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

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Abstract

Aims: We aimed to characterise 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 leguminous trees (*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 *trn*L 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 trees 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 associations, defined by fungal arbuscular structures formed within host cortical cells, in a large proportion of the world's terrestrial flora (Smith & Read 2008) including many of the more than 20,000 tree species estimated from Neotropical forests (Slik et al. 2015). However, classical morphological descriptions by Gallaud (1904) and numerous subsequent studies, reviewed by Smith and Smith (1997), confirmed that AMF form both *Arum*- and *Paris*-type colonization structures in compatible mycorrhizal plant hosts. The former are characterised as typical AMF but, in the latter, root cortical cells do not host arbuscules but are heavily colonized by intracellular hyphal coils. Tropical forest trees and herbs appear to host a predominance of *Paris*-type mycorrhizas (Alexander 1989) including the target leguminous trees at our Amazonian study site in French Guiana (Béreau & Garbaye 1994; Béreau et al. 2004; de Grandcourt et al. 2004). Whilst it appears that AMF alpha-diversity can be higher in tropical than temperate studies (Husband et al. 2002; Haug et al. 2010, 2013; Camenzind et al. 2014), this is not always the case with overlap shown in the number of AMF taxa recorded from these divergent ecosystems. It is, however, difficult to make robust comparisons due to the paucity of tropical studies coupled with

inconsistent methodologies, particularly as the field of molecular ecology advances (e.g. next generation sequencing; Shendure & Ji 2008).

Because mycorrhizal fungi form a key functional interface between plant roots and soil, they play a major role in plant nutrition (Smith & Read 2008). Leguminous plants and trees also host symbiotic nitrogen-fixing bacteria that require large amounts of phosphorus (P) for nodule development and nitrogenase functioning (e.g. Mortimer et al. 2008 and references therein). Nitrogen (N) fixation in leguminous plants in general and tropical trees in particular is therefore highly dependent upon efficient P uptake, especially in P-deficient tropical soils, which is mediated by the AMF symbiosis (Plassard & Dell 2010). In addition, AMF are known to be important in structuring plant communities with different taxa or associations of taxa having differential effects on plant growth (van der Heijden et al. 1998; Munkvold et al. 2004; Koch et al. 2006; Roger et al. 2013). For example, Kiers et al (2000) demonstrated differential responses to AMF inoculum from conspecific or heterospecific tropical tree seedlings and Pizano et al. (2011) found that AMF from tropical landslide sites had differing effects on plant growth compared to those from light-gap sites, both studies indicating the potential role of AMF in influencing plant communities. Understanding the determinants of species distributions, through studying their niche requirements, and elucidating ecological community structure is a fundamental area of research in ecology and is important to support assessment of environmental change, and inform evidence-based management of ecosystems. As AMF are obligately symbiotic organisms, both the host species (Lovelock et al. 2003; Helgason et al. 2007; Sýkorová et al. 2007; de Oliveira Freitas et al. 2014) as well as edaphic (Fitzsimmons et al. 2008; Ji et

al. 2012; de Oliveira Freitas et al. 2014) and biogeographical (Hazard et al 2011; Kivlin et al. 2011; Öpik et al. 2013) factors will influence AMF community structure but, in many cases, it is difficult to clearly disentangle these due to edaphic sorting of the host plant. Surprisingly few studies have attempted to do this (but see Fitzsimmons et al. 2008; Dumbrell et al. 2010; Ji et al. 2012) although it would clearly help in furthering our understanding of AMF community structuring.

In this study, we examined the root associated AMF fungal community on three co-occurring leguminous tree species of French Guiana where legumes form many of the commonest tree species, making a significant contribution to stand basal area (ter Steege et al. 2006). We hypothesised that: i) the *Paris*-mycorrhizal status of the target legume tree species could result from colonization by novel AMF taxa, ii) host tree and soil edaphic specific responses would be detectible in root colonizing fungal communities but iii) the co-ocurring trees would form a core AMF community with the potential to form common mycelial networks.

Methods

Study site

The study was conducted at the Nouragues Research Station (within a National Nature Reserve) in central French Guiana (Bongers et al. 2001; http://www.nouragues.cnrs.fr) with a diverse tree flora typical of much of the Guiana Shield (ter Steege et al. 2006; Gonzalez et al. 2009). The sampling was restricted to the Inselberg camp area (4°05’N; 52°41’W) in minimally disturbed tropical forest where two large sampling plots (‘Grand Plateau’ and ‘Petit Plateau’) have been delimited. These two plots have differing edaphic conditions: the

Grand Plateau is based on metamorphic geology with more fertile clay-rich soils and the Petit Plateau is based on granitic geology with more sandy soils that are less fertile, although both are Ultisols (Poszwa et al. 2009). The annual rainfall is around 2900 mm with a drier season from late August to early November.

Study species

We investigated three legume species in different tribes of the Caesalpinioideae: *Dicorynia guianensis* Amshoff (tribe Cassieae), *Tachigali melinonii* (Harms) Zarucchi & Herend. (syn. *Sclerolobium melinonii* Harms; tribe Caesalpinieae) and *Eperua falcata* Aublet (tribe Detarieae). The former two species are among the most important trees, in terms of carbon cycling, in the Amazon basin (Fauset et al. 2015). The three species have contrasting root morphologies with *Dicorynia guianensis* and *Tachigali melinonii* being similar to one another with thin, highly branched roots in contrast to *Eperua falcata* that has thicker, poorly branching root systems with short roots on long axes (Béreau & Garbaye 1994). All three species form *Paris*-type mycorrhizal associations (Béreau & Garbaye 1994; Béreau et al. 2004; de Grandcourt et al. 2004). Many Caesalp legumes possess ectomycorrhizal (EcM) associations (Smith & Read 2008) but following a careful visual assessment of sampled roots, no obvious EcM development was observed. *Dicorynia guianensis* and *Tachigali melinonii* form prominent nodules and *Eperua falcata* has nodule-like structures on the roots whose function is not entirely clear (Sprent 2001).

Field sampling

Root samples were obtained from 12-16 randomly selected trees of each of the three target species in August and September 2009 (trees were up to 1 m diameter; mean = 50.3 ± SD 20.3 cm). At

each tree, roots were exposed by careful excavation enabling them to be traced to a distance of about 1.0 to 1.5 m from the trunk. Four samples of fine root material were cut away from the surrounding friable organic soil with any soil adhering to the roots brushed off. Root samples from each tree were combined together into a single plastic vial filled with silica gel for rapid drying. Soil samples were taken from the areas immediately adjacent to root collection and also combined into a single sample. They were subsequently air-dried in the field and returned to the UK for analysis.

DNA extraction

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

centrifuging for 5 minutes at 7,000 RCF. The dried pellets were rehydrated in 25 μl TE buffer and stored at -20 ° C until use.

Host plant species validation by *trn*L amplicon fragment length analysis

Although all the root samples were visually traced during sampling, we considered it prudent to verify the identity and purity of root samples. We used a length heterogeneity PCR approach, based upon the work of Ridgway et al. (2003), to achieve this. The Genbank database was used to predict amplicon sizes for a PCR of the plastid *trn*L intron using primers c and d from Taberlet et al. (1991). PCRs were performed using the conditions described by Gonzalez et al. (2009), but the c primer was modified with CY5 on the 5' end to enable compatibility with the Beckman fragment analysis system. Amplicon lengths were measured and quantified using a Beckman CEQ 8000 automated sequencer in fragment analysis mode. Direct sequencing of some of the amplicons was performed to check the specificity of the PCR and confirm tree identities. The c and d primers (Taberlet et al. 1991) were used to obtain sequence reads using an Applied Biosystems 3730xl sequencer.

Mycorrhizal community profiling

The AMF community associated with each tree was compared using denaturing gradient gel electrophoresis (DGGE) to generate a community fingerprint from an amplified fragment of the fungal small sub-unit rRNA gene. PCR and DGGE were performed according to the method of Öpik et al. (2003), using primers AM1 (Helgason et al. 1998) and NS31 (Simon et al. 1992). A GC clamp was added to the 5’ end of NS31 primer to stabilize the melting behaviour of the DNA fragments. PCRs contained 2.5 U Taq (Bioline, London, UK), 5 μl

10 x NH_4 reaction buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of each primer, and were made up to 50 μl volume with DNA template and water; they were performed in an MJ Research PTC-200 thermal cycler following cycling parameters in Öpik et al. (2003). DGGE was carried out on the Bio-Rad DCode universal mutation detection system, using 6 % polyacrylamide gels, with urea-formamide denaturant gradients of 22 % to 35 %. Electrophoresis was run at 60 º C and 75 V for 8 hours, with 32 ng DNA loaded into each well. Gels were stained with SYBR Gold (Molecular Probes, Leiden, The Netherlands) and digitized using GeneGenius Imaging System from Syngene. We ran two DGGE gels: the first had sixteen trees of *Tachigali melinonii* ("Tachigali gel") and the second had a subset of nine of those sixteen trees plus nine *Dicorynia guianensis* and four *Eperua falcata* ("Mixed gel"). Gel images were converted to a presence/absence matrix for each band position based on a systematic procedure using the plot RGB profile function of ImageJ (Schneider et al. 2012) to extract pixel values for each lane, followed by peak detection using LabPlot (http://labplot.sourceforge.net). To correct for slight skew in the gels, coloured reference lines were added across the gel images linking lane markers and prominent reference bands before peak detection. The signatures of these lines in the RGB pixel profiles from ImageJ were used to ensure accurate alignment of lane profiles before peak detection.

Determination of mycorrhizal taxa

Whilst DGGE requires relatively short, variable PCR products to achieve good separation of bands on the gel, a longer sequence is preferable for accurate phylogenetic classification. To supplement the DGGE community analysis we therefore obtained longer DNA sequences to determine the taxonomy of numerically dominant mycorrhizal taxa in a subset of samples. For this part of the study

we used primers AML1 and AML2 that are reported to have better specificity and coverage of known AMF taxa compared to the AM1 and NS31 primers used for DGGE (Lee et al. 2008). We chose six samples: three geographically close (< 300 m) trees of each species from each of the Grand and Petit Plateaus. PCR products were produced using the protocol described by Lee et al. (2008), then cloned into *E. coli* using an Invitrogen Topo TA cloning kit. Ten positive transformants from each tree were used directly in a colony PCR (Elliott et al. 2005) using vector primers M13F and M13R to check the insert size (approximately 800 base pairs). Forty-eight successful inserts were sequenced from the T3 priming site using an Applied Biosystems 3730xl sequencer.

Sequences from forward and reverse primers were assembled using contig assembly program (Huang 1992) and single coverage regions were discarded. A total of 23 double coverage sequences were obtained and these were clustered at 99 % similarity using cd-hit-est (Huang et al. 2010) to identify unique sequences. Approximate species-level OTUs were identified using UCLUST (Edgar 2010) with a 97 % similarity threshold. A neighbour-joining phylogenetic tree was constructed from the unique sequences that exceeded 450 base pairs in length. We included the top match from MaarjAM (Öpik et al. 2010) for each of our sequences plus all sequences associated with two of our host plants (presented in Öpik et al. 2013) in addition to several globally distributed taxa to provide a wider context. We also included the three top matching sequences from a BLAST search on Genbank and any closely matching named taxa. In some cases, relevant sequences were excluded because the sequence regions did not overlap sufficiently with those reported in this study. ClustalW (Thompson et al. 1994) was used to align the sequences and all gaps were removed before creating a distance matrix

(Kimura 1980) and neighbour-joining phylogenetic tree (Saitou & Nei 1987), using the APE package (Paradis et al. 2004) for R (R Core Team 2015).

Soil analyses

All analyses were conducted in duplicate on soils that had been ground to pass a 1 mm sieve. The moisture content of the air-dried soil was determined by heating 5 g sub-samples to 105 ° C for 24 h. Soil pH was measured by adding 5 g of soil to 12.5 ml of deionised water; it was stirred and left to equilibrate for 1 h before the pH was measured with a Sartorius PB-11 pH meter. Carbon and N were determined on a LECO TruSpec elemental analyser. Total P was determined by digesting 0.25 g samples in 5 ml of concentrated sulphuric acid (with a lithium sulphate/selenium (100:1) catalyst) for 8 hours at 375 ° C. Samples were then made up to 50 ml in deionised water and analysed on a Varian Vista AX Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Cations (P, K, Ca and Mg) were extracted from 2.5 g samples that were shaken with 25 ml of Mehlich 1 solution for ten minutes before being filtered and analysed on a Thermo iCAP 6300 Duo ICP-OES.

Statistics

Rarefaction curves and Chao1 species richness estimator values were calculated (100 randomisations) in EstimateS (Colwell 2013). Redundancy analysis was performed using the Vegan package (Oksanen et al. 2015) for R (R Core Team 2015) with a backwards stepwise approach to select constraining variables. We tested whether the AMF community differed in relation to soil properties or host tree species using a PerMANOVA (Jaccard index, 999 permutations), also in the Vegan package for R.

Results

Mycorrhizal SSU amplification

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

Plant species validation by *trn*L amplicon sizes

Double-coverage *trn*L sequences were obtained from *Dicorynia guianensis* and *Eperua falcata* (GenBank accessions: KU356724-KU356727) with 99-100 % identity to publicly available *trn*L sequences from the target trees; sequencing of amplicons from *Tachigali melinonii* was unsuccessful. In almost all samples, the dominant *trn*L fragment sizes were within two base pairs of the predicted length (Table 1). We removed five samples from subsequent analyses; one had a much shorter fragment than expected (indicating that the tree was probably identified incorrectly) and four others had secondary peaks that were 10 % or more of the height of the main peak (indicating possible contamination with roots of other plants).

Mycorrhizal community profiling by DGGE

Rarefaction curves (and comparison with Chao1 values) suggest that our sampling was sufficiently extensive to describe the AMF community on *Dicorynia guianensi*s and *Tachigali melinonii* but not

Eperua falcata with around 30 bands found for the former two species and 25 for the latter (Fig. 1a) and a total of 34 bands for the community as a whole (using the Mixed gel). The Chao1 estimate for the AMF community as a whole was 34.7 ± SD 1.3 indicating extensive sampling. Within tree species, *c*. 35-55 % of the bands were rare (*i.e.* restricted to one or two individual trees) with only a small proportion (< 12 %) found on more than 80 % of the trees within a species (Fig. 1b). Around half of the bands were found on all three tree species with few restricted to a single host - mostly to *Dicorynia guianensis* (Fig. 2). Of the bands that were shared between tree species, most were rare with the exception of one band that was found on around 80 % of *Dicorynia guianensis* trees but no *Eperua falcata* trees.

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

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

The stepwise redundancy analysis model building process selected soil N as the only constraining variable describing the AMF community (Fig. 3); N also appeared to separate the host trees on

axis RDA1. The significance of N in influencing the AMF community was confirmed by PerMANOVA, explaining 8.1 % of the variation (F = 1.93, r^2 = 0.089, p = 0.039). However, different host tree species were found on soils of differing N status (Table 2) and this might have influenced the AMF community through host selection although this was not significant at the data resolution available in our study (F = 0.91, r^2 = 0.084, p = 0.64).

Phylogeny of mycorrhizal taxa

All of the sequences found on the six trees (Genbank accessions: KR706472-KR706484) were from the family Glomeraceae (within the order Glomerales), and some are likely to be erected as new virtual taxa in the MaarjAM sequence database of AMF (Öpik et al. 2010; M. Öpik pers comm). Sequences were grouped into eight approximate species-level groups with > 97 % similarity; six of these were singletons found on one tree only. The Chao 1 estimate of the number of phylogroups was 14.9 ± SD 7.5. Our sequences did not match with any named AMF taxa, and, interestingly, also did not cluster closely with sequences from two of the same host species at a site in French Guiana about 125 km distant (Öpik et al. 2013) (Fig. 4).

Discussion

There remains a critical gap in the literature relating to tropical mycorrhizal community dynamics that, in boreal and temperate biomes, are known to underpin ecosystem productivity and multifunctionality (Smith and Read, 2008; van der Heijden et al. 2015). Earlier research, that had targeted the same species-rich Amazonian forests in French Guiana, highlighted a predominance of *Paris*-, as opposed to more commonly studied, *Arum*-type

mycorrhizal colonization of leguminous trees (Béreau & Garbaye 1994; Béreau et al. 2004; de Grandcourt et al. 2004). We provide here the first report on the diversity and identity of AMF known to form *Paris*- type mycorrhiza on three co-occurring leguminous trees on differing soil types in these northern Amazonian forests.

The main aim of our study was not to exhaustively recover AMF diversity, for which we would have used a specific set of primers for each order, but to determine the abundant root-associated taxa in our study system that are likely to be symbiotically active. This approach yielded an estimate of 34 AMF taxa detected in roots on the basis of SSU-DGGE banding that has been shown, via individual band sub-cloning and sequencing, to underestimate AMF diversity (Öpik et al. 2003), although it assumes we did not have any non-specific amplification from other fungal phyla (see Kohout et al. 2014). Our estimate still compares with other tropical studies employing various classical and next generation sequencing methodologies, for example, Aldrich-Wolfe (2007) found 31 phylotypes by T-RFLP in Costa Rica, Husband et al. (2002) found 30 taxa using a cloning and Sanger sequencing approach in Panama and Camenzind et al. (2014) found 74 taxa using 454-pyrosequcncing in Ecuador. However, comparisons are difficult between studies due to different primers, sequencing platforms and clustering approaches. It appeared that our sampling was saturated with eight to ten root samples, sufficient to sample the root-colonizing AMF community fully by DGGE.

Members of the Glomeraceae dominated the AMF community with no evidence of the abundant presence of members from other families or orders in the Glomeromycota. This was somewhat surprising given the high abundance of Acualosporaceae in tropical

spore-counting studies (e.g. Lovelock et al. 2003; Stürmer & Siqueira 2011; de Oliveira Freitas et al. 2014) including at our study site (Martin et al. 2001; Oehl & Brearley, unpublished data). It should be stressed that this is not a limitation of the primers developed by Lee et al. (2008) that efficiently amplify across the phylum. At least part of this restricted phylogenetic coverage is likely due to the small number of sequences found so we should be careful not to over interpret from this small dataset. However, it raises the interesting possibility that a phylogenetically restricted subset of AMF taxa preferentially form structurally distinct *Paris*-type mycorrhizal associations including likely new virtual taxa. Öpik et al. (2013), employing 454 pyrosequencing at a similar study site in French Guiana, also found a similarly restricted subset of AMF taxa on two of our study species. *Paris*-type mycorrhizas support extensive intracellular fungal coiling (Smith and Read 2008) that could physically prevent colonization by other AMF taxa thus restricting the number of taxa due to priority effects (Hepper et al. 1988; Werner and Kiers, 2015). The restricted taxa detected on our target tree roots could, therefore, represent a natural manifestation of a phenomenon that has, to date, only been described in controlled laboratory experiments with young seedlings. Our findings also support the hypothesis of Kivlin et al. (2011) of phylogenetic clustering within sites; perhaps, in this case, due to all the host trees being within the same family. That the identified taxa found mostly formed unique clusters when compared to AMF taxa identified by Öpik et al. (2013) on two of the same host tree species additionally supports the hypothesis of Kivlin et al. (2011) of high beta diversity in AMF. Finally, there is the possibility that these are legume specialist AMF as Sheublin et al. (2004) found clear differences between the AMF communities on legumes and non-legumes in a Dutch grassland.

The clearest relationship between soil nutrients and AMF taxa representation was seen for soil N. A related study in species rich tropical montane forest found reduced AMF species richness in bulked root samples in response to N and P addition (Camenzind et al. 2014). Nitrogen input in forests will be greatly dependent on anthropogenic deposition rates but also associative and symbiotic N-fixation involving legumes. The legume tree species in this study are productive members of the community and will contribute significant organic N to the soil via litter inputs. Spatial variability in soil N concentration was shown in our species-rich forest ecosystem, and this could select for AMF taxa with differing organic N-mobilizing activities (Hodge 2014). For example, Martin et al. (2001) found soils under *Eperua falcata* to be more enriched in N than those under *Dicorynia guianensis* in common with our study. In our study, it appeared that soil N had a greater effect that host species in influencing the AMF community (although there was non-random association of tree species with particular edaphic conditions), concurring with other recent studies pointing towards edaphic factors playing a more important role than host species in structuring AMF communities (Fitzsimmons et al. 2008; Dumbrell et al. 2010; Ji et al. 2012). With regard to common mycelial networks, our data provided some evidence of potential inter- and intra-host species networking potential. Mechanisms driving restriction to limited common AMF-forming taxa in these leguminous hosts may have evolved to ensure networking within N-fixing trees in a highly resource competitive environment.

For unequivocal identification of the host species in systems with diverse vegetation, the plastid *trn*L region offers a robust and rapid marker for confirmation of root sample identity with minor species-

specific *trn*L length variations likely reflecting intra-specific variation within the study site. Zeng et al. (2015) also recently reported successful root identification of 11 tree species in a Chinese subtropical forest via *trn*L sequencing. Although many studies adopt a root-tracing approach, the important strategy taken here to confirm host species via molecular tools is rarely adopted and we promote this as a straightforward and appropriate method for verification in mixed species communities where reference material is available. As well as *trn*L (Dumbrell et al. 2010; Zeng et al. 2015), other suitable gene regions might include *trn*H-*psb*A (Jones et al. 2011), *rbc*L or *mat*K (CBOL Plant Working Group 2009).

One of the advantages to our 'classical' sequencing approach is that we detected the taxa that are more abundant in the tree roots, and therefore likely to be functionally most important in terms of mutualistic associations. Furthermore, it allows us to avoid sampling low-density 'contaminant' hyphae in the rhizoplane or spores simply present on the plant roots that would be picked up by extensive next-generation sequencing but are not forming functional AMF. If we wished to sample the soil AMF community exhaustively then next-generation sequencing or DNA metabarcoding would effectively allow this more in-depth examination of the community (e.g. Öpik et al. 2013).

What is the functional importance of root symbiotic AMF diversity and what are all these fungi doing in the ecosystem? For example, the mycorrhizal response to AMF inoculation in *Eperua falcata* is less than *Dicorynia guianensis* (de Grandcourt et al. 2004) and this may be influenced by this species' preference for nitrate (Schimann et al. 2008) mediated by root exudate influence on the rhizosphere microbial community (Michalet et al. 2013). If AMF communities

that have different functions (such as P-mining ability) are spatially separated then they have the potential to influence seedling diversity in tropical forests and hence contribute to the high diversity of these ecosystems.

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Author contributions Designed the study and obtained funding: FQB & RS; performed the study: FQB, DRE & AI; analysed the data: DRE & FQB; wrote the manuscript: FQB, RS & DRE.

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Table 1: Plastid *trn*L amplicon length predictions and measurements for three co-occurring legume tree species at Nouragues in French Guiana. The sequence accession and identity used for prediction is indicated in parentheses in the central column.

Species	*trn*L length prediction	Measurement range
Dicorynia guianensis	617 (FJ039291; *Dicorynia guianensis*)	616-618
Eperua falcata	706 (FJ039126; *Eperua falcata*)	704-705
Tachigali melinonii	578 (AF430790; *Tachigali paniculata*)	578-580

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

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

Figure 1a: Rarefaction curves and 1b: frequency distributions of the DGGE bands of arbuscular mycorrhizal fungal taxa found on three co-occurring legume tree species at Nouragues in French Guiana.

Figure 2: Venn diagram of the DGGE bands of arbuscular mycorrhizal fungal taxa found on three co-occurring legume tree species at Nouragues in French Guiana.

Figure 3: Redundancy analysis of DGGE bands of arbuscular mycorrhizal fungal taxa on three co-occurring legume tree species at Nouragues in French Guiana constrained by soil nitrogen concentration. Green circles = *Dicorynia guianensis*, Blue triangles = *Eperua falcata*, Red plus signs = *Tachigali melinonii*.

Figure 4: Phylogenetic tree (neighbour-joining) of arbuscular mycorrhizal fungi (Glomeromycota) on three co-occurring legume tree species at Nouragues in French Guiana. Filled dots denote sequences derived from this study, open triangles denote sequences from MaarjAM including *Dicorynia guianensis* and *Eperua falcata* from Öpik et al. (2013) and open squares denote sequences from Genbank. Numbers indicate bootstrap values (> 50 % shown; 100 randomisations). Host and locality of sequences are shown within round brackets (FG = French Guiana); accession numbers are given in square brackets.