Plant host and soil origin influence fungal and bacterial assemblages in the roots of woody plants

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Abstract

Microbial communities in plant roots provide critical links between above and belowground processes in terrestrial ecosystems. Variation in root communities has been attributed to plant host effects and microbial host preferences, as well as to factors pertaining to soil conditions, microbial biogeography and the presence of viable microbial propagules. To address hypotheses regarding the influence of plant host and soil biogeography on root fungal and bacterial communities we designed a trap-plant bioassay experiment. Replicate *Populus*, *Quercus*, and *Pinus* plants were grown in three soils originating from alternate field sites. Fungal and bacterial community profiles in the root of each replicate were assessed through multiplex 454 amplicon sequencing of 4 loci (i.e. 16S, SSU, ITS, LSU rDNA). Soil origin had a larger effect on fungal community composition than did host species, but the opposite was true for bacterial communities. *Populus* hosted the highest diversity of rhizospheric fungi and bacteria. Root communities on *Quercus* and *Pinus* were more similar to each other than to *Populus*. Overall, fungal root symbionts appear to be more constrained by dispersal and biogeography than by host availability.

Keywords: *Populus*, *Pinus*, *Quercus,* fungal communities, bacterial communities, phylotyping, 454 pyrosequencing, Glomeromycota, root endophytes

I. Introduction

Woody plants are major drivers in the carbon cycling of terrestrial ecosystems. While a large proportion of the carbon fixed by woody plants is transported belowground to root and soil systems (Zak *et al.*, 1993; Högberg *et al.*, 2001) biological linkages between soils, roots and the atmosphere are poorly characterized. Rhizospheric bacteria and fungi connect plants and soils, and together provide ecosystem services such as nutrient cycling and soil fertility (Kaiser *et al.*, 2010). Identifying factors influencing belowground diversity and community structure of plant-associated fungi and bacteria should

therefore be of broad interest to the management of terrestrial ecosystems. Current advances in DNA sequencing technology allows for an unprecedented ability to study plant-associated and soil microbial diversity and to assess the implication of this diversity on ecosystem functioning (Fierer *et al.*, 2012; Smith & Peay, 2014). Using high-throughput multiplex amplicon sequencing we assess here the influence of plant host species and soil origin on the assemblage of fungal and bacterial communities on roots of *Quercus*, *Pinus* and *Populus* seedlings.

Quercus and *Pinus* are EcM hosts that are widely distributed across the Northern hemisphere. These genera have been used as models to understanding EcM symbiosis and communities for well over a century (Melin & Nilsson, 1953; Newton, 1991; Frank, 2005; Jumpponen *et al.*, 2010; Talbot *et al.*, 2014). Species in these genera readily form and are dependent upon EcM roots for healthy growth. While they also are known to associate with bacterial and fungal root endophytes, the diversity and function of these root endophytes on plant health and growth has only been studied for a few selected taxa (Jumpponen & Trappe, 1998; Tedersoo *et al.*, 2009).

Populus deltoides is a woody plant species that is ubiquitous and widespread in central and eastern North America. Its native range extends from southeastern US, north to southern Canada, and across the plains to the eastern slope of the Rocky Mountains (Braatne *et al.*, 2006). This species is adapted to riparian habitats and depends on flooding events for seedling recruitment. In riparian habitats dominated by *P. deltoides,* the plant community often consists of various arbuscular mycorrhizal (AM) and ectomycorrhizal (EcM) hosts such as *Liriodendron*, *Acer*, *Platanus* and *Quercus*, *Pinus*, *Carya, Salix*, respectively.

One unique aspect of *Populus* is its ability to associate with both EcM and AM fungi (Vozzo & Hacskaylo, 1974; Karliński *et al.*, 2010). Although not fully understood, environmental conditions appear to modulate the ratio of AM:EcM formation in *Populus* (Lodge, 1989; Gehring *et al.*, 2006). Arbuscular mycorrhizal associates of *Populus* have been characterized and quantified in numerous studies (Vozzo & Hacskaylo, 1974; Walker, 1979; Beauchamp *et al.*, 2006; Obase *et al.*, 2008; Karliński *et al.*, 2010; Yang

et al., 2013) and others have reported on physiological benefits to *Populus* plants that are colonized by AM fungi (Castiglione *et al.,* 2009; Barzana *et al.,* 2012; Cicatelli *et al.,* 2012). In all of these studies AM colonization of *Populus* spp. appears to be variable and not particularly high (~ 4-25%). *Populus deltoides* is also known to produce ectomycorrhizas, however, the EcM associates of *P. deltoides* are still not well characterized and fruitbodies and root tips of of EcM fungi are not very common in *P. deltoides* dominated habitats (Jean Lodge, *personal communication;* Vilgalys and Bonito, *unpublished data*). In contrast to *P. deltoides*, other species and hybrids of *Populus* are known to associate more readily with EcM fungi, including *P. alba* x *P. grandidentata* (Stefani *et al.,* 2009) and *P. tremuloides* (Cripps & Miller, 1993; Cripps, 1997; Cripps, 2001). These observations suggest that the ectomycorrhizal habit of *Populus* may be habitat specific or may differ among *Populus* species.

High-throughput sequencing has been used to show that bacterial and fungal root endophytes of *Populus* are diverse and differ between habitats. For instance, Gottel et al. (2011) and Shakya et al. (2013) revealed differences in the community structure between bacterial and fungal root and root-endophyte communities of *P. deltoides* in upland and lowland (riparian) habitats. They found that the ecological niche (endosphere vs. root) trumped other measured factors (soil properties, season, plant genotype, etc) (upland vs. lowland) in shaping microbial communities. A dominance of endophytic fungi was found in *P. deltoides*, and several EcM species including *Cortinarius* and *Tuber* were routinely detected in *P. deltoides* roots. A high abundance of *Acidobacteria* in rhizosphere soils versus *Gamma-proteobacteria* and *Actinobacteria* in *P. deltoides* root endospheres was also reported. However, within each habitat soil factors, seasonal and geographic variation in both the fungal and bacterial communities associated with *P. deltoides* was found to best account for community level patterns (Shakya *et al.,* 2013).

Geography poses natural barriers to microbial dispersal and distribution and leads to spatial patterns where beta-diversity decays with distance (Bahram *et al.,* 2013; Talbot *et al.,* 2014). Host preferences have also been reported for some EcM plant and fungal species (Jumpponen *et al.,* 2004; Ishida *et al.,* 2007; Tedersoo *et al.,* 2008; Tedersoo *et al.,* 2013) and the chemical composition of root exudates and deposition is known to differ between plant species (Hartmann *et al.,* 2009). However, decoupling effects of soil

origin, edaphic qualities and host species has been a challenge in field-based studies, especially prior to the onset of molecular ecology methods that are scalable to allow for suitable levels of replication. Furthermore, while studies on mycorrhizal fungal taxa are plentiful, little is known about host preferences of non-pathogenic bacteria and non-mycorrhizal plant-associated fungi, which are ubiquitous on and within roots. To address these issues we use a trap-plant experiment and multiplex 454 amplicon sequencing to test the hypotheses that plant host and soil biogeography are major factors in the structuring fungal and bacterial communities in plant roots.

II. Methods

Experimental design

To discern effects of host species and soil origin on plant root microbiomes we used a trap-plant bioassay. In this approach field soils from alternate geographic areas (below) were collected and homogenized via mechanical mixing. Axenically raised host seedlings were then planted into the soils and grown as "bait" for compatible microbiota present in the target soils. Fungi and bacteria compatible with the host species are expected to associate with the host, persist and grow to the point of being detectible by DNA sequencing.

Three field soils from natural riparian habitats were used in this study and correspond to a subset of those described by Shakya et al. (2013). Soil and site properties for these sites were presented by Shakya et al (2013) and are summarized here and in Table S1. Two of the soils (NC2, NC3) were collected 17-107m away from the edge of the Yadkin River, in North Carolina (NC), USA in a mixed-species riparian forests in which *Populus* accounted for 12.5-38.5% of the tree basal area. The third soil (TN) was collected 31m from the edge of the Caney Fork River in Tennessee (TN), USA in a mixed riparian forest in which *Populus* accounted for 22.2% of the forest tree basal area. This soil corresponds to the bottomland site studied by Gottel et al. (2011), characterized by a texture of 53% sand and a pH of 6.1. Both NC soils had a texture of at least 50% silt and a pH of 5.2 but nutrient analyses show that the NC2 soil had elevated levels of C (33%), N (0.62%), P (171 ppm), K (384 ppm) and micronutrients (Ca, Mg, Zn). In our experiment, four replicates of each combination of host genus (3) and soil type (3) were

grown and harvested for a total of 36 plants. We used three plant species which co-occur naturally: *Quercus phellos* (willow oak), *Pinus taeda* (loblolly pine) and *Populus deltoides* (eastern cottonwood).

Soil collection

An auger core and spade shovel were used to collect samples of approximately 10 L of soil to a depth of 15 cm from the field sites in October 2009 (TN soil) and April 2010 (NC soils). Soils were air-dried in paper bags for 1-week then sieved through a 2-mm mesh to homogenize and remove roots. Because of a time lag between soil collections, air-dried soils from the TN site were stored at -80°C prior to setting up this experiment. Physical and chemical soil characteristics were determined by the University of Georgia's Agricultural and Environmental Services Laboratory (AESL) (http://aesl.ces.uga.edu/) on composited soil samples from three cores at each site (see Table S1).

Soils were mixed to a concentration of 30% by volume into a potting mixture before planting. The potting mixture contained vermiculite, perlite, peat and sand at a ratio of 2:2:1:1, and was autoclaved twice over a two-day period before mixing with field soil. Axenically raised plants (below) were planted in mixed soils into Cone-tainers (Stuewe and Sons, Corvallis, OR). A layer of sterile sand (1-cm) was spread across the surface of each planting to prevent cross-contamination of soils between pots.

Planting seedings and cuttings

Populus deltoides cuttings (genotypes D110, D112, D117 and the *P.deltoides* x *P.trichocarpa* hybrid 93-968) were stored at 4°C in double ziplock bags until use. Cuttings were washed in 0.1% solution of Tween 20 to remove any adhering particles, surface sterilized by submerging in 6% H_2O_2 for 10 minutes, then rinsed three times with tap water. While cuttings were not specifically screened for endophytes prior to this experiment, follow-up studies indicate that residual stem endophytes are readily replaced by soil microbiota once planted in soil (Bonito *unpublished data*). Prior to

planting the bottom half of each cutting was soaked in tap water for 24 hrs in darkness to initiate rooting. Cuttings were then planted into the various soil treatments.

Seedlings originated from *Quercus phellos* (willow oak) acorns collected locally (Durham NC) from trees before seed drop and wildtype *Pinus taeda* (loblolly pine) seeds provided by the NC State University's Forest Improvement Center. Seeds were surface sterilized by soaking in 6% H_2O_2 for 10 minutes and stratified by soaking overnight in tap water, removing water and placing seeds in sterile zip-lock bags in a dark refrigerator ($4^{o}C$) for 90 days. Prior to germination seeds were surface sterilized again and planted in autoclaved perlite. Germlings were grown under 200 µmols/m^2/s of light for approximately three weeks before inoculation.

Growth conditions, measurements and sample preparations

All experimental plant propagules were grown in the Duke Phytotron under uniform conditions of 18h days with light levels of 400 µmol/m^2/s and 6h nights. Day temperatures were $21.1^{o}C$ with humidity of 60%, while night temperatures were $15.6^{o}C$ with 85% humidity. Plant materials were watered daily and removed from the growth chamber on a monthly basis to remove fallen leaf material and racks were reordered to reduce block effects. Plants were grown between 118-165 days. After this period plant health and height (growth during experiment) was measured and plants were harvested. Plant height was divided by the number of days in the growth chamber to obtain a growth rate (cm/day).

To harvest *Populus* roots a retractable razor blade was used to cut and remove a vertical section of nearly half of the root system, rinsing the razor in ethanol and flaming between samples. Because the root systems of oaks and pines were not as extensive as those of *Populus,* the complete root system from these hosts were harvested. Sampled roots were placed in ziplock bags and washed multiple times with a 0.1% solution of Tween 20 to remove fine soil particles. Roots were then rinsed under a fine spray of tap water to remove adhered particles and were observed under a stereoscope for assessment of EcM abundance. Ectomycorrhizal colonization was scored qualitatively

into the three categories (low = 0-33%; medium = 34-66%; high = 67-100%) by visually scanning their complete root system.

DNA extractions and fungal and bacterial PCR amplification

Harvested root samples were freeze-dried and ground to a powder with a bead beater and DNA was extracted from 500 mg ground roots of each plant using CTAB method (Bruns & Gardes, 1993). A second sodium acetate precipitation helped improve the DNA quality. DNA pellets were eluted in 100 μl of 1X TE buffer and 1:3 dilutions were used for PCR.

We conducted 454 sequencing using FLX titanium chemistry and a Roche genome sequencer (Indianapolis, IN). Four loci were amplified from each samples: 16S rDNA (V4 region: 553F -1185mR) to target bacteria (and exclude plastid and mitochondrial organelles) (Hodkinson & Lutzoni, 2009; Hodkinson *et al.*, 2012); ITS rDNA (ITS1f-ITS4) to target fungi; 28S LSU rDNA (LROR-LR3) as a second fungal marker and 18S SSU rDNA (AML1-AMrev1) to target Glomeromycota (Lee *et al.*, 2008). For SSU amplification, a new reverse primer (AMrev1_GB 5'-GAA TAC TAA TGC CCC CAA CTA-3') was designed to shorten the amplicon length so that it was more compatible with 454 sequencing. Primer pairs and their sequences were modified to contain the 454 A and B primer, with one of 96 10-bp DNA barcodes on the A primer (see Table S2 for complete primer sequence information).

PCRs were carried out in 25 ul reactions and included 1 x High Fidelity PCR buffer (Invitrogen, Carlsbad, CA), 0.2 mM (each) deoxynucleoside triphosphates (dNTPs), 2 mM $MgCl_2$, 0.6 mM forward and reverse primers, 2.5 mg/ml bovine serum albumin (BSA) and 1 unit of Platinum *Taq* (Invitrogen, Carlsbad, CA). To each 25 μl reaction mixture, 1 μl of template DNA (diluted 1:3 in 1x Tris buffer) was added. Thermocycler settings were 5 min at 95°C, then 30 cycles of 95°C for 1 min, 52-62°C for 45 s (depending on primer pair) and 72°C for 1 min, with a final extension for 7 min at 72°C. Annealing temperatures were optimized with gradient PCR for each primer pair to maximize target amplification and minimize primer dimer. Annealing temperatures were 58°C for ITS, 62°C for LSU, 52°C for 16S and 55°C for SSU. PCR products were visualized through

gel electrophoresis. PCR products of the 36 samples were then titrated by locus (ITS, LSU, SSU, or 16S) to achieve equal molar concentrations of target PCR product for each sample. The ITS and SSU pools of PCR products were then titrated into a single library with the LSU and 16S pools titrated into a second library. Unincorporated primers, dNTPs and primer dimers were removed by two successive rounds of cleaning using the Agencourt AMPure purification system (Beckman Coulter, Danvers, MA). Product purity and concentration were checked with an Agilent 2100 Bioanalyzer (Santa Clara, CA). Emulsion reactions were performed in paired samples containing 2 sample PCR amplicons that were matched for template quantity and quality. The two prepared libraries were each loaded into 1/8 of a 454 plate and sequencing was performed on the GS-FLX with Titanium series reagents (Roche), sequencing from the 'A' adaptor only according to manufacturer's recommended conditions. Sequence data generated during this study have been submitted to NCBI's Sequence Read Archive under the study accession number SRP034435.

Sequence data analyses

The spilt_libraries.py script in QIIME (v1.6) (Caporaso *et al.*, 2010) was used to demultiplex the raw reads and remove primer sequences,short reads and low quality reads from the dataset. Default parameters were used with the following exceptions: homopolymers were not allowed to exceed 10 bases in length and sequences <300 bp in length were excluded. Error-correction of the sequences was done using Acacia (Bragg *et al.*, 2012) with default parameters, which has been shown to perform as well (or better than) a number of denoiser algorithms and is computationally much less intensive. The data were then analyzed separately by gene region as described below, using QIIME (v1.6) unless specified otherwise. Finally, each data set was imported into R using the phyloseq and APE packages (Paradis *et al.*, 2004; McMurdie & Holmes, 2013; Team, 2013). Permutational Multivariate Analysis of Variance Using Distance Matrices (ADONIS) (Dixon, 2003) was used to partition the variation-explained by soil origin and host plant on microbial community composition.

The ITS rDNA locus is often favored for assessing composition of fungal communities because of its ability to discern fungal species (Schoch *et al.*, 2012). This is due to the high variability of ITS1 and ITS2 regions of this locus. We used the ITS extractor to

selectively extract ITS1 and ITS2 regions from each of the sequences (Nilsson *et al.*, 2010b). Removing the conserved 5.8S domain, LSU and SSU sequences at the ends of the read allows more reliable clustering of OTUs (operational taxonomic units) and essentially removes chimeras since ITS chimeric sequences often form within the 5.8S region (Nilsson *et al.*, 2010a). ITS1 and ITS2 sequences were then clustered into OTUs by nearest neighbor based on the accepted criterion of 97% similarity (Blaalid *et al.*, 2013). Representatives of each OTU were identified taxonomically through BLAST by using the assign_taxonomy.py script and the UNITE reference dataset (Abarenkov *et al.*, 2010). Fungal community metrics of alpha-diversity and beta-diversity were calculated with QIIME (Caporaso *et al.*, 2010), with subsampling to a depth of 1200 sequences to create an even sampling depth. The statistical software Primer v6 was used for hierarchical clustering and non-metric multidimensional scaling of the samples, analyses of similarity (ANOSIM) between sample clusters and SIMPER analyses to identify fungal species that discriminate most between sample clusters (Clarke & Gorley, 2006). Analyses were carried out on normalized Bray-Curtis similarity matrices. Because sequencing was performed in the forward direction, ITS1 contained better sequencing coverage than did ITS2. For this reason we report results only for the ITS1 region.

The LSU region (28S rDNA) has long been a favored locus for fungal phylogenetics because this region (a) can be aligned across the Kingdom, (b) can be used to phylogenetically place unknown/understudied taxa, (c) amplifies and sequences easily, and (d) includes variable domains informative at the genus (and sometimes species) level for identification of taxa. Sequences belonging to the LSU rDNA region were processed in Qiime. Chimeric sequences were removed using the USEARCH algorithm (Edgar, 2010). Sequences were then mapped to a version of the eukaryotic SILVA LSU reference database that was parsed locally to include fungal and non-fungal representative sequences. Sequences mapping to non-fungal taxa were filtered from the dataset. OTUs were assigned by clustering the sequences at 99% similarity. Taxonomy was assigned to representative sequences by using a LSU reference sequences database compiled from the curated RDP dataset and SILVA 28S dataset and the taxonomic identifications of the most abundant taxa were verified by comparing sequences to the NCBI database with the BLAST algorithm. Neighbor Joining trees were built using PAUP 4.0 (Swofford, 2002). Trees were analyzed using the weighted UniFrac

metric calculated with Fast UniFrac (Hamady *et al.*, 2009) and samples were categorized according to sample host (*Quercus*, *Pinus*, or *Populus*) and soil origin (NC2, NC3 or TN).

The SSU (18S rDNA) region was sequenced with the aim of selectively detecting Glomeromycota (AM fungi). Chimeric sequences were first removed with USEARCH (Edgar, 2010). The sequences were then mapped to a version of the eukaryotic SILVA SSU reference database, which includes fungal and non-fungal representative sequences. Sequences not mapping to Glomeromycota were filtered from the dataset. Remaining OTUs were assigned by clustering the sequences at 98% similarity. To ascertain the phylogenetic placement of these OTUs, representative OTUs sequences were aligned with sequences belonging to reference taxa from across the Glomeromycota (Kruger *et al.,* 2012) with the alignment program MUSCLE (Edgar, 2004). Phylogenetic analyses were carried out in PAUP 4.0 (Swofford, 2002) as described above.

The V4 region of the 16S rDNA (SSU) was sequenced with the aim of selectively detecting bacteria (and excluding plastids and mitochondria). Chimeric sequences were removed with USEARCH (Edgar, 2010). The sequences were then identified taxonomically using a version of the eukaryotic SILVA 16S rDNA reference database and sequences identified as plastids, mitochondria, or non-Eubacteria taxa were filtered from the dataset. OTUs were assigned by clustering the sequences at 97% similarity and representative OTU sequences were given taxonomic assignments using the SILVA reference dataset. Alpha- and beta-diversity statistics were carried out in Qiime. Beta-diversity analyses were done using Bray-Curtis similarity and weighted UniFrac distance matrices that had been rarified to a common sampling depth.

Plant growth analyses

Effects of soil origin and host species on plant growth rate were tested with a 2-way ANOVA using R version 2.15.2. Normality was checked graphically with normal quantile-quantile plots and computationally with the Shapiro-Wilk test of normality. Homoscedasticity was checked by calculating the variances of the data grouped by the levels of each factor and comparing the values to see if any were more than twice any of

the others. Since this was often the case, we also used the Bartlett test of homoscedasticity, which evaluates the null hypothesis of equal variances. Differences between means in ANOVAs were checked a posteriori with the Tukey HSD test. Growth data were right-skewed slightly so were log-transformed to meet the assumptions of normality and homoscedasticity.

Linear regression analysis in R 2.15.2 was used to check for correlations between plant growth rates and bacterial and fungal diversity indices. Since no significant correlation existed, effects of host species and soil origin on microbial diversity were evaluated separately for bacteria and fungi using two 2-way ANOVAs with the alpha-diversity indices as the dependent variables. Correlations were also checked for the data split by propagation method (seed or cutting) but this did not affect the results of the regression analysis. Chi-square tests were used to check whether difference in OTUs detected between different host species and soils was significant.

III. Results

PCR and 454 sequencing

In this experiment a total of 36 plants (12 oaks, 12 pines, 12 poplar) were grown (in replicate) in three different soils to assess how host species and soil origin affect root assemblages. Two 454 libraries were prepared with amplicon pools from these 36 multiplexed samples for a total of four gene regions for each sample. The first library included ITS and SSU rDNA amplicons and resulted in 127,161 reads. The second library consisted of 16S and LSU rDNA amplicons and resulted in 106,125 reads. After error-correction and filtering for quality, length and chimeras 42,483 sequences of ITS, 55,825 of SSU (1340 of which mapped to Glomeromycota), 68,703 sequences of LSU and 17,949 sequences of 16S were recovered. Three root samples (*Pinus* NC2, *Pinus* TN and *Quercus* NC3) failed to amplify and/or sequence well, and were excluded from further analyses. The remaining 33 samples had between 226-4114 reads per sample per locus.

Assessing fungal communities with ITS sequencing

Of 42,483 total ITS1 sequences, we recovered 12,762 ITS1 sequences from *Pinus*, 15,985 from *Populus* and 13,736 from *Quercus*. These sequences were grouped into 256 OTUs: 166 OTUs for *Populus* (58 singletons); 114 OTUs for *Quercus* (39 singletons); and 95 OTUs for Pine (25 singletons) (Fig 1). Chi-square tests indicated that these differences by host are significant (P-value < 0.0001). A few abundant taxa were dominant, and for each host the most abundant 12 taxa accounted for more than 90% of the sequences. Between 5-28 OTUs were observed within each sample. A core set of 31 OTUs were shared among hosts (Fig 1), dominated by several endophytes including a novel *Atractiella* sp., *Cylindrocarpon*(=*Ilyonectria*) *pauciseptatum, Ilyonectria macrodidyma* and *Fusarium oxysporum,* along with several EcM species including Pezizaceae sp.1, *Inocybe curvipes* and *Hebeloma sacchariolens*. *Populus* had the highest number of unique OTUs (102) and fewer shared OTUs than either *Quercus* or *Pinus* (Fig 1). Unique OTUs detected for each host and their classification are presented in Table S3.

A distinct assemblage of fungal OTUs characterized each assayed soil. In total, 20,227 sequences were derived from roots of plants grown in TN soils, 12,942 from NC3 soils and 9314 from NC2 soils. Of the 256 OTUs detected, 19 OTUs were shared between the soils (Fig 1) with *C. pauciseptatum* and *Calonectria kyotensis* comprising the most abundant shared taxa. The NC3 soil had the most OTUs (136) and the TN soils had the fewest (104). The NC2 soil had 113 OTUs. Chi-square tests indicate that these differences are not significant (P-value = 0.0763). The NC3 soils had the highest number of unique OTUs (80) compared to the TN (57) and NC2 soils (56) (Fig 1). Most of the dominant unique OTUs from the NC3 site were of mycorrhizal fungi including species of *Thelephora, Laccaria*, *Scleroderma*, *Peziza*, *Tuber* and Glomeraceae. Unique sequences from NC2 were largely of EcM species in the genera *Scleroderma, Thelephora, Tomentella* and *Suillus.* Unique sequences in the TN soil included EcM species of *Hebeloma, Thelephora* and Pezizales as well as several poorly identified fungal endophytes. The two NC soils shared more OTUs with each other than they did with the TN soil. ADONIS results indicate that soil origin explained 25.68 % of the variation in fungal communities compared to 14.96% explained by plant host.

Multiple different patterns are evident in the distribution of fungal taxa between soils and hosts. Some taxa such as *C. pauciseptatum* were recovered in all soils and from all hosts assayed. Others, such as *Atractiella* sp. were found on all three hosts, but were particularly abundant in a single soil. In contrast, some fungi were abundant only on one host species, such as Chaetothyriales sp.1 that was abundant on *Populus* grown in North Carolina soils (Fig S1). SIMPER analyses of the fungal community showed that average community similarity across *Populus* samples was 35.9%, largely attributable to Chaetothyriales sp.1, *Atractiella* sp., and *C. pauciseptatum*. *Pinus* samples had an average similarity of only 11.0%, mostly attributed to Pezizaceae sp.1, *C. pauciseptatum* and *Oidiodendron* sp.1. *Quercus* had the lowest similarity between samples of 7.8%, mostly attributed to *Pezizaceae* sp.1, *Atractiella* sp.1, *C. pauciseptatum* and *Laccaria laccata* (Table S4). This high intraspecific variation is likely due to differences in species represented in the fungal propagules of the assayed soils. When fungal communities in each sample were hierarchically clustered, they grouped first by soil (TN vs NC) and then (in the case of *Populus*) by host (Fig 2). NMDS ordinations show the same pattern, whereby fungal communities of trees grown in TN soils are distinct from those grown in NC soils (with less distinction between the two NC soils) and fungal communities on *Populus* are separated cleanly from those of *Quercus* and *Pinus.* ANOSIM analyses indicate that there were significant differences between fungal communities on *Populus* compared to those on *Quercus* or *Pinus,* but no significant differences between fungal communities on *Pinus* and *Quercus.*

Assessing of fungal communities with LSU sequencing

Of the 68,703 fungal LSU rDNA sequences generated in this study, 25,774 were from *Quercus*, 24,184 were from *Pinus* and 18,740 were from *Populus*. A total of 197-269 OTUs were detected, depending on whether singletons were retained. While the 28S appears to target a wider phylogenetic range of fungal taxa than does the ITS, assigning taxonomy to these sequences is more challenging because of discrepancies and misidentifications in the reference databases. Nonetheless, rank abundances plots of the most dominant taxa detected in each host plant are fairly congruent between ITS (Fig 3) & 28S (Fig S2) datasets. For example, both ITS and LSU datasets profile *Populus* as dominated by endophytic fungi (*Ilynectria*, *Atractiella*, *Chaetothyriales*), with some EcM

taxa (*Inocybe*, *Pezizaceae*) and a low sequence abundance (<1%) of AM fungi belonging to *Rhizophagus* and *Paraglomus*. Similarly, ITS and LSU datasets both profile *Quercus* and *Pinus* as dominated by fungi with an EcM habit (Boletales, Pezizaceae, *Inocybe* on *Pinus;* Pezizaceae, *Scleroderma*, *Laccaria*, Thelephoraceae on *Quercus*) and to a lesser extent fungal root endophytes in the *Ilyonectria* and *Atractiella* (and *Oidiodendron* on *Pinus* only). Rarefaction and NMDS plots of ITS1 and LSU datasets also give comparable results (Fig S3), with between 9-40 OTUs observed per sample. While clearly not saturated, rarefaction curves based on a subsampling of 1200 iterations for hosts show that *Pinus* had the fewest number of observed OTUs (13 ± 4/ sample) while the greatest number of OTUs were observed on *Populus* (28 ± 4/ sample) (Fig S4). An average of 14 ±6 OTUs was observed per sample, with little variation in OTU number between soils.

Assessing Glomeromycota with taxon-specific sequencing of SSU rDNA

Of the 55,825 sequences recovered from PCR products aimed at specifically amplifying the Glomeromycota, the majority belonged to taxa in the Basidiomycota, with minor contributions from Chytridiomycota, Zygomycota, Ascomycota and Amoebozoa. The 1340 (2.4%) sequences assigned to the Glomeromycota were clustered into 54 OTUs. Although the region sequenced (partial 18S) was not suitable for accurately reconstructing deeper level phylogenetic relationships of the Glomeromycota (Fig 4), OTUs could be assigned to particular taxonomic groups. A single Glomeraceae OTU was detected on *Quercus*, while a cluster of 3 Glomeraceae OTUs was detected on all three hosts. The remaining 50 OTUs were detected only on *Populus.* Glomeraceae (37 OTUs) and Paraglomeraceae (10 OTUs) accounted for most of the Glomeromycota OTUs associated with *Populus*; Acaulosporaceae (3 OTUs) and Archaesporacae (2 OTUs) were also represented (Fig 4). No OTUs clustered within the Gigasporaceae. The distribution of Glomeromycota sequences in the three soils was uneven and the majority of OTUs (72%) detected were from a single soil. Only one OTU was detected in the TN soil, while 38 OTUs were detected in NC3 soils and 28 OTUs were detected in NC2 soils which had elevated levels of soil nutrients. Twenty-seven (27) of the OTUs in the NC3 soil were unique to that soil.

Assessing bacterial communities by targeting 16S rDNA

Assessing 16S rDNA diversity by targeting the V4 region resulted in 17,949 sequences representing 1094 OTUs: 624 for *Populus*, 534 for *Quercus*, 478 for *Pinus.* The roots of all three hosts were dominated by Rhizobiales, Burkholderiales and Actinomycetales (Fig 5), but ordinations of bacterial communities based on weighted UniFrac distances show that communities are structured more by host species than by soil origin (Fig S5). Core bacterial microbiomes calculated for each of the three hosts show those taxa present in at least 95% of the samples, and for *Populus* included OTUs belonging to the *Actinoplanes*, *Niastella*, *Bradyrhizobium*, Comamonadaceae and *Rhizobium*, for *Quercus* consisted of *Mycobacterium*, *Actinospica*, *Bradyrhizobium*, *Burkholderia*, and *Rhizobium*, and for *Pinus* consisted of three *Burkholderia* OTUs, two OTUs of *Bradyrhizobium*, a Rhizobiales and a Thermomonosporaceae. The most abundant OTUs were part of the core microbiome of all three hosts. Unique OTUs to *Populus* included *Oerskovia*, *Niastella*, *Salicola*, *Chitinophaga* and *Mesorhizobium*, while the root of *Pinus* was distinguished by unique OTUs of *Sphingomonas*, *Rhizobium*, *Bacillus* and *Comamamonas*. Unique Methylocystaceae and Bradyrhizobiaceae OTUs were detected on *Quercus*. ADONIS results indicate that the percent variation in bacterial communities explained by soil origin and host plant is 15.47 % and 9.2 % respectively.

Plant growth and root assessments

All seedlings survived until harvest. Qualitative assessments of seedling health and fungal colonization of the root systems after harvesting show the root systems of these plants were healthy (non-necrotic). The majority of root tips (>67%) on seedlings of *Quercus* and *Pinus* were well colonized by ectomycorrhizal fungi. In contrast, fewer (<33%) ectomycorrhizas were evident on *Populus* cuttings, although dark septate endophytes and other melanized fungal structures were conspicuous on roots.

Two-way ANOVAs showed that tree species (F=103, p=3.2e-11), soil type (F=3, p=0.0497) and the interaction between tree species and soil type (F=4, p=0.015) accounted for significant differences in seedling height. There was a strong contrast between *Quercus* and *Pinus* growth rates, with *Quercus* displaying an overall faster mean growth rate (0.15 cm/day) in all soil types as compared to the *Pinus* (0.09 cm/day)

(Fig S6). This difference was significant for every soil sample except TN (p=0.053). The smaller difference between *Quercus* and *Pinus* growth rates in TN soils as compared to those from NC shows an interaction between soil and host species. Most of this difference is from the significantly lower growth rates of the *Quercus* seedlings in the TN soils. As expected, a post-hoc Tukey test revealed that *Pinus* growth rates were overall significantly lower than those of *Populus* and *Quercus* (Fig S6). No significant correlation between plant growth with either bacterial or fungal diversity was found in linear regression analysis (Fig S7). Grouping the plants according to whether they were started from seed or cutting showed no difference among propagation types.

To examine the relationship of a diversity index to the tree species and soil types, two 2-way ANOVAs with microbial diversity (either fungal or bacterial) as the response variable were conducted. Neither soil nor host tree species had significant effects on the fungal community Shannon diversity. In contrast, host tree showed a significant effect on Shannon diversity of the bacterial community and the soil term was barely non-significant (p=0.0599). A post-hoc Tukey HSD test revealed that the difference between bacterial diversity for *Pinus* and *Populus* was significant (Fig S8), but all other pair-wise comparisons between factor levels were not.

IV. Discussion

Influence of host genus and soil origin on the structuring of fungal communities

The results from this experiment indicate that *Populus deltoides* does select for a distinct root microbiome compared to *Quercus phellos* and *Pinus taeda*, plant species that co-occur in natural communities. The influence of host species on the structuring of fungal communities continues to be a topic of interest and importance in ecology (Ishida *et al.*, 2007; Gao *et al.*, 2013; Peay *et al.*, 2013). While distinctions were evident between root fungal communities of *Populus*, *Quercus* and *Pinus*, it is interesting to note that all three fungal datasets produce here (ITS, LSU and SSU) show that soil origin (and not nutrient status or texture) had a stronger influence on the fungal community assemblage than did host species, with an evident decay by distance pattern in fungal beta-diversity. This was particularly true when contrasting between TN and NC soils, which are separated geographically by the Appalachian Mountains. Even though soils from the different sites

were collected at different times and stored under different conditions (TN soils were sampled 6 months earlier and stored at -80 °C, while NC soils were air dried 1 week prior to use), our results mirror results from a field study reported by Shakya et al. (2013) on two-dozen native *Populus* trees sampled along the Caney Fork (TN) and Yadkin (NC) rivers where watershed origin (TN vs NC) was shown to have larger effects than numerous other factors (season, genotype, soil properties, etc) on the structuring of both endosphere and rhizosphere fungal communities of *P. deltoides*. Although many fungal taxa were present across multiple soils assayed, most OTUs (including Glomeromycota) were restricted to a single soil. In contrast, a high proportion of fungal OTUs appeared to be shared between plant hosts, particularly between *Quercus* and *Pinus*. Our data also support other recent findings by Peay et al. (2012) in pine systems indicating that dispersal and availability of viable fungal propagules may be of equal or greater importance in the assembly of root fungal communities as host fidelity and preference.

In this study, *Populus* hosted nearly 50% more fungal diversity than did *Quercus* or *Pinus* irrespective of soil. However, much of this additional diversity is represented by taxa with low abundance levels. In contrast to *Pinus* or *Quercus*, fewer EcM taxa were detected on *Populus* (e.g., Pezizaeae, *Inocybe curvipes* and *Hebeloma* spp.). The majority of fungal OTUs we detected on Populus cuttings mostly likely function as root endophytes. These include species belonging to the Chaetothyriales, *Atractiella* and *Ilyonectria,* which accounted for the majority of sequences generated from *Populus*. These results are consistent with field studies on the *Populus* root/endosphere in natural habitats which indicate that species of Chaetothyriales and *Ilyonectria* (Hypocreales) are part of *Populus'* core microbiome (Shakya *et al.*, 2013). Prior field studies also showed that the basidiomycete genus *Atractiella* is naturally abundant and sometimes dominant in the roots of *Populus* (Gottel *et al.*, 2011). Why there is a higher diversity of fungi on *Populus* roots is not clear, but we speculate that one reason may be the reduced density of ectomycorrhizas on *P. deltoides* compared to either *Quercus* or *Pinus.* A lower frequency of ectomycorrhizal formation could be an adaptation of *P. deltoides* to its riparian habitat (Lodge, 1989). Fewer roots colonized by ectomycorrhizas on *P. deltoides* may also provide a greater exposed root surface for endophytic fungi to colonize. If this is true we predict that non-EcM plants should harbor high levels of fungal diversity in their roots than related EcM plant hosts.

In contrast to *Populus*, nearly half of the top ranked fungal taxa from *Quercus* and *Pinus* belonged to EcM taxa. Visual inspection of the roots of these hosts confirmed the low amount of ectomycorrhizal colonization of *P. deltoides* compared to *Quercus* and *Pinus*. The main EcM taxa on *Quercus* and *Pinus* were species of Pezizaceae, *Scleroderma*, *Laccaria*, *Hebeloma* and Thelephoraceae. Still, endophytes contributed to a significant portion of the fungal taxa detected on *Quercus* and *Pinus* roots, which have largely been ignored in mycorrhizal studies of these hosts. As mentioned above, a novel species of *Atractiella* was among the top three ranked taxa on all hosts and *Ilyonectria* species were also common on all three hosts (particularly in the TN soil). We have obtained isolates of many of these endophytic fungi including this *Atractiella* species, and ongoing studies are showing that many have beneficial plant growth promoting effects (Bonito and Vilgalys unpublished).

Arbuscular mycorrhizal communities associated with Populus

Compared to other woody plants *Populus* is fairly unique in that it can associate with both EcM and AM fungi (Karliński *et al.*, 2010). In an effort to assess and compare AM communities we used a set of primers designed to selectively amplify the SSU rDNA of Glomeromycota. Unfortunately, the majority of resulting sequences belonged to non-target taxa, nonetheless, the primers successfully amplified Glomeromycota SSU rDNA, permitting us to recover a moderate diversity of AM fungi (54 OTUs). All but one of the OTUs detected were associated with *Populus*, and a cluster of three OTUs in the Glomaceae was detected on all three hosts. This SSU dataset indicates that *Populus* associates with a relatively high diversity of AM species belonging to the Glomaceae and Paraglomaceae. ITS and LSU datasets confirm this result as well, although sequences from AM fungi were at lower relative abundance levels (<1%) in these datasets. A few OTUs belonging to Acaulosporaceae and the Archeosporales were also detected in the SSU dataset, as were two OTUs distinct (<96% similarity) from known accessions in GenBank, suggesting the presence of previously undetected Glomeromycota.

Many studies have reported low and variable incidence of AM fungi on *Populus* (Kaldorf et al., 2002; Gehring et al., 2006; Karliński et al., 2010), which has been attributed to plant genotype and environmental factors (i.e. lower AM abundance in moister soils). These factors may explain in part the low abundance of sequences belonging to AM

fungi on *Populus* in this study. AM spores have also been reported to be sensitive to freezing temperatures (Addy *et al.*, 1997), therefore it is possible that the storage of TN soils at -80 °C negatively affected AM spore viability in this treatment. There are also technical issues with selectively amplifying and pyrosequencing AM fungi from roots. Others have also had difficulty in selectively amplifying and sequencing the Glomeromycota from roots and soils using 454 pyrosequencing (Becklin *et al.*, 2012). This difficulty may in part be due to non-specific primer binding caused by long primers needed for 454 pyrosequencing or high primer homology with non-target taxa. This issue may be compounded in our study by the apparent low relative abundance of DNA from AM fungi compared to other fungi in the samples assayed (as inferred from results of ITS and LSU datasets).

Influence of host and soil origin on bacterial community structure

Community similarity metrics based on OTU composition (i.e. Bray-Curtis) and phylogenetic diversity (i.e. UniFrac) indicate that bacterial communities in the root are more tightly structured by plant host species than by soil origin. While bacteria in the Rhizobiales (α-Proteobacteria), Burkholderiales (β-Proteobacteria) and Actinomycetales were dominant on all hosts, taxa belonging to the Micromonosporineae (Actinomycetales) were particularly abundant on *Populus. Pinus* was distinguished by a relatively high abundance of Methylocystaceae (α-Proteobacteria). The prevalence of α- and β- Proteobacteria and Actinobacteria corresponds with previous reports on *Quercus* and *Populus*; however, unlike previous studies, we did not find a dominance of γ-Proteobacteria and Acidobacteria (Uroz *et al.*, 2010; Gottel *et al.*, 2011; Shakya *et al.*, 2013). It is possible that this is due to primer biases. Field studies in the same soils assayed here have shown that bacteria in the endosphere of *P. deltoides* are distinct from those present in bulk soils (Gottel *et al.,* 2011; Shakya *et al.* 2013). In bulk soils it has been suggested that bacterial community composition and diversity at higher phylogenetic levels can largely be explained by pH (Fierer & Jackson, 2006; Hartman *et al.*, 2008). Similar pH influence on bacterial communities has been observed within root analyses in native *Populus* systems (Gottel et al 2011, Shakya et al 2013). However, even within soils of similar pH and at finer phylogenetic resolutions host associations appear to have a role in moderating community structure. Analyzing microbial

community data via ADONIS, the percent variation explained by soil origin and plant host on bacterial communities was 15.47 % and 9.2 %, respectively, compared to fungal communities which were observed to have 25.68 % and 14.96 % of their variation explained based on ITS analyzed data.

Biodiversity is often considered to be beneficial to ecosystem function, but quantifying biodiversity and relating it to function is challenging because ecosystems function in many diverse ways (Setala, 2002; Fitter *et al.*, 2005). Using plant growth in a trap-plant context as a proxy for ecosystem function we found no relationship between microbial diversity in host roots and plant growth. Plant growth was best explained by host species, with *Pinus* seedlings growing significantly slower than either *Quercus* or *Populus.* Soil origin did have an effect on plant growth, but we were not able to determine whether these effects were due to differences in nutrient availability or the colonization of roots by particular microbiota that serve as plant growth modulators.

ITS vs LSU for surveying fungal communities

There has been much debate as to whether ITS or LSU is better for characterizing fungal communities and cases have been made for both loci (Lindahl *et al.*, 2013; Porras-Alfaro *et al.,* 2013). The choice of sequencing single vs. multiple loci in most studies is largely due to financial and labor constraints (Jumpponen & Jones, 2009; Tedersoo *et al.*, 2010; Danielsen *et al.*, 2012). In this study we used 454 pyrosequencing from multiple loci to compare root fungal communities. We report here that community analyses of fungal communities based on ITS and LSU regions were commensurate in detecting diversity and identifying dominant OTUs. While LSU sequence alignments can be useful for phylogenetic inferences and metrics, current reference databases for taxonomic assignments of LSU data are not as developed as those available for the ITS region (Kõljalg *et al.,* 2013). This is particularly true for early diverging fungal taxa, which often are misclassified or unclassified in the SILVA database or not well represented in the curated fungal RDP dataset (Liu *et al.*, 2012). Analysis by ADONIS revealed that ITS (25.68 %) data explain a higher percentage of variation due to soil origin than did the LSU region (15.9 %), most likely due to the better resolution and separation of strain-level OTUs by ITS over that of the more conserved LSU sequence data.

Conclusions

We were successful in using multiplex 454 pyrosequencing to assay fungal and bacterial communities in the root of woody plant species to determine the influence of plant host and soil origin on the development of the root microbiome. Using four loci we showed that while different host genera may associate with different fungi belowground in a common soil, soil origin has an even greater effect on the fungal root community composition. This difference in influence may be due to dispersal limitation in fungi, supporting the understanding that "not every fungus is everywhere" (Peay *et al.*, 2010). In contrast, bacterial communities appeared to be more tightly structured by host genus than by soil origin, potentially due to chemical or pH differences in the roots of the studied host taxa. Our results highlight the high level of promiscuity of plants in regards to their belowground fungal and bacterial associates. This could be an adaptive trait for widespread plant species and suggest a high level of functional redundancy in the root microbiome. Our results also indicate that the ecological niche and proclivity of the host plant to form EcM may be better predictors of its belowground microbial community structure than phylogenetic relatedness of host plants. Given these insights, we conclude that the trap-plant bioassay, coupled with high-throughput sequencing, provides a powerful experimental approach for decoupling complex ecological questions.

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Data Accessibility

Demultiplexed and error corrected sequence data produced in this study has been deposited in the NCBI Sequence Read Archive (SRA) under the accession: SRP034435

Final DNA sequences, sample metadata and plant growth data uploaded to Dryad doi:10.5061/dryad.2b2dj

Sampling locations and soil characterization data have been uploaded as online supplemental material.

FIGURE LEGENDS

Figure 1 – Venn diagrams show the number of unique and shared fungal OTUs among host taxa (a) and soils (b). OTUs are based on 97% similarity of the ITS1 region of the rDNA.

Figure 2 – Hierarchical community clustering of root fungal communities based on ITS1 region. Fungal communities of the TN soils cluster together and those belonging to NC soils form a second cluster. Within the TN and NC soils, communities on *Populus* cluster tightly. Within the NC soil cluster the NC2 and NC3 soils segregate, while *Pine* and *Quercus* communities are interspersed.

Figure 3 – Ranked abundance graphs of fungal species accounting for over 90% of the ITS sequences on each host. Some of these fungal taxa are abundant on all three hosts. Red bars code for putative ectomycorrhizal taxa, while bars of putative endophytes are in black.

Figure 4 – NJ phylogram of the Glomeromycota based on SSU amplicons shows the diversity and phylogenetic placement of taxa detected on *Populus* in this study (colored). OTUs belonging to the Glomeraceae are colored in blue, Acaulosporaceae are in purple, Archeosporaceae are in red and Paraglomaceae are in green. Taxa marked with an asterisk (*) were detected on *Quercus*, *Populus* and *Pinus*.

Figure 5 – Higher level taxonomic assignments of bacteria detected based on 16S sequences. Samples are separated by host and soils. Taxonomic ranks for abundant taxa are labeled on the right.

LEGENDS FOR SUPPLEMENTAL FIGURES AND TABLES

Figure S1 – Average dissimilarity of fungal communities on hosts and differences in the distribution of fungal taxa on different hosts and in different soils was detected using SIMPER analyses and NMDS. Green bubbles are scaled by abundance levels of the fungal taxon of interest in the particular soil/host sample (a) Atractiellales sp.1, (b) Pezizaceae sp.1, (c) *Cylindrocarpon pauciseptatum*. Soils are shown on NMDS plots (left) and hosts are shown on NMDS plots (right).

Figure S2 – Ranked abundance graphs of fungal species accounting for over 90% of the LSU sequences on each host. Comparing to Fig 3 it is evident that independent analysis of ITS1 and LSU identify many of the same dominant fungal taxa in these communities. Red bars code for putative ectomycorrhizal taxa, while bars of putative endophytes are in black.

Figure S3 – Rarefaction and principal coordinate ordination of fungal communities based on (a-c) ITS1 and (d-f) LSU (28S) regions present a similar view of fungal root communities and their diversity. In particular, fungal communities in TN soils segregate cleanly from those in NC soils, while communities in the two NC soils are more intermixed. Similarly, fungal communities of *Populus* segregate from fungal communities of *Pinus* and *Quercus*.

Figure S4 – Rarefaction curves for ITS (a & b) and LSU (c & d) based on resampling to a depth of 1200 sequences. Samples were rarefied by Host (a & c) and by Soil (b & d).

Figure S5 - Principal coordinate ordination of bacterial communities based on 16S rDNA Jackknifed analysis of beta diversity using weighted UniFrac metric shows that bacterial communities segregate better by host (a) than soil (b), with principal component 1 explaining 42.6% of the variation. Shaded areas represent standard deviation assessed through jackknifing the dataset to an even sampling depth. For host (a), *Populus* is shown in blue, *Quercus* in orange, and *Pinus* in red. For soil (b), TN is shown in blue, NC2 in orange, and NC3 in red.

Figure S6 – Boxplots showing the variation in growth rates (cm/day) between host (a) and soil (b). *Pinus* (pine) seedlings grew significantly slower than did *Populus* (cottonwood) or *Quercus* (oak) seedlings.

Figure S7. (a) Paired plot of plant growth rates and bacterial and fungal Shannon diversity indices. Histograms along the diagonal show the distributions of values for each variable. Scatter plots show all possible pairs of these three variables plotted against each other with the red lines tracing the trends. None of these variables were significantly correlated and a linear regression analysis showed that neither bacterial (p = 0.902) nor fungal (p = 0.644) diversity indices explained significant variation in the growth rates of the plant hosts (R^2 = -0.05866). **(b) Paired plot of growth rates and bacterial and fungal Shannon diversity indices for plants started from cuttings**. Histograms along the diagonal show the distributions of values for each variable. Scatter plots show all possible pairs of these three variables plotted against each other with the red lines tracing the trends. None of these variables were significantly correlated and a linear regression analysis showed that neither bacterial (p = 0.728) nor fungal (p = 0.633) diversity indices explained significant variation in the growth rates of the plant hosts (-0.229).**(c) Paired plot of growth rates and bacterial and**

fungal Shannon diversity indices for plants started from seed. Histograms along the diagonal show the distributions of values for each variable. Scatter plots show all possible pairs of these three variables plotted against each other with the red lines tracing the trends. None of these variables were significantly correlated and a linear regression analysis showed that neither bacterial ($p = 0.950$) nor fungal ($p = 0.859$) diversity indices explained significant variation in the growth rates of the plant hosts (-0.1514).
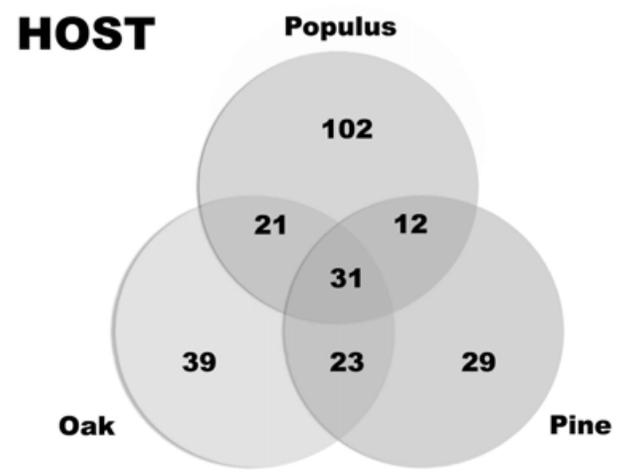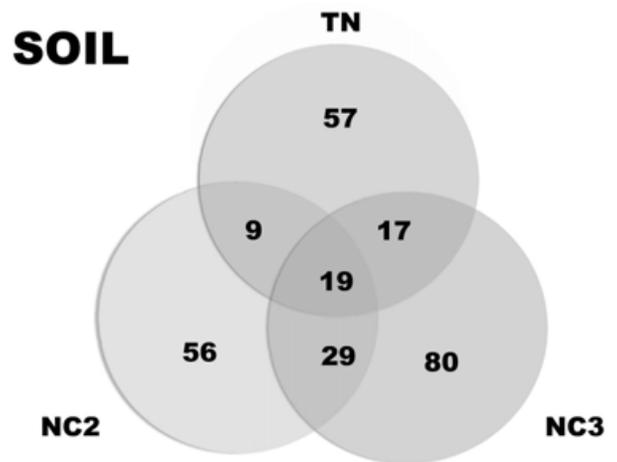
Figure S8. A post-hoc Tukey HSD test reveals that the difference between bacterial diversity for *Pinus* and *Populus* was significant but all other pair-wise comparisons between factor levels were not.

Table S1. Soil characteristics of soils used in the present study.

Table S2. 454 primer sequences and barcodes used in the present study. Primer pairs for each locus (ITS, LSU, SSU, 16S) are presented on there own sheet.

Table S3. Ranked unique fungal OTUs for each host species assayed.

Table S4. SIMPER fungal community similarity results for *Populus*, *Quercus*, and *Pinus* based on ITS1. The average similarity between samples of a given host are presented along with the contribution of each of the identified fungal taxa responsible for these contributing most to the community similarity.

SOIL

TN
57
9   17
19
56   29   80
NC2   NC3

HOST

Populus
102
21   12
31
39   23   29
Oak   Pine

Hierarchical Community Clustering

Populus

Tennessee Soils

Populus

North Carolina Soils

Populus (a)

Quercus (b)

Pinus (c)

PINUS   POPULUS   QUERCUS

Xanthomonadales

Burkholderiales

Rhizobiales

Sphingobacteriales
Actinomycetales
Acidobacteriales

TN   NC2   NC3   TN   NC2   NC3   TN   NC2   NC3