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Spheres of Influence: Host Tree Proximity and Soil Chemistry Shape rRNA, but Not DNA,
Communities of Symbiotic and Free-Living Soil Fungi in a Mixed Hardwood-Conifer Forest

A Thesis submitted in partial satisfaction of the
requirements for the degree Master of Science in
Ecology, Evolution, and Marine Biology

by

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March 2024

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June 2021

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by

Gabriel C Runte

ACKNOWLEDGEMENTS

To my *cheetah* community. Thank you for encouraging me to pursue a journey that was meaningful and fulfilling. Thank you for the support and warmth that powered me through.

ABSTRACT

Spheres of Influence: Host Tree Proximity and Soil Chemistry Shape rRNA, but Not DNA, Communities of Symbiotic and Free-Living Soil Fungi in a Mixed Hardwood-Conifer Forest

by

Gabriel C Runte

Host and symbiont diversity are inextricably linked across partnerships and ecosystems, with degree of partner reliance governing the strength of this correlation. In many forest soils, symbiotic ectomycorrhizal fungi coexist and compete with free-living saprotrophic fungi, with the outcomes of these interactions shaping resource availability and competitive outcomes for the trees aboveground. Traditional approaches to characterizing these communities rely on DNA sequencing of a ribosomal precursor RNA gene (the internal transcribed spacer region), but directly sequencing the precursor rRNA may provide a more functionally relevant perspective on the potentially active fungal communities. Here, we map ectomycorrhizal and saprotrophic soil fungal communities through a mixed hardwood-conifer forest to assess how above- and belowground diversity linkages compare across these differently adapted guilds. Using highly spatially resolved transects (sampled every 2 m) and well-mapped stands of varying host tree diversity, we sought to understand the relative influence of symbiosis versus environment in predicting fungal diversity measures. Canopy species in this forest included two oaks (*Quercus agrifolia* and *Quercus douglasii*) and one pine (*Pinus sabiniana*). At the scale of our study, spatial turnover in rRNA-based communities was much more predictable from measurable environmental attributes than DNA-based

communities. And while turnover of ectomycorrhizal fungi and saprotrophs were predictable by the presence and abundance of different canopy species, they both responded strongly to soil nutrient characteristics, namely pH and nitrogen availability, highlighting the niche overlap of these coexisting guilds and the strong influence of aboveground plants on belowground fungal communities.

1 Introduction

Forest communities are shaped by symbiosis. Plant roots engage with staggeringly diverse communities of soil organisms: many of these interactions are symbiotic, and their effects on plant health and resource movement through soils can have profound impacts on the functioning of ecosystems (Baldrian, 2017; Crowther et al., 2019). One key symbiosis structuring many forests is the ectomycorrhizal association, an essential nutritional mutualism for roughly 60% of tree stems on the planet (Steidinger et al., 2019). The diversity of ectomycorrhizal fungal mutualists available to plant roots belowground has a powerful impact on the composition and structure of the plant community aboveground (Dickie et al., 2010; Gao et al., 2013; Koide et al., 2014). This partnership between plants and fungi also influences the structure and function of soil communities: Ectomycorrhizal fungi, with their reliable source of host carbon, can inhibit decomposition and mediate soil carbon storage by competing directly with saprotrophic fungi for soil resources (Gadgil and Gadgil, 1975; Averill and Hawkes, 2016), although this effect varies substantially based on the traits of the fungi and the environment in which they interact (Fernandez et al., 2020).

When sampled at a landscape scale, plant and fungal diversity are clearly linked. In forested systems, host specificity of the ectomycorrhizal community means that turnover in aboveground host tree community membership is mirrored belowground (Tedersoo et al., 2012; Moeller et al., 2015) and ectomycorrhizal fungal species richness is known to track the genus richness of available host plants (Gao et al., 2013). This allows for some predictive linkages between aboveground plant communities and belowground function: pine forests, for example, support a distinctive set of ectomycorrhizal fungi with conserved enzymatic capabilities (Talbot et al. 2013; 2014), and exhibit distinct soil chemistry and decomposition

rates that are likely driven by these fungal associations . Yet aboveground-belowground linkages are complicated by environmental context and stochastic patterns of community assembly. Across the range of a single host tree species, the fungal community may turn over in response to climatological factors (Moeller et al., 2014; Bui et al., 2020) as well as dispersal limitation (Peay et al., 2010).

This context dependency becomes especially important when examining these above-belowground linkages at a fine spatial scale. Each tree influences and responds to a belowground rooting zone on the scale of meters, and is subject to habitat heterogeneity at that scale: it does not experience the habitat of an entire forest. The fungi, too, can extend from several centimeters to many meters belowground (Vincenot and Selosse, 2017), exposing them to substantial fine-scale variation in soil conditions. Competition among fungi for resource patches including decomposing organic matter (Fukami et al., 2010) and host root exudates (Smith et al., 2018) may also enhance community turnover at small scales (Pickles et al., 2012). Studies investigating the fine scale spatial turnover of fungal communities find that fungal community composition turns over on a scale ranging from centimeters to several meters (Lilleskov et al., 2004; Bahram et al., 2013), driven by factors such as host availability, host and fungal taxonomy, competition, and stochastic processes (Pickles et al., 2012; Bahram et al., 2013). Thus, communities of fungi and roots may be shaped strongly at the fine scale by factors that disappear when averaged across an entire landscape, obscuring the specific environmental conditions and species interactions that drive fungal diversity (Izzo et al., 2005).

Fine-scale coupling between belowground fungal communities and aboveground tree communities is shaped by how fungi interact with trees. Ectomycorrhizal fungi, reliant on

host roots for carbon, might be expected to respond more strongly to host availability than saprotrophic fungi. Trees, however, have an enormous impact on the soil, in ways that go beyond carbon subsidies to their ectomycorrhizal partners. Litter inputs, for example, can vary both in quantity and in chemical quality across different host plants (Scott and Binkley, 1997). This variation could have profound effects on both ectomycorrhizal fungi and saprotrophic fungi, since both guilds must obtain nitrogen, phosphorus, and other resources from similar substrates; the outcomes of competition between these guilds may depend strongly on litter chemistry (Smith and Wan, 2019). Highlighting the powerful but divergent influences of tree identity on these guilds, Nguyen et al. (2016b) found that that host phylogenetic diversity can be an important influence on ectomycorrhizal fungal communities, while saprotrophs may be controlled more by chemical inputs such as plant leaf nitrogen and aboveground tree biomass. However, in other settings, ectomycorrhizal and saprotrophic fungi respond similarly to aboveground vegetation, revealing only subtle differentiation between the forces structuring these groups (Tedersoo et al., 2016; Hiiesalu et al., 2017). Both guilds of fungi should be influenced by aboveground plant communities, but in fundamentally different ways. It remains unclear when these distinct mechanisms should lead these guilds to respond similarly to aboveground plant communities, and when their responses should diverge on the basis of their different trophic strategies.

To understand how ectomycorrhizal and saprotrophic fungi respond to aboveground plant diversity, we must identify the fungi making up a given community. Fungal community characterization, and ectomycorrhizal characterization specifically, has traditionally relied on either sporocarp surveys or soil environmental DNA for the description of communities. As high-throughput sequencing technologies have become increasingly accessible, amplicon

sequencing of the fungal internal transcribed spacer (ITS) region, a gene encoding a precursor component of mature ribosomal RNA, has become a standard for community characterization at the species level. Sequencing DNA, however, is an imperfect way to measure fungal communities: DNA can be preserved in soils for months to years (Nielsen et al., 2006), both as extracellular molecules sorbed to clays (Ogram et al., 1988; Morrissey et al., 2015), and within necromass and dormant propagules (Lennon and Jones, 2011; Carini et al., 2016). Fungal spores, too, can make it difficult to detect active community members using DNA, since fungi can disperse over great distances (Peay and Bruns, 2014; Horton, 2017), and their spores may persist in a dormant but intact state for many years (Bruns et al., 2009). To limit the influence of these forms of inconsistent data, researchers in the past have selected root tips from ectomycorrhizal plants and sequenced the individual fungi on each tip using Sanger sequencing (Moeller, Peay, and Fukami 2014; Janowski et al. 2019; Kumar and Satyanarayana 2002). This practice, though, is labor intensive and can be biased toward species with more conspicuous mycorrhizas. Additionally, saprotrophic fungi cannot be assessed in a parallel way because they cannot be isolated by visual identification of host association.

Ribosomal RNA-based community profiling can produce a more functionally relevant snapshot of microbial community composition (Dlott et al., 2015; Sorensen and Shade, 2020), allowing researchers to characterize which members of a community have the greatest protein synthesis potential -- that is, the greatest number of ribosomes (Blazewicz et al., 2013). Thus, rRNA-based community profiling can reflect the potentially active, functional community at a site at the time of sampling, while DNA-based profiling of rRNA genes, like ITS, reveals all the fungi whose DNA is present at the site, whether or not they are active members of the

community (Baldrian et al., 2012; Barnard et al., 2013; Liao et al., 2014; Žifčáková et al., 2016; Wutkowska et al., 2019). The ITS region is particularly well suited to capturing the truly active members of a fungal community, because it is a precursor rRNA molecule absent even from dormant spores (van der Linde and Haller, 2013). The ITS region itself is spliced out before a ribosome becomes active (Schoch et al., 2012), so any fungi with measurable ITS rRNA molecules are in the process of producing new ribosomes. These fungi are more likely to be active than fungi present only as DNA, since they are actively building the protein synthesis enzymes that drive their ecological functions.

Here, we use both precursor rRNA and DNA-based ITS communities to examine the connections between the spatial turnover in aboveground plant communities and the turnover in soil fungal communities, comparing ectomycorrhizal (symbiotic) and saprotrophic (free-living) fungi across four transects with varied aboveground plant community composition. Using high resolution sampling and mapping of above-ground canopy trees, we created a spatially explicit set of influences on each sequenced soil community. Each sample had a unique set of soil chemical characteristics and continuous ectomycorrhizal host availability data. We extracted and sequenced amplicons from both RNA and DNA in each soil sample in order to compare turnover at the meter scale within both potentially active (precursor rRNA-based) and more broadly inclusive DNA-based community profiles. We anticipated that host plant diversity would have a more pronounced effect on the potentially active, RNA-based community than the longer-term, DNA-based community, and that the RNA-based community composition would be more variable across our samples. We also hypothesized that ectomycorrhizal fungal communities would be much more responsive to the availability

of host trees than saprotrophs, but that both guilds of fungi would respond to soil resource and chemical properties.

2 Methods

Sampling Site and Sample Collection

We conducted our study at the Sedgwick Reserve, part of the University of California Natural Reserve System, in Santa Barbara County, California in January, 2020. This southern California site experiences a Mediterranean climate of hot, dry summers and cool, wet winters (Davis, Tyler, and Mahall 2011), leading us to sample in the winter wet season when fungi could be expected to be most active. We selected four 30m transects with varied aboveground plant community composition within 0.2km of one another (Fig. 1).

The canopy species on our transects were gray pine (*Pinus sabiniana*, abbreviated PISA hereafter), blue oak (*Quercus douglasii*, abbreviated QUDO), and coast live oak (*Q. agrifolia*, abbreviated QUAG). These two oak species are more phylogenetically distinct from the pine than one another, though blue oaks are deciduous and belong to the white oak group while coast live oaks are evergreen and belong to the red oak group (Plumb and Gomez 1983). Previous studies have described distinct differences in the ectomycorrhizal (but not saprotrophic) communities associated with such phylogenetically diverged oaks (Morris et al. 2008), as well as between oaks and co-occurring pine species (Smith et al. 2009; Suz et al. 2017; Rasmussen, Busby, and Hoeksema 2018).

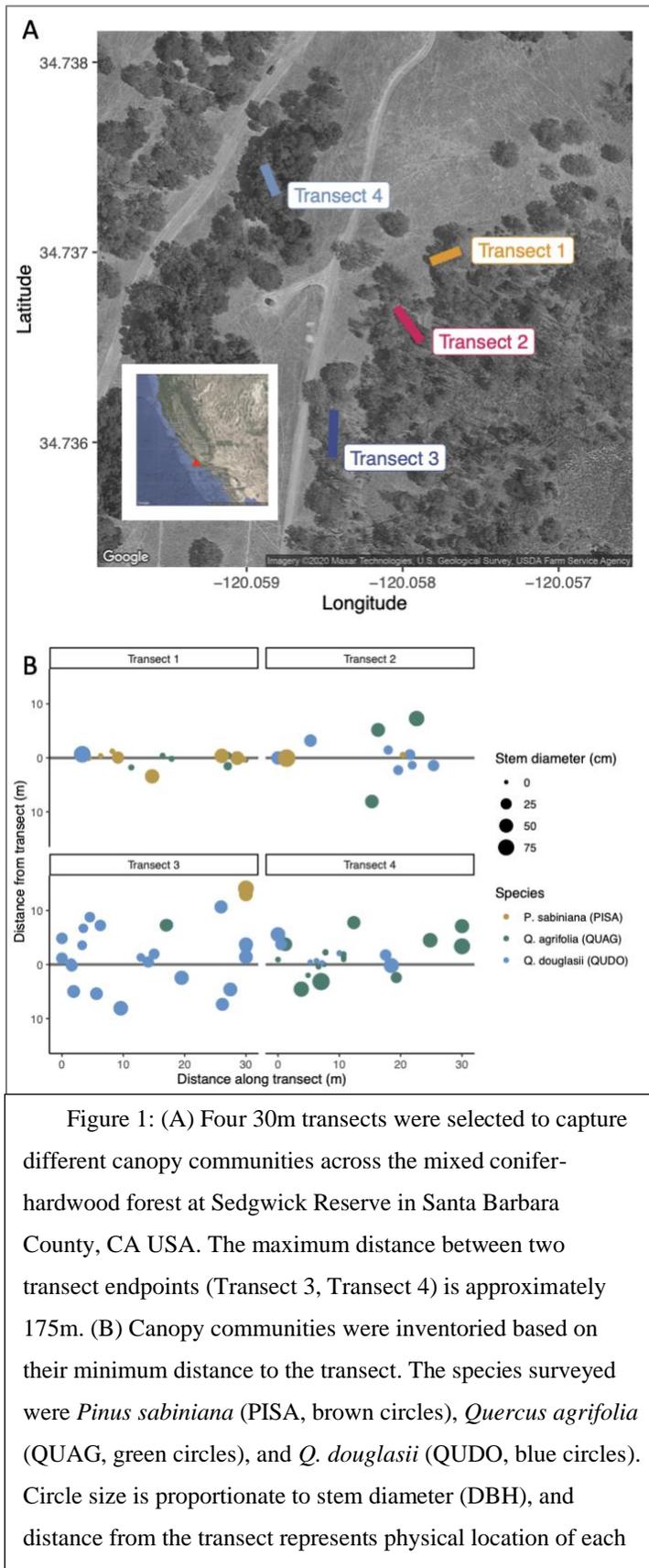


Figure 1: (A) Four 30m transects were selected to capture different canopy communities across the mixed conifer-hardwood forest at Sedgwick Reserve in Santa Barbara County, CA USA. The maximum distance between two transect endpoints (Transect 3, Transect 4) is approximately 175m. (B) Canopy communities were inventoried based on their minimum distance to the transect. The species surveyed were *Pinus sabiniana* (PISA, brown circles), *Quercus agrifolia* (QUAG, green circles), and *Q. douglasii* (QUDO, blue circles). Circle size is proportionate to stem diameter (DBH), and distance from the transect represents physical location of each

Soils across the four transects derived from the same alluvium parent materials and were Chamise shaly loams (transects one, three, and half of two: Clayey-skeletal, mixed, active, thermic Ultic Palexerolls), Elder loams (transect four: Coarse-loamy, mixed, superactive, thermic Cumulic Haploxerolls), and Positas fine sandy loams (half of transect two: fine, smectitic, thermic Mollic Palexeralfs) (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture). We sampled each 30m transect every 2m (n=16 soil cores per transect, including 0m and 30m end points) by removing surface vegetation and using a hand auger to a depth of 25cm. We emptied auger contents into an ethanol-sterilized bin and homogenized them by passing through a 4mm soil sieve and mixing before collecting a sample for nucleic acid extraction. These samples were weighed into sterile 15 mL tubes (1-2g soil per sample) and flash frozen on dry ice; samples were placed in a -80°C freezer within 24 hours of collection. A separate 1-quart homogenized soil sample from the same core was placed in a clean plastic bag for chemical analysis and stored in a cooler with ice until placement in a 4°C refrigerator within 24 hours.

We quantified aboveground plant community composition for each soil core by surveying host trees of all three species whose canopies extended to within 10m of the transect ([Day et al., 2010](#)). For each individual tree, we measured diameter at breast height (DBH) and position relative to the transect (by measuring the distance of the tree to the nearest location on the transect tape to the nearest centimeter). This allowed us to create a high-resolution map of aboveground plant diversity. To compute the host influence on each soil core, we calculated the angular size of each tree relative to each sampling point ([Hein et al., 2018](#)), expressing the potential influence of each tree species as the percentage of each core's 360° panoramic field of view that was occupied by that host species. If a host tree was too far from a given soil core

to include that core in its rooting zone -- that is, if the core was outside the tree's sphere of influence -- we excluded that tree from the calculation. We computed this distance as 1/2 of the tree's maximum rooting diameter, calculated using a formula from a metaanalysis linking maximum rooting radius (r) to DBH ([Day et al., 2010](#)):

$$r = 526.6 \times (1 - e^{-0.096 \times DBH})$$

Soil Chemical Analysis

Within one week of sampling, we subsampled and weighed refrigerated soils, then dried them to constant mass in an oven at 45-60°C and reweighed them to determine percent moisture. The remainder of our soil analyses were performed at Brookside Laboratories Inc on dried soil. (Ohio, USA). Briefly, total carbon and total nitrogen were evaluated via dry combustion on an elemental vario EL cube and divided for site C:N ratios. Phosphorus was extracted via the Bray II method and other trace nutrients were extracted with Mehlich III extractions (data not shown). Soil pH was evaluated using a 1:1 soil to water slurry.

We compared soil properties between transects using analysis of variance (ANOVA) tests with a Tukey's honest significant difference post-hoc test to assign significance groups. We used linear regression to evaluate correlation between tree-driven parameters and soil chemical properties, selecting our models based on lowest AIC score when grouping all transects' data. From this point, we tested for Pearson's correlation coefficient between selected parameters to choose which would be included in later analyses.

Nucleic Acid Extraction and Sequencing

We co-extracted RNA and DNA from 1 to 2 grams of soil using the Qiagen RNeasy Powersoil Total RNA Kit and RNeasy Powersoil DNA Elution Kit (Qiagen, Hilden,

Germany), according to the manufacturer's instructions. We reverse transcribed cDNA from the RNA extracts using the Qiagen OneStep RT-PCR kit and used Promega GoTaq Green mastermix for the DNA extracts. We amplified all samples with the ITS1 primer pair ITS1F-KYO1 and ITS2-KYO1 (Toju et al., 2012) and the following thermal cycler steps: 95°C for 3 min, 35 rounds of 95°C for 30s, 50°C for 30s, 72°C for 30s, then a final elongation step of 5 min at 72°C. We ligated Illumina indices with 3 min at 95°C, 35 cycles of 95°C for 30s, 47°C for 30s, 72°C for 30s, then a final elongation step of 72°C for 5 min. Finally, we purified samples with AMPure XP beads and diluted them to 10ng/uL before multiplexing all samples for sequencing in a single run. Amplicons were sequenced on the Illumina MiSeq platform with 250-bp paired-end reads at the California NanoSystems Institute (UC Santa Barbara).

Bioinformatics Pipeline

We processed amplicon sequences using the DADA2 pipeline (Callahan et al., 2016). To assess the broadest fungal community, we processed only forward sequences, as described in Pauvert et al. (2019). We removed adapter and primer sequences using the 'cutadapt' package and removed chimeras before filtering (Martin, 2011). Our *DADA2::filterandtrim* settings removed any sequences with an 'N' assignment and truncated reads at the first Q-score of 2 or lower, with a minimum read length of 50-bp. We chose not to rarefy our sequences due to the potential for false positives in differential abundance testing and our intention to use a consistent dataset through both richness and turnover (beta diversity) estimates (McMurdie and Holmes 2014). We finally clustered our amplicon sequence variants (ASVs) at a 97-percent similarity threshold using the *DECIPHER* package before making taxon assignments on these operational taxonomic units (OTUs) with the UNITE database (Nilsson et al., 2019; Wright, 2020). We assigned fungal functional guilds using the

FUNGuild database and retained OTUs called as either ‘probable’ or ‘highly probable’, further filtering for any OTU with ‘Ectomycorrhizal’ or ‘Saprotroph’ in its guild assignment to be in that guild for our analyses (Nguyen et al., 2016a). Finally, we removed any samples with fewer than 500 reads after guild assignment and also removed any OTU with only a single read to maintain the power of later analyses to infer differences between samples.

Statistical Analyses

We compared DNA and RNA amplicon communities from the whole library and as subsets assigned (through FUNGuild) to ectomycorrhizal or saprotrophic fungal guilds. To understand patterns of diversity between transects and nucleic acid extracts, we assessed the whole library for species richness as the total number of OTUs and the Shannon’s diversity index using the *vegan* package in R . To quantify spatial community turnover (beta diversity), we also computed Bray-Curtis OTU community dissimilarities based on relative taxon abundance data with the *distance* function in the *Phyloseq* package (McMurdie and Holmes, 2013). We calculated differences across transects with linear regression and Tukey’s honest significant difference tests. Comparisons of DNA to RNA diversity metrics within transects were assessed with two-sample t-tests.

To visualize patterns of fungal community turnover as they related to tree identity, we created non-metric multidimensional scaling (NMDS) plots of Bray-Curtis dissimilarity using the *metaMDS* function in the *vegan* package, looking specifically at the ectomycorrhizal and saprotroph guilds across RNA- and DNA-extracted community profiles. We used permutational analysis of variance tests (PERMANOVA; *adonis2* function in the *vegan* package) to quantify the influence of pine (PISA angular size) and oak (QUDO plus QUAG angular size) on community composition, grouping at the genus level to assess broader above-

ground plant influence. In order to understand how much of the overall fungal community turnover could be attributed to forest canopy changes or soil core proximity, we employed Mantel tests on OTU and tree availability Bray-Curtis dissimilarity matrices using the *mantel* function in the *vegan* package.

To investigate how aboveground plant composition and soil chemistry interacted to drive fungal community turnover, we compared community dissimilarity matrices against environmental dissimilarity matrices including soil chemical data and canopy survey data, analyzing each of the same four groups as in the NMDS plots (ectomycorrhizal DNA, ectomycorrhizal RNA, saprotroph DNA, saprotroph RNA). For these analyses, we used the *bioenv* function to select the subset of environmental factors that were most strongly correlated with community turnover. In total, the list of factors selected for one or more groups included PISA angular size, QUAG angular size, QUDO angular size, pH, nitrogen percentage, soil phosphorus concentration, litter depth, and C:N ratio. From this set of predictors, we removed correlated predictors (C:N ratio, correlated with PISA, and litter depth, correlated with pH) and used all remaining predictors in a PERMANOVA test of each group to assess relative predictive power between canopy and environmental factors. For these tests, we included transect as a random predictor.

3 Results

Site properties and aboveground plant diversity

Across our soil cores, we captured substantial diversity in tree species influence and soil chemistry (Fig. 1b, Fig. 2). On average, cores in Transects 1 and 2 had the greatest influence by gray pine (PISA), with very limited to zero pine influence in transects 3 and 4, respectively. Cores in transects 1, 2, and 4 (but not 3) exhibited considerable influence of coast live oak

(QUAG), and all transects had large amounts of blue oak (QUDO), though substantial within-transect variation existed. Soil chemistry varied subtly but significantly in our study. We found significantly lower phosphorus levels in Transects 1 and 3 than in Transects 2 and 4. Nitrogen was variable across transects (Fig. 2e), with Transect 1 having the lowest percent nitrogen and Transect 3 having the highest. We found small but consistent transect-specific differences in pH, though generally all sites were near neutral (Fig. 2f).

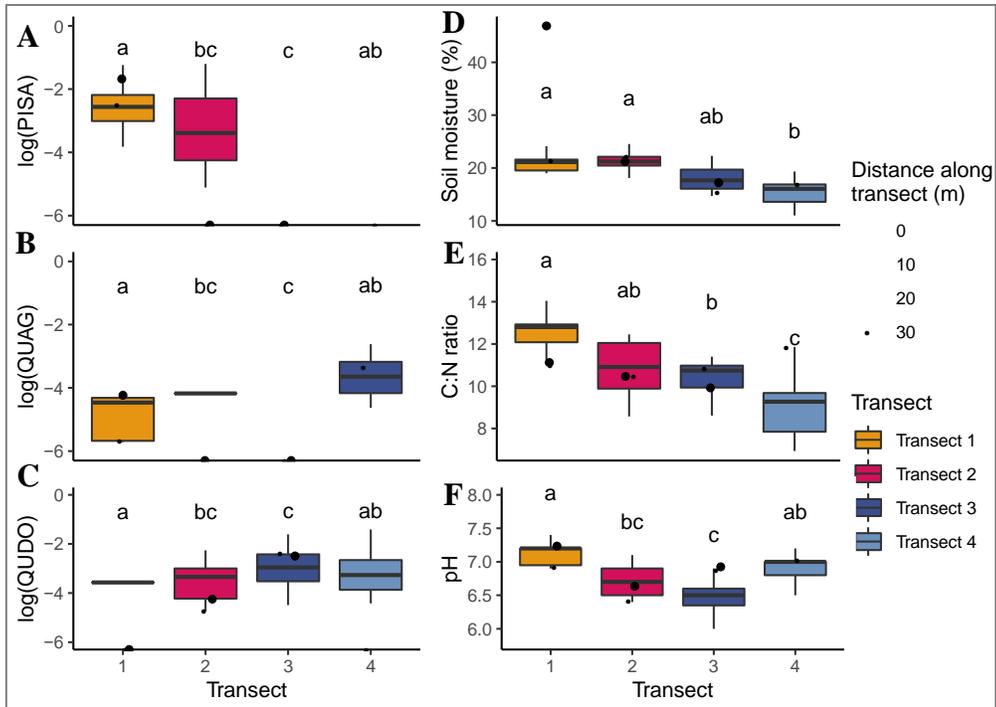


Figure 2: Aboveground plant and soil properties of transects. Panels a-c: Transects varied in the composition of their aboveground tree communities, measured in proportion of each soil core's field-of-view represented by each host type (note log transformation of the y-axis). Soil chemical properties were relatively consistent across transects with the greatest variation arising in C:N ratio (correlated with PISA). Boxplots span the 25th to 75th percentile with median values marked with the horizontal line. Whiskers depict at maximum +/- 1.5 times the IQR from the lower hinge. Data from individual soil cores are shown as points overlaid on each boxplot. Letters indicate statistically significant differences in transect means at the $p < 0.05$ level (ANOVA with Tukey's HSD correction for multiple hypothesis tests).

Both pH and C:N ratio were strongly correlated with tree-driven factors. The influence of pine was correlated with C:N with a Pearson's r value of 0.491 and a p-value of $p < 0.001$. Similarly, deeper litter depth was correlated with lower pH with a Pearson's r value of 0.392 and a p-value of $p < 0.01$. To avoid including tightly correlated parameters in later analyses, we chose to drop C:N ratio and litter depth in favor of retaining PISA angular size and pH, respectively, in our PERMANOVA tests of community dissimilarity.

Belowground fungal diversity

We successfully extracted, amplified, and sequenced 61 of 64 DNA samples and 62 of 64 RNA samples. (The five unsequenced samples were randomly distributed among our study samples.) After filtering, our dataset contained just over 15.7 million reads, with an average of roughly 127,000 sequences per sample. We found 5,264 unique OTUs in 123 samples (see Supplemental Figure 1 for species accumulation curves). Of these OTUs, 3,192 were observed in the RNA samples, 4,153 were observed in the DNA samples, and 2,450 were observed in both. Subsetting our data to ectomycorrhizal and saprotroph OTUs reduced our OTU count to 430 and 396 OTUs and 1.85million and 1.58million reads, respectively (see Supplemental Figure 2 for a visualization of all fungi in dataset). RNA and DNA samples accounted for 46 and 54 percent of the total read counts, respectively. Species accumulation data suggest that read depth was closer to saturation in the RNA communities and that the subset of saprotrophic fungi were closer than either the ectomycorrhizal guild or the total community to saturating OTU richness (Fig. S1).

OTU richness was stable across transects in the DNA communities, with no significant differences ($p < 0.05$), though Transect 3 had greater OTU abundance than the other transects

in the RNA communities ($p < 0.001$, Figure 3a). DNA communities were generally more OTU-rich than their RNA counterparts, with significantly higher richness in Transects 1 and 4 ($p < 0.05$ and $p < 0.001$, respectively). Shannon diversity of DNA and RNA within transects was similar except for Transect 2 where the RNA community was significantly more diverse ($p < 0.01$) than in the DNA samples (Figure 3b). Richness and Shannon diversity trends were similar when rarefied data were analyzed with the most notable difference being a relative increase in richness in RNA samples versus DNA (Supplemental Figure 3). The most consistent difference in measured diversity was in fungal turnover (beta diversity). The DNA communities again were indistinguishable between transects but RNA varied widely (Figure 3c). Transect 1 had the highest spatial turnover (significantly higher than T2 and T3, $p < 0.05$ and $p < 0.001$, respectively) and Transect 3 the lowest (significantly lower than T1 and T4, $p < 0.001$ and $p < 0.05$, respectively). Across transects, each RNA community had significantly greater spatial turnover than the corresponding DNA community ($p < 0.001$ for all transects).

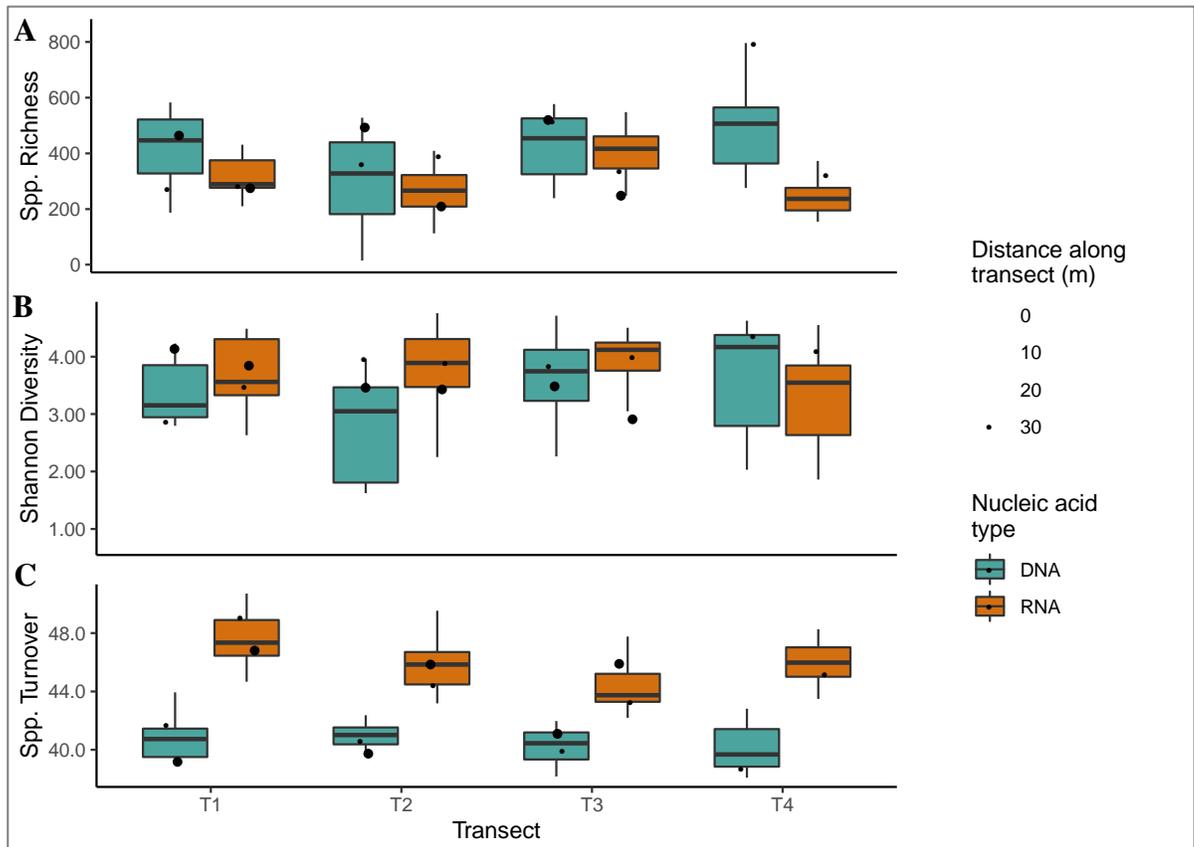


Figure 3: Alpha and beta diversity of transects. (A) Transects did not differ significantly in species richness, though DNA communities generally captured greater richness. (B) Shannon diversity was consistent among transects and nucleic acid types. (C) species turnover (beta diversity) was much higher in RNA communities than DNA communities. Boxplots span the 25th to 75th percentile with median values marked with the horizontal line. Whiskers depict at maximum +/- 1.5 times the IQR from the lower hinge. Points are scattered individual data points shaded by distance along the transect.

Drivers of spatial turnover in belowground communities

In our NMDS plots, aboveground plant diversity drove turnover in belowground diversity in fungal RNA but not DNA communities (Figure 4, Supplemental Figure 2). In the RNA samples, communities closer to a pine host separated from those closer to an oak host. Our host influence PERMANOVA revealed that, in the RNA communities, ectomycorrhizal fungal community turnover was significantly, but not strongly, predicted by both pine influence (pseudo-F = 2.794, $R^2 = 0.050$, $p < .001$) and oak influence (pseudo-F = 1.658, R^2

= 0.030, $p < .05$). The saprotrophic fungal community behaved similarly, although the influence of pine hosts was not statistically significant (pine: pseudo-F = 1.500, $R^2 = 0.027$, $p = .055$; oak: pseudo-F = 2.201, $R^2 = 0.040$, $p < 0.01$). Similar analysis of DNA-based communities did not reveal significant structuring by pine or oak host influence (Supplemental Table 1).

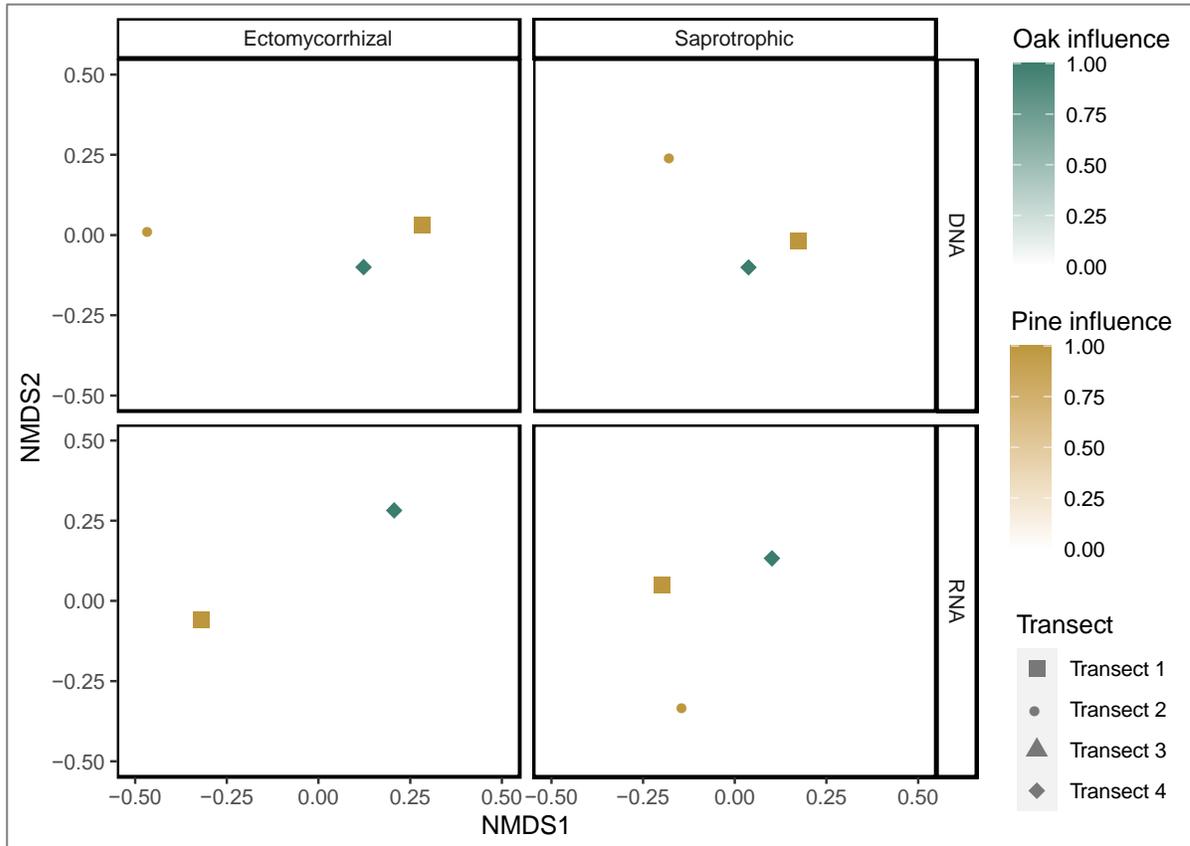


Figure 4: NMDS plots show a separation by aboveground influence in both ectomycorrhizal and saprotrophic communities when displaying RNA but not DNA community data. Colors are scaled to the maximum influence of each canopy genus in any soil core. NMDS plots were constructed using Bray-Curtis dissimilarity.

In RNA communities, the spatial turnover in fungal community composition was significantly predicted by spatial turnover in aboveground plant community composition. This relationship between above- and below-ground diversity was strongest when analyzing all

fungi in the data set (Mantel $r = 0.25992$, $p < 0.01$), although it was also significantly predictive for ectomycorrhizal fungi ($r = 0.11021$, $p < 0.05$) and showed a weaker trend for saprotrophic fungi ($r = 0.1519$, $p = 0.056$). The DNA fungal communities did not respond significantly to aboveground plant turnover (Supplemental Table 2). RNA communities from samples taken closer to one another were not significantly correlated in Mantel tests of any community subset (Supplemental Table 3). DNA-based fungal communities did not exhibit any significant spatial autocorrelation (Supplemental table 3).

Table 1: PERMANOVA results show the overlap in predictive parameters between saprotrophic and ectomycorrhizal fungi. Parameters were significantly predictive in both RNA communities but neither DNA community. Tests were performed on all transects together with transect identity integrated as a random effect.

	DNA				RNA			
	Saprotroph		Ectomycorrhizal		Saprotroph		Ectomycorrhizal	
	R^2	Pr ($>F$)	R^2	Pr ($>F$)	R^2	Pr ($>F$)	R^2	Pr ($>F$)
Gray pine (PISA)	0.0175	0.815	0.0095	0.988	0.0192	0.268	0.0269	0.028
Coast live oak (QUAG)	0.0168	0.859	0.0284	0.242	0.0202	0.235	0.0212	0.121
Blue oak (QUDO)	0.0191	0.754	0.027	0.278	0.0393	0.004	0.0223	0.095
pH	0.0136	0.964	0.0232	0.405	0.0329	0.009	0.0489	0.001
Phosphorus (mg/kg)	0.0169	0.877	0.0181	0.704	0.0186	0.348	0.0322	0.01
Percent nitrogen	0.02	0.674	0.0219	0.511	0.0434	0.002	0.0488	0.001
Residual	0.8867	NA	0.8684	NA	0.8048	NA	0.7576	NA
Total	1	NA	1	NA	1	NA	1	NA

Examining spatial turnover with the PERMANOVA allowed us to identify significant drivers of community turnover in the RNA samples, but not in the DNA samples (Table 1). None of our predictors explained more than 5% of the variation in spatial turnover for RNA-based fungal communities, however, and the entire model only accounted for 20% and 25% of the variation in saprotrophic and ectomycorrhizal communities, respectively. RNA community turnover was significantly predicted in both saprotroph and ectomycorrhizal communities by pH and soil nitrogen levels (Table 1). Additionally, saprotroph RNA communities were significantly predicted by the influence of QUDO hosts, while

ectomycorrhizal communities were correlated with PISA influence and soil phosphorus. A similar model with transect as a predictor (instead of a random effect) yielded similar results, although transect identity explained approximately 8% of the variation in all communities when included explicitly as a predictor, and effects of soil chemistry and aboveground plant community were less strong than in the main analysis (Supplemental Table 4).

4 Discussion

Understanding the ways in which aboveground diversity maps onto belowground patterns of spatial turnover in fungal communities is an important step toward predicting both the function and the vulnerabilities of forested ecosystems. We show here that both aboveground tree community composition and soil chemistry influence fungal communities regardless of fungal trophic mode, but that the chemical factors affecting symbiotic ectomycorrhizal fungi are not identical to those influencing free-living saprotrophs. These patterns were clearly discernible in the precursor rRNA communities, but were not significant for DNA communities.

Although both nucleic acid types allowed us to detect similar levels of fungal OTU richness, the precursor rRNA-based communities responded much more predictably to measured soil and plant factors than the DNA-based communities, which appeared to be mostly stochastic. This is likely due, at least in part, to the fact that precursor rRNA represents the metabolically active component of the fungal community (van der Linde and Haller, 2013; Wutkowska et al., 2019), and may therefore more closely mirror local environmental conditions. Supporting this possibility, other studies that have examined both nucleic acids in soils have also found that rRNA-based communities respond to a greater number of deterministic factors than DNA-based communities, or respond to those factors more strongly

(Romanowicz et al., 2016; Lüneberg et al., 2018; Lasa et al., 2019; Meyer et al., 2019; Nawaz et al., 2019; Wutkowska et al., 2019). In some cases, like the present study, rRNA communities are the only ones in which deterministic effects on community structure are statistically detectable (Ragot et al., 2016; Gill et al., 2017). DNA- and precursor rRNA-based communities can exhibit more similar structures and environmental responses, but studies that find this tend to be conducted at a much larger spatial scale and to target substantially greater habitat diversity than ours did (Baldrian et al., 2012; Žifčáková et al., 2016). At the relatively fine spatial scale of our study, the potentially active fungal community (precursor rRNA-based) provided more useful ecological insight than the DNA-based community.

We found that soil pH, soil nutrients, and canopy species all had significant capacity to predict community turnover in the potentially active community for both saprotrophs and ectomycorrhizal fungi, but the variance explained by these factors was small (Table 1). This may be attributable to the fact that the levels of these factors did not, themselves, vary substantially at the relatively small spatial scale of this study. In this context, it is perhaps a testament to the importance of these soil factors that their influence was detectable even within this fairly limited range of values. Detecting these factors, which subtly but significantly influenced the structure of potentially active ectomycorrhizal and saprotrophic fungal communities, allowed us to disentangle how these distinct trophic guilds responded to their shared environment. We hypothesized that symbiotic ectomycorrhizal communities would track aboveground plant composition more closely than free-living saprotrophs, but this hypothesis was only partially supported by our precursor rRNA community data. In our study system, ectomycorrhizal community turnover was significantly predicted by pine influence, a result consistent with prior work on ectomycorrhizal root tips in mixed woodlands (Smith et

al. 2009, Suz et al. 2017). The spatial turnover in saprotrophic fungi, by contrast, was significantly predicted by oaks (Table 1). Other belowground factors, such as nitrogen availability and pH, were significant predictors of both communities.

In part, effects on these two fungal guilds likely arise from the cascading effects of host trees on the soil community. While ectomycorrhizal host plants directly supply their belowground partners with carbon, they also indirectly influence the whole fungal community through root exudates and leaf litter (Aponte et al., 2010; Hugoni et al., 2018). For example, blue oak litter shapes the seasonality of nutrient deposition (Callaway & Nadkami 1991) and understory productivity (Callaway et al. 1991) in California woodlands. Indeed, in our study, we found that the pine availability correlated with soil C:N ratios, and the depth of surface litter correlated with soil pH. Soil pH is a dominant factor predicting soil microbial community structure (van der Linde et al., 2018), and soil C:N ratios indicate the relative cost of decomposition of soil organic matter and, thus, its bioavailability to free-living saprotrophs (Manzoni et al., 2010; Smith and Wan, 2019). The overlap in these resource-availability predictors between ectomycorrhizal fungi and saprotrophs underscores the niche similarity of these fungal guilds (Bödeker et al., 2016), despite their different trophic strategies. Our results emphasize the profound influence that aboveground tree communities can have on belowground fungal assemblages, both directly, through symbiotic interactions, and indirectly, by mediating soil chemistry with litter inputs and root exudates. Ectomycorrhizal and saprotrophic fungi share an environment and are affected by similar environmental factors, but these guilds perform fundamentally distinct ecosystem functions (Talbot et al., 2013; Averill et al., 2014; Lindahl and Tunlid, 2015; Bödeker et al., 2016). Understanding

how these guilds will be differentially affected by ecological stressors will be important for predicting their contributions to ecosystem function moving forward.

The strength of host identity effects in our ecosystem is consistent with results from prior studies in California woodlands, particularly studies showing the effects of oak host identity using DNA-based sampling of ectomycorrhizal root tips (Morris et al. 2008; Smith et al. 2009). Further, ectomycorrhizal communities diverged more strongly between oak and pine hosts, than between oak species, consistent with the phylogenetic distances of the host trees (Tedersoo et al. 2013). Host effects may be particularly strong in these woodlands, where the water-limited environment creates a patchwork of trees and grass cover (Figures 1, 2; Borchert et al. 1991). This habitat mosaic results in greater spatial separation (and reduced rooting zone overlap) among host trees, potentially increasing our ability to detect differences in fungal community composition because of physical separation between hosts. In denser canopies, or more speciose systems, such signals might be more difficult to detect.

Despite the influences of these deterministic environmental factors on precursor rRNA-based communities, our analyses were unable to explain more than 25% of the variance in spatial turnover of potentially active fungal assemblages. The large amount of unaccounted-for variation likely stems from a combination of deterministic factors, such as unmeasured environmental variables and biotic interactions belowground, and the stochastic and partially-stochastic processes well known to play important roles in community assembly, such as dispersal, ecological drift, and historical contingency (Peay and Bruns, 2014; Fukami, 2015; Vellend, 2016; Bogar and Peay, 2017). Although studies tend to agree that, for instance, pH and nitrogen are important in structuring fungal communities, it is not uncommon for much of the variance in fungal communities to remain unaccounted for even after modeling the

influence of soil and plant traits, especially at relatively fine spatial scales (Romanowicz et al., 2016; Ren et al., 2018) or when focusing on functionally specific subsets of the total fungal community (Kivlin and Hawkes, 2016; Glassman et al., 2017; Daws et al., 2020). Because dispersal limitation likely played only a modest role in determining community membership at the fine spatial scale of our study (tens of meters) (Peay et al., 2012; Horton, 2017), it is especially striking that we found consistent, significant variation in the potentially active fungal communities predictable with soil and plant factors. Both tree identity and soil chemistry were significant factors structuring the active fungal communities in this study, even at a spatial scale where stochastic processes also likely exerted powerful influence.

5 Conclusions

In our study, active fungal communities, characterized with precursor rRNA (ITS), responded significantly to measured ecological factors, providing insight into the linkages between aboveground canopy composition and the community structure of symbiotic ectomycorrhizal and free-living saprotrophic fungi. DNA-based communities provided little ecological insight at this scale. The turnover of ectomycorrhizal and saprotrophic communities were differentially predicted by the availability of canopy species, though both were driven by similar soil properties. The overlap in predictive factors between the ectomycorrhizal and saprotrophic guilds reiterates their degree of niche overlap and the critical nature of symbiosis in structuring these communities. Our study suggests that, at relatively fine (meter) scales, precursor rRNA may allow researchers to identify ecological factors that affect fungal community function, while DNA would obscure these relationships. Future work should investigate whether precursor rRNA-based communities are, in fact, consistently more responsive to deterministic predictors than DNA-based communities, and at what spatial and

temporal scales this is most true. In addition, manipulative experiments that isolate direct plant influences, such as symbiotic associations, from indirect plant effects on the soil environment, such as litter input, would provide valuable insight into the mechanisms driving differences between ectomycorrhizal and saprotrophic fungal community dynamics.

6 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

7 Author Contributions

LB, GR, and HM designed the study and led the sample collection effort; AS extracted all nucleic acids and prepared sequencing libraries with GR, and developed the host influence metric. LB and GR designed the analyses, and GR conducted all data processing and statistical analyses. All authors contributed to manuscript preparation.

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10 Data Availability Statement

All analyses were performed in R (version 3.6.3, [\(R Core Team, 2020\)](#)) using the R Studio interface (version 1.2.5001, [\(RStudio Team, 2020\)](#)). Scripts and data are available on [Github](#). Data files not stored on Github (due to size constraints) are available upon request from GR. Sequences are available through GenBank with Bioproject accession number PRJNA684619.

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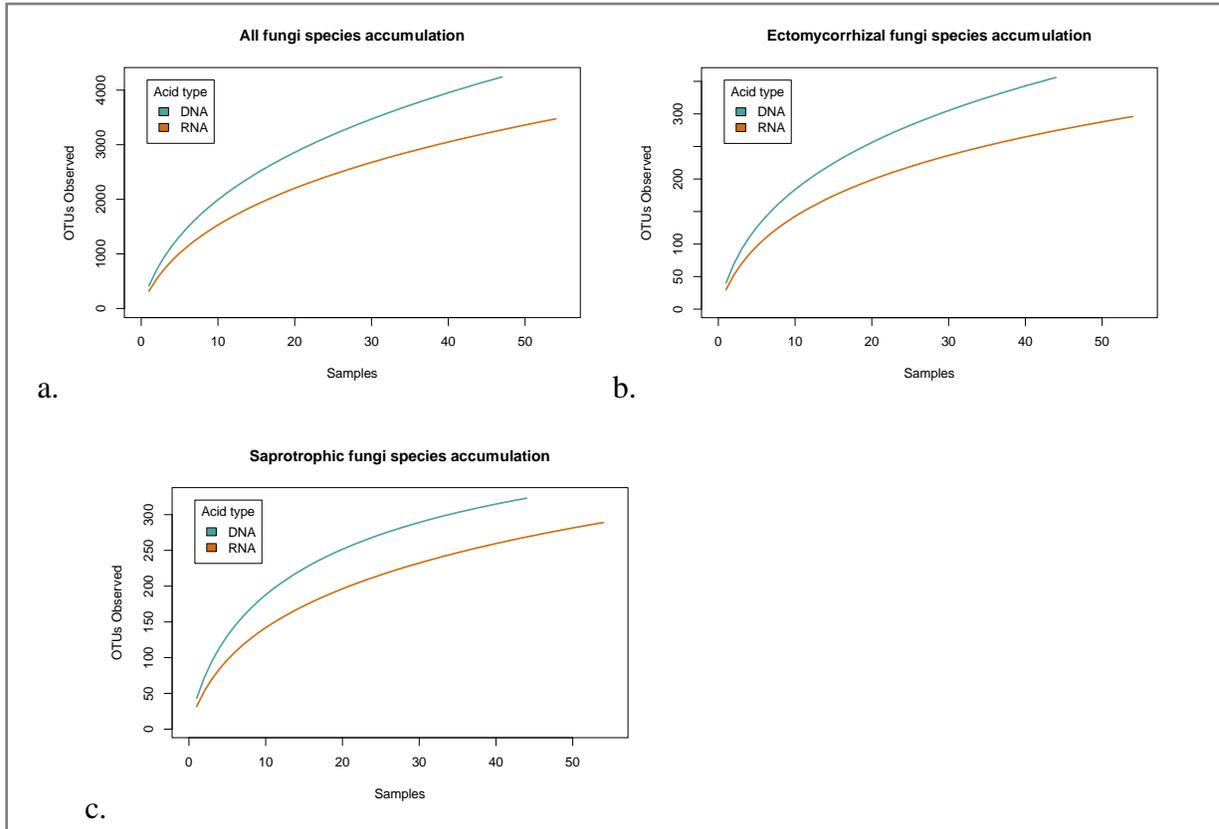
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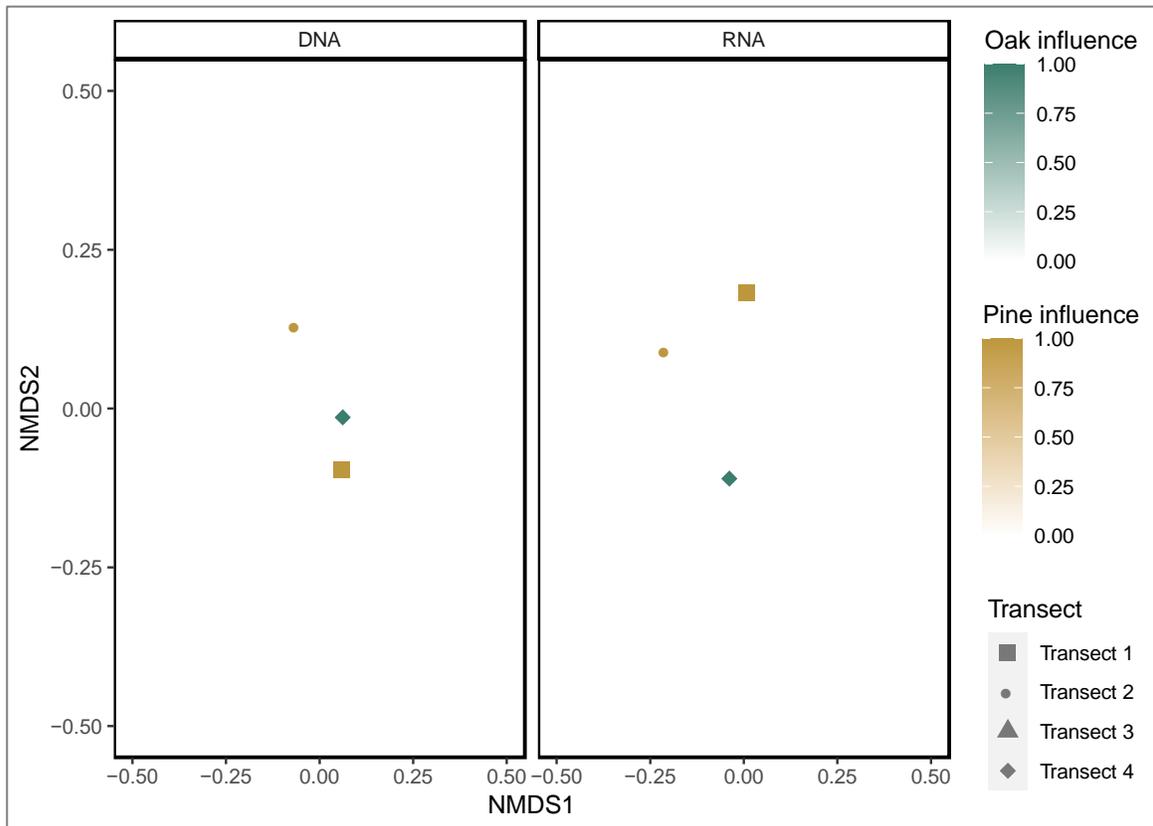
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Appendix – Supplementary Material



Supplemental Figure 1: Species accumulation curves for all fungal OTUs (a.), ectomycorrhizal OTUs (b.), and saprotrophic OTUs (c.). Neither the whole dataset nor the ectomycorrhizal communities were near to saturating OTUs, though saprotrophic species appear closer to saturation. Lines are species accumulation estimates with shaded regions showing +/- the standard error.



Supplemental Figure 2: An NMDS of all fungi, regardless of guild, shows turnover as predicted by canopy species in the RNA community but not the DNA community. These findings are similar to those found when subsetting to ‘Ectomycorrhizal’ and ‘Saprotroph’ fungi, supporting the idea that the reads that were retained through UNITE and FUNGuild were representative of the greater community.

Supplemental table 1: PERMANOVA results show the significant influence of oak on both saprotrophic and ectomycorrhizal RNA, but not DNA, community turnover. Pine influence is highly significant and marginally significant in the ectomycorrhizal and saprotrophic RNA communities, respectively. DNA community turnover was not predicted by above-ground influence. Tests were performed on all transects together with transect identity integrated as a random effect.

	DNA				RNA			
	Saprotroph		Ectomycorrhizal		Saprotroph		Ectomycorrhizal	
	R2	Pr(>F)	R2	Pr(>F)	R2	Pr(>F)	R2	Pr(>F)
Pine	0.016	0.894	0.0138	0.916	0.0274	0.057	0.0502	0.001
Oak	0.0212	0.565	0.0283	0.24	0.0402	0.007	0.0298	0.02
Residual	0.9627	NA	0.9569	NA	0.9303	NA	0.9155	NA
Total	1	NA	1	NA	1	NA	1	NA

Supplemental table 2: Mantel test results show that each of the RNA communities (all, ectomycorrhizal, and saprotrophic) were significantly positively correlated with above-ground community turnover. No DNA community turnover was significantly correlated with above-ground turnover.

	All fungi		Ectomycorrhizal fungi		Saprotrophic fungi	
	DNA	RNA	DNA	RNA	DNA	RNA
Mantel r	0.0152	0.2599	-0.0904	0.1085	0.1008	0.1785
P-value	0.412	0.004	0.934	0.048	0.821	0.043

Supplemental table 3: Mantel test results found only marginal significance in the correlations between soil core proximity and community turnover in the RNA communities. The R^2 values for these correlations were low. No DNA communities were correlated with soil core proximity.

	All fungi		Ectomycorrhizal fungi		Saprotrophic fungi	
	DNA	RNA	DNA	RNA	DNA	RNA
Mantel r	-0.0791	0.0743	-0.0672	0.0627	0.1061	0.0379
P-value	0.881	0.063	0.890	0.075	0.965	0.189