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 *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 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

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 fungi form both *Arum*- and *Paris*-type colonization structures in compatible mycorrhizal plant hosts. The former type 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 forest 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 ecosystems (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 credible 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 a metamorphic geology with more fertile clay-rich soils and the Petit Plateau is based on a 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 had thicker, poorly branching root systems with short roots on long axes (Béreau & Garbaye 1994; Supplementary Figure 1). 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 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 (Supplementary Figure 1a). 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 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), 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 water and DNA template; 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 % polyacrilamide gels, with urea-formamide denaturant gradients of 22 to 35 %. Electrophoresis was run at 60 ºC 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, for accurate phylogenetic classification a longer sequence is preferable. For this part of the study, we therefore 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 cutoff 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 generation of the 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 & 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 were calculated in EstimateS (100 randomisations). 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.

Results

Arbuscular 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* (*c*. 75 %) and *Tachigali melinonii* (100 %). Difficulties amplifying the host *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: PENDING) 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 probable 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 a 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 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 and extractable P and cations whereas those surrounding *Dicorynia guianensis* were lowest in all measured nutrients (Table 2).

The step-wise redundancy analysis model building process selected soil N as the only constraining variable describing the AMF community structure (Fig. 3); N also appeared to separate the host trees on axis RDA1. The significance on N on the AMF community was confirmed by PerMANOVA (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 a significant determinant of at the data resolution available in this 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). They 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 17: this was half that estimated from the DGGE bands although in closer agreement with the number

predicted when rarefied to six samples (24.6 ± SD 1.7). Our sequences did not match with any named AMF taxa, and, interestingly, did not cluster closely with sequences from two of the same host species in 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 root-colonizing diversity (Öpik et al. 2003), although it assumes we did not have any non-specific amplification from other fungal phyla (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 differing primers, sequencing platforms and clustering approaches. It appeared that our sampling was saturated with eight to ten 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. 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 appear to support extensive intracellular fungal coiling (Smith and Read 2008) that could physically prevent colonization by other AMF taxa thus restricting functional taxa representation 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 highly-supported 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 (Camenzind et al. 2014) in species rich tropical montane forest found reduced AMF species richness in bulked root samples in response to N and P. Nitrogen input in native 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 N content was shown in these species-rich forest systems that could select for AMF taxa with enhanced potential organic N-mobilizing activities (Hodge 2014). For example, Martin et al. (2001) showed soils under *Eperua falcata* to be more enriched in N than those under *Dicorynia guianensis* in common with this study. In our study, it appeared that soil N had a greater effect that host species (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 productive 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) 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 certainty 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, 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. 2012) 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.

Acknowledgements

We thank Centre National de la Recherche Scientifique for managing the Nouragues Research Station and funding this study through Project Amazonie and Manchester Metropolitan University for providing a research assistantship to DRE. We are very grateful to Jérôme Chave for his support of this project and Philippe Gaucher and Patrick Châtelet who were of great help during fieldtrip preparation and sampling. Stavros Veresoglou, John Davison and anonymous reviewers are thanked for their comments on the manuscript. This project was also partly funded from "Investissement d’Avenir" grants managed by Agence Nationale de

la Recherche (CEBA: ANR-10-LABX-25-01 and ANAEE-France: ANR-11-INBS-0001).

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.

References

Aime MC, Brearley FQ (2012) Tropical fungal diversity: closing the gap between species estimates and species discovery. Biodivers Conserv 21:2177-2180. doi:10.1007/s10531-012-0338-7.

Aldrich-Wolfe L (2007) Distinct mycorrhizal communities on new and established hosts in a transitional tropical plant community. Ecology 88:559-566. doi:10.1890/05-1177.

Alexander IJ (1989) Mycorrhizas in tropical forests. In: Mineral Nutrients in Tropical Forest and Savanna Ecosystems. Ed. J Proctor. pp. 169-188. Blackwell Scientific Publications, Oxford, UK.

Béreau M, Garbaye J (1994) First observations on the root morphology and symbioses of 21 major tree species in the primary tropical rain forest of French Guyana. Ann Sci For 51:407-416. doi: 10.1051/forest:19940406.

Béreau M, Louisanna E, Garbaye J (2004) Mycorrhizal symbiosis in the tropical rainforest of French Guiana and its potential contribution to tree regeneration and growth. In: Ecology and Management of a Neotropical Rainforest (eds S Gourlet-Fleury, J-M Guehl, O Laroussinie), pp. 114-119. Elsevier, Paris, France.

Bongers F, Charles-Dominique P, Forget PM, Théry M (2001) Nouragues: Dynamics and Plant-Animal Interactions in a Neotropical Rainforest. Kluwer Academic Publishers, Dordrect, The Netherlands.

Camenzind T, Hempel S, Homeier J, Horn S, Velescu A, Wilcke W, Rillig MC (2014) Nitrogen and phosphorus additions impact arbuscular mycorrhizal abundance and molecular diversity in a tropical montane forest. Glob Chang Biol 20:3646-3659. doi:: 10.1111/gcb.12618.

CBOL Plant Working Group (2009) A DNA barcode for land plants. Proc Natl Acad Sci USA 106:12794-12797. doi:10.1073/pnas.0905845106.

de Grandcourt A, Epron D, Montpied P, Louisanna E, Béreau M, Garbaye J, Guehl J-M (2004) Contrasting responses to mycorrhizal inoculation and phosphorus availability in seedlings of two tropical rainforest tree species. New Phytol 161:865-875. doi:10.1046/j.1469-8137.2004.00978.x.

de Oliveira Freitas R, Buscardo E, Nagy L, dos Santos Maciel AB, Carrenho R, Luizão RCC (2014) Arbuscular mycorrhizal fungal communities along a pedo-hydrological gradient in a Central Amazonian terra firme forest. Mycorrhiza 24:21-32. doi: 10.1007/s00572-013-0507-x.

Dirzo R, Raven PH (2003) Global state of biodiversity and loss Annu Rev Environ Resour 28:137-167. doi:10.1146/annurev.energy.28.050302.105532.

Dumbrell AJ, Nelson M, Dytham C, Helgason T, Fitter AH (2010) Relative roles of niche and neutral processes in structuring a soil microbial community. ISME J 4:337-345. doi:10.1038/ismej.2009.122.

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460-2461. doi: 10.1093/bioinformatics/btq461.

584 Elliott DR, Wilson M, Buckley CMF, Spratt DA (2005) Cultivable oral microbiota of
585 domestic dogs. J Clin Microbiol 43:5470-5476. doi:10.1128/JCM.43.11.5470-
586 5476.2005.
587 Fauset S et al. (2015) Hyperdominance in Amazonian forest carbon cycling. Nat
588 Commun 6:6857. doi: 10.1038/ncomms7857.
589 Fitzsimons MS, Miller RM, Jastrow JD (2008) Scale-dependent niche axes of
590 arbuscular mycorrhizal fungi. Oecologia 158:117-127. doi: 10.1007/s00442-
591 008-1117-8.
592 Gallaud I. (1904). Études sur les Mycorrhizes Endotrophes. Le Bigot Frères,
593 Lille, France.
594 Ghazoul J, Sheil D (2010) Tropical Rain Forest Ecology, Diversity, and
595 Conservation. Oxford University Press, UK.
596 Gibson L, Lee TM, Koh LP, Brook BW, Gardner TA, Barlow J, Peres CA, Bradshaw
597 CJA, Laurance WF, Lovejoy TE, Sodhi NS (2011) Primary forests are
598 irreplaceable for sustaining tropical biodiversity. Nature 478 : 378–381.
599 doi:10.1038/nature10425.
600 Gonzalez MA, Baraloto C, Engel J, Mori SA, Pétronelli P, Riéra B, Roger A, Thébaud
601 C, Chave J (2009) Identification of Amazonian trees with DNA barcodes.
602 PLoS ONE 4:e7483. doi:10.1371/journal.pone.0007483.
603 Haug I, Wubet T, Weiß M, Aguirre N, Weber M, Günter S, Kottke I (2010) Species-
604 rich but distinct arbuscular mycorrhizal communities in reforestation plots
605 on degraded pastures and in neighboring pristine tropical mountain rain
606 forest. Trop Ecol 51:125-148. DOI
607 Haug I, Setaro S, Suárez JP (2013) Reforestation sites show similar and nested
608 AMF communities to an adjacent pristine forest in a tropical mountain area
609 of South Ecuador. PLOS ONE 8:e63524. doi:10.1371/journal.pone.0063524.
610 Hazard C, Gosling P, van der Gast CJ, Mitchell DT, Doohan FM, Bending GD (2013)
611 The role of local environment and geographical distance in determining
612 community composition of arbuscular mycorrhizal fungi at the landscape
613 scale. ISME J 7:498-508. doi:10.1038/ismej.2012.127.
614 Heinonsalo J, Jørgensen KS, Sen R (2001) Microcosm-based analyses of Scots
615 pine seedling growth, ectomycorrhizal fungal community structure and
616 bacterial carbon utilization profiles in boreal forest humus and underlying
617 illuvial mineral horizons. FEMS Microbiol Ecol 36:73-84. doi:10.1111/j.1574-
618 6941.2001.tb00827.x.
619 Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW (1998) Ploughing up the
620 wood-wide web? Nature 394:431. doi:10.1038/28764.
621 Helgason T, Merryweather JW, Young JPW, Fitter AH. (2007) Specificity and
622 resilience in the arbuscular mycorrhizal fungi of a natural woodland
623 community. J Ecol 95: 623-630. doi:10.1111/j.1365-2745.2007.01239.x.
624 Hepper CM, Azcon-Aguilar C, Rosendahl S, Sen R (1988) Competition between
625 three species of *Glomus* used as spatially separated introduced and
626 indigenous mycorrhizal inocula for leek (*Allium porrum* L.). New Phytol
627 110:207-215. doi:10.1111/j.1469-8137.1988.tb00254.x.
628 Hodge, A. (2014) Interactions between arbuscular mycorrhizal fungi and organic
629 material substrates. Adv Appl Microbiol 89:47-99. doi:10.1016/B978-0-12-
630 800259-9.00002-0.
631 Huang X (1992) A contig assembly program based on sensitive detection of
632 fragment overlaps. Genomics 14:18-25. doi:10.1016/S0888-7543(05)80277-
633 0.
634 Huang Y, Niu B, Gao Y, Fu L, Li W (2010) CD-HIT Suite: a web server for clustering
635 and comparing biological sequences. Bioinformatics 26:680-682.
636 doi:10.1093/bioinformatics/btq003.

Husband R, Herre EA, Turner SL, Gallery R, Young JPW (2002) Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. Mol Ecol 11:2669-2678. doi:10.1046/j.1365-294X.2002.01647.x.

Jackson CR, Roden EE, Churchill PF (2000) Denaturing gradient gel electrophoresis can fail to separate 16S rDNA fragments with multiple base differences. Mol Biol Today 1:49-51. DOI

Ji B, Bentivenga SP, Caspar BB (2012) Comparisons of AM fungal spore communities with the same hosts but different soil chemistries over local and geographical scales. Oecologia 168:187-197. doi:10.1007/s00442-011-2067-0.

Jones FA, Erickson DL, Bernal MA, Bermingham E, Kress WJ, Herre EA, Muller-Landau HC, Turner BL (2011) The roots of diversity: below ground species richness and rooting distributions in a tropical forest revealed by DNA barcodes and inverse modeling. PLoS ONE 6:e24506. doi:10.1371/journal.pone.0024506.

Kiers ET, Lovelock CE, Krueger EL, Herre EA (2000) Differential effects of tropical arbuscular mycorrhizal fungal inocula on root colonization and tree seedling growth: implications for tropical forest diversity. Ecol Lett 3:106-113. doi: 10.1046/j.1461-0248.2000.00126.x.

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16, 111-120.

Kivlin SN, Hawkes CV, Treseder KK (2011) Global diversity and distribution of arbuscular mycorrhizal fungi. Soil Biol Biochem 43: 2294-2303. doi:10.1016/j.soilbio.2011.07.012.

Koch AM, Croll D, Sanders IR. (2006) Genetic variability in a population of arbuscular mycorrhizal fungi causes variation in plant growth. Ecol Lett 9:103-110. doi: 10.1111/j.1461-0248.2005.00853.x.

Kohout P, Sudová R, Janoušková M, Čtvrtlíková M, Hejda M, Pánková H, Slavíková R, Štajerová K, Vosátka M, Sýkorová Z (2014) Comparison of commonly used primer sets for evaluation of arbuscular mycorrhizal fungal communities: Is there a universal solution? Soil Biol Biochem 68:482-493. doi:10.1016/j.soilbio.2013.08.027.

Lee J, Lee S, Young JPW (2008) Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiol Ecol 65:339-49. doi:10.1111/j.1574-6941.2008.00531.x.

Lovelock CE, Andersen K, Morton JB (2003) Arbuscular mycorrhizal communities in tropical forests are affected by host tree species and environment. Oecologia 135:268-279. doi:10.1007/s00442-002-1166-3.

Martin J, Béreau M, Louisanna E, Ocampo JA (2001) Arbuscular mycorrhizas in *Dicorynia guianensis* and *Eperua falcata* trees from primary tropical rain forest of French Guiana. Symbiosis 31:283-291. DOI

Michalet S, Rohr J, Warshan D, Bardon C, Roggy J-C, Domenach A-M, Czarnes S, Pommier T, Combourieu B, Guillaumand N, Bellvert F, Comte G, Poly F (2013) Phytochemical analysis or mature tree root exudates *in situ* and their role in shaping soil microbial communities in relation to tree N-acquisition strategy. Plant Physiol Biochem 72:169-177. doi:10.1016/j.plaphy.2013.05.003.

Mortimer PE, Pérez-Fernández MA, Valentine AJ (2008) The role of arbuscular mycorrhizal colonization in the carbon and nutrient economy of the tripartite symbiosis with nodulated *Phaseolus vulgaris*. Soil Biol Biochem 40:1019-1027. doi:10.1016/j.soilbio.2007.11.014.

Munkvold L. Kjøller R, Vestberg M, Rosendahl S, Jakobsen I (2004) High functional diversity within species of arbuscular mycorrhizal fungi. New Phytol 164:357-364. doi:10.1111/j.1469-8137.2004.01169.x.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. (2015) vegan: Community Ecology Package. R package version 2.2-1. http://cran.r-project.org/package=vegan

Öpik M, Moora M, Liira J, Kõljalg U, Zobel M, Sen R. (2003) Divergent arbuscular mycorrhizal fungal communities colonize roots of *Pulsatilla* spp. in boreal Scots pine forest and grassland soils. New Phytol 160:581-593. doi:10.1046/j.1469-8137x.2003.00917.x.

Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier Ü, Zobel M (2010) The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol 188:223-241. doi:10.1111/j.1469-8137.2010.03334.x.

Öpik M, Zobel M, Cantero JJ, Davison J, Facelli JM, Hiiesalu I, Jairus T, Kalwij JM, Koorem K, Leal ME, Liira J, Metsis M, Neshataeva V, Paal J, Phosri C, Põlme S, Reier Ü, Saks Ü, Schimann H, Thiéry O, Vasar M, Moora M (2013) Global sampling of plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. Mycorrhiza 23:411-430. doi:10.1007/s00572-013-0482-2.

Paradis E, Claude G, Strimmer K (2004) APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289-290. doi: 10.1093/bioinformatics/btg412.

Pizano C, Mangan SA, Herre EA, Eom AH, Dalling JW (2011) Above- and belowground interactions drive habitat segregation between two cryptic species of tropical trees. Ecology 92:47-56. doi:10.1890/09-1715.1.

Plassard C.,Dell B. (2010) Phosphorus nutrition of mycorrhizal trees. Tree Physiol 30:1129-1139. doi: 10.1093/treephys/tpq063.

Poszwa A, Ferry B, Pollie B, Grimaldi C, Charles-Dominique P, Loubet M, Dambrine E (2009) Variations of plant and soil $^{87}Sr/^{86}Sr$ along the slope of a tropical inselberg. Ann For Sci 66:512. doi:10.1051/forest/2009036.

R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austrai. http://www.R-project.org/.

Ridgway KP, Duck JM, Young JPW (2003) Identification of roots from grass swards using PCR-RFLP and FFLP of the plastid *trn*L (UAA) intron. BMC Ecol 3:8. doi:10.1186/1472-6785-3-8.

Roger A, Colard A, Angelard C, Sanders IR (2013) Relatedness among arbuscular mycorrhizal fungi drives plant growth and intraspecific fungal coexistence. ISME J 7:2137-2146. doi:10.1038/ismej.2013.112.

Saitou N, Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406-425.

Sheublin TR, Ridgway KR, Young JPW, van der Heijden MGA et al. (2004) Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. Appl Environ Microbiol 70:6240-6246. doi: 10.1128/AEM.70.10.6240-6246.2004.

Schimann H, Ponton S, Hättenschwiler S, Ferry B, Lensi R, Domenach A-M, Roggy J-C (2008) Differing nitrogen use strategies of two tropical rainforest late successional tree species in French Guiana: evidence from ^{15}N natural abundance and microbial activities. Soil Biol Biochem 40:487-494. doi:10.1016/j.soilbio.2007.09.011.

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671-675. doi:10.1038/nmeth.2089.

Shendure J, Ji H (2008) Next-generation DNA sequencing. Nat Biotechnol 26:1135-1145. doi:10.1038/nbt1486.
Simon L, Lalonde M, Bruns TD (1992) Specific amplification of 18S fungal ribosomal genes from vesicular–arbuscular endomycorrhizal fungi colonizing roots. Appl Environ Microbiol 58: 291-295. DOI
Slik JWF et al. (2015) An estimate of the number of tropical tree species. Proc Natl Acad Sci USA 112:7272:7477. doi:10.1073/pnas.1423147112.
Smith SE, Read DJ (2008) Mycorrhizal Symbiosis, 3rd edn. Academic Press, New York, USA.
Smith, SE, Smith FA (1997) New Phyt. 137: 373–388.
Sprent JI (2001) Nodulation in Legumes. Royal Botanic Gardens, Kew, UK.
Stürmer SL, Siqueira JO (2011) Species richness and spore abundance of arbuscular mycorrhizal fungi across distinct land uses in Western Brazilian Amazon. Mycorrhiza 21:255-267. doi: 10.1007/s00572-010-0330-6.
Sýkorová Z, Wiemken A, Redecker D. (2007) Cooccurring *Gentiana verna* and *Gentiana acaulis* and their neighboring plants in two Swiss upper montane meadows harbor distinct arbuscular mycorrhizal fungal communities. Appl Environ Microbiol 73: 5426-5434. doi: 10.1128/AEM.00987-07.
Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol. 17:1105-1109. doi:10.1007/BF00037152.
ter Steege H, Pitman NCA, Phillips OL, Chave J, Sabatier D, Duque A, Molino J-F, Prévost M-F, Spichiger R, Castellanos H, von Hildebrand P, Vásquez R (2006) Continental-scale patterns of canopy tree composition and function across Amazonia. Nature 443:444-447. doi:10.1038/nature05134.
Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl Acids Res 22:4673-4680. doi:10.1093/nar/22.22.4673.
van der Heijden MGA, Boller T, Wiemken A, Sanders IR (1998) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology 79:2082-2091. doi:10.1890/0012-9658(1998)079[2082:DAMFSA]2.0.CO;2
van der Heijden MGA, Martin FM, Selosse M-A, Sanders IR (2015) Mycorrhizal ecology: the past, the present, and the future. New Phytol 205:1406-1423. doi:10.1111/nph.13288.
Vandenkoornhuyse P, Husband R, Daniell TJ, Watson IJ, Duck JM, Fitter AH, Young JPW (2002) Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. Mol Ecol 11:1555-1564. doi:10.1046/j.1365-294X.2002.01538.x.
Werner GDA, Kiers ET (2015) Order of arrival structures arbuscular mycorrhizal colonization of plants. New Phytol 205: 1515-1524. doi: 10.1111/nph.13092.
Zeng W, Zou B, Lei P, Zeng Y, Liu Y, Liu C, Xiang W (2015) A molecular method to identify species of fine roots and to predict the proportion of a species in mixed samples in subtropical forests Front Plant Sci 6:313. doi: 10.3389/fpls.2015.00313.

Table 1: *trn*L amplicon length predictions and measurements for three co-occurring legume trees 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 trees 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 trees at Nouragues in French Guiana.

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

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

Figure 4: Phylogenetic tree (neighbour-joining) of arbuscular mycorrhizal fungi (Glomeromycota) on three co-occurring legume trees 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).

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