

Arbuscular mycorrhizal community structure on co-existing tropical legume trees in French Guiana

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Abstract

Aims: We aimed at a characterisation of the arbuscular mycorrhizal fungal (AMF) community structure and potential edaphic determinants in the dominating, but poorly described, root-colonizing *Paris*-type AMF community on co-occurring Amazonian leguminous trees.

Methods: We targeted three highly productive co-occurring leguminous species (*Dicorynia guianensis*, *Eperua falcata* and *Tachigali melinonii*) in species-rich forests on contrasting soil types at the Nouragues Research Station in central French Guiana. Abundant AMF SSU rRNA amplicons (NS31-AM1 & AML1-AML2 primers) from roots identified via *trnL* profiling were subjected to denaturing gradient gel electrophoresis (DGGE), clone library sequencing and phylogenetic analysis.

Results: Classical approaches targeting abundant SSU amplicons highlighted a diverse root-colonizing symbiotic AMF community dominated by members of the Glomeraceae. DGGE profiling indicated that, of the edaphic factors investigated, soil nitrogen was most important in influencing the AMF community and this was more important than any host tree species effect.

Conclusions: Dominating *Paris*-type mycorrhizal leguminous tree species in Amazonian soils host diverse and novel taxa within the Glomeraceae that appear under edaphic selection in the investigated tropical forests. Linking symbiotic diversity of identified AMF taxa to ecological processes is the next challenge ahead.

Introduction

Tropical forests are exceptionally species rich, holding over half the world's species (Dirzo & Raven 2003; Gibson et al. 2011). Most ecological studies in tropical forests have examined above-ground communities (Ghazoul & Sheil 2010) whilst microscopic taxa found below-ground such as fungi and bacteria have received considerably less attention, at least partly due to their cryptic nature (Aime & Brearley 2011). Compared to other microscopic taxa, arbuscular mycorrhizal fungi (AMF; phylum Glomeromycota) have been relatively well studied. These fungi form beneficial root symbiotic

44 associations, defined by fungal arbuscular structures formed within
45 host cortical cells, in a large proportion of the world's terrestrial flora
46 (Smith & Read 2008) including many of the more than 20,000 tree
47 species estimated from Neotropical forests (Slik et al. 2015).
48 However, classical morphological descriptions by Gallaud (1904) and
49 numerous subsequent studies, reviewed by Smith and Smith (1997),
50 confirmed that AMF fungi form both *Arum*- and *Paris*-type
51 colonization structures in compatible mycorrhizal plant hosts. The
52 former type are characterised as typical AMF but, in the latter, root
53 cortical cells do not host arbuscules but are heavily colonized by
54 intracellular hyphal coils. Tropical forest trees and forest herbs
55 appear to host a predominance of *Paris*-type mycorrhizas (Alexander
56 1989) including the target leguminous trees at our Amazonian study
57 site in French Guiana (Béreau & Garbaye 1994; Béreau et al. 2004;
58 de Grandcourt et al. 2004). Whilst it appears that AMF alpha-
59 diversity can be higher in tropical than temperate ecosystems
60 (Husband et al. 2002; Haug et al. 2010, 2013; Camenzind et al.
61 2014), this is not always the case with overlap shown in the number
62 of AMF taxa recorded from these divergent ecosystems. It is,
63 however, difficult to make robust comparisons due to the paucity of
64 tropical studies coupled with inconsistent methodologies,
65 particularly as the field of molecular ecology advances (e.g. next
66 generation sequencing; Shendure & Ji 2008).

67

68 Because mycorrhizal fungi form a key functional interface between
69 plant roots and soil, they play a major role in plant nutrition (Smith &
70 Read 2008). Leguminous plants and trees also host symbiotic
71 nitrogen-fixing bacteria that require large amounts of phosphorus (P)
72 for nodule development and nitrogenase functioning (e.g. Mortimer
73 et al. 2008 and references therein). Nitrogen (N) fixation in
74 leguminous plants in general and tropical trees in particular is

75 therefore highly dependent upon efficient P uptake, especially in P-
76 deficient tropical soils, which is mediated by the AMF symbiosis
77 (Plassard & Dell 2010). In addition, AMF are known to be important
78 in structuring plant communities with different taxa or associations
79 of taxa having differential effects on plant growth (van der Heijden
80 et al. 1998; Munkvold et al. 2004; Koch et al. 2006; Roger et al.
81 2013). For example, Kiers et al (2000) demonstrated differential
82 responses to AMF inoculum from conspecific or heterospecific
83 tropical tree seedlings and Pizano et al. (2011) found that AMF from
84 tropical landslide sites had differing effects on plant growth
85 compared to those from light-gap sites, both studies indicating the
86 potential role of AMF in influencing plant communities.
87 Understanding the determinants of species distributions, through
88 studying their niche requirements, and elucidating ecological
89 community structure is a fundamental area of research in ecology
90 and is important to support credible assessment of environmental
91 change, and inform evidence-based management of ecosystems. As
92 AMF are obligately symbiotic organisms, both the host species
93 (Lovelock et al. 2003; Helgason et al. 2007; Sýkorová et al. 2007; de
94 Oliveira Freitas et al. 2014) as well as edaphic (Fitzsimmons et al.
95 2008; Ji et al. 2012; de Oliveira Freitas et al. 2014) and
96 biogeographical (Hazard et al 2011; Kivlin et al. 2011; Öpik et al.
97 2013) factors will influence AMF community structure but, in many
98 cases, it is difficult to clearly disentangle these due to edaphic
99 sorting of the host plant. Surprisingly few studies have attempted to
100 do this (but see Fitzsimmons et al. 2008; Dumbrell et al. 2010; Ji et
101 al. 2012) although it would clearly help in furthering our
102 understanding of AMF community structuring.

103

104 In this study, we examined the root associated AMF fungal
105 community on three co-occurring leguminous tree species of French

106 Guiana where legumes form many of the commonest tree species,
107 making a significant contribution to stand basal area (ter Steege et
108 al. 2006). We hypothesised that: i) the *Paris*-mycorrhizal status of
109 the target legume tree species could result from colonization by
110 novel AMF taxa, ii) host tree and soil edaphic specific responses
111 would be detectible in root colonizing fungal communities but iii) the
112 co-occurring trees would form a core AMF community with the
113 potential to form common mycelial networks.

114

115

Methods

116 Study site

117 The study was conducted at the Nouragues Research Station (within
118 a National Nature Reserve) in central French Guiana (Bongers et al.
119 2001; <http://www.nouragues.cnrs.fr>) with a diverse tree flora typical
120 of much of the Guiana Shield (ter Steege et al. 2006; Gonzalez et al.
121 2009). The sampling was restricted to the Inselberg camp area
122 (4°05'N; 52°41'W) in minimally disturbed tropical forest where two
123 large sampling plots ('Grand Plateau' and 'Petit Plateau') have been
124 delimited. These two plots have differing edaphic conditions: the
125 Grand Plateau is based on a metamorphic geology with more fertile
126 clay-rich soils and the Petit Plateau is based on a granitic geology
127 with more sandy soils that are less fertile, although both are Ultisols
128 (Poszwa et al. 2009). The annual rainfall is around 2900 mm with a
129 drier season from late August to early November.

130

131 Study species

132 We investigated three legume species in different tribes of the
133 Caesalpinioideae: *Dicorynia guianensis* Amshoff (tribe Cassieae),
134 *Tachigali melinonii* (Harms) Zarucchi & Herend. (syn. *Sclerolobium*
135 *melinonii* Harms; tribe Caesalpinieae) and *Eperua falcata* Aublet

136 (tribe Detarieae). The former two species are among the most
137 important trees, in terms of carbon cycling, in the Amazon basin
138 (Fauset et al. 2015). The three species have contrasting root
139 morphologies with *Dicorynia guianensis* and *Tachigali melinonii*
140 being similar to one another with thin, highly branched roots in
141 contrast to *Eperua falcata* that had thicker, poorly branching root
142 systems with short roots on long axes (Béreau & Garbaye 1994;
143 Supplementary Figure 1). All three species form *Paris*-type
144 mycorrhizal associations (Béreau & Garbaye 1994; Béreau et al.
145 2004; de Grandcourt et al. 2004). Many Caesalp legumes possess
146 ectomycorrhizal (EcM) associations (Smith & Read 2008) but
147 following a careful visual assessment of sampled roots, no obvious
148 EcM development was observed. *Dicorynia guianensis* and *Tachigali*
149 *melinonii* form prominent nodules and *Eperua falcata* has nodule-like
150 structures on the roots whose function is not entirely clear (Sprent
151 2001).

152

153 **Field sampling**

154 Root samples were obtained from 12-16 randomly selected trees
155 each of the three target species in August and September 2009
156 (trees were up to 1 m diameter; mean = $50.3 \pm \text{SD } 20.3$ cm). At
157 each tree, roots were exposed by careful excavation enabling them
158 to be traced to a distance of about 1.0 to 1.5 m from the trunk
159 (Supplementary Figure 1a). Four samples of fine root material were
160 cut away from the surrounding friable organic soil with any soil
161 adhering to the roots brushed off. Root samples from each tree were
162 combined together into a single plastic vial filled with silica gel for
163 rapid drying. Soil samples were taken from the areas immediately
164 adjacent to root collection and combined into a single sample. They
165 were subsequently air-dried in the field and returned to the UK for
166 analysis.

167

168 **DNA extraction**

169 In the laboratory, dried root material was finely chopped and
170 homogenised aseptically using a sterile scalpel and larger diameter
171 sections were removed until all fragments were less than 2 mm in
172 length. No obvious spore contamination in the rhizoplane was
173 detected in these root fragments. DNA extraction was performed on
174 the homogenised roots using a modification of the method of
175 Heinonsalo et al. (2001) developed for highly pigmented Scots pine
176 roots/mycorrhizas. Briefly, two extractions were made from each
177 root sample starting with 5 mg material each. The roots were
178 further ground using a micro-pestle and fine quartz sand, then 1 ml
179 CTAB buffer with 1 % PVP was added and the sample was
180 periodically ground during an incubation at 65 °C for 1 hour. The
181 two extractions were then centrifuged at 16,000 RCF for 5 minutes
182 and the supernatants were separately extracted twice with equal
183 volumes of chloroform. After extraction, the two aqueous layers
184 were combined and precipitated together with an equal volume of
185 chilled isopropanol. The DNA was collected by centrifuging at
186 16,000 RCF for 30 minutes then removing the supernatant, then the
187 pellet was washed twice by applying 200 µl chilled 70 % ethanol and
188 centrifuging for 5 minutes at 7,000 RCF. The dried pellets were
189 rehydrated in 25 µl TE buffer and stored at -20°C until use.

190

191 **Host plant species validation by *trnL* amplicon fragment** 192 **length analysis**

193 Although all the root samples were visually traced during sampling,
194 we considered it prudent to verify the identity and purity of root
195 samples. We used a length heterogeneity PCR approach, based
196 upon the work of Ridgway et al. (2003), to achieve this. The
197 Genbank database was used to predict amplicon sizes for a PCR of

198 the plastid *trnL* intron using primers c and d from Taberlet et al.
199 (1991). PCRs were performed using the conditions described by
200 Gonzalez et al. (2009), but the c primer was modified with CY5 on
201 the 5' end to enable compatibility with the Beckman fragment
202 analysis system. Amplicon lengths were measured and quantified
203 using a Beckman CEQ 8000 automated sequencer in fragment
204 analysis mode. Direct sequencing of some of the amplicons was
205 performed to check the specificity of the PCR and confirm tree
206 identities. The c and d primers Taberlet et al. (1991) were used to
207 obtain sequence reads using an Applied Biosystems 3730xl
208 sequencer.

209

210 **Mycorrhizal community profiling**

211 The AMF community associated with each tree was compared using
212 denaturing gradient gel electrophoresis (DGGE) to generate a
213 community fingerprint from an amplified fragment of the fungal
214 small sub-unit rRNA gene. PCR and DGGE were performed according
215 to the method of Öpik et al. (2003), using primers AM1 (Helgason et
216 al. 1998) and NS31 (Simon et al. 1992). A GC clamp was added to
217 the 5' end of NS31 primer to stabilize the melting behaviour of the
218 DNA fragments. PCRs contained 2.5 U Taq (Bioline), 5 µl 10 x NH₄
219 reaction buffer, 1.5 mM MgCl₂, 200 µM of each dNTP, 0.2 µM of each
220 primer, and were made up to 50 µl volume with water and DNA
221 template; they were performed in an MJ Research PTC-200 thermal
222 cycler following cycling parameters in Öpik et al. (2003). DGGE was
223 carried out on the Bio-Rad DCode universal mutation detection
224 system, using 6 % polyacrilamide gels, with urea-formamide
225 denaturant gradients of 22 to 35 %. Electrophoresis was run at 60
226 °C and 75 V for 8 hours, with 32 ng DNA loaded into each well. Gels
227 were stained with SYBR Gold (Molecular Probes, Leiden, The
228 Netherlands) and digitized using GeneGenius Imaging System from

229 Syngene. We ran two DGGE gels: the first had sixteen trees of
230 *Tachigali melinonii* ("Tachigali gel") and the second had a subset of
231 nine of those sixteen trees plus nine *Dicorynia guianensis* and four
232 *Eperua falcata* ("Mixed gel"). Gel images were converted to a
233 presence/absence matrix for each band position based on a
234 systematic procedure using the plot RGB profile function of ImageJ
235 (Schneider et al. 2012) to extract pixel values for each lane, followed
236 by peak detection using LabPlot (<http://labplot.sourceforge.net>). To
237 correct for slight skew in the gels, coloured reference lines were
238 added across the gel images linking lane markers and prominent
239 reference bands before peak detection. The signatures of these
240 lines in the RGB pixel profiles from ImageJ were used to ensure
241 accurate alignment of lane profiles before peak detection.

242

243 **Determination of mycorrhizal taxa**

244 Whilst DGGE requires relatively short, variable PCR products to
245 achieve good separation of bands on the gel, for accurate
246 phylogenetic classification a longer sequence is preferable. For this
247 part of the study, we therefore used primers AML1 and AML2 that
248 are reported to have better specificity and coverage of known AMF
249 taxa compared to the AM1 and NS31 primers used for DGGE (Lee et
250 al. 2008). We chose six samples: three geographically close (< 300
251 m) trees of each species from each of the Grand and Petit Plateaus.
252 PCR products were produced using the protocol described by Lee et
253 al. (2008), then cloned into *E. coli* using an Invitrogen Topo TA
254 cloning kit. Ten positive transformants from each tree were used
255 directly in a colony PCR (Elliott et al. 2005) using vector primers
256 M13F and M13R to check the insert size (approximately 800 base
257 pairs). Forty-eight successful inserts were sequenced from the T3
258 priming site using an Applied Biosystems 3730xl sequencer.

259

260 Sequences from forward and reverse primers were assembled using
261 contig assembly program (Huang 1992) and single coverage regions
262 were discarded. A total of 23 double coverage sequences were
263 obtained and these were clustered at 99 % similarity cutoff using cd-
264 hit-est (Huang et al. 2010) to identify unique sequences.
265 Approximate species-level OTUs were identified using UCLUST
266 (Edgar 2010) with a 97 % similarity threshold. A neighbour-joining
267 phylogenetic tree was constructed from the unique sequences that
268 exceeded 450 base pairs in length. We included the top match from
269 MaarjAM (Öpik et al. 2010) for each of our sequences plus all
270 sequences associated with two of our host plants (presented in Öpik
271 et al. 2013) in addition to several globally distributed taxa to provide
272 a wider context. We also included the three top matching
273 sequences from a BLAST search on Genbank and any closely
274 matching named taxa. In some cases, relevant sequences were
275 excluded because the sequence regions did not overlap sufficiently
276 with those reported in this study. ClustalW (Thompson et al. 1994)
277 was used to align the sequences and all gaps were removed before
278 generation of the distance matrix (Kimura 1980) and neighbour-
279 joining phylogenetic tree (Saitou & Nei 1987), using the APE
280 package (Paradis et al. 2004) for R (R Core Team 2015).

281

282 **Soil analyses**

283 All analyses were conducted in duplicate on soils that had been
284 ground to pass a 1 mm sieve. The moisture content of the air-dried
285 soil was determined by heating 5 g sub-samples to 105 °C for 24 h.
286 Soil pH was measured by adding 5 g of soil to 12.5 ml of deionised
287 water; it was stirred and left to equilibrate for 1 h before the pH was
288 measured with a Sartorius PB-11 pH meter. Carbon and N were
289 determined on a LECO TruSpec elemental analyser. Total P was
290 determined by digesting 0.25 g samples in 5 ml of concentrated

291 sulphuric acid (with a lithium sulphate/selenium (100:1) catalyst) for
292 8 hours at 375 °C. Samples were then made up to 50 ml in
293 deionised water and analysed on a Varian Vista AX Inductively
294 Coupled Plasma Optical Emission Spectrometer (ICP-OES). Cations
295 (P, K, Ca & Mg) were extracted from 2.5 g samples that were shaken
296 with 25 ml of Mehlich 1 solution for ten minutes before being filtered
297 and analysed on a Thermo iCAP 6300 Duo ICP-OES.

298

299 **Statistics**

300 Rarefaction curves were calculated in EstimateS (100
301 randomisations). Redundancy analysis was performed using the
302 Vegan package (Oksanen et al. 2015) for R (R Core Team 2015) with
303 a backwards-stepwise approach to select constraining variables. We
304 tested whether the AMF community differed in relation to soil
305 properties or host tree species using a PerMANOVA (Jaccard index,
306 999 permutations), also in the Vegan package.

307

308

Results

309 **Arbuscular mycorrhizal SSU amplification**

310 PCR success varied and was limited for more recalcitrant samples of
311 *Eperua falcata* that had thick and highly pigmented roots (c. 40 %)
312 when compared with *Dicorynia guianensis* (c. 75 %) and *Tachigali*
313 *melinonii* (100 %). Difficulties amplifying the host *trnL* marker
314 mirrored difficulties amplifying the fungal SSU; therefore AMF
315 amplification failure was most likely due to PCR inhibition rather
316 than absence of fungi in the samples.

317

318 **Plant species validation by *trnL* amplicon sizes**

319 Double-coverage *trnL* sequences were obtained from *Dicorynia*
320 *guianensis* and *Eperua falcata* (GenBank accessions: PENDING) with

321 99-100 % identity to publicly available *trnL* sequences from the
322 target trees; sequencing of amplicons from *Tachigali melinonii* was
323 unsuccessful. In almost all samples, the dominant *trnL* fragment
324 sizes were within two base pairs of the predicted length (Table 1).
325 We removed five samples from subsequent analyses; one had a
326 much shorter fragment than expected (indicating that the tree was
327 probably identified incorrectly) and four others had secondary peaks
328 that were 10 % or more of the height of the main peak (indicating
329 probable contamination with roots of other plants).

330

331 **Mycorrhizal community profiling by DGGE**

332 Rarefaction curves (and comparison with Chao1 values) suggest that
333 our sampling was sufficiently extensive to describe the AMF
334 community on *Dicorynia guianensis* and *Tachigali melinonii* but not
335 *Eperua falcata* with around 30 bands found for the former two
336 species and 25 for the latter (Fig. 1a) and a total of 34 bands for the
337 community as a whole (using the Mixed gel). The Chao1 estimate
338 for the AMF community as a whole was $34.7 \pm \text{SD } 1.3$ indicating
339 extensive sampling. Within a species, c. 35-55 % of the bands were
340 rare (*i.e.* restricted to one or two individual trees) with only a small
341 proportion (< 12 %) found on more than 80 % of the trees within a
342 species (Fig. 1b). Around half of the bands were found on all three
343 tree species with few restricted to a single host - mostly to *Dicorynia*
344 *guianensis* (Fig. 2). Of the bands that were shared between species,
345 most were rare with the exception of one band that was found on
346 around 80 % of *Dicorynia guianensis* trees but no *Eperua falcata*
347 trees.

348

349 **Root-colonizing mycorrhizal community responses to tree** 350 **species and soil chemistry**

351 The soils were acidic and low in nutrients but there were significant
352 differences between the Grand and Petit Plateau, with the Petit
353 Plateau soils slightly more acidic and lower in the major plant
354 nutrients (Table 2). Consequently, there were also differences
355 between the tree species in their surrounding edaphic variables
356 (Table 2) as *Dicorynia guianensis* was more commonly sampled on
357 the Petit Plateau whereas *Eperua falcata* and *Tachigali melinonii*
358 were more commonly sampled on the Grand Plateau. In particular,
359 soils surrounding *Eperua falcata* were highest in C, N and
360 extractable P and cations whereas those surrounding *Dicorynia*
361 *guianensis* were lowest in all measured nutrients (Table 2).

362

363 The step-wise redundancy analysis model building process selected
364 soil N as the only constraining variable describing the AMF
365 community structure (Fig. 3); N also appeared to separate the host
366 trees on axis RDA1. The significance on N on the AMF community
367 was confirmed by PerMANOVA ($F = 1.93$, $r^2 = 0.089$, $p = 0.039$).
368 However, different host tree species were found on soils of differing
369 N-status (Table 2) and this might have influenced the AMF
370 community through host selection although this was not a significant
371 determinant of at the data resolution available in this study ($F =$
372 0.91 , $r^2 = 0.084$, $p = 0.64$).

373

374 **Phylogeny of mycorrhizal taxa**

375 All of the sequences found on the six trees (Genbank accessions:
376 KR706472-KR706484) were from the family Glomeraceae (within the
377 order Glomerales). They were grouped into eight approximate
378 species-level groups with > 97 % similarity; six of these were
379 singletons found on one tree only. The Chao 1 estimate of the
380 number of phylogroups was 17: this was half that estimated from
381 the DGGE bands although in closer agreement with the number

382 predicted when rarefied to six samples ($24.6 \pm \text{SD } 1.7$). Our
383 sequences did not match with any named AMF taxa, and,
384 interestingly, did not cluster closely with sequences from two of the
385 same host species in a site in French Guiana about 125 km distant
386 (Öpik et al. 2013) (Fig. 4).

387

388

Discussion

389 There remains a critical gap in the literature relating to tropical
390 mycorrhizal community dynamics that, in boreal and temperate
391 biomes, are known to underpin ecosystem productivity and
392 multifunctionality (Smith and Read, 2008; van der Heijden et al.
393 2015). Earlier research, that had targeted the same species-rich
394 Amazonian forests in French Guiana, highlighted a predominance of
395 *Paris*-, as opposed to more commonly studied, *Arum*-type
396 mycorrhizal colonization of leguminous trees (Béreau & Garbaye
397 1994; Béreau et al. 2004; de Grandcourt et al. 2004). We provide
398 here the first report on the diversity and identity of AMF known to
399 form *Paris*- type mycorrhiza on three co-occurring leguminous trees
400 on differing soil types in these northern Amazonian forests.

401

402 The main aim of our study was not to exhaustively recover AMF
403 diversity, for which we would have used a specific set of primers for
404 each order, but to determine the abundant root-associated taxa in
405 our study system that are likely to be symbiotically active. This
406 approach yielded an estimate of 34 AMF taxa detected in roots on
407 the basis of SSU-DGGE banding that has been shown, via individual
408 band sub-cloning and sequencing, to underestimate AMF root-
409 colonizing diversity (Öpik et al. 2003), although it assumes we did
410 not have any non-specific amplification from other fungal phyla
411 (Kohout et al. 2014). Our estimate still compares with other tropical

412 studies employing various classical and next generation sequencing
413 methodologies, for example, Aldrich-Wolfe (2007) found 31
414 phylotypes by T-RFLP in Costa Rica, Husband et al. (2002) found 30
415 taxa using a cloning and Sanger sequencing approach in Panama
416 and Camenzind et al. (2014) found 74 taxa using 454-
417 pyrosequencing in Ecuador. However, comparisons are difficult
418 between studies due to differing primers, sequencing platforms and
419 clustering approaches. It appeared that our sampling was saturated
420 with eight to ten samples, sufficient to sample the root-colonizing
421 AMF community fully by DGGE.

422

423 Members of the Glomeraceae dominated the AMF community with
424 no evidence of the abundant presence of members from other
425 families or orders in the Glomeromycota. This was somewhat
426 surprising given the high abundance of Acaulosporaceae in tropical
427 spore-counting studies (e.g. Lovelock et al. 2003; Stürmer & Siqueira
428 2011; de Oliveira Freitas et al. 2014) including at our study site
429 (Martin et al. 2001; Oehl & Brearley, unpublished data). It should be
430 stressed that this is not a limitation of the primers developed by Lee
431 et al. (2008) that efficiently amplify across the phylum. At least part
432 of this restricted phylogenetic coverage is likely due to the small
433 number of sequences found so we should be careful not to over-
434 interpret from this small dataset. However, it raises the interesting
435 possibility that a phylogenetically restricted subset of AMF taxa
436 preferentially form structurally distinct *Paris*-type mycorrhizal
437 associations. Öpik et al. (2013), employing 454 pyrosequencing at a
438 similar study site in French Guiana, also found a similarly restricted
439 subset of AMF taxa on two of our study species. *Paris*-type
440 mycorrhizas appear to support extensive intracellular fungal coiling
441 (Smith and Read 2008) that could physically prevent colonization by
442 other AMF taxa thus restricting functional taxa representation due to

443 priority effects (Hepper et al. 1988; Werner and Kiers, 2015). The
444 restricted taxa detected on our target tree roots could, therefore,
445 represent a natural manifestation of a phenomenon that has, to
446 date, only been described in controlled laboratory experiments with
447 young seedlings. Our findings also support the hypothesis of Kivlin
448 et al. (2011) of phylogenetic clustering within sites; perhaps, in this
449 case, due to all the host trees being within the same family. That
450 the identified taxa found mostly formed highly-supported unique
451 clusters when compared to AMF taxa identified by Öpik et al. (2013)
452 on two of the same host tree species additionally supports the
453 hypothesis of Kivlin et al. (2011) of high beta diversity in AMF.
454 Finally, there is the possibility that these are legume specialist AMF
455 as Sheublin et al. (2004) found clear differences between the AMF
456 communities on legumes and non-legumes in a Dutch grassland.

457

458 The clearest relationship between soil nutrients and AMF taxa
459 representation was seen for soil N. A related study (Camenzind et
460 al. 2014) in species rich tropical montane forest found reduced AMF
461 species richness in bulked root samples in response to N and P.
462 Nitrogen input in native forests will be greatly dependent on
463 anthropogenic deposition rates but also associative and symbiotic N-
464 fixation involving legumes. The legume tree species in this study
465 are productive members of the community and will contribute
466 significant organic N to the soil via litter inputs. Spatial variability in
467 N content was shown in these species-rich forest systems that could
468 select for AMF taxa with enhanced potential organic N-mobilizing
469 activities (Hodge 2014). For example, Martin et al. (2001) showed
470 soils under *Eperua falcata* to be more enriched in N than those
471 under *Dicorynia guianensis* in common with this study. In our study,
472 it appeared that soil N had a greater effect than host species
473 (although there was non-random association of tree species with

474 particular edaphic conditions) concurring with other recent studies
475 pointing towards edaphic factors playing a more important role than
476 host species in structuring AMF communities (Fitzsimmons et al.
477 2008; Dumbrell et al. 2010; Ji et al. 2012). With regard to common
478 mycelial networks, our data provided some evidence of potential
479 inter- and intra- host species networking potential. Mechanisms
480 driving restriction to limited common AMF-forming taxa in these
481 productive leguminous hosts may have evolved to ensure
482 networking within N-fixing trees in a highly resource competitive
483 environment.

484

485 For unequivocal identification of the host species in systems with
486 diverse vegetation, the plastid *trnL* region offers a robust and rapid
487 marker for confirmation of root sample identity with minor species-
488 specific *trnL* length variations likely reflecting intra-specific variation
489 within the study site. Zeng et al. (2015) recently reported
490 successful root identification of 11 tree species in a Chinese
491 subtropical forest via *trnL* sequencing. Although many studies adopt
492 a root-tracing approach, the important strategy taken here to
493 confirm host species via molecular tools is rarely adopted and we
494 promote this as a straightforward and appropriate method for
495 certainty in mixed species communities where reference material is
496 available. As well as *trnL* (Dumbrell et al. 2010; Zeng et al. 2015),
497 other suitable gene regions might include *trnH-psbA* (Jones et al.
498 2011), *rbcL* or *matK* (CBOL Plant Working Group 2009).

499

500 One of the advantages to our 'classical' sequencing approach is that
501 we detected the taxa that are more abundant in the tree roots and,
502 therefore, functionally most important in terms of mutualistic
503 associations; furthermore, it allows us to avoid sampling low-density
504 'contaminant' hyphae in the rhizoplane or spores simply present on

505 the plant roots that would be picked up by extensive next-
506 generation sequencing but are not forming functional AMF. If we
507 wished to sample the soil AMF community exhaustively then next-
508 generation sequencing or DNA metabarcoding would effectively
509 allow this more in-depth examination of the community (e.g. Öpik et
510 al. 2013).

511

512 What is the functional importance of root symbiotic AMF diversity
513 and what are all these fungi doing in the ecosystem? For example,
514 the mycorrhizal response to AMF inoculation in *Eperua falcata* is less
515 than *Dicorynia guianensis* (de Grandcourt et al. 2004) and this may
516 be influenced by this species' preference for nitrate (Schimann et al.
517 2012) mediated by root exudate influence on the rhizosphere
518 microbial community (Michalet et al. 2013). If AMF communities
519 that have different functions (such as P-mining ability) are spatially
520 separated then they have the potential to influence seedling
521 diversity in tropical forests and hence contribute to the high
522 diversity of these ecosystems.

523

524

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539 FQB & RS; performed the study: FQB, DRE & AI; analysed the data:
540 DRE & FQB; wrote the manuscript: FQB, RS & DRE.

541

542

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791 **Table 1: *trnL* amplicon length predictions and measurements**
792 **for three co-occurring legume trees at Nouragues in French**
793 **Guiana. The sequence accession and identity used for**
794 **prediction is indicated in parentheses in the central column.**

Species	<i>trnL</i> length prediction	Measurement range
<i>Dicorynia guianensis</i>	617 (FJ039291; <i>Dicorynia guianensis</i>)	616-618
<i>Eperua falcata</i>	706 (FJ039126; <i>Eperua falcata</i>)	704-705
<i>Tachigali melinonii</i>	578 (AF430790; <i>Tachigali paniculata</i>)	578-580

795

796 **Table 2: Soil chemical characteristics (mean \pm standard**
 797 **error) found around three co-occurring legume trees on two**
 798 **soil types ('Grand Plateau' and 'Petit Plateau') at Nouragues**
 799 **in French Guiana. Significant differences (*t*-test, $p < 0.05$)**
 800 **between plateaus are marked with an asterisk and**
 801 **significant differences (Tukey's test, $p < 0.05$) between tree**
 802 **species are noted with letters; absence of asterisk or stars**
 803 **indicates no significant differences.**

	Grand Plateau		Petit Plateau	<i>Dicorynia guianensis</i>	<i>Eperua falcata</i>	<i>Tachigali melinonii</i>
pH	4.39 \pm 0.10		4.26 \pm 0.06	4.28 \pm 0.06	4.38 \pm 0.26	4.36 \pm 0.10
C (%)	7.74 \pm 0.73		6.05 \pm 0.62	5.79 \pm 0.53 a	10.04 \pm 1.05 b	6.78 \pm 0.71 a
N (%)	0.56 \pm 0.03	*	0.39 \pm 0.03	0.38 \pm 0.02 a	0.64 \pm 0.05 b	0.52 \pm 0.04 b
Tot. P ($\mu\text{g g}^{-1}$)	430 \pm 33.5	*	104 \pm 9.2	124 \pm 27 a	325 \pm 55 ab	419 \pm 58 b
Extr. P ($\mu\text{g g}^{-1}$)	16.1 \pm 2.6		12.6 \pm 2.1	10.3 \pm 0.9 a	25.4 \pm 4.6 b	13.9 \pm 2.4 a
Extr. K ($\mu\text{g g}^{-1}$)	167 \pm 15	*	105 \pm 11	100 \pm 8.0 a	204 \pm 10.3 b	149 \pm 18.0 a
Extr. Ca ($\mu\text{g g}^{-1}$)	965 \pm 322	*	384 \pm 95	401 \pm 105	1607 \pm 896	598 \pm 153
Extr. Mg ($\mu\text{g g}^{-1}$)	250 \pm 37		175 \pm 17	175 \pm 19 a	324 \pm 79 b	208 \pm 32 ab

804

805 **Figure 1a: Rarefaction curves and 1b: frequency**
806 **distributions of the DGGE bands of arbuscular mycorrhizal**
807 **fungal taxa found on three co-occurring legume trees at**
808 **Nouragues in French Guiana.**
809
810

811 **Figure 2: Venn diagram of the DGGE bands of arbuscular**
812 **mycorrhizal fungal taxa found on three co-occurring legume**
813 **trees at Nouragues in French Guiana.**

814

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816

817 **Figure 3: Redundancy analysis of DGGE bands of arbuscular**
818 **mycorrhizal fungal taxa on three co-occurring legume trees**
819 **at Nouragues in French Guiana constrained by soil nitrogen**
820 **concentration. Circles (green) = *Dicorynia guianensis*,**
821 **triangles (blue) = *Eperua falcata*, Plus-signs (red) = *Tachigali***
822 ***melinonii*.**
823
824

825 **Figure 4: Phylogenetic tree (neighbour-joining) of arbuscular**
826 **mycorrhizal fungi (Glomeromycota) on three co-occurring**
827 **legume trees at Nouragues in French Guiana. Filled dots**
828 **denote sequences derived from this study, open triangles**
829 **denote sequences from MaarjAM including *Dicorynia***
830 ***guianensis* and *Eperua falcata* from Öpik et al. (2013) and**
831 **open squares denote sequences from Genbank. Numbers**
832 **indicate bootstrap values (> 50 % shown; 100**
833 **randomisations).**

834

835

836 **Supplementary Figure: a) Roots of *Dicorynia guianensis* *in***
837 ***situ* b) Roots of *Eperua falcata* c) Roots of *Tachigali melinonii***
838 **with spherical nodules.**
839