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TRANSCRIPTOMIC AND GENOMIC ANALYSES OF COMMUNITIES

The role of soil chemistry and plant neighbourhoods in structuring fungal communities in three Panamanian rainforests

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Summary

1. Fungi play critical roles in ecosystem processes and interact with plant communities in mutualistic, pathogenic, and commensal ways. Fungal communities are thought to depend on both associated tree communities and soil properties. However, the relative importance of the biotic and abiotic drivers of soil fungal community structure and diversity in lowland tropical forests remains poorly understood.

2. We examined the community structure of trees and fungi at different levels of phosphorus (0·17–16·3 mg kg^{-1}) in moist tropical forests in Panama. We predicted that arbuscular mycorrhizal (AM) fungal composition would be more strongly associated with soil properties than with local tree communities while the composition of other fungal clades would be more strongly correlated with local tree communities than soil properties. We also predicted that fungal operational taxonomic unit (OTU) richness would be negatively correlated with soil fertility and positively correlated with tree species diversity within and among forests.

3. We characterized soil chemistry, fine root biomass, and sequenced the ITS1 barcode region to describe fungal community composition from 70 soil cores across three 1-ha tropical rainforest sites in Panama. The sites vary in soil chemistry, including P, and in tree species community composition, but experience similar annual rainfall.

4. AM fungal community composition was partially correlated with soil chemistry ($r = 0{\cdot}32$, $P \leq 0{\cdot}001$), but not with local tree communities, while non-AM fungal communities were nearly equally correlated with soil chemistry (Partial Mantel test, $r = 0{\cdot}38$, $P \leq 0{\cdot}001$) as with tree communities ($r = 0{\cdot}36$, $P \leq 0{\cdot}001$). Linear models showed that AM OTU richness was not explained by any independent variable. For non-AM fungi, phosphorus, pH, and soil moisture better predicted OTU richness across all cores than other biotic and abiotic factors.

5. *Synthesis*. Our results show that AM fungal structure is driven primarily by soil chemistry. For non-AM fungi, soil properties and the local tree community can play a joint role in structuring communities. Furthermore, we found that more diverse local tree communities did not harbour more fungal species. Our results suggest that soil properties act as an environmental filter for both trees and fungi, setting the stage for interactions between the two.

Key-words: ITS1, metabarcoding, microbial ecology, mycorrhizal fungi, Panama, plant–soil (below-ground) interactions, soil phosphorus

Introduction

Many lowland tropical rainforests are strongly phosphorus (P) depleted (Sollins 1998; Mirmanto *et al.* 1999; Vitousek *et al.* 2010; Wright *et al.* 2011) and they harbour some of the highest plant diversity on the planet (Wright 2002). Biotic forces, including plant-associated soil fungi, play a key role in promoting plant diversity (Klironomos 2002; Bell, Freckleton & Lewis 2006; Petermann *et al.* 2008; Mangan *et al.* 2010; Schnitzer & Klironomos 2011). Fungi interact with plant

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communities in mutualistic, pathogenic, and commensal ways (Rosenblueth & Martínez-Romero 2006; Smith & Read 2008b; Van Der Heijden, Bardgett & Van Straalen 2008; Dodds & Rathjen 2010). Fungi play critical roles in ecosystem processes including decomposition and nutrient cycling, and they have been hypothesized to play a critical role in the maintenance of plant diversity via pathogen-mediated negative density dependent survival (Janzen 1970; Connell 1971; Augspurger 1983; Clark & Clark 1984; Carson *et al.* 2008; Timothy Paine *et al.* 2008; Hol *et al.* 2013; Bagchi *et al.* 2014; Comita *et al.* 2014; but also see Hyatt *et al.* 2003) and plant-soil feedbacks (Bever 2002; Mangan *et al.* 2010; Philippot *et al.* 2013). Despite their recognized role in these processes, the relative importance of the abiotic and biotic drivers of soil fungal community structure and diversity in lowland tropical forests remains poorly understood.

Soil properties can determine soil fungal communities and host-microbe interactions (Lauber *et al.* 2008; Vinale *et al.* 2008). For example, arbuscular mycorrhizal (AM) fungi (subphylum Glomeromycotina) form associations with 80% of plant species worldwide (Wang & Qiu 2006; Brundrett 2009), and enhance the uptake of water, inorganic P, and other relatively immobile nutrients by effectively increasing root surface area and therefore increasing the volume of soil explored (Smith, Anderson & Smith 2015). The symbiosis between plants and AM fungi can be facultative and dependent on the plant's soil nutrient requirements and resource availability (Heijden & Kuyper 2001; Lambers *et al.* 2008; Dumbrell *et al.* 2009; Albornoz *et al.* 2016a). Similarly, P and other soil properties can affect non-AM fungal communities, such as ectomycorrhizal fungi (Albornoz *et al.* 2016b), saprophytic fungi (Kerekes *et al.* 2013), and pathogens (Toljander *et al.* 2006; Tedersoo *et al.* 2016). Nevertheless, fungi are only partially driven by soil properties, and other factors, such as biotic interactions, simultaneously structure fungal communities.

Plant communities can also affect their associated fungal communities (Johnson *et al.* 2004; Prescott & Grayston 2013) due to differences in fungal host specificity, defined as the taxonomic range of plant host species (Molina & Trappe 1982; Botnen *et al.* 2014). For example, obligate pathogens experience high selection pressure to infect hosts and reciprocal natural selection can occur between hosts and pathogens. This leads to an 'evolutionary arms race' between pathogen effectors and plant immunity genes, resulting in increased host specificity of pathogens (Jones & Dangl 2006; Dodds & Rathjen 2010; Brown & Tellier 2011). In contrast, AM fungi are thought to be generalists in forming host associations (i.e. a broad range of hosts; Davison *et al.* 2015) compared to other fungal groups such as saprophytes, pathogens, and endophytes (Tedersoo *et al.* 2010). Because of the host specificity that many fungal clades exhibit, they are thought to be strongly coupled with plant communities (Davey *et al.* 2015; Gao *et al.* 2015). However, the effects of plant community composition relative to soil chemistry in structuring fungal communities remains largely unknown, and studies that quantitatively determine the relative impact of each are scarce.

In this study, we aimed to disentangle the effects of soil chemistry and tree communities in structuring fungal communities by studying forests across a strong P gradient in Panama. Using massively parallel sequencing of a fungal metabarcode region, we evaluated fungal communities associated with three forest sites with contrasting soil chemistry and tree community richness and composition. We hypothesized that (i) the turnover of AM fungal operational taxonomic units (OTUs) among sites would be lower relative to non-AM fungi, (ii) AM fungal composition would more strongly correlate with soil properties than with local tree community composition while non-AM fungal composition would be more strongly correlated with local tree community composition than soil properties, and (iii) fungal OTU richness, and in particular non-AM fungi, would be negatively correlated with soil fertility and positively correlated with local tree diversity across soil cores and among sites.

Materials and methods

SITE DESCRIPTION

We chose three 1-ha mature secondary and old-growth forest sites located in Central Panama (Pyke *et al.* 2001) to examine the relative roles of soil nutrients and tree diversity on fungal community structure. Mean annual rainfall in these forests varies from c. 2482 to 3053 mm, and the sites are distinct in their tree species composition and richness, with an average of 12·2% species shared between the three forests (range = 4·8–17·9%) (Engelbrecht *et al.* 2007; Condit *et al.* 2013a). These sites were chosen to minimize differences in annual rainfall and maximize differences in soil P such that the chosen sites vary by two orders of magnitude in resin P concentrations (Table 1). These differences in soil P represent the relative range of variation that is found across a network of plots on the Isthmus of Panama (0·1–22·8 mg kg^{-1}) (Condit *et al.* 2013a). Within each site, all stems >10 cm diameter had previously been measured, mapped, tagged, and identified to species (Pyke *et al.* 2001; Engelbrecht *et al.* 2007). Soils have been described previously (Turner & Engelbrecht 2011; Condit *et al.* 2013a). Soils from Campo Chagres are Alfisols on limestone from the Alajuela formation, soils from Santa Rita Ridge are Oxisols on pre-Tertiary basalt, and soils from Pipeline Road are Oxisols on the Gatuncillo formation (i.e. marine sedimentary rock).

SITE SELECTION AND SAMPLE COLLECTION

Within each of the sites, soil cores were collected at 25 locations spaced 20 m apart in a grid formation. Samples were taken to a depth of 20 cm in increments of 10 cm using a soil corer with 9·25 cm diameter. Samples within each site were collected on the same day except for six samples at the Pipeline site that were collected 10 days after the rest. All samples were taken during the 2015 wet season between June 24 and July 22. Two adjacent cores were taken at each of the 25 coring locations, one for soil nutrient chemistry and one for fine root measurements and fungal community analyses. Soils for nutrient analyses were analyzed fresh the same day as collection and soil for fine root extraction were frozen for up to 15 days. We homogenized soil in each core and collected 2·5 mL of soil in microcentrifuge tubes, which were then frozen at −80 °C. From the same cores, we retrieved all fine roots (<3 mm diameter) by dry-sieving.

Table 1. Site characteristics including rainfall, parent material, soil order, soil nutrients, local tree community diversity (Shannon-Weaver index) and richness, and dry fine root biomass per litre of soil (mean ± 95% CI). Soil nutrient and fine root biomass measurements are for individual soil cores, tree community metrics are for 10-metre radii around each core, and mean rainfall estimates are from Engelbrecht *et al.* (2007). Letters show significant ($P \leq 0{\cdot}005$) differences among sites based on a *post hoc* Tukey test. Vegetation data was obtained from Pyke *et al.* (2001) and Engelbrecht *et al.* (2007)

	Site		
	Santa Rita Ridge	Pipeline	Campo Chagres
Annual rainfall (mm)	3053	2325	2482
Parental material	Pre-Tertiary basalt	Marine sedimentary	Calcareous sandstone
Soil order (Soil Taxonomy)	Oxisol	Oxisol	Alfisol
Soil moisture (g g^{-1} dry soil)	34·1 a	18·2 b	34·3 a
pH (H_2O)	4·50 ± 0·03 a	4·82 ± 0·04 b	6·46 ± 0·08 c
pH ($CaCl_2$)	4·02 ± 0·03 a	3·98 ± 0·03 a	6·42 ± 0·08 b
Mehlich-III P (mg kg^{-1})	0·82 ± 0·09 a	1·23 ± 0·06 b	21·62 ± 3·47 c
Resin P (mg kg^{-1})	0·17 ± 0·03 a	1·69 ± 0·19 b	16·34 ± 2·31 c
NH_4 (mg N kg^{-1})	0·81 ± 0·12 a	1·12 ± 0·17 a	2·27 ± 0·56 b
NO_3 (mg N kg^{-1})	0·50 ± 0·12 a	0·80 ± 0·16 a	3·87 ± 1·87 b
Ca (mg kg^{-1})	69 ± 11 a	193 ± 28 b	8969 ± 500 c
Cu (mg kg^{-1})	0·51 ± 0·11 a	0·90 ± 0·06 b	1·21 ± 0·13 b
Fe (mg kg^{-1})	113·37 ± 8·73 a	82·83 ± 5·08 b	119·19 ± 7·35 a
K (mg kg^{-1})	31·38 ± 1·91 a	28·46 ± 3·83 a	117·52 ± 21·47 b
Mg (mg kg^{-1})	31·95 ± 4·22 a	104·17 ± 9·23 b	446·78 ± 37·41 c
Mn (mg kg^{-1})	5·10 ± 1·87 a	24·438 ± 2·85 b	90·60 ± 8·74 c
Zn (mg kg^{-1})	0·73 ± 0·12 a	1·49 ± 0·15 b	4·67 ± 1·01 c
Vegetation properties			
Fine root biomass (g L^{-1})	1·90 ± 0·19 a	1·73 ± 0·19 ab	1·31 ± 0·12 b
Shannon diversity index	2·08 ± 0·06 a	1·9 ± 0·07 a	1·54 ± 0·09 b
Plant species richness	11·52 ± 0·63 a	8·8 ± 0·56 b	6·88 ± 0·49 c

Then, roots were washed to remove soil particles, oven-dried (50 °C for 48 h), and weighed. This was replicated at all three forest sites for a total of 75 coring locations. Tree community neighbourhood composition and Shannon diversity indices (hereafter termed 'local tree composition' and 'local tree diversity', respectively) were calculated from the summed diameter of stems for each species within a 10-m radius of each soil core location. A 10-m radius neighbourhood was chosen because previous work that examined tree neighbourhood and fungal community structure in a nearby forest plot on Barro Colorado Island showed that plant neighbourhoods at 10 m radii were more strongly correlated with fungal and bacterial community structure than neighbourhoods <10 m (Barberán *et al.* 2015). Tree community data was obtained from the most recent censuses for each site (Condit *et al.* 2013b).

SOIL NUTRIENT ANALYSES

Soil nutrients and pH were measured using procedures described previously (Turner & Romero 2009). Briefly, soil pH was determined in a 1 : 2 soil/water ratio with a glass electrode in water and 0·01 M $CaCl_2$. Resin P was determined by extraction with anion exchange membranes, while ammonium and nitrate were determined by extraction in 2 M KCl, with detection in both cases by automated colorimetry on a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO, USA). Extractable Al, base cations (Ca, K, Mg), P, and micronutrients (Cu, Fe, Mn, Zn) were determined by extraction in Mehlich-III solution and detection by inductively-coupled plasma optical-emission spectrometry (ICP–OES) on an Optima 7300 DV (Perkin Elmer, Inc., Shelton, CT, USA). Soil moisture was obtained by measuring the gravimetric water content of 1 g of soil, defined as the ratio of soil water mass to soil dry weight.

DNA EXTRACTION, AMPLIFICATION AND HIGH-THROUGHPUT SEQUENCING

We extracted DNA from 25 mg of soil per core using the PowerSoil DNA isolation kit (MO BIO Laboratories, Carlsbad, CA, USA), following the manufacturer's instructions. Four core locations were eliminated because soil for DNA extraction was not collected. PCR amplification of the ITS1 barcode region was performed with primers ITS1F (CTTGGTCATTTAGAGGAAGTAA; Gardes & Bruns 1993) and ITS2 (GCTGCGTTCTTCATCGATGC; White *et al.* 1990). Amplification was carried out using a mixture of 12·5 μL of KAPA HiFi HotStart Ready Mix (Kapa Biosystems, Wilmington, MA, USA), 1·25 μL of both forward and reverse primers at 10 nM concentration, and 2·5 μL of extracted DNA sample. Amplification was conducted on Bio-Rad T100 thermocyclers (Bio-Rad, Hercules, CA, USA) with the following settings: 95 °C for 5 m, and 30 cycles of 98 °C for 20 s, 65 °C for 15 s, 72 °C for 30 s, and finally 72 °C for 5 min. DNA extractions were additionally diluted (1/10) to test for the presence of amplification inhibitors. PCR products were then evaluated for quantity and quality via electrophoresis with 2% agarose (w/v) gel. Positive and negative controls were used to evaluate potential environmental contamination during DNA amplification. PCR product clean-up, ligation of Illumina Nexterra XT indices and adapters, and final library purification and pooling was conducted following manufacturers' protocols by the Center for Genome Research and Biocomputing at Oregon State University. Amplicons were sequenced using the Illumina MiSeq platform with 250-bp paired-end reads.

BIOINFORMATICS

Raw paired sequence reads were demultiplexed, trimmed to remove adapter and primer sequences, and filtered to remove reads that fell

below a mean quality score of 15 (99·9% accuracy) using a sliding window of 4 bp with Trimmomatic v.0·36 (Bolger, Lohse & Usadel 2014). One sample with fewer than 10 000 total reads was dropped from the analysis (Fig. S1a, Supporting Information). Paired reads were merged using Pandaseq (Masella *et al.* 2012). Sequences were then dereplicated at 100% identity across their full length and clustered at a 97% identity threshold using the UPARSE (Edgar 2013) algorithm implemented in USEARCH (Edgar 2013) to identify OTUs. Chimeras were removed prior to clustering and OTUs comprised of fewer than two sequences were filtered out. While choosing to remove singletons is somewhat arbitrary, several similar studies have used the same approach (Kerekes *et al.* 2013; Bálint *et al.* 2014; Barberán *et al.* 2015; Tedersoo *et al.* 2016; Malacrinò *et al.* 2017). After quality checks, an OTU table was created in USEARCH by identifying the number of sequences of each OTU in each core. Consensus sequences of each OTU were queried against the UNITE fungal database (Kõljalg *et al.* 2005; Abarenkov *et al.* 2010) at 75% similarity using USEARCH (Edgar 2013) and the single best match was retained.

FUNGAL COMMUNITY COMPOSITION

We used a Wilcoxon rank sum test to determine whether there were significant differences in the proportion of total reads among sites for each taxonomic group after false discovery rate correction, and we visualized the log fold change in reads among sites per taxon with metacodeR (Foster, Sharpton & Grunwald 2016; Methods S1). We examined differences in community composition of both AM and non-AM fungi among sites by calculating Jaccard dissimilarity among all cores and using non-metric multidimensional scaling (NMDS). To address our first hypothesis, we tested for differences in fungal community composition among sites using permutational multivariate analysis of variance (PERMANOVA). To assess our second hypothesis, we tested for a correlation between fungal communities and both tree community and soil chemistry among all cores. To do this, we performed two partial Mantel tests to examine: (i) the correlation between soil chemistry (i.e. pH and nutrient concentrations) and fungal composition while controlling for the partial effect of tree community composition, and (ii) the correlation between tree community composition and fungal composition controlling for the partial effect of soil chemistry.

FUNGAL RICHNESS

In order to evaluate differences in OTU richness between AM and non-AM fungi across all cores and sites, we first rarefied OTU richness based on the minimum number of sequences observed per core (AM: $N = 34$; non-AM: $N = 15{,}407$) and dropped three cores because they had fewer than four OTUs. We tested for differences in mean OTU richness among sites using generalized least square models. To test for correlations between OTU richness (AM and non-AM) and soil properties, local tree diversity, and fine root biomass, we used linear mixed effects models (Pinheiro & Bates 2000; Zuur *et al.* 2009) with site as a random effect. For all models, residuals were inspected visually for assumptions of normality and homoscedasticity. When assumptions were not met, variance structures that accounted for heteroscedasticity among sites and/or non-normal residual distributions were utilized in a second model and residuals were again inspected visually for violations (Methods S1). We performed model selection by fitting a full model with all explanatory variables and reduced models with *a priori* combinations of explanatory variables of biological significance, including soil nutrients, pH and moisture, local tree diversity, and fine root biomass (Methods S1; Table S1). Models were compared using Akaike information criterion and likelihood ratio tests (Zuur *et al.* 2009; Table S1; Table S2). All statistical analyses were conducted and figures were produced using the R statistical software package (R Core Team 2016). Methods S1 lists all functions and packages used in this study.

Results

TREE AND SOIL COMPOSITION

Soil chemistry differed greatly among sites (PERMANOVA, $P \leq 0{\cdot}003$; Fig. 2d). Specifically, soil pH at the Santa Rita Ridge and Pipeline sites was 1·96 and 1·64 units less than at Campo Chagres site, respectively (Table 1; Tukey *post hoc* tests, $P \leq 0{\cdot}01$). Resin P differed by two orders of magnitude among sites, with Campo Chagres showing the highest concentrations (c. 16 mg kg^{-1}) and Santa Rita Ridge the lowest (c. 0·17 mg kg^{-1}) (Table 1; Tukey *post hoc* test, $P \leq 0{\cdot}01$). Similarly, Ca, Mg, Mn, and Zn significantly increased from the lowest (Santa Rita Ridge) to the highest (Campo Chagres) (Table 1; Tukey *post hoc* test, $P \leq 0{\cdot}01$). Nitrogen and K did not differ between the Santa Rita Ridge and Pipeline sites, but did differ between Campo Chagres and the other two sites (Table 1; Tukey *post hoc* test, $P \leq 0{\cdot}05$). Lastly, gravimetric soil moisture was 53% lower at Pipeline relative to the Campo Chagres and Santa Rita Ridge sites, and the latter two were not significantly different from each other (Table 1; Tukey *post hoc* test, $P \leq 0{\cdot}05$).

Local tree composition was different among sites (PERMANOVA, $P \leq 0{\cdot}003$; Fig. 2c). Furthermore, local tree diversity was c. 35% and 23% greater at the Santa Rita Ridge and Pipeline sites compared to Campo Chagres, respectively (Table 1; Tukey *post hoc* test, $P \leq 0{\cdot}01$). Local tree species richness was 40% and 22% greater at Santa Rita Ridge and Pipeline compared to Campo Chagres (Table 1; Tukey *post hoc* test, $P \leq 0{\cdot}01$). Finally, fine root biomass was greatest at Santa Rita Ridge and lowest at Campo Chagres (Table 1; Tukey *post hoc* test, $P \leq 0{\cdot}01$).

FUNGAL OTU COMPOSITION

We obtained 4,245,975 fungal sequences from 70 soil cores, comprising 2,853 unique fungal OTUs. Within soil cores, we recovered 444 unique OTUs at Campo Chagres, 292 at Pipeline, and 150 at Santa Rita Ridge. Overall, Ascomycota was the most abundant fungal phylum across all sites with 1,598 OTUs, while Chytridiomycota was the least abundant with 21 OTUs (Fig. 1). There were 37 AM OTUs (1·6% of total OTUs) across all sites and only two cores out of 70 contained unique AM fungal OTUs (i.e. rare OTUs not found in other cores), both found at the Campo Chagres site. Additionally, among sites, there was little change in mean rarified OTU richness of AM fungi (range = 1–5·24), but a large difference of non-AM fungi (range = 162·4–563·6). Similarly, the sequence abundance of AM fungi did not vary among sites while several Ascomycota

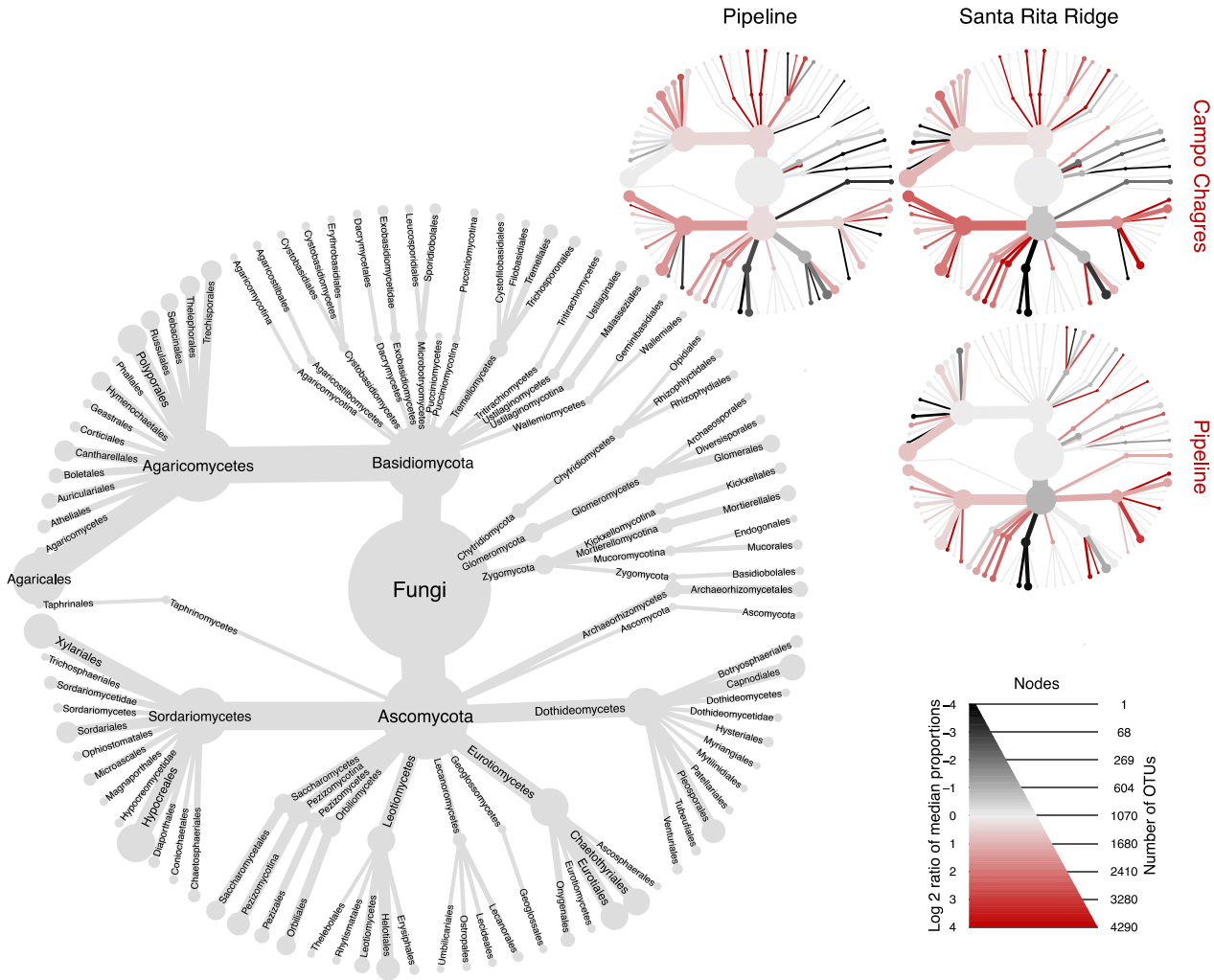

Fig. 1. Bottom left: Taxonomic tree of fungal taxa found across all cores. Node size indicates number of sequences belonging to each taxon. Upper right: Matrix of taxonomic trees comparing abundance of fungal taxa between pairs of sites. Black or red indicates that the proportion of the median number of sequences clustered to each taxa is different than 1 based on a Wilcoxon rank test ($P \leq 0{\cdot}05$) with a Holm multiple comparison correction. Colour intensity represents the proportion of median sequences for each taxa for the sites being compared. Red indicates a significantly higher proportion of sequences found at the site shown in red letters, black indicates a higher proportion of reads for the site shown in black letters. [Colour figure can be viewed at wileyonlinelibrary.com]

and Basidiomycota (non-AM) clades differed greatly among sites (Fig. 1). For example, the number of sequences from the Eurotiales clade, in the Ascomycota phylum, was about three times greater at the Santa Rita Ridge site compared to the Campo Chagres and Pipeline sites (Fig. 1). From the Basidiomycota phylum, sequences from Thelephorales were roughly 2·7 times more abundant at the Campo Chagres site than at the Pipeline site (Fig. 1).

Among all sites, AM fungal community composition was similar (PERMANOVA, $P = 0{\cdot}26$; Fig. 2a), while non-AM fungal communities differed in OTU composition (PERMANOVA, $P \leq 0{\cdot}0003$; Fig. 2b). When controlling for differences in soil chemistry, community composition of AM fungi was not correlated with local tree composition across all cores and sites (Partial Mantel, $r = -0{\cdot}02$; $P = 0{\cdot}70$; Table 2). However, non-AM fungal community composition was correlated with local tree composition, but only when all cores were pooled into a single analysis (Partial Mantel $r = 0{\cdot}36$; $P \leq 0{\cdot}001$; Table 2). When controlling for the effect of local tree community composition in the partial Mantel tests, soil nutrient chemistry was strongly correlated with both AM and non-AM fungi, again only when considering all sites together (Table 2; AM: partial Mantel $r = 0{\cdot}32$; $P \leq 0{\cdot}001$; Non-AM: partial Mantel $r = 0{\cdot}38$; $P \leq 0{\cdot}001$).

FUNGAL OTU RICHNESS

Variance partitioning analyses showed that differences among cores explained 94% of total variation in AM fungal richness (Table S3). In contrast, site and core explained 38% and 55% of total variation of non-AM fungal richness, respectively (Table S3). Arbuscular mycorrhizal OTU richness did not vary among sites (*t*-test, $P = 0{\cdot}40$), but non-AM richness did, with the Santa Rita Ridge site having c. 55% fewer OTUs than the other two sites (*t*-test, $P \leq 0{\cdot}0001$). For AM fungi. the model selection analysis showed that the best supported

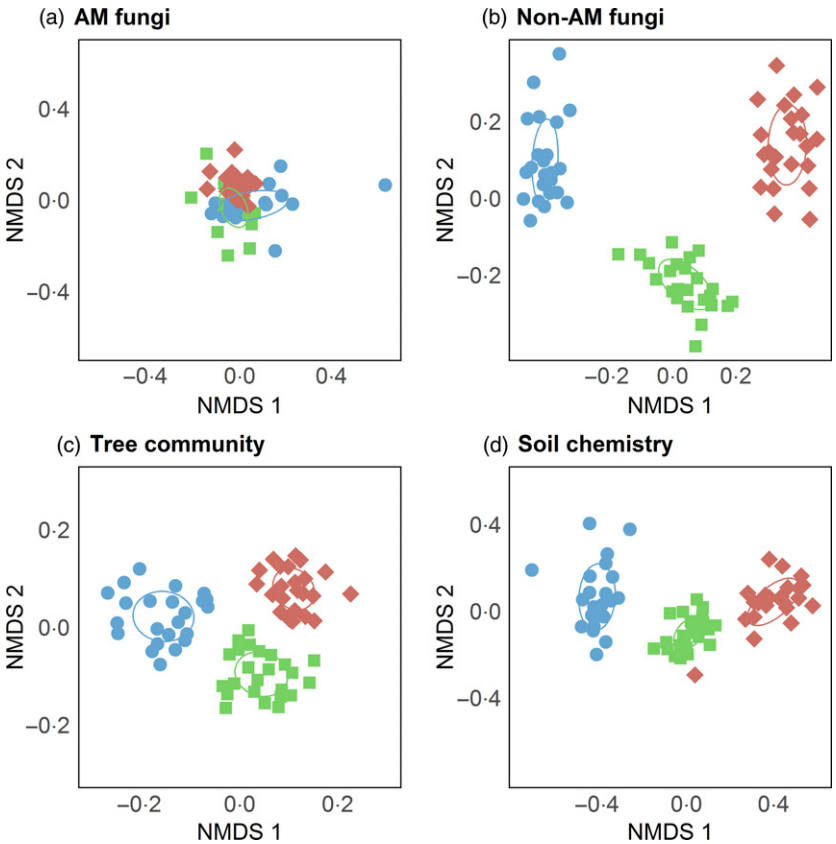

Fig. 2. Nonmetric multidimensional scaling ordination plot showing differences in community composition of (a) arbuscular mycorrhizal (AM) fungi, (b) non-AM fungi, (c) tree community, and (d) soil chemistry across three contrasting sites (red diamond – Santa Rita Ridge; green square – Pipeline; blue circle – Campo Chagres). Ellipses represent 95% confidence area around the mean centroid for each site using Jaccard dissimilarity values. Statistical differences ($P \leq 0{\cdot}05$) in composition were determined using PERMANOVA and pairwise comparisons with Holm correction (Holm 1979). [Colour figure can be viewed at wileyonlinelibrary.com]

Table 2. Partial Mantel test correlations of arbuscular mycorrhizal (AM) and non-AM fungi with tree community and soil chemistry at three forest sites in Central Panama. Differences in community composition were estimated using Jaccard distances. Statistics correspond to partial Mantel tests controlling for differences in soil chemistry or tree community, respectively. Bold and asterisks are significant correlations ($P \leq 0{\cdot}001$)

	Tree community		Soil chemistry	
	Correlation	*P*-value	Correlation	*P*-value
AM fungi				
Santa Rita Ridge	−0·08	0·75	0·04	0·40
Pipeline	−0·11	0·92	0·1	0·16
Campo Chagres	0·20	0·29	−0·03	0·60
All sites	−0·02	0·70	**0·32**	**0·001***
Non-AM fungi				
Santa Rita Ridge	−0·03	0·60	0·08	0·28
Pipeline	0·07	0·20	0·19	0·06
Campo Chagres	0·06	0·31	−0·002	0·5
All sites	**0·36**	**0·001***	**0·38**	**0·001***

model contained none of the explanatory variables (Tables 3 and S2). Finally, the best supported model showed that non-AM richness increased with increasing soil pH, resin P, and moisture among all soil cores (Table 3).

Discussion

Our results provide support for our first hypothesis that AM fungal communities show low turnover and variation among sites and non-AM fungi show greater differences among sites. We found that AM fungal communities showed high overlap in species composition among sites despite marked variation in vegetation composition and soil chemistry, including P availability. Non-AM fungal community structure differed strongly among sites. Our results agree with previous studies that have found most AM fungi to be generalists (Öpik *et al.* 2006; Davison *et al.* 2015) while other groups like Ascomycota and Basidiomycota show some degree of host specificity (Ferrer & Gilbert 2003; Tedersoo *et al.* 2010). Our second hypothesis, that AM fungi would be better explained by abiotic soil chemistry and non-AM better explained by local biotic variables, was partially supported by the results of the partial Mantel tests. These tests showed that variation in AM fungi among cores was correlated with soil chemistry but not local tree neighbourhoods. The partial Mantel test revealed that non-AM fungal communities were roughly equally correlated with soil chemistry and local tree community composition. Finally, our results do not support our third hypothesis that fungal OTU richness within soil cores was negatively correlated with soil P and positively correlated with tree diversity. Instead, we found no relationship between AM richness and any biotic or abiotic variable. We did find a positive relationship between non-AM richness and resin P, soil pH, and soil moisture, which was contrary to our initial hypothesis. Overall, our study suggests that AM fungal community structure varies at fine spatial scales and is generally correlated with soil properties. For other fungi, soil properties and the local tree community play equivalent roles in structuring their communities.

Table 3. Summary statistics for two linear mixed effects models examining the relationship of rarefied OTU richness of (i) arbuscular mycorrhizal (AM) fungi and (ii) non-AM fungi with pH, log of resin Phosphorus (kg ha^{-1}), local tree diversity (Shannon index), log of dry fine root biomass (g) and gravimetric soil moisture content (g). Site was used as a random effect. Pseudo *r*-squared value is for the entire model. Bold denotes a statistically significant relationship ($P \leq 0{\cdot}05$)

Response variable	Pseudo *r*-squared	Explanatory variable	Estimate	SE	d.f.	*t*-value	*P*-value
Rarefied AM OTU richness	0	Intercept	2·87	0·13	62	22·34	<0·001
Rarefied non-AM OTU richness	0·81	**Log of resin P**	**26·41**	**6·00**	**64**	**4·40**	**<0·001**
		pH	**96·49**	**28·12**	**64**	**3·43**	**<0·001**
		Gravimetric soil moisture	**4·50**	**1·49**	**64**	**3·02**	**<0·004**

FUNGAL OTU COMPOSITION

There was no correlation between the composition of AM fungi and local tree community composition when accounting for soil chemistry. Consistent with our results, previous studies have found that AM fungi are widely distributed with 90% of OTUs found on multiple continents (Öpik *et al.* 2006; Davison *et al.* 2015). Other studies have found that AM fungal communities can be determined by plant community composition, but these were either conducted within artificial mesocosms in temperate grasslands (Burrows & Pfleger 2002; Johnson *et al.* 2004), or with a small subset of plant species (Vandenkoornhuyse *et al.* 2002; Mummey, Rillig & Holben 2005). While AM composition was not correlated with local tree composition, it was correlated with soil properties. Previous studies have shown that AM fungal communities can be influenced by soil properties (Lekberg *et al.* 2007; Sheldrake *et al.* 2017). For example, soil pH (Hazard *et al.* 2013; Rodríguez-Echeverría *et al.* 2016) and P (Johnson *et al.* 2010; Krüger *et al.* 2015) can be strong abiotic drivers of AM fungal and bacterial communities (Fierer & Jackson 2006). Therefore, given their wide geographic distribution (Öpik *et al.* 2006; Davison *et al.* 2015) and low host specificity (Morton & Benny 1990; Fitter & Moyersoen 1996; Klironomos 2000; but see Husband *et al.* 2002; Smith & Read 2008a), it is likely that AM fungal communities in the forests we studied are more strongly driven by soil properties rather than host-symbiont interactions.

We expected to find a stronger correlation between non-AM fungi and local tree community composition among sites relative to soil properties. Instead, we found that their composition was almost equally explained by soil chemistry and local tree composition. The non-AM fungi in this study were comprised of taxa with a large variety of ecological functions, including saprophytes, pathogens and symbionts, and there was marked turnover among sites in many clades (Fig. 1). While we cannot infer host specificity for these fungal guilds from OTUs sequenced from ambient soil, we found that non-AM fungal composition co-varied and was in part explained by local tree composition, suggesting some degree of host-specific plant-fungal interactions in these forests. Kivlin & Hawkes (2016) found that community composition of fungi in the phyla Ascomycota, Basidiomycota and Chytridiomycota differed among experimental monoculture plots of different tropical tree species. Furthermore, there is evidence for narrow host specificity in wood decomposing fungi (Ferrer & Gilbert 2003), leaf endophytes (Peršoh 2013), seedling pathogens (Gilbert & Webb 2007), seed pathogens (Beckman & Muller-Landau 2011), and in general for plant pathogens via reciprocal selection (Brown & Tellier 2011; Marone *et al.* 2013). Conversely, many tropical fungi have been shown to have broad host ranges (Lindblad 2000; Rodriguez *et al.* 2009). However, the Mantel tests show that soil chemistry explained an equal amount of variation in non-AM fungal community composition as the local tree community around the soil core. Many saprophytic and ectomycorrhizal fungi respond to soil chemistry, including N availability (Peter, Ayer & Egli 2001) and soil pH (Smith, Anderson & Smith 2015). Hence, both soil properties and tree diversity simultaneously drive non-AM fungal communities, and the relative effect of each might differ among fungal guilds.

Plant communities and soil properties are often strongly correlated (Peltzer *et al.* 2010; Condit *et al.* 2013a; Zemunik *et al.* 2016), which can obscure the relative effect of each on fungal communities. Here, we found that local tree community composition and soil chemistry were strongly correlated (Partial Mantel test, $r = 0{\cdot}62$; $P \leq 0{\cdot}001$; Table S2). Strong relationships between plant and fungal communities could be misleading when the partial effects of soil nutrients are not accounted for. For example, Peay, Baraloto & Fine (2013) found a strong relationship between fungal and plant communities in the Amazon basin. In that study, the authors acknowledge the strong correlation of both fungal and tree community composition with soil properties, but did not examine the relative effects of each. When we performed a Mantel test without controlling for the partial effect of soil chemistry, we also found a correlation between AM fungal community composition and local tree composition ($r = 0{\cdot}22$; $P \leq 0{\cdot}001$; Table S2) that was stronger than when soil chemistry was partially controlled. Because plant composition and soil nutrients often co-vary (Peltzer *et al.* 2010; Condit *et al.* 2013a; Zemunik *et al.* 2016) methods that explicitly account for the effects of both are required to determine the potential drivers of soil fungal community composition. While our analyses were able to correct for the effects of soil properties and tree community composition statistically, work that experimentally isolates the individual effects of each on fungal composition would contribute to our understanding of the causative mechanisms in this tripartite relationship.

FUNGAL OTU RICHNESS

There was no evidence that AM fungal richness was associated with any soil property or local tree diversity. This lack of association with soil properties contradicts several studies that have shown the strong effects of pH (Verbruggen *et al.* 2012; Brundrett & Ashwath 2013; Wardle & Lindahl 2014; Carrino-Kyker *et al.* 2016) and other soil properties (Camenzind *et al.* 2014, 2016; Sheldrake *et al.* 2017) on AM communities. The lack of relationship between any soil property and AM richness is somewhat unexpected but consistent with the high species overlap observed among cores. The low variation of AM richness among cores may be a consequence of the functional redundancy of AM fungi (Gosling, Jones & Bending 2016). For example, Kivlin & Hawkes (2016) found that vegetation communities had no effect on species richness of AM fungi or any individual fungal clade across tropical secondary forest plots in Costa Rica.

We found evidence that non-AM OTU richness was associated with a number of abiotic independent variables but not with local tree diversity. Soil pH, resin P and soil moisture all showed a positive relationship with non-AM richness, suggesting that soil properties impose abiotic filtering on fungi such that relatively few taxa are able to survive on acidic soils with low P and moisture (Koide, Fernandez & Petprakob 2011; Kivlin *et al.* 2014). Several studies have shown how abiotic factors can affect fungal richness (McGuire *et al.* 2012; Kerekes *et al.* 2013; Albornoz *et al.* 2016b). McGuire *et al.* (2012) studied the effects of annual precipitation, soil nutrients and plant diversity in structuring fungal communities in Panamanian forests close to the ones studied here and found that only mean annual rainfall was positively correlated with fungal richness. Here, we examined within-site variation in fungal communities, soil chemistry, and tree neighbourhoods at the scale of 20 m. McGuire *et al.* (2012) pooled their soil samples to determine a single site value, which could explain why we were able to detect a significant correlation of non-AM richness with pH and resin P, as well as soil moisture. With respect to tree diversity, previous studies have also found no relationship with non-AM richness (McGuire *et al.* 2012; Kivlin & Hawkes 2016; but see Peay, Baraloto & Fine 2013). Hence, the relationship between tree and fungal richness is not clear, and other factors, such as soil properties are likely to be the main determinants of fungal richness. Our results indicate that more diverse tree communities may not harbour more fungal species *per se*, but rather abiotic factors likely drive non-AM fungal diversity through environmental filtering.

There were a number of limitations in our study. First, previous studies have shown that timing of sampling can have significant effects on microbial community composition (Koranda *et al.* 2013; Voříšková & Baldrian 2013). We collected soil samples during the wet season over a 1-month period and data from nearby plots in Panama show that the temporal variation in nutrients such as pH, N, P and Ca within sites is less than variation among sites (Turner *et al.* 2013). In addition, microbial biomass varies relatively little during the wet season, with the main changes occurring during the dry season when microbial biomass and enzyme activities decline (Turner & Wright 2014). Another limitation is the potential for primer bias against rare species of AM fungi. However, we found a similar number of AM OTUs (37) compared to other studies conducted in Panamanian forests (50 AM OTUs; Kerekes *et al.* 2013), including one that used AM-specific primers (30 AM RFLP phylotypes; Husband *et al.* 2002). We note that caution should be taken when interpreting molecular data from ambient soil as only a subset of the inoculum pool actively colonizes plant roots at any given time (Herre *et al.* 2005; Hempel, Renker & Buscot 2007). The tree community data from these forests includes only trees with DBH larger than 10 cm, excluding smaller trees and understory species in the census data. However, large trees possess more extensive root systems and can affect greater soil volume than smaller plants. Furthermore, we note that a previous study in a similar forest found that plant communities at distances >10 m radius from a soil core better predict fungal and bacterial communities than smaller plant neighbourhoods that, by their nature, have fewer large trees (Barberán *et al.* 2015). Finally, it is likely that we found weak correlations between fungal communities and both soil properties and tree communities when each site was analyzed separately (N = 23, 23, and 24 for Santa Rita Ridge, Chagres, and Pipeline, respectively) due to less statistical power than when all sites were combined (N = 70).

In conclusion, our results suggest that the composition of AM fungi varies at fine-spatial scales and is driven primarily by soil properties, while both soil properties and tree communities jointly structure non-AM fungal communities in lowland tropical forests of Panama. Together with results from McGuire *et al.* (2012), we show that environmental drivers such as rainfall and soil properties can partly determine fungal community composition and richness, but that the mechanistic functional relationship between tree and fungal diversity remains less clear. Condit *et al.* (2013a) showed a strong effect of moisture and soil chemistry, in particular P, in determining tree species distribution and abundance across these forests. Thus, it appears that underlying biogeochemical processes act as an environmental filter for both tree and fungal species and set the stage for biotic interactions between the two. Future work could experimentally examine how soil chemistry may jointly limit tree and fungal species distributions and modulate the host-symbiont interactions that have long been hypothesized to play a critical role in the maintenance of plant species diversity in tropical forests.

Authors' contributions

T.S., A.J. and B.T. conceived the study and designed methodology; T.S., F.A., A.N., R.C. and B.T. collected the data; T.S. and F.A. analyzed the data; T.S. and F.A. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Conflict of interest

The authors of this study declare they have no conflict of interest.

Data accessibility

Sequencing data are available in GenBank (accession number: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA363090/). Tree neighbourhood, OTU table, root biomass, and soil property data are available in Dryad Digital Repository: https://doi.org/10.5061/dryad.sc38s (Schappe *et al*. 2017).

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Supporting Information

Details of electronic Supporting Information are provided below.

Methods S1. List of packages for R statistical software used for data analysis.

Table S1. Summary of models and metrics used for model selection procedure.

Table S2. Mantel test correlations of soil properties and arbuscular mycorrhizal (AM) with tree communities.

Fig. S1. Fungal OTU accumulation curves of each core for all sites.