

Transfer of ^{13}C between paired Douglas-fir seedlings reveals plant kinship effects and uptake of exudates by ectomycorrhizas

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1 **Transfer of ^{13}C between paired Douglas-fir seedlings reveals plant kinship**
2 **effects and uptake of exudates by ectomycorrhizas**

3

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25

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27 **Summary**

28

29 • Processes governing the fixation, partitioning, and mineralization of carbon in soils are
30 under increasing scrutiny as we develop a more comprehensive understanding of global
31 carbon cycling. Here we examined fixation by Douglas-fir seedlings and transfer to
32 associated ectomycorrhizal fungi, soil microbes, and full-sibling or non-sibling
33 neighbouring seedlings.

34

35 • Stable isotope probing with 99% ^{13}C -CO₂ was applied to trace ^{13}C -labelled
36 photosynthate throughout plants, fungi, and soil microbes in an experiment designed to
37 assess the effect of relatedness on ^{13}C -transfer between plant pairs. The fixation and
38 transfer of ^{13}C -label to plant, fungal, and soil microbial tissue was examined in biomass
39 and PLFAs.

40

41 • After a 6-day chase period, approximately 26.8% of the ^{13}C remaining in the system
42 was translocated belowground. Enrichment was proportionally greatest in
43 ectomycorrhizal biomass. The presence of mesh barriers (0.5 or 35 μm) between
44 seedlings did not restrict ^{13}C -transfer.

45

46 • Fungi were the primary recipients of ^{13}C -labelled photosynthate throughout the system,
47 representing 60–70% of total ^{13}C -enriched phospholipids. Full-sibling pairs exhibited
48 significantly greater ^{13}C -transfer to recipient roots in two of four Douglas-fir families,
49 representing 3- and 4-fold increases (+ approx. 4 μg excess ^{13}C) compared to non-
50 sibling pairs. The existence of a root/mycorrhizal exudation – hyphal uptake pathway
51 was supported.

52

53 **Key words** (5-8): Carbon allocation / ectomycorrhizas / host relatedness / interior Douglas-fir /
54 PLFA / stable-isotope probing.

55 Introduction

56 Accurate estimates of belowground carbon cycling are critical to linking terrestrial
57 ecosystems with biogeochemical processes and making useful predictions about how these
58 may change under future climates (Richter & Billings, 2015). In temperate forests, the
59 estimated quantities of atmospheric carbon sequestered via fixation are globally-relevant (~73-
60 159 Pg C aboveground, and 153-195 Pg C belowground; Reichstein, 2007; Lorenz & Lal,
61 2010), with humid and warm evergreen forests displaying the highest gross primary production
62 of any temperate or boreal forest types (Luyssaert *et al.*, 2007). Photosynthate is mainly
63 incorporated into plant biomass, but also supports a diverse microbial soil community either
64 directly, via mycorrhizal fungi, or more generally, via scavenging of root exudates (Nehls *et*
65 *al.*, 2007; Phillips *et al.*, 2011) and rhizodeposits (Jones *et al.*, 2009). Studying the
66 belowground ecology governing carbon cycling is challenging due to the complexity of these
67 communities and soil systems in general (De Deyn *et al.*, 2008; Bardgett *et al.*, 2013), and
68 requires quantitative data on the allocation of photosynthate to plant biomass and its transfer to
69 mycorrhizal fungi and microbes (Kaiser *et al.*, 2015). Here we undertook a multifaceted
70 exploration of carbon allocation within and between paired interior Douglas-fir (*Pseudotsuga*
71 *menziesii* var. *glauca*) seedlings, their mycorrhizal symbionts, and soil microbiota.

72 While the majority of plants form mycorrhizas, less than 5% of plant species are
73 estimated to associate with ectomycorrhizal fungi (EMF). However, globally, these interactions
74 are abundant in all forest biomes (Brundrett, 2009). Soils in ecosystems where ectomycorrhizal
75 plants dominate exhibit higher C:N ratios compared to soils where they do not (Averill *et al.*,
76 2014), and ectomycorrhizal hosts have been found to allocate 10% – 30% of their
77 photosynthate to mycorrhizas (Söderström, 1992; Leake *et al.*, 2006; Högberg & Read, 2006).
78 EMF incorporate photosynthate into their biomass, enhancing carbon sequestration by
79 synthesising recalcitrant carbon compounds like chitin (Clemmensen *et al.*, 2013; Kashian *et*
80 *al.*, 2013), especially in poorly oxygenated soil where decomposition is slow (Wallander *et al.*,
81 2001). Additionally, EMF add mineral nutrients to soils through the breakdown of rock with
82 organic acids (Hoffland *et al.*, 2003; Plassard & Fransson, 2009), and promote soil aggregate
83 formation and carbon sequestration by exuding extracellular proteins and compounds that bind
84 mineral particles (Rillig & Mummey, 2006; Graf & Frei, 2013). However, not all mycorrhizal
85 activity is a carbon sink. In addition to respiration of host-derived photosynthate, some EMF
86 species can decompose plant litter to acquire limiting nutrients, thus releasing soil carbon back
87 into the atmosphere at rates comparable to saprotrophic fungi (Talbot *et al.*, 2008; Rineau *et*
88 *al.*, 2013; Phillips *et al.*, 2014). Therefore, to assess how these processes jointly influence the

89 dynamics of soil carbon cycling in complex belowground systems, quantification of the fate of
90 photosynthate in EMF-dominated systems is required.

91 An emergent property of mycorrhizal systems is the common mycorrhizal network,
92 which arises when a fungal mycelium connects multiple plant hosts belowground across scales
93 of cm² to at least tens of m² (Selosse *et al.*, 2006; Beiler *et al.*, 2010; Simard *et al.*, 2012).
94 Ectomycorrhizal networks have been demonstrated to transfer water, nitrogen, and small
95 quantities of carbon between interior Douglas-fir hosts (Simard *et al.*, 1997a; Teste *et al.*,
96 2009; Bingham & Simard, 2011). Evidence that EMF display trait heritability based on host
97 and fungal genotype (Rosado *et al.*, 1994a,b; Karst *et al.*, 2008), and that closely related plants
98 display greater arbuscular mycorrhizal network size and root colonisation (File *et al.*, 2012;
99 Dudley *et al.*, 2013), raises the possibility of preferential connectivity of kin through
100 compatibility of parent-mycorrhiza-offspring genotypes. This has not previously been explored
101 in conifers. In arbuscular mycorrhizal grassland plant species, root exudates play a role in ‘kin
102 recognition’ (Dudley *et al.*, 2013) by moderating intra- and inter-specific plant root behaviour
103 (Semchenko *et al.*, 2014), suggesting that plant relatedness may influence nutrient uptake or
104 transfer by altering root growth and hence mycorrhizal formation. The finding that root
105 exudates are important in kin recognition suggests that mycorrhizas are involved in recognition
106 mechanisms in temperate forests, where trees are comprehensively mycorrhizal. If nutrient
107 transfer through ectomycorrhizal networks can also differ with host relatedness, then fitness,
108 and thereby forest stand composition, may be altered (Simard, 2009). Thus, to assess the
109 potential for host relatedness effects in the experimental system, the extent of carbon transfer
110 between ‘kin’ (full sibling) and ‘non-kin’ (no shared parent) seedling pairs was quantified.

111 Although mycorrhizal fungi are known to transfer labelled carbon between plants
112 (Finlay & Read, 1986; Simard *et al.*, 1997b), interpretations are split between those suggesting
113 retention of labelled carbon by fungi within their biomass (Graves *et al.*, 1997; Fitter *et al.*,
114 1998; Wu *et al.*, 2001) and those indicating small (Simard *et al.*, 1997a; Teste *et al.*, 2010;
115 Philip *et al.*, 2010) or large (Klein *et al.*, 2016) degrees of carbon transfer through fungal
116 mycelium and into plant biomass. The transfer of carbon between plants may also occur via
117 uptake of root exudates along an ‘exudation-dissolved organic carbon-mycorrhizal hyphae’
118 pathway (Robinson & Fitter, 1999), and these hypotheses regarding the mechanism of transfer
119 need not be mutually exclusive. In this experiment we established a size-hierarchy between
120 seedlings of different ages together in the same pot, separated by a mesh barrier to prevent
121 direct root interaction, and labelling the larger (older) seedling with ¹³C-CO₂.

122 A small number of stable isotope probing (SIP) studies, in which a plant is exposed to
123 ^{13}C -labelled CO_2 and the distribution of ^{13}C -photosynthate is examined after a specific time
124 period, have investigated interconnections between the Pinaceae and their EMF symbionts (see
125 Epron *et al.*, 2012). Here, for the first time in a paired seedling model system, we investigated
126 the fungal and microbial communities active in plant photosynthate assimilation and transfer,
127 applying SIP methods to quantify ^{13}C allocation to plant and fungal biomass and the
128 phospholipid fatty acids (PLFA) of plants, fungi, and bacteria (Boschker *et al.*, 1998). Our
129 study examined paired interior Douglas-fir seedlings with the following objectives: (i) to
130 quantify the distribution of ^{13}C -labeled photosynthate throughout plant and soil carbon pools
131 and within microbial biomass, (ii) to determine the proportion of carbon transferred (if any)
132 between seedlings by EMF symbionts, and (iii) to determine whether relatedness has a role in
133 carbon transfer between conifer seedlings.

134

135 **Materials and methods**

136 *Seed and soil*

137 Seeds from four ‘families’ (cross-bred from four different pairs of known parents) of
138 interior Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *glauca* (Beissn.) Franco) were
139 sourced from the B.C. Ministry of Forests, Lands, and Natural Resource Operations
140 (Kalamalka Research Station, B.C.). To encourage EMF colonization, seeds were grown in a
141 soil mixture with the following specifications: 1:1 mix (approximately 3.4 L total) of
142 autoclaved potting soil (1.7 L 1:1 mix of peat to perlite) and forest soil (1.7 L). Forest soil was
143 classified as Dystric Brunisol (Inceptisol in U.S. soil taxonomy) with moderate humus and
144 sandy loam texture (Soil Classification Working Group, 1998). Soil was collected from two
145 sub-locations within a mono-specific interior Douglas-fir stand (120.58°W, 49.43°N) in the
146 Dry, Cool Interior Douglas-fir (IDFdk) biogeoclimatic subzone (Pojar *et al.*, 1987). Following
147 the removal of the litter layer, the fermentation layer, humus layer, and mineral soil were
148 sampled to a total depth of 10-15 cm. Large debris was removed during collection and soil
149 was homogenised in clean conditions before sub-sampling to create experimental units.

150

151 *Experimental design and seedling growth*

152 Each experimental unit was a 3.8 L pot containing a pair of seedlings of different ages
153 to establish a carbon gradient, which were spaced approximately 8 cm apart and separated by a
154 nylon mesh bag (Plastok® Meshes and Filtration Ltd., Birkenhead, UK). One ‘donor’ seedling
155 was established from seed planted in March 2012 in the 805 g (11.9 s.e.m.) dry weight soil

156 outside the mesh bag. One ‘recipient’ seedling was established from seed planted in November
157 2012 inside the 8 x 18 cm mesh bag containing 403 g (5.9 s.e.m.) dry weight soil. Seedlings
158 were grown in a glasshouse without supplementary light or fertiliser, in order to encourage
159 mycorrhizal formation. Pots were watered to field capacity once per week following an early
160 germination period of light daily watering. A fine gravel layer was applied to soil surfaces to
161 discourage infection and mortality by pathogenic soil fungi (e.g. *Fusarium*, *Phytophthora*,
162 *Pythium*, *Rhizoctonia*; colloquially called ‘damping off’ fungi). Harvesting took place in the
163 first week of February 2013, when donor and recipient seedlings were 11 and 3 months old,
164 respectively (Asay, 2013).

165 Seedling relatedness was manipulated through seed origin: seedling pairs were either
166 full siblings (same parents; ‘kin’) or non-siblings (different parents; ‘non-kin’). Kin seed was
167 obtained from four pairs of parent trees to assess whether any relatedness effects were a
168 general observation or family specific. Sufficient seed was provided to establish 10 kin pairs
169 from each of the four sets of parents (n=40). Non-kin seed was more readily available so
170 additional pairs were planted (n=60). Hyphal connection was manipulated via mesh bags:
171 seedling pairs were separated by a root-blocking barrier that either allowed the passage of EMF
172 hyphae (35 µm mesh) or was expected to restrict or prevent the passage of EMF hyphae (0.5
173 µm) (Teste *et al.* (2006). Mesh sizes were divided equally among the relatedness treatments
174 resulting in the following experimental factors: kin-unrestricted (n=20), kin-restricted (n=20),
175 non-kin-unrestricted (n=30), non-kin-restricted (n=30).

176

177 ¹³CO₂ isotope labelling

178 Donor plants were pulse-labelled with 99 atom% ¹³C-CO₂ eleven months after they
179 were established in pots. Surviving pairs in each treatment were assigned to subsequent
180 analyses as follows. To estimate the initial uptake and fixation of ¹³C-label, we assessed
181 incorporation of ¹³C-photosynthate into plant and fungal biomass 1 day after labelling (“1-day
182 chase”): non-kin-unrestricted (n=3), non-kin-restricted (n=3), non-labelled controls (n=3). To
183 examine the transfer and incorporation of ¹³C-photosynthate into biomass 6 days after labelling
184 (“6-day chase”): kin-unrestricted (n=9), kin-restricted (n=8), non-kin-unrestricted (n=10), non-
185 kin-restricted (n=10). Unlabelled controls consisted of kin-unrestricted (n=9), kin-restricted
186 (n=8), non-kin-unrestricted (n=7), non-kin-restricted (n=7).

187 Immediately before labelling, all donor seedlings were sealed using Tuck® Contractors
188 Sheathing Tape inside plastic Foodsaver® vacuum bags (6 L). Bags were fitted with an
189 injection valve and inflated with ambient air. Non-labelled control seedlings were bagged in

190 the same manner and stored with a minimum separation distance of 4 m from the nearest
191 labelled seedling. Three injections of ^{13}C -CO₂ were received by labelled seedlings at equal
192 time intervals through the 10 h pulse period, totalling 50 mL of ^{13}C -CO₂ (with maximum
193 concentrations of 2500-3000 ppm). An additional seedling was used to monitor bag CO₂
194 concentration using a portable infrared gas analyser (Qubit Systems, Kingston, Canada).
195 Ambient greenhouse CO₂ levels (394 ppm) were checked prior to labelling, 5 h after labelling,
196 and 10 h after labelling and showed no significant variation. After the final pulse, when bag
197 CO₂ concentrations had dropped below 300 ppm, labelling bags were removed.

198

199 *Sampling of plant and soil pools*

200 Eleven distinct plant and soil pools were examined in this experiment, with donor (D)
201 and recipient (R) seedling samples collected as described in Figure 1. Due to growth stage
202 differences between donor and recipient seedlings, root sections were collected from donor
203 transport fine roots (McCormack *et al.*, 2015) and the recipient's main tap root, which were of
204 equivalent diameter and structure (i.e. no absorptive fine roots were included in these samples).
205 Samples were kept on dry ice after weighing, and stored at -80 °C. All fine root tips were
206 sampled from each seedling, morphotyped based on ectomycorrhizal structures or their absence
207 (Goodman *et al.*, 1998), then counted and weighed prior to subsampling for fungal
208 identification via amplified internal transcribed spacer (ITS) sequences. All root tips from six
209 plant pairs were kept on dry ice after weighing, and stored at -80 °C. Soil for PLFA analyses
210 was immediately frozen in liquid nitrogen before storage at -80 °C. Samples were lyophilized
211 prior to DNA or PLFA extraction and isotopic analysis. Remaining plant biomass from each
212 pool was oven-dried and weighed.

213

214 *Isotopic measurements*

215 Total carbon and nitrogen content and carbon isotopic composition of samples were
216 measured with combustion analysis using an elemental analyzer (Elementar, Hanau, Germany)
217 in C, N mode, interfaced with an isotope-ratio mass spectrometer (IRMS; Isoprime, Cheadle,
218 UK). Samples were considered enriched if their $\delta^{13}\text{C}$ value was greater than the upper 99%
219 confidence interval of the control mean $\delta^{13}\text{C}$ (natural abundance) and all control sample $\delta^{13}\text{C}$
220 values. Atom %¹³C excess was calculated for each pool as per Leake *et al.* (2006). Teste *et al.*'s
221 (2009) modification of Boutton's (1991) isotopic calculations was applied to convert $\delta^{13}\text{C}$ into
222 "excess ¹³C" as ¹²C-equivalent (mg), the mass of labelled carbon compensating for the one
223 Dalton difference in mass of ¹²C.

224 Isotopic composition of PLFAs was analysed using gas chromatography-IRMS,
225 following extraction according to Bligh & Dyer (1959), as detailed in Churchland *et al.* (2013),
226 with the following exceptions: (i) methyl undecanoate (c11:0) was the internal standard, and
227 (ii) quantitation was performed based on average values derived from serial dilution of
228 undecanoate, nonadecanoate (c19:0), and methyl cis-13-docosenoate (c22:1 ω 9). Peak
229 identification was based on retention time compared to two reference standards: bacterial acid
230 methyl-ester standard 47080-0 (Sigma–Aldrich, St. Louis, USA) and a 37-Component fatty
231 acid methyl-ester mix (47885-U). Unidentifiable ¹³C-enriched peaks were included in analysis
232 if they met the following conditions: i) detection in > 3 samples, ii) average $\delta^{13}\text{C} > +50\text{‰}$.
233 Taxonomic affiliations of specific PLFAs were assigned as per Högberg *et al.* (2013), with
234 c18:1 ω 9 and c18:3 ω 6 added as fungal markers, according to Ruess & Chamberlain (2010).

235

236 *Fine root tip fungal DNA extraction-sequencing*

237 Fungal DNA was extracted from multiple representative root tips of each EMF
238 morphotype, from which adhering soil had been carefully removed, using ITS1 (White *et al.*,
239 1990) and ITS4/ITS4B primers (Gardes & Bruns, 1993) following the protocol provided in
240 Supporting Methods S1. Raw sequence data were analysed using SEQUENCHER Version 3.0
241 (Gene Codes Corp., Ann Arbor, USA) and converted into FASTA format prior to comparison
242 with the UNITE database (Kõljalg *et al.*, 2013), using the BLAST algorithm to identify each
243 fungal species. Sequence data were deposited in the GenBank database as accession numbers
244 KT314836 to KT314861. Three samples from the rhizosphere soil partition were selected for
245 metatranscriptomic sequencing to assess activity of root-associated communities (Supporting
246 Methods S2).

247

248 *Statistical analyses*

249 All analyses were performed using R 3.2.3 (R Core Team, 2015) unless otherwise
250 stated. Data was square root or \log_{10} transformed where necessary to meet parametric
251 assumptions, with highly influential data points (≥ 3 st. dev. from the treatment median) treated
252 as statistical outliers and removed prior to analysis. Differences between treatments in the
253 excess ¹³C content of recipient pools were assessed by fitting linear mixed models in R-
254 packages “nlme” (Pinheiro *et al.*, 2016) and “lme4” (Bates *et al.*, 2015). The fixed factors in
255 each model were: seedling relatedness (kin or non-kin), hyphal restriction (35 μm or 0.5 μm),
256 recipient family (A, B, C, or D), and their two-way interactions. In all models, donor family
257 was included as a random factor. The response variables examined were “excess ¹³C as ¹²C

258 equivalent” of the R_{bulk} , $R_{\text{rhizosphere}}$, R_{ECM} , and R_{root} pools. Model fit was determined using R-
259 package “piecewiseSEM” (Lefcheck, 2016). Standardised coefficients, a measure of
260 standardised effect size (SES) (Schielzeth, 2010), were estimated for each model (regression
261 coefficients divided by two times their standard deviation).

262 Linear models were used to assess whether excess ^{13}C in kin and non-kin recipient
263 pools displayed different relationships to potentially explanatory biological factors (D_{shoot} ^{13}C -
264 enrichment, D_{ECM} ^{13}C -enrichment, D_{ECM} abundance). SES was measured using Cohen’s *d*
265 (Cohen, 1988). False discovery rate (FDR) correction (Verhoeven *et al.*, 2005) was applied
266 where data was regressed against multiple factors.

267 Enriched fungal biomass (^{13}C per g tissue dry weight) was calculated by converting
268 from mg enriched fungal PLFAs, using the conversion factor provided by Joergensen &
269 Wichern (2008). Average carbon incorporation into fungal biomass was calculated for D_{ECM}
270 (reflecting the hartig net, mantle, and extramatrical mycelium of EMF) and D_{root} (to account for
271 the presence of fungal endophytes and any potential extension of EMF hyphae into transport
272 fine roots; see Kaiser *et al.*, 2010).

273 Seedling EMF community data was examined with the Sørensen (Bray-Curtis) distance
274 measure using nonparametric multi-dimensional scaling (NMDS) and multi-response
275 permutation procedures (MRPP) in PC-Ord 5 (MjM Software, Gleneden Beach, USA).

276 All data used in this analysis, along with a custom script for processing SIP-PLFA data,
277 can be found at the stable URL: <https://github.com/roli-wilhelm>.

278

279 **Results**

280 *Partitioning of ^{13}C -labeled photosynthate*

281 Every plant and soil pool exhibited elevated levels of ^{13}C -labelled carbon relative to the
282 natural abundance in unlabelled controls (Table 1). No significant differences in $\delta^{13}\text{C}$ were
283 observed among unlabelled controls. ^{13}C -enrichment of R_{shoot} , the most distant pool from donor
284 plants, was significant in 4 kin and 2 non-kin samples (6/37). The decrease in ^{13}C -labelled
285 carbon, from D_{shoot} to R_{shoot} , revealed the scope and scale of carbon flow through the
286 belowground system (Table 1; Supplementary Figure S1a). As expected, in each plant D_{ECM}
287 and R_{ECM} contained significantly more ^{13}C -labelled carbon as a percentage of their total carbon
288 content compared to all other pools, illustrating their assimilation of this carbon
289 (Supplementary Figure S1b).

290 Of the total mass of pulsed ^{13}C -labelled carbon (29.02 mg), approximately 75.4%
291 (21.88 mg) was fixed in donor plant tissue after the 1-day chase. Following the 6-day chase

292 period, approximately 44.1% (9.64 mg) of the fixed label (33.2% of the pulse) was detected
 293 across all biomass pools. The total transfer to measured belowground and recipient pools was
 294 approximately 12% of the ^{13}C -label fixed, amounting to 26.8% of the total ^{13}C -label detected
 295 in the 6-day chase. For an account of all individual pools see Table 1. The remaining
 296 unaccounted ^{13}C -label was either not fixed or fixed and respired during the labelling period.

297 A strong inverse relationship was observed between $D_{\text{shoot}} \delta^{13}\text{C}$ and donor biomass ($r^2 =$
 298 0.66, $P < 0.001$), while the total mass of excess ^{13}C in D_{shoot} did not vary significantly with
 299 donor biomass ($r^2 < 0.01$, $P = 0.29$). Thus larger plants had a lower ^{13}C -content relative to total
 300 seedling biomass than smaller plants.

301

302 *Role of seedling relatedness and ectomycorrhizal hyphae in carbon transfer*

303 Analysis using linear mixed models revealed a seedling relatedness effect on R_{root}
 304 excess ^{13}C , with the significant model interaction term revealing variation between the four
 305 recipient families (Table 2). Significantly greater R_{root} excess ^{13}C was observed in kin
 306 recipients from families A and B than in non-kin recipients (Fig. 2a), whereas there was no
 307 significant difference based on relatedness in families C and D. In terms of μg excess ^{13}C this
 308 represented a 3-fold increase in kin of family A (kin mean 5.7 μg ; non-kin mean 1.9 μg), and a
 309 4-fold increase in kin of family B (kin mean 5.6 μg ; non-kin mean 1.4 μg). Carbon transfer to
 310 other recipient pools was not significantly different between relatedness treatments (Table 2).
 311 Linear regression analysis revealed that $R_{\text{root}} \delta^{13}\text{C}$ enrichment increased with increasing D_{ECM}
 312 abundance in kin pairs only (Fig. 3a). In both kin and non-kin pairs, $R_{\text{bulk}} \delta^{13}\text{C}$ enrichment
 313 increased with increasing D_{ECM} abundance (both: $r^2 = 0.34$, kin: $P = 0.014$; non-kin: $P =$
 314 0.007). In kin pairs only, R_{ECM} excess ^{13}C was positively associated with that of both D_{ECM}
 315 (Fig. 3b) and D_{shoot} (Fig. 3c).

316 Hyphal exclusion did not reduce colonisation of recipient roots, or significantly reduce
 317 overall sub-surface carbon transfer. However, analysis using linear mixed models (Table 2)
 318 indicated a reduction in R_{bulk} excess ^{13}C with hyphal exclusion in recipient family D (SES =
 319 0.60-0.66), and reduced $R_{\text{rhizosphere}}$ excess ^{13}C among kin recipients in families C (SES = 0.58)
 320 and D (SES = 0.62). Conversely, increased R_{root} excess ^{13}C with hyphal exclusion was
 321 observed for kin in recipient family A and regardless of relatedness in family D (Fig. 2b).
 322 Carbon transfer to other recipient pools did not differ between mesh treatments (Table 2).

323 To assess whether the observed relatedness effects could be due to differences in
 324 belowground biomass allocation between families, the same linear mixed models were
 325 performed for donor and recipient root: shoot ratio, and the biomass of D_{root} , R_{root} , D_{ECM} and

326 R_{ECM}. The only significant factor across these models was that family C exhibited a lower root:
327 shoot ratio than the other three families. No other fixed or interactive factors proved to be
328 significant (data not shown).

329

330 *Assimilation of ¹³C-photosynthate by the microbial community*

331 Total ¹³C-incorporation into PLFAs in all pools amounted to 1% of belowground
332 carbon transfer (0.023 mg ¹³C in PLFAs / 2.31 mg total ¹³C transferred). Fungal PLFA ¹³C-
333 enrichment was 5.57 mg fungal C g⁻¹ dry weight in D_{ECM} and 1.97 mg fungal C g⁻¹ dry weight
334 in D_{root}. Estimated D_{ECM} PLFA ¹³C-enrichment was strongly correlated with ¹³C-enrichment of
335 D_{ECM} tissue ($r^2 = 0.844$, $P < 0.001$), and D_{ECM} PLFAs contained the highest total excess ¹³C
336 measured (Supplementary Figure S2). The D_{root} pool contained the next highest level of PLFA
337 ¹³C-enrichment, originating from the plant root and associated fungi and bacteria. Fungal
338 PLFA markers were the most ¹³C-enriched in all pools, containing ~70% of assimilated ¹³C-
339 label. The second most ¹³C-enriched taxonomic group were the 'higher eukaryotes,' a
340 heterogeneous category of long-chain fatty acids, indistinguishable between fungi, plants, and
341 other eukaryotic species.

342 Microbes closely associated with host roots were less diverse than those in soil (~27
343 PLFAs), based on the average number of enriched PLFAs (D_{root}: 12, D_{ECM}: 9, R_{ECM}: 13).
344 Differences between root-associated and soil-associated communities were evident in ¹³C-
345 enriched PLFA profiles (Supplementary Figure S3) and unidentified fatty acid profiles
346 (Supplementary Figure S4). The D_{coarse} community was the most distinct, displaying increased
347 ¹³C-enrichment of medium-length fatty acids between c14:0 – c16:1ω9. Fungi assimilated the
348 vast majority of photosynthate based on total ¹³C-enrichment of PLFAs; however, other
349 taxonomic groups in the rhizosphere exhibited substantial assimilation rates (Fig. 4). In the
350 D_{root} pool bacteria incorporated ¹³C-label at rates comparable to that of fungi (i.e. relative to
351 their biomass). Gram-negative bacteria assimilated significant amounts of ¹³C-exudate across
352 all donor pools in every sample assayed, whereas gram-positive bacteria did not assimilate
353 detectable ¹³C-exudate in ectomycorrhizal pools.

354

355 *Fungal root-tip community*

356 Seedlings were primarily colonised by *Rhizopogon vinicolor*, and an ectomycorrhizal
357 Pyrenomataceae sp. (Table 3), both of which were also detected in a preliminary
358 metatranscriptomic analysis of three rhizosphere soil samples (Supporting Methods S1 and Fig.
359 S5). The abundance of these EMF species on recipient seedlings was positively related to their

360 abundance on donor seedlings regardless of treatment. MRPP analysis following NMDS
361 ordination (Supporting Fig. S6) revealed that the only significant difference between EMF
362 communities was weak and occurred between donor and recipient seedlings, rather than
363 treatments ($A = 0.131$, $P < 0.001$; where $A > 0.3$ is considered an ecologically relevant effect).
364 Notably, in the sole case where a plant lacked *Rhizopogon* sp. ectomycorrhizas, ^{13}C -enrichment
365 was not detected in recipient pools.

366

367 Discussion

368 Fungi dominated the assimilation of photosynthetic carbon in all belowground
369 experimental pools, with ectomycorrhizal fungi serving as major agents of carbon transfer.
370 EMF incorporation of photosynthate from host plants is hypothesised to be a major factor in
371 carbon sequestration in coniferous forests (Clemmensen *et al.*, 2013). An estimated 26.8% of
372 the ^{13}C -label remaining in the system was recovered from belowground pools (primarily donor
373 roots), of which 6.3% was assimilated by, or transferred through, EMF. Carbon transfer from
374 donor to recipient seedlings was significantly greater to sibling roots than non-sibling roots in
375 two of the Douglas-fir families, indicating a host relatedness effect that was most likely
376 mediated by EMF. The transfer of ^{13}C -label in the presence of a hyphae-restricting mesh
377 implies that labile ^{13}C -compounds were exuded into soil by donor roots and/or EMF, before
378 being taken up by recipient hyphae. Overall we observed that a diverse microbial community
379 was actively assimilating ^{13}C -labeled photosynthate.

380

381 *Scale and significance of belowground partitioning of Douglas-fir photosynthate*

382 Carbon allocation to donor plant root and ectomycorrhizal root tip biomass (~23% of
383 the total recovered) was within the range of previously estimated allocation values for
384 ectomycorrhizal seedlings of other species: Norway spruce, Scots pine, and silver birch (13-
385 24%; Pumpanen *et al.*, 2008), Scots pine (31%; Heinonsalo *et al.*, 2010), and willow (47%;
386 Durall *et al.*, 1994). Furthermore, ^{13}C -enrichment of fungal-specific PLFAs indicated
387 significant carbon allocation to fungi within donor transport fine roots, most likely attributable
388 to intra-root EMF biomass (Kaiser *et al.*, 2010) and/or fungal endophytes.

389 Mycorrhizal networks in mature forests can be extensive (Beiler *et al.*, 2010), offering
390 the potential for substantial carbon transfer among plants. Yet the net benefit of seedling-to-
391 seedling carbon transfer remains poorly understood. Previous research indicates that EMF and
392 their mycorrhizal networks mediate the transfer of variable amounts of carbon (Simard *et al.*,
393 1997a; Teste *et al.*, 2009; Philip *et al.*, 2010), water (Allen, 2007; Plamboeck *et al.*, 2007;

394 Bingham & Simard, 2011), and nitrogen (He *et al.*, 2003; Read & Perez-Moreno, 2003; Teste
395 *et al.*, 2009) between plants. The quantities of seedling-seedling carbon transfer measured in
396 our experiment (~0.1% of total recovered ^{13}C -label across all pools) and elsewhere (Teste *et*
397 *al.*, 2009, 2010) are small, and unlikely to represent a substantial nutritional source. However,
398 research on the EMF host-symbiont interface reveals that EMF produce signalling compounds,
399 which are translocated into plant cell nuclei. For example, *Laccaria bicolor* produces the
400 MiSSP7 protein, which alters the host transcriptome to promote ectomycorrhizal formation and
401 reduce jasmonic acid production (Plett *et al.*, 2011, 2014). Our observations may therefore
402 represent the transfer of signalling compounds through a fungal mycelium, or their uptake by
403 roots or fungal hyphae following exudation. The stimulation of physiological responses in
404 recipient plants by potential signalling compounds has previously been observed in arbuscular
405 mycorrhizal systems (Babikova *et al.*, 2013; Song *et al.*, 2014) and EMF systems involving
406 interior Douglas-fir and ponderosa pine (Song *et al.*, 2015).

407

408 *Host relatedness*

409 Intriguingly, kin pairs exhibited increased carbon transfer to the R_{root} pool in two of the
410 four Douglas-fir families, with excess ^{13}C in those families 3 to 4-fold greater than in non-kin
411 pairings. The absolute quantities of increased excess ^{13}C involved in this relatedness effect
412 were small (+ 4 μg), but represented a large proportion of total recipient plant excess ^{13}C
413 content (overall recipient plant mean: 6.4 μg). The strong positive relationship between R_{root}
414 $\delta^{13}\text{C}$ and D_{ECM} abundance in kin pairs demonstrated that the genetic relatedness effect on
415 carbon transfer involved donor plant EMF. This was further reinforced by the positive
416 relationships between excess ^{13}C in the R_{ECM} pool and both the D_{shoot} and D_{ECM} pools in kin
417 pairs. Further investigation is required, but we propose that the establishment of an
418 ectomycorrhizal symbiosis between an individual fungal mycelium and a host plant may
419 increase both the likelihood that the fungus will successfully colonise other hosts of a similar
420 genotype, and the efficiency of carbon transfer through its mycelium. In our system,
421 *Rhizopogon* spp. and the ectomycorrhizal Pyrenomataceae sp. are the most likely candidate
422 fungi due to their abundance on seedling roots and their detected activity in soil
423 metatranscriptomes. We further hypothesise that increased carbon transfer among kin
424 seedlings may have resulted from (i) increased inter-root EMF biomass between compatible
425 host genotypes (Rosado *et al.*, 1994a,b; Dudley *et al.*, 2013), and/or (ii) increased inter-root
426 activity due to increased transfer of signalling compounds and/or micronutrients (Plett *et al.*,
427 2011; Babikova *et al.*, 2013). Alternative explanations for the observed carbon transfer are

428 certainly possible, but would have to account for the significant interaction between relatedness
429 and family (e.g., differences in belowground carbon allocation between families would not
430 explain why increased transfer of ^{13}C to roots in families A and B only occurred in the
431 presence of kin).

432

433 *Hyphal restriction*

434 Transfer of ^{13}C -carbon to recipient pools occurred regardless of hyphal exclusion,
435 demonstrating that ^{13}C -compounds can be transferred in the absence of a direct linkage
436 between mycorrhizal hyphae (e.g. Robinson & Fitter, 1999). Carbon transfer was clearly
437 associated with mycorrhizas, since: (i) exclusion reduced transfer to bulk soil in some of the
438 recipient families; (ii) donor and recipient EMF abundance was positively associated for
439 several EMF species; (iii) D_{ECM} abundance was associated with enrichment of recipient pools;
440 and (iv) D_{ECM} and R_{ECM} biomass contained proportionally more ^{13}C -label than all other pools.
441 Previous studies similarly reported small quantities of ^{13}C transfer across a 0.5 μm mesh in
442 both ectomycorrhizal (Teste *et al.*, 2009; Philip *et al.*, 2010; Deslippe & Simard, 2011) and
443 arbuscular mycorrhizal (Fitter *et al.*, 1998) systems. Thus, the mesh-bagging treatment may not
444 be effective for preventing mycorrhizal-mediated carbon transfer. This is potentially due to: (i)
445 recipient EMF hyphae scavenging donor ^{13}C -exudates that diffused through the mesh
446 (Robinson & Fitter, 1999; Johnson & Gilbert, 2015), (ii) hyphae fusing across the mesh, or (iii)
447 hyphae breaching the mesh, possibly degrading it via secreted organic acids (Plassard &
448 Fransson, 2009). No breaches were observed in our experiment, but consistent with
449 possibilities (i) and (ii), there were regions of mesh with adjacent patches of hyphae on either
450 side. Future experiments could employ the in-growth core rotation method (Johnson *et al.*,
451 2001) to reduce the possibility of (ii) and (iii), although it is unlikely to prevent (i), which
452 represents an alternative belowground transfer pathway in natural systems (Simard *et al.*
453 1997b; Robinson & Fitter, 1999; Philip *et al.*, 2010; Deslippe & Simard, 2011).

454

455 *Conclusions*

456 Our stable isotope approach successfully elucidated the pattern and scale of
457 mycorrhiza-mediated carbon transfer between interior Douglas-fir seedlings, and the
458 incorporation of enriched carbon into microbial biomass. EMF symbionts, specifically
459 *Rhizopogon* spp. and Pyronemataceae sp., were the primary external beneficiaries of host-
460 derived photosynthate, and were able to take it up despite the presence of a hyphae-restricting
461 mesh. The small quantities of carbon transferred between seedlings suggest that it is unlikely

462 to be an important nutritional source, although the timing and transfer of micronutrients or
463 signalling compounds may have a substantial ecological impact. We report evidence that
464 relatedness influences carbon transfer between donor and recipient plants, and that the presence
465 of this effect varied between families, raising the possibility of a mosaic of relatedness effects
466 at larger scales. These findings require further exploration in the field, however, the
467 implications for forest ecology are substantial.

468

469 **Conflict of interest statement**

470 The authors declare no conflict of interest.

471

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480

481 **Author contributions**

482 A.K.A. and S.W.S. planned the seedling kin-recognition experiment, which A.K.A. performed.
483 B.J.P., R.W., and S.W.S. designed the SIP experiment, with B.J.P. analysing the excess ^{13}C data
484 and implementing all experimental models, and R.W. analysing the $\delta^{13}\text{C}$ data. R.W. and
485 W.W.M. planned the analysis of ^{13}C -labelled PLFA, which R.W. performed and analysed.
486 B.J.P. and A.K.A. collected the EMF colonisation data, which B.J.P. analysed. A.S.H. generated
487 the metatranscriptome data, which A.S.H. and R.W. analysed. A.K.A., B.J.P., R.W., and A.S.H.
488 performed the experiments. B.J.P. led the writing of the manuscript with significant
489 contributions from R.W. All authors reviewed and commented on the manuscript.

490

491 **Supporting Information**

492 Additional supporting information may be found in the online version of this article.

493 **Supporting Methods S1.** EMF DNA extraction and Sanger sequencing details.

494 **Supporting Methods S2.** Metatranscriptome analysis extraction-sequencing details.

495 **Supporting Figure S1.** Boxplots of excess ^{13}C ; a) biomass, b) proportion.

- 496 **Supporting Figure S2.** Incorporation of ^{13}C -label by microbial groups.
497 **Supporting Figure S3.** PLFA profiles of root and soil pools.
498 **Supporting Figure S4.** Profiles of unidentified PLFAs in root and soil pools.
499 **Supporting Figure S5.** Metatranscriptomic data based on Silva taxonomy.
500 **Supporting Figure S6.** NMDS ordination of seedling root tip fungal communities.

501

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723 **Figure headings**

724 **Figure 1.** Illustration of the plant and soil carbon pools sampled in each experimental unit,
 725 which consisted of (a.) one donor (D) and one full-sibling or unrelated recipient (R)
 726 *Pseudotsuga menziesii* var. *glauca* seedling in one pot separated by a nylon mesh bag (dark grey
 727 dashed cylinder) with holes of either 0.5 μm or 35 μm . In panels b-f, red and blue fill represents
 728 the donor and recipient pools, respectively. **b.** Above-ground plant biomass, divided into donor
 729 shoot pool (D_{shoot}) and recipient shoot pool (R_{shoot}). **c.** Below-ground plant biomass, divided into
 730 donor root pool (D_{root}) and recipient root pool (R_{root}). **d.** Below-ground plant-fungal biomass,
 731 divided into donor ectomycorrhizal root tip pool (D_{ECM}) and recipient ectomycorrhizal root tip
 732 pool (R_{ECM}). **e.** Soil attached to roots following their removal from soil and light shaking,
 733 divided into donor rhizosphere pool ($D_{\text{rhizosphere}}$) and recipient rhizosphere pool ($R_{\text{rhizosphere}}$); the
 734 donor rhizoplane pool ($D_{\text{rhizoplane}}$), very tightly adhering soil washed from root surface following
 735 vigorous vortexing in sterile water and subsequently pelleted by centrifugation, is approximated
 736 with solid black lines. **f.** Soil not attached to roots following their removal from soil and light
 737 shaking, divided into donor coarse soil pool (D_{coarse}) and recipient bulk soil pool (R_{bulk}).

738 **Figure 2.** Relatedness and hyphal restriction effects on *Pseudotsuga menziesii* var. *glauca*
 739 seedling R_{root} pool enrichment (excess ^{13}C as ^{12}C -equivalent), and their variation among
 740 recipient families. **a-b,** Standardised regression coefficients for linear mixed models (refer to
 741 Table 2), illustrating the differences between treatment levels in each recipient family in terms of
 742 their standardised effect size (SES). **a.** difference between kin and non-kin within mesh
 743 treatment (positive values indicate greater enrichment in kin pairs). **b.** difference between 35
 744 μm and 0.5 μm mesh within relatedness treatment (positive values indicate greater enrichment in
 745 pairs separated by 35 μm mesh). Circles indicate average estimates, lines are 95% confidence
 746 intervals. Filled circles indicate significant difference between treatment levels, open points
 747 indicate no significant difference.

748 **Figure 3.** Linear regression of kin (left panels) and non-kin (right panels) ^{13}C -enrichment in
 749 *Pseudotsuga menziesii* var. *glauca* seedlings, revealing the positive relationships between: **a.**
 750 R_{root} $\delta^{13}\text{C}$ and D_{ECM} abundance (kin significant), **b.** R_{ECM} and D_{ECM} excess ^{13}C as ^{12}C -equivalent
 751 (mg) (kin significant), and **c.** R_{ECM} and D_{shoot} excess ^{13}C as ^{12}C -equivalent (mg) (kin significant).
 752 Circles represent data points. Black lines indicate significant linear relationship, grey lines
 753 indicate non-significant relationship. Note that kin and non-kin panels are scaled separately.

754 **Figure 4.** Incorporation of ^{13}C -label into PLFAs by different taxonomic groups in each biomass
 755 pool based on the average $\delta^{13}\text{C}$ of PLFAs. Error bars correspond to standard error. Dotted red
 756 line indicates natural abundance value of $\delta^{13}\text{C}$. Dashed vertical black line indicates separation

757 between donor and recipient pools by mesh bag. Text indicates whether biomass in the pool was
758 primarily derived from plant material (*Pseudotsuga menziesii* var. *glauca*), plant and fungal
759 material, the interface between plant/fungi and soil, or soil alone. PLFA identities are provided
760 in Supporting Figures S2 and S3.
761

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762 **Table 1.** Partitioning of plant-assimilated ^{13}C -labelled carbon in aboveground and belowground pools of interior Douglas-fir associated with
 763 ectomycorrhizal fungi six days after the start of a 10-h $^{13}\text{CO}_2$ labelling period

Pool	^{13}C atom% excess (APE) APE (s.e.m.)	Total amount of excess ^{13}C ($\mu\text{g plant}^{-1}$)				Excess ^{13}C incorporated of the mean total fixed (%)			
		Total μg (s.e.m.)	n	Kin μg (s.e.m.)	n	Non-kin μg (s.e.m.)	n	Kin % (s.e.m.)	Non-kin % (s.e.m.)
D _{shoot}	0.439 (0.037)	7050.67 (325.77)	35	6858.59 (521.51)	16	7212.41 (418.62)	19	31.345 (2.383)	32.962 (1.913)
D _{root}	0.238 (0.020)	1980.24 (159.76)	35	1878.00 (229.32)	17	2076.80 (226.67)	18	8.583 (1.048)	9.491 (1.036)
D _{ECM}	0.979 (0.068)	136.21 (27.57)	31	156.72 (56.54)	14	119.31 (20.52)	17	0.716 (0.258)	0.545 (0.094)
D _{rhizoplane}	0.076 (0.010)	231.81 (21.54)	36	241.61 (33.26)	17	223.04 (28.65)	19	1.104 (0.152)	1.019 (0.131)
D _{rhizosphere}	0.035 (0.004)	167.07 (20.08)	35	122.88 (22.96)	16	204.29 (29.39)	19	0.562 (0.105)	0.934 (0.134)
D _{coarse}	0.014 (0.002)	53.74 (11.29)	33	60.44 (18.81)	15	48.15 (13.69)	18	0.276 (0.086)	0.220 (0.063)
R _{bulk}	0.001 (0.000)	3.96 (0.50)	27	5.35 (0.83)	13	2.68 (0.39)	14	0.024 (0.004)	0.012 (0.002)
R _{rhizosphere}	0.004 (0.001)	4.90 (1.06)	33	4.80 (1.67)	16	4.98 (1.39)	19	0.022 (0.008)	0.023 (0.006)
R _{ECM}	0.119 (0.021)	2.43 (0.48)	34	2.48 (0.86)	16	2.39 (0.52)	18	0.011 (0.004)	0.011 (0.002)
R _{root}	0.013 (0.001)	3.76 (0.47)	33	4.96 (0.80)	16	2.64 (0.35)	17	0.023 (0.004)	0.012 (0.002)
R _{shoot}	0.006 (0.001)	2.76 (1.11)	6	3.46 (1.59)	4	1.37 (0.59)	2	0.016 (0.007)	0.006 (0.003)
Plant and soil mean values									
D _{plant}		9167.11		8893.31		9408.52		40.64	43.00
D _{soil}		452.62		424.93		475.48		1.94	2.17
R _{soil}		8.86		10.15		7.66		0.05	0.03
R _{plant}		8.95		10.89		6.40		0.05	0.03

764 APE = atom percent excess; n = number of significantly enriched samples; Total amount added during pulse = 29 020 μg excess ^{13}C ; Mean
 765 amount fixed after 1-day chase = 21 881.08 μg excess ^{13}C

766 **Table 2.** Effects of hyphal restriction and donor relatedness treatments on excess ^{13}C as ^{12}C equivalent (μg) of recipient *Pseudotsuga menziesii*
 767 var. *glauca* seedling biomass pools, using maximum likelihood analysis of linear mixed-effects models¹ and likelihood ratio tests

Pool	\mathbf{R}_{bulk}			$\mathbf{R}_{\text{rhizosphere}}$			\mathbf{R}_{ECM}			\mathbf{R}_{root}		
Data transformation	-			sqrt			-			log ₁₀		
Marginal R^2	0.605			0.565			0.325			0.673		
Factor	df	F-value	P-value	df	F-value	P-value	df	F-value	P-value	df	F-value	P-value
Intercept	1,15	97.53	<0.0001	1,15	283.15	<0.0001	1,15	50.41	<0.0001	1,15	110.65	<0.0001
Relatedness (Re)	1,15	1.81	0.199	1,15	0.15	0.708	1,15	0.08	0.787	1,15	6.47	0.020
Hyphal (Hy)	1,15	3.65	0.077	1,15	3.29	0.090	1,15	0.79	0.387	1,15	5.38	0.032
Recipient Family (Rfam)	3,15	2.79	0.079	3,15	2.14	0.138	3,15	0.31	0.816	3,15	0.50	0.687
Re*Hy	1,15	0.14	0.710	1,15	0.31	0.585	1,15	1.82	0.197	1,15	2.34	0.143
Re*Rfam	3,15	0.37	0.775	3,15	0.61	0.619	3,15	1.55	0.243	3,15	4.59	0.015
Hy*Rfam	3,15	3.36	0.050	3,15	3.54	0.041	3,15	0.03	0.991	3,15	4.14	0.021

768 Values in bold are significant at the $\alpha < 0.05$ level.

769 Marginal R^2 = model fit based on fixed factors alone. Inclusion of random factor did not increase model fit.

770 ¹Model form: (Pool) ~ (Re)*(Hy) + (Re)*(Rfam) + (Hy)*(Rfam), random ~ 1| (Donor family)

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771 **Table 3.** Identity and relative abundance of root-associated fungi on kin and non-kin *Pseudotsuga menziesii* var. *glauca* seedlings based on root
 772 tip morphotyping and ITS region taxonomic classification. No significant differences in colonisation were observed between mesh sizes.

Fungal morphotype ID	Seedling	Relative abundance		Accession	Sequence match (NCBI accession)	% Identity
		Kin	Non-kin			
<i>Rhizopogon vinicolor</i> (Morphotype 1)	Donor	0.46	0.58	KT314836	<i>Rhizopogon vinicolor</i> (AF263933)	652/656 (99%)
	Recipient	0.44	0.57			
<i>Rhizopogon vinicolor</i> (Morphotype 2)	Donor	0.24	0.11	KT314840	<i>Rhizopogon vinicolor</i> (HQ385848)	529/535 (99%)
	Recipient	0.14	0.03			
Pyronemataceae sp.	Donor	0.12	0.09	KT314854	uncultured Pyronemataceae (GU452518)	524/524 (100%)
	Recipient	0.26	0.29			
<i>Wilcoxina</i> sp.	Donor	0.08	0.05	-	Sequencing failed – Taxonomic ID	-
	Recipient	0.13	0.09			
Uncolonised root tips	Donor	0.05	0.15	N/A	N/A	N/A
	Recipient	0.00	<0.01			
<i>Fusarium</i> sp.	Donor	0.03	0.01	KT314859	<i>Fusarium acuminatum</i> (KP068924)	478/478 (100%)
	Recipient	0.03	0.02	KT314860	<i>Fusarium oxysporum</i> (KP132221)	451/451 (100%)
<i>Rhizopogon</i> sp.	Donor	0.01	0.01	KT314850	<i>Rhizopogon fragrans</i> (AM085523)	619/621 (99%)
	Recipient	< 0.01	< 0.01			
<i>Rhizopogon ochraceisporus</i>	Donor	0.01	0.00	KT314851	<i>Rhizopogon ochraceisporus</i> (AF366389)	603/609 (99%)
	Recipient	0.00	0.00			

773 N/A = not applicable (uncolonised root tips which did not generate fungal DNA).

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