental Studies, University of Zürich, Zürich, Switzerland; [4] School of Biological Sciences, University of Aberdeen, Aberdeen, Scotland, UK; [5] Department of Plant Sciences, University of Oxford, Oxford, UK; [6] Forest Research Centre, Sabah Forestry Department, Sandakan, Sabah, Malaysia; [7] Swiss Federal Research Institute for Forest, Snow and Landscape, Birmensdorf, Switzerland

+ = Joint first authors

*Corresponding author. Email: f.q.brearley@mmu.ac.uk

Abstract

Background: Connections between mature trees and seedlings *via* ectomycorrhizal (EcM) hyphal networks existing in dipterocarp-dominated tropical rain forests of South-east Asia could have strong implications for seedling growth and survival and the maintenance of high diversity in such forests.

Aim: To test whether EcM hyphal network connections are important for the growth and survival of dipterocarp seedlings.

Methods: We conducted four independent experiments that prevented contact of experimental seedlings with an EcM network by using a series of fine meshes and/or plastic barriers. We measured the growth and survival (and foliar $\delta^{13}C$ in one

experiment) of seedlings of six dipterocarp species over intervals ranging from 11 to 29 months.

Results: Seedling growth (diameter, height or leaf number) was unaffected by exclusion from the EcM network in three experiments and there were no differences in foliar $\delta^{13}C$ values in the fourth. Seedling survival was reduced following exclusion from the EcM network in one experiment. Our results give little support to the hypothesis that dipterocarp seedlings growing in the shaded forest understorey benefit from being connected, through a common EcM network, to surrounding trees.

Conclusions: We suggest that our negative results, in contrast to studies conducted in low diversity boreo-temperate or tropical forests, are due to these high diversity forests lacking host species-specific EcM fungi, and therefore providing little opportunity for adaptive support of seedlings *via* hyphal networks.

Keywords: Borneo, dipterocarps, ectomycorrhizas, mycorrhizal networks, source-sink relationships

42 **Introduction**

43 Mycorrhizas are a symbiotic association between specialised root-inhabiting fungi and
44 the roots of living plants. The plant provides the fungus with carbon derived from
45 photosynthesis, and, in return, the fungus may improve the nutrient uptake, growth,
46 water relations, pathogen and heavy metal resistance of the plant (van der Heijden and
47 Sanders 2002; Smith and Read 2008 and references therein). Although the majority of
48 tropical trees form arbuscular mycorrhizal (AM) associations, an important minority
49 form ectomycorrhizal (EcM) associations including members of the Dipterocarpaceae
50 (Brearley 2012). Dipterocarp trees dominate the forests of South-east Asia (Slik et al.
51 2003, 2009), and there are more than 250 species on the island of Borneo alone (Ashton
52 2004). Their seeds are produced every 3-8 years in mast-fruiting events (Curran et al.
53 1999; Sakai et al. 2006; Brearley et al. 2007a) after which they germinate and become
54 colonised rapidly by EcM fungi (Lee and Alexander 1996). The main method of
55 colonisation is from the hyphae of fungi already present and forming network in the soil
56 radiating out from roots of adjacent adult trees (Alexander et al. 1992) – during the
57 process of EcM colonisation seedlings become 'connected' to this network. After a
58 mast-fruiting event, dipterocarp seedlings are found at high densities close to parent
59 trees forming seedling banks where they are limited in their growth and survival in the
60 shaded forest understorey.

61 Numerous studies have shown the existence of EcM networks in various forest
62 ecosystems with shared fungal species linkages between adults and seedlings (Beiler et
63 al. 2010; Diédhiou et al. 2011; Michaëlla Ebenye et al. in press) and Connell and
64 Lowman (1989) hypothesised that the dominance of dipterocarps in South-east Asian
65 lowland evergreen rain forests was linked to the ability of newly germinated seedlings
66 to link into this EcM-mediated resource acquisition network. Studies conducted in

67 lowland tropical forests of Cameroon found that isolation of seedlings of *Paraberlinia*
68 *bifoliolata* (Leguminosae) from roots and EcM fungi reduced seedling biomass and
69 survival (Onguene and Kuyper 2002), and a similar study in Guyana showed that
70 *Dicymbe corymbosa* (Leguminosae) had reduced growth and survival when isolated
71 from an EcM hyphal network using fine meshes (McGuire 2007). Contrasting with
72 these findings, seedlings of only one of three Caesalpinioideae legume species in
73 Cameroon had a higher growth rate in the presence of adult trees and their associated
74 roots and EcM fungi (Newbery et al. 2000). The cause of this difference in outcome
75 between studies in different locations is unknown, and further research is required to
76 extend the range of environments where this is examined including both high and low
77 diversity sites. Whether the connection into an EcM hyphal network has implications
78 for the high species richness observed in dipterocarp-dominated tropical rain forests
79 remains unsolved, and clearly, then, it is important to improve our knowledge of the
80 role of EcM networks in facilitating the regeneration of tropical forest trees.
81 The benefits of being connected into this hyphal 'wood-wide web' have been
82 reported from boreo-temperate forests (Simard et al. 2012). For example, carbon has
83 been shown to move between plants or seedlings that form a hyphal network in a
84 'source-sink' fashion whereby plants that are photosynthesising at a rapid rate, such as
85 those under higher irradiance, pass carbon to those that have lower rates of
86 photosynthesis, such as those which are strongly shaded (Francis and Read 1984;
87 Simard et al. 1997; Klein et al. 2016). Support *via* an EcM hyphal network may
88 therefore be beneficial for the survival of seedlings that are growing below the light
89 compensation point in shaded understorey environments. Francis and Read (1984) were
90 the first to show that carbon could move between plants via an AM hyphal network, but
91 not until the milestone study of Simard et al. (1997) was net movement of carbon in

EcM systems shown: they found that 6.6% of carbon fixed in *Betula papyrifera* (Betulaceae) was transferred to *Pseudotsuga menziesii* (Pinaceae) and that 45% of this transferred carbon was found in the plant shoots (*i.e.* not fungal structures). Most recently, Klein et al. (2016) showed transfer of carbon from *Picea abies* (Pinaceae) adult trees to roots of adjacent EcM species. However, the ecological importance of this network has been under considerable debate as inter-plant carbon transfer is a complex and variable process. From a phytocentric view, there is a challenge in explaining how this process could be adaptive as it is only likely to be selected for if adults are transferring beneficial compounds, such as carbon, to kin. If considered mycocentrically, however, then the fungus will simply be moving compounds to where they are most required at a given point in time.

EcM colonisation in shade tolerant dipterocarps has been shown to improve the growth of seedlings under nursery conditions although far fewer studies have shown a similar benefit under natural field conditions (Brearley 2011, 2012). We report four independent studies on the island of Borneo, using seedlings of six dipterocarp species with contrasting ecological characteristics. We hypothesised that seedlings that were experimentally excluded from an EcM network would display slower growth rates and reduced survival than seedlings that were connected to the network.

Materials and methods

Rationale

In the first three experiments reported, we planted seedlings surrounded by meshes of various pore size with the intention of creating a series of barriers to in-growth by plant roots and fungal hyphae. Therefore, the control treatments allowed free access to fine roots and fungal hyphae, a large mesh treatment had a fine pore-size mesh (35-50 μm)

117 to prevent the access of fine roots but allow access by fungal hyphae and a small mesh
118 treatment had a very fine pore-size mesh (0.5-1.0 μm) and/or a severing treatment to
119 prevent access to both roots and fungal hyphae. It was assumed that seedlings in which
120 fungal hyphae were allowed access through the meshes had the potential to become
121 colonised by hyphae present in the soil outside the meshes, and therefore connect into
122 the EcM hyphal network, whereas those seedlings in the treatments where hyphal access
123 was restricted would only be able to form EcMs *via* spores or hyphal fragments present
124 within their enclosed rooting volume, and would therefore not connect into the EcM
125 network outwith the meshes. This approach has been used successfully to control
126 mycorrhizal colonisation and partition of soil respiration fluxes in previous experiments
127 (Johnson et al. 2001; Heinemeyer et al. 2007; Vallack et al. 2012). A number of the
128 seedlings were raised in a nursery before being transplanted into the forest and, based
129 on prior observations (Brearley 2003), we are confident they were all colonised by EcM
130 fungi, albeit those more common of nursery conditions (*e.g.* Brearley 2006; Brearley et
131 al. 2003, 2007b; Saner et al. 2011). Whilst 'priority effects' of EcM colonisation have
132 often been found to affect subsequent competitive replacement by other EcM species
133 (Kennedy et al. 2009), replacement of nursery EcMs with those present in forest soil has
134 been seen within six months for studies in Peninsular Malaysia (Chang et al. 1994,
135 1995) and, given that the length of all our studies was over at least 11 months, we do
136 not consider this to have affected our results.

137 In one experiment we tested whether carbon was measurably transferred from
138 adult trees to seedlings through an EcM hyphal network by trenching the seedlings in
139 order to isolate them from the EcM network and then determining the $\delta^{13}C$ values of
140 newly produced leaves. This approach is based on the fact that canopy leaves have a
141 less negative $\delta^{13}C$ signature than seedlings due to differences in the atmospheric-to-

intercellular carbon dioxide ratio (O'Leary 1988; Farquhar et al. 1989) and the isotopic signature of the source carbon dioxide in the ambient air taken up for photosynthesis (Medina and Minchin 1980; Medina et al. 1986, 1991; Buchman et al. 1997). For example, if the isotopic difference between adult trees and seedlings were 5‰, using a two-source mixing model, receipt of 10% of carbon by seedlings from adult trees would result in those connected to the EcM network having a foliar $\delta^{13}C$ value 0.5 ‰ closer to adults than trenched seedlings.

Study species

Six dipterocarp species (Table 1) were selected, based on their differences in shade tolerance and maximum growth rates (Experiments 1-3), edaphic preferences (Experiment 3), and on their availability at the start of the experiments (Experiments (1-4).

Experiment 1. EcM-network exclusion and fungicide addition effects on two dipterocarp species

This experiment was carried out in the northern part of the Kabili-Sepilok Forest Reserve, on alluvial soils (5° 52' N, 117° 56' E; Fox 1973; Nilus 2004). Four plots of ca. 7 m x 7 m were cleared of the understorey vegetation and some smaller trees to reduce heterogeneity in the light environment within and between plots. Six-month-old seedlings of *Hopea nervosa* and *Parashorea tomentella* obtained from the INFAPRO nursery, Danum Valley, Sabah that had been potted in forest-derived soil (see Saner et al. 2011 and Paine et al. 2012a for nursery conditions), were planted into the four plots in March 2000. In each plot, 30 seedlings of each of the two species were randomly allocated to planting locations ca. 50 cm apart. Three treatments and two controls were

applied to the seedlings: (1) Control: no meshes were used, fungal hyphae and other roots could fully interact with the planted seedling; (2) Sub-Control: a 1 mm pore-size polyester mesh cylinder was installed around the seedling; the aim of this mesh was to attempt to provide some rigidity and to protect the smaller pore-sized meshes in the other treatments from larger soil invertebrates; (3) Root exclusion (-R): one layer of 35 μm pore-size nylon mesh (within the 1 mm pore-size polyester mesh cylinder) was installed around the seedlings to allow connection to a mycorrhizal hyphal network; (4) Root and mycorrhizal exclusion (-RM): two layers of 0.5 μm pore-size nylon mesh (within the 1 mm pore-size polyester mesh cylinder) were installed around the seedlings; the cylinders were twisted slightly every four weeks to break any hyphal connections that might have occurred through the meshes; (5) Fungicide (-RM+F): as the -RM treatment but with the addition of Mancozeb fungicide (Bio-Dithane 945, PBI Home & Garden Ltd., Enfield, Middlesex, UK) bi-weekly at a rate of 0.08 g per seedling in 50 ml of water to control the growth of EcMs on the seedling roots (Brearley 2003). All the mesh barriers were sewn into cylinders of 7 cm diameter with a lip of 2 cm above ground to prevent hyphal entry and dug into the soil to a depth of 25 cm using an auger to create a hole; they remained open at the bottom. All meshes were obtained from, and sewn by, Plastok Associates Ltd. (Birkenhead, Wirral, UK). Apart from the -RM+F treatment all other treatments were given 50 ml of water bi-weekly to control for the addition of water with the fungicide. Other than this bi-weekly fungicide solution or water addition, the seedlings were given supplemental water twice weekly for the first month following planting. Leaf litter and twigs lying across the meshes were removed at monthly intervals to prevent fungal hyphae entering the cylinders *via* this potential pathway. Other vegetation was hand-weeded from the plots throughout the experimental period.

192

193 *Experiment 2. EcM-network exclusion and distance to adult tree effects on two*
194 *dipterocarp species*

195 This experiment was conducted in the Malua Forest Reserve (5º 05' N, 117º 38' E) that
196 was selectively logged for timber in the 1980s (Marsh and Greer 1992). Twenty large
197 trees (mean dbh = 69.7 ± SD 15.1 cm) of either *Dryobalanops lanceolata* or *Shorea*
198 *parvifolia* were chosen within the Sabah Biodiversity Experiment (Hector et al. 2011;
199 Saner et al. 2012). Trees were only selected if they were among the largest trees and no
200 other large dipterocarp or Fagaceae trees were within 15 m of the plots to ensure that
201 the EcM network of the focal tree was closest to the planted seedlings. At every focal
202 tree, one plot (ca. 1.5 m x 2 m) was cleared of understorey vegetation to reduce within
203 and between plot heterogeneity in the light environment under the tree canopy (2-4 m
204 away from the trunk) and one plot was established and cleared of understorey
205 vegetation outside the tree canopy (15-17 m away from the trunk), based on the
206 assumption that the tree canopy approximately reflected the extension of the rooting
207 system (Baillie and Mamit 1983; Katayama et al. 2009). One control and two treatments
208 were applied to the seedlings: (1) Control: no mesh or tube was used, fungal hyphae and
209 other roots could fully interact with the planted seedling; (2) Root exclusion (-R):
210 seedlings were planted into a PVC tube (15 cm diameter x 70 cm depth) covered at the
211 bottom with 50-µm pore-size mesh allowing fungal hyphae to grow into the tube; (3)
212 Root and mycorrhiza exclusion (-RM): seedlings were planted into a PVC tube as above
213 but with a 1-µm pore-size mesh to prevent the entry of fungal hyphae. The meshes were
214 made of monofilament PET (Sefar PETEX, Heiden, Switzerland) and were glued
215 between the bottom of the PVC tube and an additional PVC ring (15 cm diameter x 5
216 cm depth) with silica and aluminium tape. In every plot, 12 seedlings were planted at a

217 spacing of ca. 50 cm and dug into the soil to a depth of 70 cm. Six seedlings were the
218 same species as the focal tree and six seedlings were of the other tree species. All
219 seedlings where raised in a local nursery at the Malua Field Station, Malua Forest
220 Reserve, Sabah, with conditions similar to those at the INFAPRO nursery noted earlier,
221 and ca. 6 months old and 0.5 m tall when planted into the field. Seedlings were
222 randomly allocated and planted in September 2006. Seedlings were watered once at the
223 beginning of the experiment. Leaf litter and twigs lying across the meshes were
224 removed at monthly intervals to prevent fungal hyphae entering the cylinders. Other
225 vegetation was hand-weeded from the plots throughout the experimental period. An
226 index of light interception (% of canopy openness at the plot level) was measured at the
227 beginning, middle (6 months) and end (11 months) of the experiment, using a Spherical
228 Densiometer Model A.
229
230 *Experiment 3. EcM-network exclusion and soil type effects on four dipterocarp species*
231 This experiment was carried out in the northern and central parts of Kabili-Sepilok
232 Forest Reserve on two contrasting soil types (Nilus 2004; Dent et al. 2006). Ten
233 understorey plots of ca. 5 m x 5 m were chosen within both the sandstone and the
234 alluvial soil types respectively, and understorey vegetation cleared to reduce
235 heterogeneity in the light environment within and between plots. Within each plot,
236 seedlings of *Shorea beccariana*, *S. multiflora* (both sandstone soil specialists),
237 *Dryobalanops lanceolata* and *Parashorea tomentella* (both alluvial soil specialists)
238 were planted in April 2003 at an equal spacing of ca. 1 m (seedlings were grown from
239 seeds collected within the Kabili-Sepilok Forest Reserve during the 2002 mast-fruiting
240 event and were ca. 6 months old when transplanted). They were subjected to three
241 treatments and one control: (1) Control: no tube or mesh was used, fungal hyphae and

242 other roots could fully interact with the planted seedling; (2) Sub-Control: seedlings
243 were planted in PVC tubes of 15 cm in diameter and 35 cm in depth that were open at
244 the bottom (with 5 cm above the soil surface). Three rectangular windows of 7 cm
245 width x 20 cm depth were made in the tube, allowing both mycorrhizal hyphae and
246 plant roots to penetrate. Six small holes (of 5 mm diameter) were cut in the tubes at the
247 level of the soil surface to aid in drainage. (3) Root exclusion (-R): seedlings were
248 planted in PVC tubes as above and the windows were covered in 35 μm pore-size mesh
249 (Plastok Associates Ltd., Birkenhead, Wirral, UK), allowing only mycorrhizal hyphae
250 to penetrate. (4) Root and mycorrhizal exclusion (-RM): Seedlings were planted in PVC
251 tubes but there were no rectangular windows in the tubes and a knife was used to cut
252 around the edges of the tubes once per week to sever any fungal hyphae that might have
253 entered through the small drainage holes. Once planted, seedlings were not given
254 additional water and there were no on-going manipulations (such as removal of leaf
255 litter and twigs lying across the piping or weeding of vegetation). The two sandstone
256 species (*Shorea beccariana* and *S. multiflora*) grown in the alluvial plots were harvested
257 in July 2004 (after 15 months) due to high mortality rates; all other seedling/soil type
258 combinations were followed for 29 months. An index of light interception (% of canopy
259 openness) was measured at the beginning of the experiment with hemispherical
260 photography using a Minolta X-700 camera with a Rokkor 7.5 mm fisheye lens; images
261 were subsequently analysed using Gap Light Analyser (Frazer et al. 1999).
262
263 *Experiment 4. EcM-network effects on carbon isotope ratios on one dipterocarp species*
264 Twenty areas with seedling banks of *Shorea multiflora* were selected in March 2000 in
265 two separate areas of Kabili-Sepilok Forest Reserve. Ten areas were in the vicinity of
266 research plots in the northern part of the Reserve and another ten were along a trail

267 running north-south through the Reserve. In each area, a circular plot of 68.5 cm
268 diameter was trenched to a depth of 5-10 cm (varying with the local microtopography)
269 and a plastic barrier was placed in the trench. An equally-sized and shaped plot
270 (situated between 0.45-3.2 m from the trenched plot; mean: 1.25 m) was marked out
271 using a circle of plastic, lain on the forest floor but remained otherwise unaltered in
272 order to act as a control. Each plot contained a mean of 13.5 (± 4.9 SD) seedlings of
273 which 11.8 (± 4.7 SD) were *Shorea multiflora*. The number of leaves and height of each
274 seedling was recorded so that after 13 months, one leaf that had been produced during
275 that interval was randomly selected from one seedling within each plot. The leaves
276 were dried at 50° C for at least one week, ground in liquid nitrogen and a sample of 1
277 mg was analysed for $\delta^{13}C$ (PDZ Europa ANCA-GSL preparation module connected to a
278 20-20 isotope ratio mass spectrometer, Northwich, Cheshire, UK). Isotope ratios were
279 calculated as: $\delta^{13}C$ (‰) = $(R_{sample}/R_{standard} -1) \times 1000$ where R is the isotope ratio of
280 $^{13}C/^{12}C$ of either the sample or the standard (Pee Dee Belemnite). In addition, one leaf
281 was collected from the canopy of eight large individuals of *Shorea multiflora* (40-45 m
282 tall; C. R. Maycock pers. comm.; R. N. Thewlis pers. comm.) and analysed for $\delta^{13}C$ as
283 above.
284
285 *Seedling measurements*
286 Non-destructive measurements of seedling height (to the apical meristem), basal
287 diameter and leaf number as well as survival rate were taken periodically. In
288 Experiment 1, six measurements were taken over 24 months (March 2000-February
289 2002), in Experiment 2, three measurements were taken over 11 months (September
290 2006-August 2007), in Experiment 3, 10 measurements were taken over 29 months
291 (April 2003-September 2005). Seedlings that died or were severely damaged by

292 mammals or tree/branch falls, where the meshes were damaged or where there was poor
293 drainage and the tubes became waterlogged (Experiment 2 only) were removed from
294 the growth analyses. For the individual growth analyses a total of n=233 (Experiment 1),
295 n=267 (Experiment 2), and n=317 (Experiment 3) observations were included. Only
296 seedlings grown under dark conditions (<5% canopy openness) were included in the
297 analysis for Experiment 2.
298
299 *Statistical analyses*
300 Based on an initial screen, we assumed linear growth, as individual seedlings showed
301 relatively constant increases in diameter, height and leaf number over time. The linear
302 model was fitted for every seedling and the individual regression slope (r) extracted.
303 The slopes were then standardised by dividing by the mean height, diameter or leaf
304 number of the last measurement, termed in this paper as relative growth rate (Paine et al.
305 2012b). A linear mixed-effects model for each study was carried out in R 3.2.0 (R
306 Development Core Team 2015), using the *nlme* library (Pinheiro and Bates 2000).
307 Treatment and species (all experiments), plus planting distance (Experiment 2), or
308 habitat (soil) type (Experiment 3) were treated as fixed effects; as we were specifically
309 interested in selected species effects they were included as fixed, rather than random,
310 effects, plot was included as a random effect. Unequal variance was observed and
311 accounted for by defining a linear increase in variance with time (Experiment 1) or light
312 level by species (Experiments 2 and 3). In the case of Experiment 2, adding
313 conspecificity/heterospecificity did not significantly improve the fit of the model (in all
314 cases: $\chi^2<16.5$, $P>0.15$) so this variable was removed for ease of comparison with the
315 other studies. Analysis of survival rates was made on binomial count data of seedlings
316 that survived compared to those that died, including the same structure of fixed and

317 random effects as outlined above, with the function *glmer()* and a binomial distribution
318 in the *lme4* library (Bates et al. 2015). The statistical tests are reported based on the
319 analysis of relative growth rates for all three non-destructive measurements (height,
320 diameter and leaf number) and for survival, but for simplicity only the increase in
321 diameter is shown graphically (for additional graphical representation of all non-
322 destructive measurements see supplementary material). We present the *F*-test or Chi-
323 square (survival analysis) statistic with associated *P*-values obtained through the
324 *anova()* command and *t*-test statistic with associated *P*-values obtained through the
325 *summary()* command for main effects and their interactions as outlined in Tables 2, 4
326 and 5. Note that with non-orthogonal designs in complex models the outcome from the
327 *anova()* command and the *summary()* command may differ slightly (Hector et al. 2010;
328 Hector 2015). Experiment 4 was analysed using a straightforward one-way ANOVA to
329 compare foliar $\delta^{13}C$ values between large trees and trenched and untrenched seedlings.
330
331 **Results**
332 *Experiment 1*
333 *Diameter growth.* For relative diameter growth rate, significant main effects of
334 treatment and species were observed (treatment: $F_{4,220}$=12.8, P<0.0001; species:
335 $F_{1,220}$=19.2, P<0.001) and there was also a significant interaction between treatment and
336 species ($F_{4,220}$=2.7, P<0.05). For *Hopea nervosa*, fungicide addition (-RM+F)
337 significantly reduced growth by 40% (mean ± 95% CI: 5-75%) compared to the
338 seedlings of the root exclusion treatment (-R) ($t_{4,220}$=2.2, P<0.05), however for
339 *Parashorea tomentella*, fungicide addition (-RM+F) did not affect diameter growth rate.
340 In contrast to our hypothesis, seedlings of *Parashorea tomentella* in the root and
341 mycorrhizal exclusion treatment (-RM) grew significantly ($t_{4,220}$=3.1, P<0.01) faster

342 than seedlings of the root exclusion treatment (-R) (mean ± 95% CI: 38% ± 19-50%)
343 (Figure 1 and Table 2, supplementary material Figure S1).
344
345 *Height growth.* There was no effect of the treatments on height growth rates but
346 *Parashorea tomentella* showed a significantly faster relative height growth rate than
347 *Hopea nervosa* ($F_{1,220}$=4.1, P<0.05) (Table 2, Figure S2 a and b).
348
349 *Leaf growth.* Relative growth rate in leaf number showed significant main effects of the
350 treatment ($F_{4,220}$=5.0, P<0.001) and species ($F_{1,220}$=116.3, P<0.0001). *Hopea nervosa*
351 seedlings grew significantly faster those of *Parashorea tomentella* ($t_{1,220}$=6.0,
352 P<0.0001). No significant treatment effects were observed for *Parashorea tomentella,*
353 however for *Hopea nervosa*, control seedlings grew significantly faster than both
354 seedlings of the root (-R) and mycorrhizal exclusion (-RM) treatment ($t_{4,220}$=2.2,
355 P<0.05 and $t_{4,220}$=2.9, P<0.01 respectively). Fungicide addition significantly reduced
356 growth compared to control seedlings ($t_{4,220}$=4.2, P<0.0001) (Table 2, Figure S3 a and
357 b).
358
359 *Survival.* No effects of the treatments were observed for seedling survival but seedlings
360 of *Hopea nervosa* showed a significantly greater survival rate compared to *Parashorea*
361 *tomentella* (χ^2=6.4, P=0.01) (Tables 2 and 3).
362
363 *Experiment 2*
364 *Diameter growth.* There was no effect with respect to either the treatment or the
365 planting distance from the large trees but *Dryobalanops lanceolata* seedlings showed

significantly greater relative diameter growth rates than *Shorea parvifolia* seedlings ($F_{1,238}$=10.2, P<0.01; Figure 2 and Table 4, Figure S4).

Height growth. There was no effect of connection to an EcM network or species on height growth. However, the root exclusion (-R) treatment of *Dryobalanops lanceolata* showed a 73% increase in height growth rate when planted close to a large tree compared to those that were planted away from the tree ($t_{1,238}$=2.5, P<0.05) (Table 4, Figure S5 a and b).

Leaf growth. Leaf growth in *Shorea parvifolia* was significantly reduced in the root and mycorrhizal exclusion treatment (-RM) compared to the root exclusion (-R) treatment ($t_{2,238}$=2.4, P<0.05) (Table 4, Figure S6 a and b).

Survival. A significant treatment effect (χ^2=13.3, P=0.001) was found, as seedlings with the root and mycorrhizal exclusion treatment (-RM) showed a lower survival rate than the root exclusion (-R) treatment and the control seedlings. Seedlings of *Dryobalanops lanceolata* showed a significantly higher survival rate compared to *Shorea parvifolia* (χ^2=4.4, P<0.05) (Tables 3 and 4).

Experiment 3

Diameter growth. A significant interaction between treatment and soil type ($F_{3,276}$=2.7, P<0.05) and between species and soil type ($F_{3,276}$=5.4, P<0.01) was found. Seedlings with the root and mycorrhizal exclusion treatment (-RM) of three species (*Parashorea tomentella*, *Shorea beccariana* and *S. multiflora)* grew faster in the sandstone soil type compared to seedlings with only the root exclusion treatment (-R). Seedlings of

391 *Dryobalanops lanceolata* with the root exclusion treatment (-R) grew marginally faster
392 on the alluvial soil type ($t_{1,285}$=1.7, P<0.10) and also showed more rapid growth
393 compared to seedlings with the root and mycorrhizal exclusion treatment (-RM)
394 ($t_{1,285}$=2.2, P<0.05) (Figure 3 and Table 5, Figure S7).
395
396 *Height growth.* Seedlings of all four dipterocarp species showed significantly different
397 height growth rates ($F_{3,276}$ = 3.6, P<0.05). *Parashorea tomentella* seedlings with the
398 root exclusion treatment (-R) grew faster on the sandstone soil type ($t_{1,276}$=2.1, P<0.05)
399 (Table 5, Figure S8 a and b).
400
401 *Leaf growth.* A significant interaction between species and soil type was observed for
402 relative leaf growth rates ($F_{3,276}$ = 4.7, P<0.01). *Dryobalanops lanceolata* seedlings
403 grew significantly faster on the alluvial compared to the sandstone soil type ($t_{1,276}$=2.0,
404 P<0.05); for all other species there were no differences between the soil types (Table 5,
405 Figure S9 a and b).
406
407 *Survival.* A marginal species effect (χ^2=7.2, P<0.10) and a significant soil type effect
408 (χ^2=6.0, P=0.01) were found, however no effect of the treatments was observed after 15
409 months (Tables 3 and 5). Notably, the sandstone specialists *Shorea beccariana* and *S.*
410 *multiflora* showed lower survival rates on alluvial soil but the species by soil type
411 interaction was not significant.
412
413 *Experiment 4*
414 There was no difference between the foliar $\delta^{13}C$ values of seedlings grown in trenched
415 (-35.05‰ ± 0.22 SE) or untrenched (-35.00‰ ± 0.22 SE) plots but both were

significantly more negative than the value of -30.31‰ ± 0.34 SE obtained from the canopy leaves of large trees ($F_{2,45}$=79.06, P<0.001). No effect of the treatment (trenched vs. untrenched) on seedling survival rate was observed (Table 3).

Discussion

Several studies have addressed the benefits to seedlings of tropical forest trees of being in contact with EcM hyphae radiating out from tree roots (Alexander et al. 1992; Yasman 1995; Newbery et al. 2000), but few have tested the importance of incorporation into a common EcM network under field conditions. Two independent prior studies by Onguene and Kuyper (2002) and McGuire (2007) reported significant increases in seedling mass (35%) and height growth (73%) respectively, that they related to incorporation into the EcM networks of Caesalpinioideae trees in studies in Cameroon and Guyana, respectively. In contrast, the key result from our analysis across four complementary experiments with dipterocarps in South-east Asia is that there are minimal effects of experimentally imposed treatments that alter seedling incorporation into an EcM hyphal network on measures of dipterocarp seedling growth in understorey conditions. Only two growth measures (the number of leaves of *Shorea parvifolia* in Experiment 2 and the diameter of *Dryobalanops lanceolata* in Experiment 3) suggested any importance of an EcM hyphal network. There was some evidence that exclusion from the EcM network reduced seedling survival, as, in Experiment 2, seedling survival was lower in the –RM treatment compared to the –R treatment and the control, although there is the possibility that this was due to waterlogging. In our combined studies we thus did not detect any benefit to seedlings from being connected, through a common EcM network, to surrounding mature trees.

441 We suggest that the lack of any effect on seedling growth of being connected to
442 an EcM network, in contrast to boreo-temperate forests (Simard et al. 2012) and low
443 diversity tropical forest (McGuire 2007) is because our lowland dipterocarp forest study
444 sites have high tree diversity and low species preference of EcM fungi. Peay et al.
445 (2015) showed 'extreme host generalism' of EcM fungi in similar tropical forests in
446 northern Borneo and it has been found that there is little evidence for host preference by
447 EcM fungal species in other tropical forests with high diversity of trees and a substantial
448 proportion of EcM trees (Tedersoo et al. 2010; Diédhiou et al. 2010; Smith et al. 2011).
449 If considered from a phytocentric perspective, an absence of host-specific EcM
450 associations removes the selective advantage of supporting seedlings *via* an EcM hyphal
451 network because there can be no guarantee that the supported seedling would be
452 conspecific kin.
453
454 Overall, the majority of measurements showed no effect (positive or negative) of
455 inclusion into an EcM network on seedling growth. However, in some cases,
456 experiment-specific findings argue for species-specific growth patterns, sometimes even
457 across the experiments. *Parashorea tomentella* seedlings in Experiments 1 and 3
458 showed increased growth rates when isolated from a common EcM network, suggesting
459 that EcM networks could even have detrimental effects on seedling growth and survival.
460 Two additional species (*Shorea beccariana* and *S. multiflora*) showed this effect in
461 Experiment 3 but only on the sandstone soil type. This result may not be entirely related
462 to an EcM network but in this case we hypothesise that providing exclusive access to
463 EcM hyphae associated with the seedlings to the rooting space inside the mesh tubes
464 prevented competition with hyphae from outside. It could also indicate that the
465 artificially induced limitation of root competition over scarce resources could be

466 directly beneficial for seedling growth (Coomes and Grubb 2000). Furthermore, there
467 was some evidence in Experiment 1 that fungicide addition limited diameter and leaf
468 growth in *Hopea nervosa*, but not in *Parashorea tomentella*. Fungicide addition
469 reduced the growth rate of this one species even though there was no significant
470 reduction in EcM colonisation (Brearley 2003). Clearly, the application of fungicide
471 will have additional effects other than simply reducing EcM colonisation such as effects
472 on soil nutrient status and impacts on pathogenic fungal populations (Newsham et al.
473 1994; Brearley 2003; Teste et al. 2006). In a similar experiment under high light
474 conditions (gaps), Brearley (2003) found that fungicide addition did reduce EcM
475 colonisation but this had a greater impact on seedling nutrient status than on seedling
476 growth. Other aspects of our experimental manipulations that may not have created
477 seedlings that were entirely disconnected from an EcM network include the depth of
478 barriers that were variable among experiment designs (*i.e.* possibly too shallow in
479 Experiment 4) and their open-bottomed nature in some experiments that might have
480 allowed colonisation by EcM hyphae from deeper soil layers (Pickles and Pither 2014).
481 In addition, there is the possibility of confounding the experimental treatments with
482 colonisation by different EcM fungal species; seedling roots isolated from the EcM
483 network would be more likely to be colonised by spore-forming fungi (and perhaps
484 retain initial greenhouse colonising fungi for longer) whereas those connected to the
485 EcM network would be more likely to become colonised via hyphal connections.
486 However, despite the potential for priority effects (Kennedy et al. 2009), there is a rapid
487 turnover of the EcM community on dipterocarp seedlings (Chang et al. 1994,1995; Lee
488 and Alexander 1996). Indeed, it would have been highly beneficial to have determined
489 the EcM fungi present on the seedlings' roots in each of the treatments (both at the
490 beginning and end of the experiments), in addition to those on adult trees, to provide

491 additional support for the efficacy of our experimental manipulations, as well as
492 comparing our different experimental designs. Importantly, it would also provide
493 support for our hypothesis of low EcM host specificity and this should be the key target
494 of future research.
495
496 Whilst we do not question the benefit to seedlings coming into contact with
497 EcM hyphae already present in the soil allowing them to rapidly form EcM associations
498 (Alexander et al. 1992), we did not find any importance of the EcM network for growth
499 of seedlings although survival was affected in one experiment. Whilst the main
500 mechanism through which connections to an EcM network have been hypothesised to
501 benefit seedlings is the provisioning of carbon for seedling growth in low light
502 environments, it could be questioned whether incorporation into an EcM network
503 provides other benefits that we have not measured. These could include improved
504 resistance to herbivores (Booth 2004), drought tolerance through hydraulic uplift
505 (Egerton-Warburton et al. 2007; Bingham and Simard 2011), or access to nutrients
506 being taken up from a larger volume of soil - possibly being more important where light
507 is less limiting. Bingham and Simard (2011) found a greater importance of an EcM
508 network under drought conditions; our sites rarely experience drought but it could be
509 informative to test the effect of EcM networks under an experimentally induced drought
510 or along a climatic gradient. Under very low light conditions, such that light was highly
511 limiting to growth (i.e. below the light compensation point), seedling survival is
512 arguably more important than seedling growth in determining future community
513 composition. In our experiment, light levels were above the light compensation point
514 for seedling growth (Eschenbach et al. 1998) such that growth was a more relevant
515 measure than survival although we did see some suggestions that the EcM network was

important for seedling survival. We altered light conditions by removal of some vegetation - this might have influenced our results but as the majority of these would have been AM species the impact of this is considered minor. An isotope labelling study (^{13}C) would be the next step to truly confirm if this lack of importance of an EcM hyphal network is indeed the case although, clearly, this is logistically challenging (Philip and Simard 2008, but see Klein et al. 2016).

In conclusion, we found that incorporation into a common EcM network has few measurable beneficial effects on dipterocarp seedling growth. That is not to say that the EcM network is unimportant, but, that within the constraints of short-term experiments (< 2 ½ years), we could not detect a signal of its influence on seedling growth. We did determine suggestions of an effect on seedling survival but this was only in one experiment and may have been an experimental artefact. We recommend that further studies should focus on the role that EcM networks play in resilience to drought periods or nutrient limitation of dipterocarp seedlings. In addition, we propose a working hypothesis, that needs further experimental testing, that the high tree species diversity and lack of benefit to trees of supporting heterospecific seedlings through a generalist EcM network is the reason for the minimal effects seen here. We welcome additional experiments and note that they need to be supported by identification of EcM fungi on seedling roots to aid interpretation. Currently, incorporation into an EcM network cannot categorically be invoked as affecting dipterocarp seedling growth or determining patterns of community diversity in dipterocarp-dominated tropical forests of Borneo.

Acknowledgements

For assistance with experimental design, fieldwork and comments on earlier versions of the manuscript we thank: Udin bin Ladin and the Malua field station team, Adzimi Madran, Adzley Madran, Justin Tabai, Dainold Yudat, Daulin Yudat, Rineson Yudat, Karin Saner, Ian Alexander, Yann Hautier, Jan Jansa, Lee Su See, Robert Ong, Malcolm Press and Glen Reynolds. This research is manuscript no. 15 of the Sabah Biodiversity Experiment and part of the Royal Society South-East Asia Rainforest Research Programme (Project No. RS243). All experiments complied with the laws of the country they were conducted in (Malaysia) at the time of the studies.

This project was financially supported through the British Ecological Society, the Ishizaka Foundation, the Darwin Initiative (United Kingdom Department for Environment, Food and Rural Affairs) and the University of Zürich.

Disclosure statement

The authors declare that they have no conflicts of interest. The authors acknowledge that they have no financial interest or benefit arising from the direct applications of this research.

Notes on Contributors

Francis Q. Brearley is an ecologist interested in the functional importance of plant-soil interactions for ecological processes in tropical forests

Philippe Saner is an environmental scientist with a main interest in tropical plant community ecology and the restoration of tropical forests

565 **Ayuho Uchida** was a Ph.D. student examining the importance of root competition and
566 ectomycorrhizal fungi for dipterocarp seedling growth
567 **David F.R.P. Burslem** is interested in the community and ecosystem ecology of
568 tropical forests with a particular focus on the maintenance of species diversity and the
569 conservation of tropical forests
570 **Andy Hector** is a community ecologist interested in biodiversity loss and its
571 consequences for the stability and functioning of ecosystems and the provision of
572 ecological services
573 **Reuben Nilus** is an ecologist working on the diversity, distribution and conservation of
574 the forests of Sabah
575 **Julie D. Scholes** is a physiologist/molecular biologist interested in the role of pathogens
576 and mycorrhizas in the maintenance of dipterocarp diversity in tropical forests
577 **Simon Egli** has a main interest in mycorrhizal fungi and how they support the
578 resistance and resilience of forest ecosystems in a changing environment
579

580 **References**

581 Alexander IJ, Ahmad N, Lee SS. 1992. The role of mycorrhizas in the regeneration of
582 some Malaysian forest trees. Philosophical Transactions of the Royal Society of
583 London Series B-Biological Sciences 335:357–367.
584 Ashton PS. 2004. Dipterocarpaceae. In: Soepadmo E, Saw LG, Chung RCK (editors).
585 Tree Flora of Sabah and Sarawak. Volume 5. Sandakan, Kepong, Kuching
586 (Malaysia): Sabah Forestry Department, Forest Research Institute Malaysia and
587 Sarawak Forestry Department. p. 63–388.
588 Baillie IC, Mamit JD. 1983. Observations on rooting in mixed dipterocarp forest,
589 central Sarawak. Malaysian Forester 46:369–374.
590 Bates D, Mächler M, Bolker BM, Walker SC. 2015. Fitting linear mixed-effects models
591 using lme4. Journal of Statistical Software arXiv:1406.5823.

592 Beiler KJ, Durall, Simard SW, Maxwell SA, Kretzer AM. 2010. Architecture of the
593 wood-wide web: *Rhizopogon* spp. genets link multiple Douglas-fir cohorts. New
594 Phytologist 185:543-553.
595 Bingham MA, Simard, SW 2011. Do mycorrhizal network benefits to survival and
596 growth of interior Douglas-fir seedlings increase with soil moisture stress?
597 Ecology and Evolution 1:306-316.
598 Booth MG. 2004. Mycorrhizal networks mediate overstorey-understorey competition in
599 a temperate forest. Ecology Letters 7:538–546.
600 Brearley FQ. 2003. The Role of Ectomycorrhizas in the Regeneration of Dipterocarp
601 Seedlings [Doctoral Thesis]. [Sheffield]: University of Sheffield.
602 Brearley FQ, Press MC, Scholes JD. 2003. Nutrients obtained from leaf litter can
603 improve the growth of dipterocarp seedlings. New Phytologist 160:101–110.
604 Brearley FQ. 2006. Differences in the growth and ectomycorrhizal community of
605 *Dryobalanops lanceolata* (Dipterocarpaceae) seedlings grown in ultramafic and
606 non-ultramafic soils. Soil Biology and Biochemistry 38:3407–3410.
607 Brearley FQ, Proctor J, Suriantata Nagy L, Dalrymple G, Voysey BC. 2007a.
608 Reproductive phenology over a 10-year period in a lowland evergreen rain forest
609 of central Borneo. Journal of Ecology 95:828–839.
610 Brearley FQ, Scholes JD, Press MC, Palfner G. 2007b. How does light and phosphorus
611 fertilisation affect the growth and ectomycorrhizal community of two contrasting
612 dipterocarp species? Plant Ecology 192:237–249.
613 Brearley FQ. 2011. The importance of ectomycorrhizas for the growth of dipterocarps
614 and the efficacy of ectomycorrhizal inoculation schemes. In: Rai M, Varma A
615 (editors). Diversity and Biotechnology of Ectomycorrhizae. Berlin (Germany):
616 Springer-Verlag. p. 3–17.
617 Brearley FQ. 2012. Ectomycorrhizal associations of the Dipterocarpaceae. Biotropica
618 44:637–648.
619 Buchmann N, Guehl JM, Barigah TS, Ehleringer JR. 1997. Interseasonal comparison of
620 CO_2 concentrations, isotopic composition, and carbon dynamics in an Amazonian
621 rainforest (French Guiana). Oecologia 110:120–131.
622 Chang YS, Lapeyrie FF, Lee SS. 1994. The survival and competitiveness of *Pisolithus*
623 *tinctorius* on outplanted seedlings of Shorea glauca in Malaysia. In: S Appanah,
624 KC Khoo (editors). Proceedings of the Fifth Round Table Conference on
625 Dipterocarps. Kepong (Malaysia): Forest Research Institute. p. 165–169.

626 Chang YS, Lee SS, Lapeyrie FF, Yazid SM. 1995. The competitiveness of two strains
627 of *Pisolithus tinctorius* on seedlings of three species of dipterocarps under nursery
628 and field conditions: preliminary results. In: Wickneswari R, Yahya AZ, Shariff
629 AHM, Haji Ahmad D, Khoo KC, Suzuki K, Sakurai S, Ishii K (editors).
630 Proceedings of the International Workshop of BIO-REFOR, Kangar, 1994. Tokyo
631 (Japan) & Kepong (Malaysia): BIO-REFOR, IUFRO-SPDC & Forest Research
632 Institute Malaysia. p. 208–212.
633 Connell JH, Lowman MD. 1989. Low-diversity tropical rain forests: some possible
634 mechanisms for their existence. The American Naturalist 134:88–119.
635 Coomes DA, Grubb PJ. 2000. Impacts of root competition on forests and woodlands: a
636 theoretical framework and review of experiments. Ecological Monographs
637 70:171–207.
638 Curran LM, Caniago I, Paoli GD, Astianti D, Kusneti M, Leighton M, Nirarita CE,
639 Haeruman H. 1999. Impact of El Niño and logging on canopy tree recruitment in
640 Borneo. Science 286:2184–2188.
641 Dent DH, Bagchi R, Robinson D, Majalap-Lee N, Burslem DFRP. 2006. Nutrient
642 fluxes via litterfall and leaf litter decomposition vary across a gradient of soil
643 nutrient supply in a lowland tropical rain forest. Plant and Soil 288:197–215.
644 Diédhiou AG, Selosse M-A, Galiana A, Diabaté M, Dreyfus B, Bâ AM, de Faria SM,
645 Béna G. 2010. Multi-host ectomycorrhizal fungi are predominant in a Guinean
646 tropical rainforest and shared between canopy trees and seedlings. Environmental
647 Microbiology 8:2219–2232.
648 Egerton-Warburton LM, Querejeta JI, Allen MF. 2007. Common mycorrhizal networks
649 provide a potential pathway for the transfer of hydraulically lifted water between
650 plants. Journal of Experimental Botany 58:1473-1483.
651 Eschenbach C, Glauner R, Kleine M, Kappen L. 1998. Photosynthesis rates of selected
652 tree species in lowland dipterocarp rainforest of Sabah, Malaysia. Trees 12:356–
653 365.
654 Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and
655 photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology
656 40:503–537.
657 Fox JED. 1973. Kabili-Sepilok Forest Reserve, Sabah Forest Record 9. Malaysia:
658 Borneo Literature Bureau.
659 Francis R, Read DJ. 1984. Direct transfer of carbon between plants connected by

660 vesicular-arbuscular mycorrhizal mycelium. Nature 307:53–56.
661 Frazer GW, Canham CD, Lertzman KP. 1999. Gap Light Analyzer (GLA) Version 2.0:
662 Imaging software to extract canopy structure and gap light transmission indices
663 from true-colour fisheye photographs, users manual and program documentation.
664 Burnaby, British Columbia (USA) & Millbrook, New York (USA): Simon Fraser
665 University, & The Institute of Ecosystem Studies.
666 Hector A, von Felten S, Schmid B. 2010. Analysis of variance with unbalanced data: an
667 update for ecology & evolution. Journal of Animal Ecology 79:308–316.
668 Hector A, Philipson CD, Saner P, Chamagne J, Dzulkifli D, O'Brien M, Snaddon JL,
669 Ulok P, Weilenmann M, Reynolds G, et al. 2011. The Sabah Biodiversity
670 Experiment: a long-term test of the role of tree diversity in restoring tropical
671 forest structure and functioning. Philosophical Transactions of the Royal Society
672 of London Series B-Biological Sciences 366:3303–3315.
673 Hector A. 2015. The New Statistics with R. Oxford (UK): Oxford University Press.
674 Heinemeyer A, Hartley IP, Evans SP, Carreira de la Fuente JA, Ineson P. 2007. Forest
675 soil CO_2 flux: uncovering the contribution and environmental responses of
676 ectomycorrhizas. Global Change Biology 13:1786–1797.
677 Johnson D, Leake JR, Read DJ. 2001. Novel in-growth core system enables functional
678 studies of grassland mycorrhizal mycelial networks. New Phytologist 152:555–
679 562.
680 Katayama A, Kume T, Komatsu H, Ohashi M, Nakagawa M, Yamashita M, Otsuki K,
681 Suzuki M, Kumagai T. 2009. Effect of forest structure on the spatial variation in
682 soil respiration in a Bornean tropical rainforest. Agricultural and Forest
683 Meteorology 149:1666–1673.
684 Kennedy P, Peay KG, Bruns TD. 2009. Root tip competition among ectomycorrhizal
685 fungi: are priority effects a rule or an exception? Ecology 90:2098–2107.
686 Klein T, Siegwolf RTW, Körner C. 2016. Belowground carbon trade among tall trees in
687 a temperate forest. Science 352:342–344.
688 Lee SS, Alexander IJ. 1996. The dynamics of ectomycorrhizal infection of *Shorea*
689 *leprosula* seedlings in Malaysian rain forests. New Phytologist 132:297–305.
690 Marsh CW, Greer AG. 1992. Forest land-use in Sabah, Malaysia: an introduction to
691 Danum Valley. Philosophical Transactions of the Royal Society of London Series
692 B-Biological Sciences 335:331–339.

693 McGuire KL. 2007. Common ectomycorrhizal networks may maintain monodominance
694 in a tropical rain forest. Ecology 88:567–574.
695 Medina E, Minchin P. 1980. Stratification of $\delta^{13}C$ values of leaves in Amazonian rain
696 forests. Oecologia 45:377–378.
697 Medina E, Montes G, Cuevas E, Rokzandic Z. 1986. Profiles of CO_2 concentration and
698 $\delta^{13}C$ values in tropical rain forests of the upper Rio Negro Basin, Venezuela.
699 Journal of Tropical Ecology 2:207–217.
700 Medina E, Sternberg L, Cuevas E. 1991. Vertical stratification of $\delta^{13}C$ values in closed
701 natural and plantation forests in the Luquillo mountains, Puerto Rico. Oecologia
702 87:369–372.
703 Meijer W, Wood GHS. 1964. Dipterocarps of Sabah (North Borneo), Sabah Forest
704 Record 5. Sandakan (Malaysia): Forest Department.
705 Michaëlla Ebenye HC, Taudière A, Niang N, Ndiaye C, Sauve M, Onguene Awana N,
706 Verbeken M, De Kesel A, Séne S, Diédhiou AG, Sarda V, Sadio O, Cissoko M,
707 Ndoye I, Selosse M-A, Bâ AM. in press. Ectomycorrhizal fungi are shared
708 between seedlings and adults in a monodominant *Gilbertiodendron dewevrei* rain
709 forest in Cameroon. Biotropica.
710 Newbery DM, Alexander IJ, Rother JA. 2000. Does proximity to conspecific adults
711 influence the establishment of ectomycorrhizal trees in rain forest? New
712 Phytologist 147:401–409.
713 Newman MF, Burgess PF Whitmore TC. 1996. Manuals of Dipterocarps for Foresters:
714 Borneo Island Light Hardwoods. Edinburgh (UK): Royal Botanic Garden.
715 Newman MF, Burgess PF Whitmore TC. 1998. Manuals of Dipterocarps for Foresters:
716 Borneo Island Medium and Heavy Hardwoods. Edinburgh (UK): Royal Botanic
717 Garden.
718 Newsham KK, Fitter AH, Watkinson AR. 1994. Root pathogenic and arbuscular
719 mycorrhizal fungi determine fecundity of asymptomatic plants in the field.
720 Journal of Ecology 82:805–814.
721 Nilus R. 2004. Effect of Edaphic Variation on Forest Structure, Dynamics, Diversity
722 and Regeneration in a Lowland Tropical Rain Forest in Borneo. [Doctoral Thesis].
723 [Aberdeen]: University of Aberdeen.
724 O'Leary MH. 1988. Carbon isotopes in photosynthesis. Bioscience 38:328–336.
725 Onguene NA, Kuyper TW. 2002. Importance of the ectomycorrhizal network for
726 seedling survival and ectomycorrhiza formation in rain forests of south Cameroon.

727 Mycorrhiza 12:13–17
728 Paine CET, Stenflo M, Philipson CD, Saner P, Bagchi R, Ong RC, Hector A. 2012a.
729 Differential growth responses in seedlings of ten species of Dipterocarpaceae to
730 experimental shading and defoliation. Journal of Tropical Ecology 28:377–384.
731 Paine CET, Marthews TR, Vogt DR, Purves D, Rees M, Hector A, Turnbull LA. 2012b.
732 How to fit nonlinear plant growth models and calculate growth rates: an update
733 for ecologists. Methods in Ecology and Evolution 3:245–256.
734 Peay KG, Russo SE, McGuire KL, Lim Z, Chan JP, Tan S, Davies SJ. 2015. Lack of
735 host specificity lead to independent assortment of dipterocarps and
736 ectomycorrhizal fungi across a soil fertility gradient. Ecology Letters 18:807–816.
737 Philip LJ, Simard SW. 2008. Minimum pulses of stable and radioactive carbon isotopes
738 to detect belowground carbon transfer between plants. Plant and Soil 308:23–35.
739 Pickles BJ, Pither J. 2014. Still scratching the surface: how much of the 'black box' of
740 soil ectomycorrhizal communities remains in the dark? New Phytologist
741 201:1101–1105.
742 Pinheiro JC, Bates DM. 2000. Mixed-effects Models in S and S-Plus. New York
743 (USA): Springer Verlag.
744 R Development Core Team. 2015. R: A Language and Environment for Statistical
745 Computing. R Foundation for Statistical Computing. Vienna (Austria): R
746 Foundation for Statistical Computing.
747 Sakai S, Harrison RD, Momose K, Kuraji K, Nagamasu H, Yasunari T, Chong L,
748 Nakashizuka T. 2006. Irregular droughts trigger mass flowering in aseasonal
749 tropical forests in Asia. American Journal of Botany 93:1134–1139.
750 Saner P, Philipson CD, Ong RC, Majalap N, Egli S, Hector A. 2011. Positive effects of
751 ectomycorrhizal colonization on growth of seedlings of a tropical tree across a
752 range of forest floor light conditions. Plant and Soil 338:411–421.
753 Saner P, Loh YY, Ong RC, Hector A. 2012. Carbon stocks and fluxes in tropical
754 lowland dipterocarp rainforests in Sabah, Malaysian Borneo. PLoS One 7:e29642.
755 Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R. 1997. Net
756 transfer of carbon between ectomycorrhizal tree species in the field. Nature
757 388:579–582.
758 Simard SW, Beiler KJ, Bingham MA, Deslippe JR, Philip LJ, Teste FP. 2012.
759 Mycorrhizal networks: mechanisms, ecology and modeling. Fungal Biology
760 Reviews 26:39–60.

761 Slik JWF, Poulsen AD, Ashton PS, Cannon CH, Eichhorn KAO, Kartawinata K,
762 Lanniari I, Nagamasu H, Nakagawa M, van Nieuwstadt MGL, et al. 2003. A
763 floristic analysis of the lowland dipterocarp forests of Borneo. Journal of
764 Biogeography 30:1517–1531.
765 Slik JWF, Raes N, Aiba SI, Brearley FQ, Cannon CH, Meijaard, E, Nagamasu H, Nilus
766 R, Paoli G, Poulsen AD, Sheil D, Suzuki E, van Valkenburg JLCH, Webb CO,
767 Wilkie P, Wulfraat S. 2009. Environmental correlates for tropical tree diversity
768 and distribution patterns in Borneo. Diversity and Distributions 15:523–532.
769 Smith ME, Henkel TW, Aime MC, Fremier A, Vilgalys R. 2011. Ectomycorrhizal
770 fungal diversity and community structure on three co-occurring leguminous
771 canopy tree species in a Neotropical rainforest. New Phytologist 192:699–712.
772 Smith SE, Read DJ. 2008. Mycorrhizal Symbiosis, 3rd edn. London (UK): Academic
773 Press.
774 Tedersoo L, Sadam A, Zambrano M, Valencia R, Bahram M. 2010. Low diversity and
775 high host preference of ectomycorrhizal fungi in Western Amazonia, a neotropical
776 biodiversity hotspot. The ISME Journal 4:465–746.
777 Teste FP, Karst J, Jones MD, Simard SW, Durall DM. 2006. Methods to control
778 ectomycorrhizal colonization: effectiveness of chemical and physical barriers.
779 Mycorrhiza 17:51–65.
780 Vallack HW, Leronni V, Metcalfe DB, Högberg P, Ineson P, Subke J-A. 2012.
781 Application of nitrogen fertilizer to a boreal pine forest has a negative impact on
782 the respiration of ectomycorrhizal hyphae. Plant and Soil 352:405–417.
783 van der Heijden, MGA, Sanders IR. 2002. Mycorrhizal Ecology, Ecological Studies 157.
784 Berlin (Germany): Springer-Verlag.
785 Yasman I. 1995. Dipterocarpaceae: Tree-Mycorrhizae-Seedling Connections. [Doctoral
786 Thesis]. Wageningen: University of Wageningen.

Figure legends

Figure 1: Effect of fungicide addition, but no effect of exclusion from an ectomycorrhizal hyphal network on the relative diameter growth rate (RGR) of two species of dipterocarp seedlings (top: *Hopea nervosa* and bottom: *Parashorea tomentella*) over a 24-month period at Kabili-Sepilok Forest Reserve in Sabah (Malaysian Borneo). The box indicates the data range from the lower quartile (25%) to the upper quartile (75%) and covers 50% of the data with the solid horizontal line within the box indicating the median. Whiskers indicate the data range from the lower 10% to the upper 90% (1.5 times the lower or upper quartile); outliers are indicated separately with a dot. See text for full details of experimental treatments.

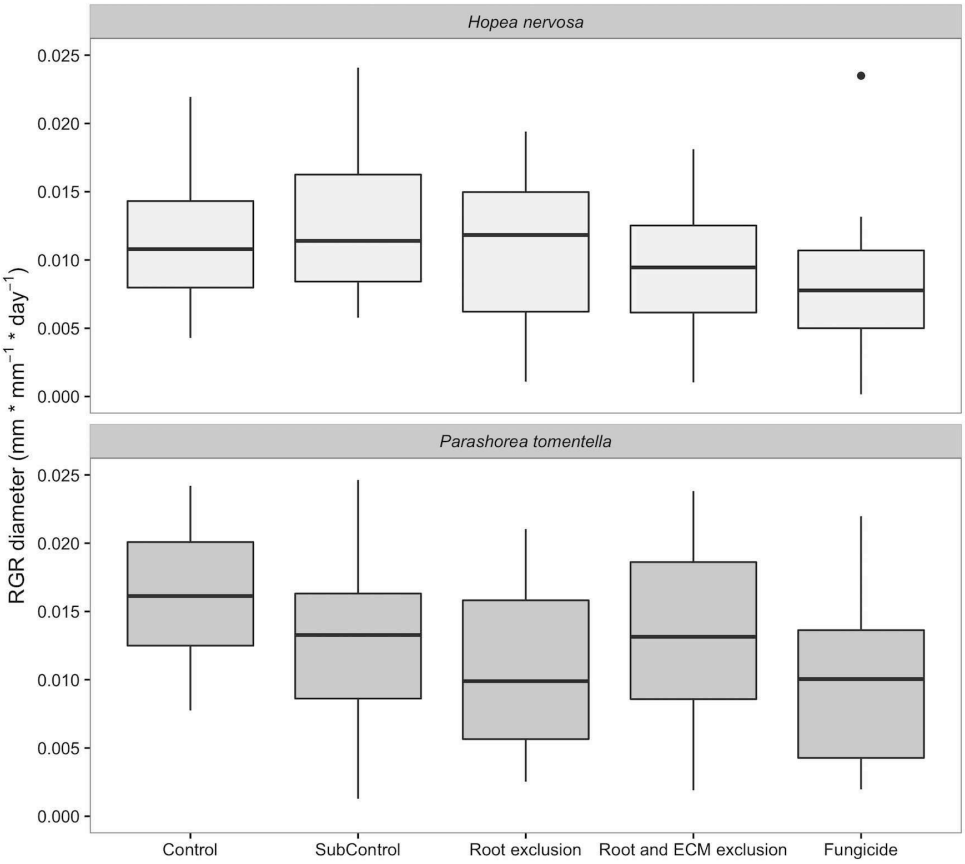


Figure 2: No effect of exclusion from an ectomycorrhizal hyphal network on the relative diameter growth rate (RGR) of two species of dipterocarp seedlings by distance from adult tree (top: *Dryobalanops lanceolata* and bottom: *Shorea parvifolia*) over an 11-month period at Malua Forest Reserve in Sabah (Malaysian Borneo). The box indicates the data range from the lower quartile (25%) to the upper quartile (75%) and covers 50% of the data with the solid horizontal line within the box indicating the median. Whiskers indicate the data range from the lower 10% to the upper 90% (1.5 times the lower or upper quartile); outliers are indicated separately with a dot. See text for full details of experimental treatments.

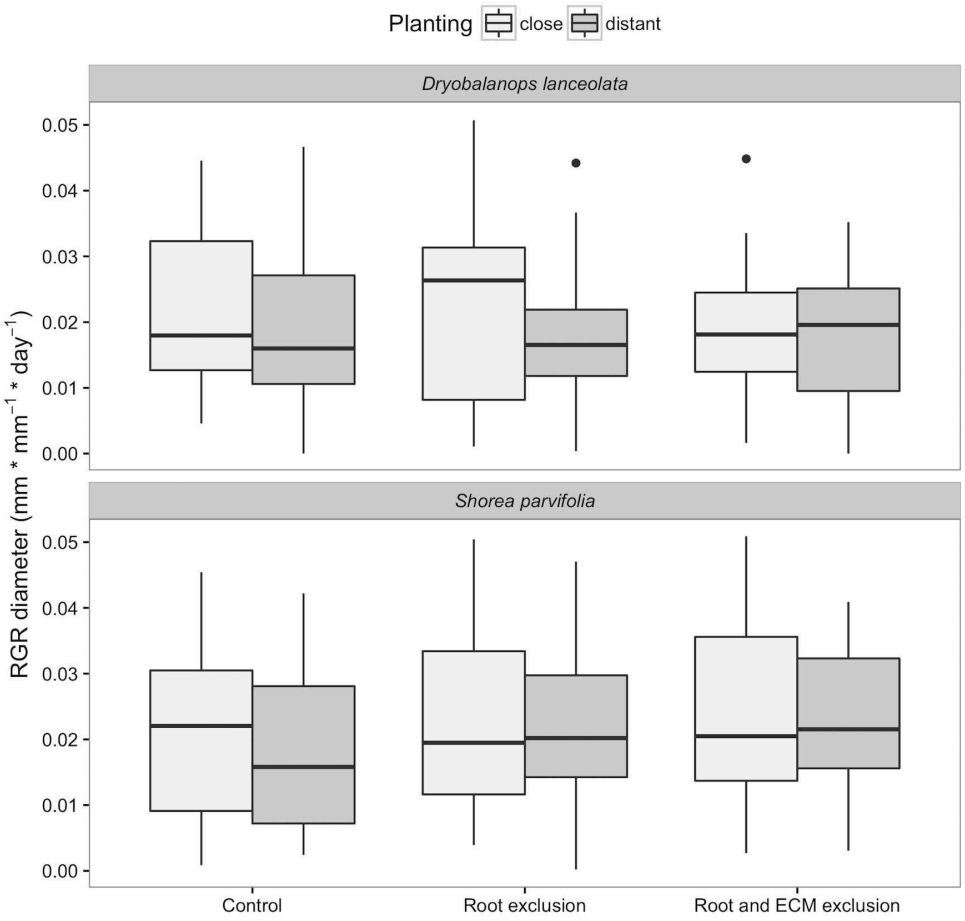
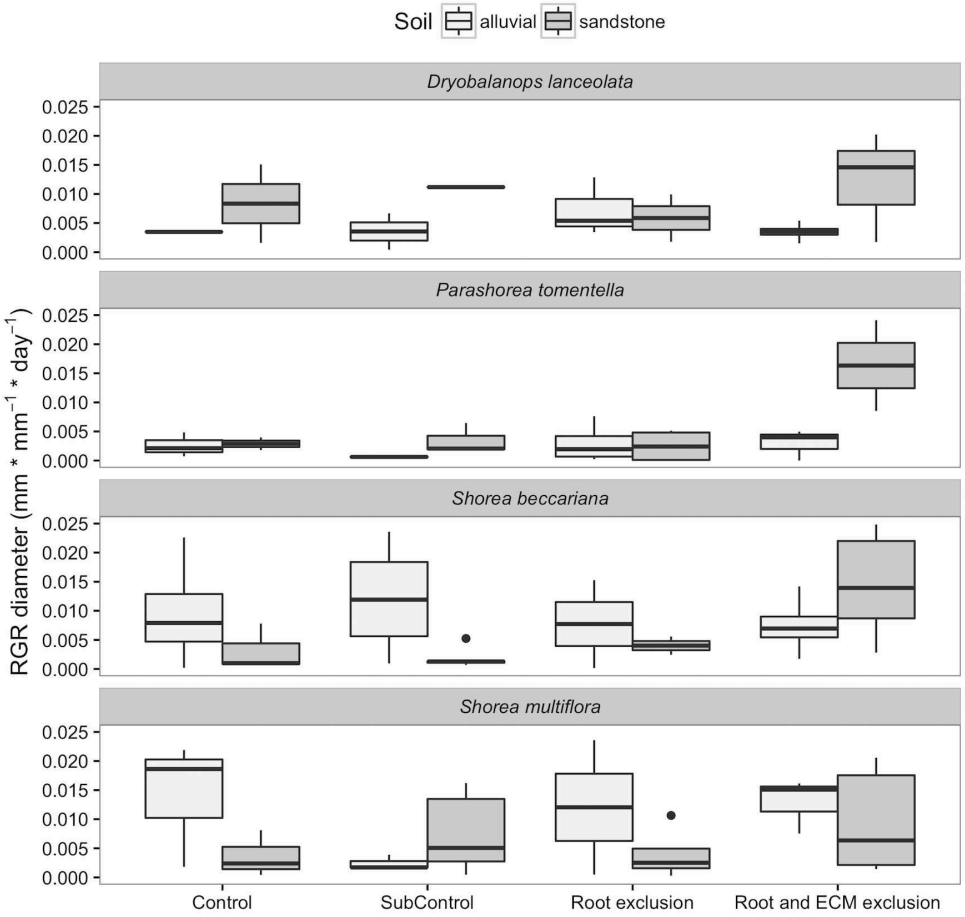


Figure 3: No effect of exclusion from an ectomycorrhizal hyphal network on the relative diameter growth rate (RGR) of four species of dipterocarp seedlings (top: *Dryobalanops lanceolata*, middle top: *Parashorea tomentella*, middle bottom: *Shorea beccariana* and bottom: *Shorea multiflora*) across soil types over a 29-month period at Kabili-Sepilok Forest Reserve in Sabah (Malaysian Borneo). Note that *S. beccariana* and *S. multiflora* growing in the alluvial soil type were harvested after 15 months due to high mortality rates. The box indicates the data range from the lower quartile (25%) to the upper quartile (75%) and covers 50% of the data with the solid horizontal line within the box indicating the median. Whiskers indicate the data range from the lower 10% to the upper 90% (1.5 times the lower or upper quartile); outliers are indicated separately with a dot. See text for full details of experimental treatments.



817 **Table 1:** Ecological information on seedlings of six dipterocarp species used to
818 experimentally assess the important of incorporation into a common EcM network on
819 seedling growth in tropical forests of Malaysian Borneo.

Name	Size	Wood density	Distribution	Experiment(s)
Dryobalanops lanceolata	Very large emergent	Medium / heavy	Common on fertile clay-rich soils in lowland northern Borneo	2,3
Hopea nervosa	Medium-sized	Heavy	Locally common in eastern Sabah	1
Parashorea tomentella	Large emergent	Light	Locally common on fertile lowland soils with occasional flooding (only on the east coast of northern Borneo)	1,3
Shorea beccariana	Medium-sized to large	Light	Common in northern Borneo on sandy soils and particularly ridge-tops associated with sandstone rocks.	3
Shorea multiflora	Small to medium-sized	Light	Common throughout Borneo on nutrient-poor or sandy soils and coastal hill slopes.	3,4
Shorea parvifolia	Large emergent	Light	Common throughout Borneo on better-drained clay soils.	2

820 Information collated from Ashton (2004), Meijer and Wood (1964), Newman *et al*. (1996, 1998) and
821 personal observations.
822

Table 2: Experiment 1: Statistical summary table and biological interpretation of exclusion from an ectomycorrhizal hyphal network on relative growth rates (diameter, height and number of leaves) and survival for two species of dipterocarp seedlings (*Hopea nervosa* and *Parashorea tomentella*) over a 24-month period at Kabili-Sepilok Forest Reserve in Sabah (Malaysian Borneo). -R = Root exclusion, -RM = Root and ectomycorrhiza exclusion, -RM+F = Root and ectomycorrhiza exclusion plus fungicide addition.

Effect	F_{df}	***P***	**Interpretation**
Diameter			
Treatment	$F_{4,220}$=12.8	<0.0001	-RM+F reduced growth of *Hopea nervosa* -RM treatment showed faster growth rate compared to -R in *Parashorea tomentella*
Species	$F_{1,220}$=19.2	<0.0001	Seedlings of *Parashorea tomentella* grew faster than *Hopea nervosa*
Treatment x Species	$F_{4,220}$=2.7	<0.05	Slower growth with =RM+F for *Hopea nervosa* but not *Parashorea tomentella*
Height			
Treatment	$F_{4,220}$=1.4	ns	No ectomycorrhizal network effect
Species	$F_{1,220}$=4.1	<0.05	*Parashorea tomentella* grew faster than *Hopea nervosa*
Treatment x Species	$F_{4,220}$=0.8	ns	No significant interaction term
Leaves			
Treatment	$F_{4,220}$=5.0	<0.001	*Hopea nervosa* control seedlings grew faster than -R and -RM -RM+F significantly reduced growth in *Hopea nervosa*
Species	$F_{1,220}$=116.3	<0.0001	*Hopea nervosa* seedlings grew faster than *Parashorea tomentella* seedlings
Treatment x Species	$F_{4,220}$=1.8	ns	No significant interaction term
Survival			
Treatment	$\chi^2_{3,7}$=4.0	ns	No effect of treatment on survival
Species	$\chi^2_{6,7}$=6.4	<0.01	Seedlings of *Hopea nervosa* showed higher survival compared to *Parashorea tomentella*
Treatment x Species	$\chi^2_{7,11}$=4.2	ns	No significant interaction term

832 **Table 3:** Survival rates (%) of dipterocarp seedlings following exclusion from an
833 ectomycorrhizal hyphal network in four independent experiments conducted in Borneo.
834 See text for full details of experimental set-up in each experiment. Dash (-) indicates
835 that the treatment noted was not present in the given experiment. Asterisk (*) indicates
836 15 months to harvest whilst all other values are for the entire experimental period. -R =
837 Root exclusion, -RM = Root and ectomycorrhiza exclusion, -RM+F = Root and
838 ectomycorrhiza exclusion plus fungicide addition.

	Control	**Sub-Control**	**-R**	**-RM**	**-RM+F**
Experiment 1					
Parashorea tomentella	79	83	80	71	80
Hopea nervosa	96	92	100	88	83
Experiment 2					
Near					
Dryobalanops lanceolata	98	-	97	95	-
Shorea parvifolia	98	-	97	89	-
Far					
Dryobalanops lanceolata	99	-	100	97	-
Shorea parvifolia	98	-	98	90	-
Experiment 3					
Alluvial					
Dryobalanops lanceolata	50	70	60	70	-
Shorea beccariana	30*	30*	30*	20*	-
Shorea multiflora	20*	30*	40*	10*	-
Parashorea tomentella	20	20	40	20	-
Sandstone					
Dryobalanops lanceolata	40	40	50	60	-
Shorea beccariana	60	60	40	30	-
Shorea multiflora	50	30	40	30	-
Parashorea tomentella	40	70	60	30	-
Experiment 4					
Shorea multiflora	92	-	-	88	-

839

840 **Table 4:** Experiment 2: Statistical summary table and biological interpretation of
841 exclusion from an ectomycorrhizal hyphal network and distance from adult tree on
842 relative growth rates (diameter, height and number of leaves) and survival for two
843 species of dipterocarp seedlings (*Dryobalanops lanceolata* and *Shorea parvifolia*) over
844 an 11-month period at the Malua Forest Reserve in Sabah (Malaysian Borneo).
845 Interaction terms not included were not statistically significant for any of the parameters
846 measured. -R = Root exclusion, -RM = Root and ectomycorrhiza exclusion.

Effect	F_{df}	***P***	**Interpretation**
Diameter			
Treatment	$F_{2,238}$=0.6	ns	No ectomycorrhizal network effect
Distance	$F_{1,238}$=1.1	ns	No effect of distance from adult tree
Species	$F_{1,238}$=10.2	<0.01	*Dryobalanops lanceolata* grew faster than *Shorea parvifolia*
Treatment x Species	$F_{2,238}$=0.3	ns	No significant interaction term
Height			
Treatment	$F_{2,238}$=1.2	ns	No ectomycorrhizal network effect
Distance	$F_{1,238}$=9.3	<0.01	*Dryobalanops lanceolata* grew faster closer to adult trees
Species	$F_{1,238}$=0.1	ns	No species differences
Treatment x Species	$F_{2,238}$=2.5	<0.10	*Shorea parvifolia* control seedlings grew marginally faster than -R and significantly faster than -RM but no effect on *Dryobalanops lanceolata*
Leaves			
Treatment	$F_{2,238}$=3.1	<0.05	*Shorea parvifolia* -RM seedlings grew slower than the -R treatment
Distance	$F_{1,238}$=0.9	ns	No effect of distance from adult tree
Species	$F_{1,238}$=0.1	ns	No species differences
Treatment x Species	$F_{2,238}$=3.2	<0.05	*Dryobalanops lanceolata* -RM seedlings grew slower, but no effect on *Shorea parvifolia*
Survival			
Treatment	$\chi^2_{3,5}$=13.3	<0.0001	-RM showed significantly lower survival for both species
Distance	$\chi^2_{5,6}$=1.0	ns	No effect of distance from adult tree
Species	$\chi^2_{4,5}$=4.4	<0.05	Survival rate in *Shorea parvifolia* lower than in *Dryobalanops lanceolata*
Treatment x Species	$\chi^2_{5,7}$=0.4	ns	No significant interaction term

847

Table 5: Experiment 3: Statistical summary table and biological interpretation of exclusion from an ectomycorrhizal hyphal network and soil type on relative growth rates (diameter, height and number of leaves) for four species of dipterocarp seedlings (*Dryobalanops lanceolata*, *Parashorea tomentella*, *Shorea beccariana* and *Shorea multiflora*) over a 29-month period at Kabili-Sepilok Forest Reserve in Sabah (Malaysian Borneo). The three-way interaction term is not included as it was not statistically significant for any of the parameters measured. -R = Root exclusion, -RM = Root and ectomycorrhiza exclusion.

Effect	F_{df}	***P***	**Interpretation**
Diameter			
Treatment	$F_{3,276}$=2.6	<0.10	See interactions below
Soil type	$F_{1,276}$=0.8	ns	No soil type effect
Species	$F_{3,276}$=0.8	ns	No species effect
Treatment x Species	$F_{9,276}$=1.3	ns	No significant interaction term
Treatment x Soil type	$F_{3,276}$=2.7	<0.05	-RM of *Parashorea tomentella*, *Shorea beccariana* and *Shorea multiflora* grew faster on sandstone soil than -R for all three species
Species x Soil type	$F_{3,276}$=5.4	<0.01	-R of *Dryobalanops lanceolata* grew faster on alluvial soil and overall faster than –RM
Height			
Treatment	$F_{3,276}$=1.0	ns	No ectomycorrhizal network effect
Soil type	$F_{1,276}$=0.9	ns	No soil type effect
Species	$F_{3,276}$=3.6	<0.05	-R of *Parashorea tomentella* grew faster on sandstone soil
Treatment x Species	$F_{9,276}$=1.0	ns	No significant interaction term
Treatment x Soil type	$F_{3,276}$=1.1	ns	No significant interaction term
Species x Soil type	$F_{3,276}$=0.4	ns	No significant interaction term
Leaves			
Treatment	$F_{3,276}$=1.1	ns	No ectomycorrhizal network effect
Soil type	$F_{1,276}$=0.1	ns	No soil type effect
Species	$F_{3,276}$<0.1	ns	No species effect
Treatment x Species	$F_{9,276}$=1.4	ns	No significant interaction term
Treatment x Soil type	$F_{3,276}$=2.0	ns	No significant interaction term
Species x Soil type	$F_{3,276}$=4.7	<0.01	*Dryobalanops lanceolata* seedlings grew faster on alluvial soil than sandstone soil
Survival			
Treatment	$\chi^2_{6,9}$=1.2	ns	No effect of treatment on survival
Soil type	$\chi^2_{8,9}$=6.0	<0.01	Survival on alluvial soil was significantly lower compared to sandstone soil
Species	$\chi^2_{6,9}$=7.2	<0.10	Seedlings of *Shorea multiflora* and *Shorea beccariana* showed lowest survival after 15 months
Treatment x Species	$\chi^2_{9,18}$=4.3	Ns	No significant interaction term
Treatment x Soil type	$\chi^2_{9,12}$=2.3	ns	No significant interaction term
Species x Soil type	$\chi^2_{9,12}$=4.6	ns	No significant interaction term