SOIL MICROBIAL ASSEMBLY AND FUNCTION IN AGROECOSYSTEMS UNDER VARIOUS MANAGEMENT AND DISTURBANCE REGIMES

by

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A DISSERTATION

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DISSERTATION APPROVAL PAGE

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Title: Soil Microbial Assembly and Function in Agroecosystems Under Various Management and Disturbance Regimes

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DISSERTATION ABSTRACT

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Title: Soil Microbial Assembly and Function in Agroecosystems Under Various Management and Disturbance Regimes

Soil microbes are key to the functioning of terrestrial ecosystems through their roles in nutrient cycling, carbon sequestration, and interactions with plants along a mutualistic to parasitic continuum. Management practices in agricultural systems impose significant disturbances to ecosystems, which can influence soil microbial assembly patterns in the rhizosphere of crop plants with implications for functional outcomes of agroecosystem productivity and stability under different management regimes. Plant-soil interactions and community composition can vary under changing environmental contexts, making it difficult to predict agroecosystem functional responses to subsequent disturbances such as climate change pressures or pathogen invasion. Drawing general conclusions regarding soil microbial responses to agricultural management across environmental and ecological contexts thus requires cross-scale investigations of soil microbial communities in agroecosystems under different management, in different regions, and under cultivation of different crop species.

My dissertation aims to unveil generalizable patterns in soil microbial assembly and function in agroecosystems under different management regimes, varying in disturbance intensity and environmental context. In Chapter II, I determine how soil microbial assembly patterns vary depending on the season and spatial scale at which microbial composition is examined in Oregon vineyard agroecosystems under different management. In Chapter III, I investigate soil fungal composition and emergent function in Mexican coffee agriculture under various disturbance regimes, drawing connections between specific functional groups of fungi and their roles governing nutrient cycling functions. My final chapter elucidates bipartite assembly patterns in plant-mycorrhizal fungal communities, and how plant community diversity relates to mycorrhizal fungal nutritive function in a prairie-pasture grass ecosystem; this ecosystem is an essential component of food systems, and together with cropland makes up more than half of global land under management. Understanding how specific practices affect plant-associated bacterial and fungal communities in agricultural soils will enhance our ability to predict agroecosystem stability under climate change, identifying vulnerable regions and cropping systems pertinent to food security and sustainability goals.

This dissertation includes previously submitted and co-authored material.

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For Maxwell, whose kindness and resilience through thick and thin
have inspired me to be a better person.

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CHAPTER I

INTRODUCTION

The stability and functioning of terrestrial ecosystems are becoming increasingly threatened under frequent and intense disturbances due to climate change, especially in agricultural systems[1]. This is because land use for agriculture has negative impacts on the diversity of all biological communities, in turn negatively impacting essential agroecosystem functions such as crop productivity and resilience to biotic and abiotic stressors[2,3]. Plant-associated soil microbial communities govern essential ecosystem functions such as nutrient cycling dynamics and plant community productivity and response to disturbances through their roles as decomposers, pathogens and mutualists. How and why soil communities respond to aboveground declines in biodiversity under land use and climate pressures, however, is not well-characterized. Efforts to enhance soil microbial functioning through sustainable agricultural practices may buffer agricultural systems against climate change pressures[4], and there is strong evidence supporting the notion that low-input agricultural management can promote complex soil microbial communities that increase resistance to drought in intensively-managed systems[5]. Despite the importance of plant-associated microbes for maintaining agricultural stability and productivity in a changing world, our current understanding of the factors that control shifts in soil microbial reveal context-dependency in ecological patterns, making predictions for stability of agricultural soils in the future difficult. A crucial component of better understanding general patterns across different ecological contexts is to identify

patterns and processes acting across broader ranges of spatial and temporal scales in diverse agroecosystems[6]. Further, understanding how shifts in microbial composition translate to emergent functional properties of ecosystems is a pernicious issue in microbial ecology, with few studies addressing both the soil microbiome and its contribution to ecosystem functions[7].

The overarching goal of my dissertation is to draw general conclusions regarding the biotic and abiotic controls governing soil microbial community assembly across diverse agricultural systems and spatial scales. To address this aim, I studied plant-associated microbes in three agroecosystems, representing some of the most valuable and prevalent cropping systems globally: vineyards, coffee plantations, and a prairie-pasture grass system. For all of these systems, I work towards linking shifts in community composition with microbially-mediated agroecosystem functions (nutrient cycling, transfer of nutrients between plants via common mycorrhizal networks, wine grape microbiomes across spatial scales). One of my predominant aims in the following chapters is to take particular care to consider how different functional groups of soil microbes such as mycorrhizal mutualists, pathogens and saprotrophs respond differently to environmental factors. This is because most studies to date examine whole-community responses to perturbations or environmental gradients, but do not address the fact that separate functional guilds and individual species likely respond differently to environmental factors and have divergent impacts on ecosystem function. The analyses and results presented herein have implications for enhancing sustainability in agricultural systems, ultimately contributing to our understanding of the response of food systems to changing climate and environmental conditions.

In the first chapter of my dissertation, I characterize the hierarchical structuring of agricultural soil microbiomes across spatial scales using a survey of fungal and bacterial communities in vineyard soils collected seasonally from 79 sites across the state of Oregon. This chapter is titled "Management-induced shifts in vineyard soil microbial composition are generalizable across scales" and is coauthored by me, Brett Youtsey, Terra Hiebert, Kyle Meyer, Emily Hill and Krista McGuire. This is the largest survey of agricultural soil microbiomes in vineyards to date. For this study, I hypothesized that the relative influence of biotic and abiotic controls over vineyard soil microbial composition depend on spatial scale, with factors such as season and regional climate contributing to spatial heterogeneity on larger scales, and soil properties and plant properties on smaller, nested scales[6,7]. Specifically, I aimed to illustrate the interactions between these factors and specific agricultural management practices, and how those interactions shift soil microbial composition across spatial scales and with grapevine phenology throughout the growing season. Through detailed analysis of soil fungal and bacterial community change under perturbations such as tillage and mineral fertilizer application, I describe the relative strength of management differences relative to regional distinctiveness and seasonality in vine-associated microbiomes.

The second chapter of my dissertation is titled "Coffee farm management drives shifts in soil enzymatic potential and fungal community composition in Chiapas, Mexico" and is currently in review with *Agriculture, Ecosystems and Environment*. This work describes the fungal communities in coffee farm soils under different management regimes and adjacent undisturbed forest soils in relation to emergent soil functional potential. This work is coauthored by me, Stacy Philpott, Rosie Gradoville, Caitlyn

Gillikin and Krista McGuire. We aimed to understand the ecological consequences of coffee farm management intensity, where increasing intensification directly correlates with declines in aboveground diversity. The overarching hypothesis for this chapter was that loss of aboveground diversity with increasing management intensity would be reflected in soil fungal communities, which would in turn reduce the nutrient cycling potential in soils for functions mediated by soil fungi. This study provides evidence for coffee farm management-induced shifts in saprotrophic fungi and their controls over essential nutritive functions in agricultural soils.

The third chapter of my dissertation examines how obligate plant symbionts – arbuscular mycorrhizal (AM) fungi – co-assemble with host plants across environmental gradients, and what differences in plant-fungal assembly patterns means for common mycorrhizal network (CMN) function. This work is a key component to a large collaborative project aiming to characterize nutrient transport between plants facilitated by AM fungi in prairie and pasture grasses. This chapter is coauthored by Hilary Dawson, Paul Reed, Scott Bridgham and Lucas Silva, with further collaboration with Jennifer Pett-Ridge and colleagues, and is titled "Local- and regional-scale mycorrhizal network assembly in an experimental pasture system". In this study, I aimed to better understand how arbuscular mycorrhizal fungal-plant co-occurrences vary across latitude, drought treatment, plant species and plant functional groups. We hypothesized that more closely related plants would share similar consortia of AM fungi, and sharing of nutrients would occur in greater magnitude within more closely related plants. Through network analyses and stable isotope probing, I provide evidence for preferential partner selection in plant-

AM fungal networks at regional-scales, and identify a subset of AM fungal taxa facilitating nutrient sharing amongst plants at the plot-level.

CHAPTER I

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CHAPTER II

MANAGEMENT-INDUCED SHIFTS IN VINEYARD SOIL MICROBIAL COMPOSITION ARE GENERALIZABLE ACROSS SCALES

Contributions

This manuscript is coauthored by Katherine L. Shek, Brett Youtsey, Terra C. Hiebert, Kyle M. Meyer, Emily Hill and Krista McGuire. I am responsible for all of the writing with comments and edits from coauthors. I also performed the majority of field and lab work for the data presented here, and all bioinformatics work for microbial sequence processing. Data analyses were collaborative effort by myself and coauthors. Supplementary material for this chapter found in Appendix A.

INTRODUCTION

Soil microbes govern fundamental ecosystem processes such as C and N cycling through decomposition, maintenance of soil stability and carbon storage, and interact directly with plants along a mutualistic-parasitic continuum[1]. These plant-microbe interactions are integral to sustainable cropping systems, and influence the resiliency of agroecosystems under environmental pressures related to climate change. Land use intensification is widely documented as having a major influence on the compositional and functional diversity of soil communities; specifically, agricultural soil management such as mechanical perturbations and increased nutrient inputs are known to strongly shift soil composition and decrease diversity[2].

The relative impacts of biotic and abiotic controls over soil microbial composition depend on spatial scale, with geophysical factors such as topography and climate contributing to community variability on larger spatial scales, and plant species identity and soil properties on smaller, nested scales [1,3]. How agricultural disturbances interact with these factors in the context of soil microbial assembly, however, is not well-characterized. Most research to date has focused on small-scale spatial variability in agricultural soil microbiomes [2,4-7]. Larger-scale interactions (i.e., across regions and climate zones) between agricultural intensification and factors related to climate and soil type remain elusive, leaving major knowledge gaps when trying to predict an agricultural system's response to shifts in regional climate patterns and management regimes. As global climate models project major shifts in annual temperature and precipitation patterns across agriculturally important regions, it is essential to strengthen our ability to predict the stability and productivity of agroecosystems under climate change[8]. Prediction can be done by identifying how management practices, climate and soil properties interact to determine shifts in soil microbial composition and function across distinct regions at a large spatial scale.

Low-input agricultural practices aim to maintain endogenous microbial functions essential to agroecosystem stability and productivity. These aims are based upon substantial evidence that agricultural intensification can have negative consequences on below-ground community dynamics. For instance, external inputs such as mineral fertilizer additions can shift the nature of soil resource pools, altering trophic dynamics in the soil microbiome and shifting plant-soil interactions in the rhizosphere via altered nutrient economy dynamics between plants and their beneficial symbionts[9]. Additionally,

practices such as intensive tilling are often regarded as beneficial to agroecosystem productivity by removing competitive weedy plant species and increasing soil aeration; however, tillage mechanically disrupts the soil environment, increases soil erosion and loss, and eliminates certain soil fungi important for nutrient cycling dynamics and soil stability[10]. Research characterizing the effects of agricultural intensification on soil microbial community structure and function has produced conflicting evidence for whether low-input agricultural practices result in greater functional diversity [11] or are completely dependent on environmental context [12,13]. Further, different functional groups of soil microbes such as mycorrhizal mutualists, pathogens and saprotrophs, may respond differently to shifts in climate and management practices, necessitating a detailed examination of spatiotemporal patterns in agricultural soil microbiomes separated by functional guilds.

Globally, low-input farming practices are becoming increasingly popular, with 71.5 million hectares under organic agricultural management in 2018, a 546% increase since 1999 [14]. The cultivation of wine grapes (*Vitis vinifera*) is one prominent example of a perennial cropping system with high incentive for transitioning towards low-input management. Wine grapes are long-lived "permanent" crops typically cultivated in shallow, well-drained soils that are low in nutrients and frequently receive fungicide or pesticide applications to counter *Vitis*-specific pathogen stress [15]. Additionally, *Vitis* plants are highly reliant on their partnerships with arbuscular mycorrhizal (AM) fungi for growth and nutrition [16]. Vineyards are clonally planted in distinct geographic regions delineated by their climate, soils and topographies (known in the US as American

Viticultural Areas, or AVAs), allowing independent comparisons to be made across climatic, edaphic and management factors.

While wine grapes are cultivated in 96 countries across the world, production in the Pacific Northwest (PNW) of the United States has been growing steadily in the last 6 decades and is now considered an emerging leader in the wine industry. There is strong momentum for vineyards in the PNW to transition to low-input practices, and nearly half of the planted acres in Oregon are currently certified sustainable or organic [17]. Cultivation of wine grapes is notoriously sensitive to changes in climate, and PNW vineyard management practices may need to adapt to increasing climate change pressures as the region is warming, causing substantial variation in wine production and quality [18,19]. Previous research has examined the effects of viticultural management practices on soil microbial communities in well-established wine growing regions such as California, upstate New York, and Europe [15,20-24], but no studies to date have extensively studied vineyard soil microbiomes across climate gradients in the PNW. Additionally, these studies are limited to single growing regions, climate zones or individual groups of microbes, and did not examine the hierarchy of spatial and environmental variables on multiple soil microbial functional groups under variable management regimes.

Recently, the application of machine learning (ML) algorithms to analyze large microbial datasets has enabled predictions of ecological outcomes such as disease occurrence in crop plants[25] and patterns of microbial responses to environmental perturbations[26,27]. There are several benefits of ML applications in microbial community analysis; first, these algorithms can identify subtle patterns in large, sparse datasets by training models on a subset of the data to learn patterns, and testing the model's ability to

predict patterns on different subsets of data. Second, ML models can identify specific features, or microbial taxa, that drive accurate predictions of patterns, ultimately distilling high-dimensional datasets containing thousands of taxa to those that are distinguishing characteristics under different environmental conditions. Using this feature selection component of ML analyses, microbial taxa contributing to variation in agricultural soil microbiomes under different management regimes can be observed even if they are relatively rare in the community. Applying ML modeling to a microbial dataset across spatial scales thus enables simultaneous identification of underlying patterns of soil microbial responses to agricultural management under varying regional climate contexts, while selecting the microbial taxa that play major roles in driving management-induced differences in soil microbial composition.

Here, we present a concomitant investigation of regional and management-induced shifts in soil microbial composition across four seasons of growth in PNW vineyard agroecosystems. We applied a ML approach to address the overarching question of whether the vineyard management practices alter soil fungal and bacterial communities in generalizable patterns across different regional and seasonal environmental contexts. We asked whether vineyard soil microbial communities are hierarchically structured, where vine genotype (cultivar, rootstock) and edaphic properties are nested within season and climate as determinants of soil microbial structure (Fig. 1A). We then aimed to identify which microbial groups are changing in relative abundances across specific management practices, including tillage and mineral fertilizer application. Using high-throughput sequencing of bacterial and fungal communities in Oregon vineyard soils collected from the same sites four times throughout the 2018

growing season, we aimed to address the following hypotheses: (1) management effects on soil microbial composition are detectable across all seasons and regions; (2) vineyard soil microbiomes are otherwise hierarchically structured, with season as the strongest predictor of shifts in soil microbial composition followed by growing region and site-level differences in soil and climate variables; (3) more intensive management practices (tillage, mineral fertilizer application) shift the composition of fungal functional groups, specifically plant pathogens, AM fungi and saprotrophs.

By analyzing shifts in soil fungal and bacterial composition across sites representing different levels of management intensity as well as climatic and edaphic gradients, we enable local- and regional-scale conclusions regarding the effects of viticultural management regimes on soil microbial assembly patterns.

METHODS

Site selection and sampling design

Soils were collected from the vine rows of 79 vineyard sites across 17 distinct growing regions in Oregon, USA (Fig. 1B). Samples were collected four times throughout the 2018 growing season to more closely examine shifts in soil microbial communities across seasons and with changes in vine phenology. Sampling began in February 2018 during the winter season until late March (vines dormant); spring samples were collected between late April and mid-May (vine buds appear); summer samples in the month of July (grapes appear/verasion); and fall samples were collected from October to late November (grapes mature, harvest).

We explicitly sampled from a variety of vineyards that ranged in management regimes including differences in tillage, fertilizer application and farm certification (i.e., certified organic, biodynamic or uncertified). An electronic survey and personal interviews with vineyard managers and owners provided specific information on management practices, as practices do not always correlate with certifications. For instance, biodynamic and organic vineyards in Oregon utilize compost-based fertilizers while uncertified, conventionally managed vineyards apply chemical fertilizers, but some uncertified vineyards use low-input practices. Low-input vineyards limit the usage of chemical fertilizers and some pesticides while leaving at least a small portion of the land unmanaged or managed with intention to promote biodiversity.

We primarily focused on sampling soil from Pinot noir, Pinot gris and Chardonnay vines as these are the most widely planted grape varieties in Oregon. However, for sites that did not grow these varieties we sampled Albarino, Syrah, Tempranillo, Barbera, Riesling and Cabernet sauvignon vines. Nearly all grapevines in Oregon are grafted onto rootstocks, and rootstock genotypes are consistent across the aforementioned grape varieties that we sampled; for instance, vines grafted to rootstock 101-14 (*V. riparia* x *V. rupestris*) are represented by all above-ground grape varieties represented in this study.

Each season, soil cores were collected to a depth of 10cm from directly next to the base of grapevines at each site for a total of 1,803 samples. We chose two rows in each vineyard block and randomly selected vines to sample along 50m-transects; all soils were collected in sterile bags with ethanol-sterilized soil cores and stored on ice in coolers until

transport back to the lab. All samples were stored at -20ºC until processing for DNA extraction and molecular analysis.

Microbial analyses

All DNA extractions were performed using the Qiagen DNeasy Powersoil HTP kits (Qiagen, Hilden, Germany) following the manufacturer's protocol. Fungal and bacterial communities were selectively amplified using the ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') primer pair for fungi [28,29], and the 16S V4 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACNVGGGTWTCTAAT-3') primers for bacteria [30]. The ITS2 and 515F primers were modified with Illumina adapter, linker and primer pad sequences with error-correcting barcodes following Earth Microbiome Project protocols, as described in [31]. Addition of two unique Nextera 8-bp barcodes on the opposite primers permitted multiplexing approximately 800 samples in each Illumina library. PCRs were performed in duplicate 25uL reactions per sample, each containing 12.5uL GoTaq Green Master Mix (Promega Corp., Madison, WI), 0.5µL each primer, 0.05µL bovine serum albumin (BSA), 9.95µL PCR-grade water, and 1µL template DNA. PCRs were run on a BioRad T100 thermal cycler (BioRad, Hercules, CA, USA) following Earth Microbiome Project thermal cycler protocols[32]. Duplicate reactions were pooled and run on a 1% agarose gel to confirm amplification and expected amplicon length. PCR products were quantified on a SpectraMax M5E Microplate Reader (Molecular Devices, San Jose, CA, USA) using the Quant-iT™ PicoGreen dsDNA Assay Kit (Invitrogen, Waltham, MA, USA).

Amplicons were pooled in equimolar concentrations and purified using the QIAquick PCR Purification kit (Qiagen). Paired-end 150bp sequencing was performed in parallel on the Illumina HiSeq 4000 platform (Illumina Inc., San Diego, CA, USA) at the University of Oregon Genomics and Cell Characterization Core Facility (Eugene, OR, USA).

Bioinformatics and sequence processing

All raw sequence reads were demultiplexed using QIIME v1.9.1 [33] before removal of primer and adapter sequences using cutadapt v1.10.1 [34]. Sequences were then quality filtered and assembled into amplicon sequence variants (ASVs) using the DADA2 pipeline (v1.10.1) with standard filtering parameters and paired-end reads merged with >= 12bp overlap and 100% sequence similarity [35]. Taxonomy was assigned using the UNITE database for fungi [36] and the SILVA database for bacteria [37].

To normalize differences in ASV count data across samples, we rarefied reads to 120,000 reads/sample for ITS data, and 72,000 reads/sample for 16S data. The final ASV table for ITS data was used to assign putative functional groups of fungi using the FUNGuild algorithm [38].

Climate and soils data

Climate data for all sites by month (elevation, precipitation, minimum and maximum temperature, mean dewpoint and minimum and maximum vapor pressure deficit) were obtained by entering site latitude and longitude data into the continuously updated

Parameter-elevation Regressions on Independent Slopes Model (PRISM) database for both 2017 and 2018[39].

General soil type data (0-25cm) were acquired for each site using GeoTIFF grids aggregated from the USDA-NCSS soil survey database (SSURGO, STATSGO) for the following variables: bulk density, cation exchange capacity, % clay, % sand, % silt, electrical conductivity, organic matter, plant available water, and pH.

Supervised machine learning

To determine whether vineyard management practices and region could be predicted based on microbial composition, we applied four different types commonly used supervised machine learning algorithms to rarified ASV tables. All models were generated and evaluated within scikit-learn (0.21.3). Two different logistic regression solver methods (L1 and L2 regularized), a support vector machine method (linear kernel), and random forest were trained to predict Till/No-Till on the same 75% split of samples collected in Fall. ROC curves were calculated based on predicted probability estimates of the remaining 25% of Fall samples. A standard scaler was applied to the ASV tables for both Logistic Regression and SVM models. After AUC values were evaluated for the four different models, L1 regularized Logistic Regression (LASSO) was chosen for all downstream analyses using a bootstrap aggregation procedure.

For bootstrap aggregation, each ASV table was partitioned into thirds. For each partition, a LASSO regression model was trained. Accuracy for each model was calculated by testing data from the other two partitions. In order to build a feature consensus of important ASVs between the three models, we established the following

criteria: a feature must have all non-zero coefficient values across the three models, and its standard deviation is less than the mean. Thereby, our bootstrap aggregation procedure selects ASVs which are relevant and have a clear positive or negative effect on prediction. We ran bootstrap aggregation of LASSO regression models separately for bacteria, fungi, and also chose three groupings of fungi to more closely examine how functional groups respond differently to regional and management variables: arbuscular mycorrhizal fungi, plant pathogens and saprotrophs.

For all models, we report results of three bootstrapped models within each subset of data and variable tested. First, we trained individual L1-regularized logistic regression models using several different groupings of data: all fungi (whole ITS dataset), all bacteria (whole 16S rRNA gene dataset), and three functional groups of fungi subsetted from the ITS dataset: AM fungi, plant pathogens and saprotrophs. Models were built for all seasons together and each season separately to test the average predictive power for growing region (AVA), vineyard certification, till/no till, and mineral fertilizer application variables. We compared these average model accuracies with three other types of classifier ML models: Random forest, support vector machine learning, and finally L2-regularized logistic regression solvers are similar to the L1 method described above, but vary slightly in how coefficients are assigned to each ASV.

We subsetted the ASVs that were consistently chosen by all three bootstrapped models for further analyses; hereafter, ‘feature taxa’ analyses. This was done using the coefficients assigned by each model to features of the microbial datasets at the ASV level. All machine learning analyses were performed in python (3.6.0).

Unsupervised/traditional multivariate statistical analyses

All ASV tables were transformed to Bray-Curtis dissimilarities using the 'distance' function in the phyloseq package[40]. We tested whether microbial composition within each season varied significantly across growing regions, management categories (vineyard certification, till vs. no till, and presence/absence of mineral fertilizer application), grape variety and rootstock using permutational multivariate analysis of variance (PERMANOVA) with the 'adonis2' function in the vegan R package [41]. PERMANOVA models were run with 999 permutations blocked by AVA with the following order of variables added sequentially: AVA, vineyard certification, till/no till, mineral fertilizer (presence/absence), grape variety, rootstock, and the interaction between all variables. Since sample numbers are not equal across all factors of each variable, we adjusted the PERMANOVA R^2 values using the 'RsquareAdj' function in vegan.

All statistical analyses were performed in R (version 3.5.3), and figures were produced using ggplot2 and ggpubr in R [42].

RESULTS

Both fungal and bacterial communities varied significantly across seasons, growing regions, vineyard management regimes, grape varieties and vine rootstocks throughout the 2018 growing season (Tables S1, S2). We observed these patterns using both traditional multivariate approaches (PERMANOVA) in addition to supervised machine learning analyses in which models are trained to classify samples into categorical variables based on microbial composition, and measures of accuracy and

precision of these classifications indicate underlying variation in microbial composition (Table S1).

Permutational multivariate analysis of variance across microbial groups

When we applied traditional multivariate analyses to test whether vineyard soil fungal and bacterial community composition varied significantly across variables, there were significant differences across seasons, which was largely driven by the major differences in winter compared to all other seasons (Table S2). This result was further supported by the aforementioned decrease in average accuracies across ML models for the winter season compared to other seasons for nearly all AVA and management variables (Fig. S1).

ML model performance

We first validated our choice of machine learning algorithm by testing the performance of several types of ML models on the Fall ASV dataset, including two different logistic regression solver methods (L1 and L2 regularized), random forest, and a support vector machine method. All model types performed well classifying samples into till/no till categories with the full fungal dataset, producing consistent and high values for accuracy, precision and recall across models (AUC values >0.94, Fig. S1). We chose the L1-regularized logistic regression solver for running all further ML analyses because the regularization methods reduce overfitting, constrains parameter complexity and is relatively straightforward assigning coefficients to 'feature' ASVs, or ASVs that serve as meaningful predictors in classification[26]. The L1-regularization method – also known as

lasso regression - was selected over the L2 method (ridge regression) – due to nearly identical accuracy results (Fig. S2), and the more straightforward interpretation of feature taxa selected by L1-regularized models.

We show that AVA (growing region) of a vineyard site could be predicted with 78-90% accuracy for all fungi and 81-88% accuracy for bacteria across all seasons (Fig. 2, top left panel). The functional groupings of fungi had slightly lower average accuracy, ranging from 69-75% for AM fungi, 76-84% for plant pathogens and 77-86% for saprotrophs. For all groups except AM fungi, accuracy of models predicting AVA was lowest in the winter season, with most models performing significantly better in the spring, summer and fall seasons; this seasonal trend was consistent across the management variable models as well (Fig. 2). Vineyard management certification model performance was relatively low, with accuracies ranging between 29-74% across seasons for all microbial groups (Fig. 2, top right panel). However, model performance for classifying certain management variables - tillage and application of mineral fertilizer - was high for all groups across all seasons, ranging from 63-84% accuracy (Fig. 2 bottom panels). When interpreting ML results, it is important to consider not just the classification accuracy of each model, but also the precision and recall performance metrics, accounting for type I (false positive) and type II (false negative) errors. We report the accuracy, precision and recall for all L1-regularized models in Table S1.

Feature selection by ML models

An added benefit of the L1 regularized logistic regression approach is the straightforward selection and interpretation of microbial ASVs that drive underlying differences across

different treatments, also known as 'feature ASVs'. For the bacteria dataset, tillage models selected 133 feature ASVs and mineral fertilizer models selected 139 feature ASVs (Fig. 3A). For the 'all fungi' dataset, there were 71 feature ASVs selected across seasons for till/no till models and 91 feature ASVs for models classifying samples into mineral fertilizer/no mineral fertilizer categories (Fig. 3C). Notably, most of the feature ASVs selected for till/no till and mineral fertilizer models were relatively rare in the dataset, with half of the feature taxa appearing with <3200 reads and <1200 reads across all samples for fungi and bacteria, respectively (Fig. 3). This importance of rare ASVs was further supported by a significant decrease in model performance when we applied several prevalence thresholds to the data; removing ASVs that were not present in at least 10% of samples in a dataset decreased classification accuracy by approximately 5% (Fig. S3).

We then tested whether the relative abundances of feature ASVs varied with environmental variables, including monthly climate variables and site-level soil physicochemical properties such as pH, texture and bulk density (Fig. 4). Several fungal feature ASVs correlated significantly with soil physicochemical variables, namely cation exchange capacity, bulk density, and electrical conductivity (Fig. 4A). There were fewer bacterial feature ASVs that significantly correlated with environmental variables, most of which belong to the Proteobacteria and correlated positively with all climate variables except precipitation (Fig. 4B).

Fungal functional groups

We ran the same L1-models for fungal data subsetted for three major functional groups of fungi identified by the FunGUILD algorithm[38] (AM fungi, plant pathogens and saprotrophs) and selected feature ASVs as previously mentioned. We present the feature importance for all functional group feature ASVs and the category of tillage and mineral fertilizer variables each ASV correlated with in each model, both of which are representations of the magnitude and direction (respectively) of the coefficients for each ASV across models (Figs. 5, 6, 7). For plant pathogens and saprotrophs, there were several feature ASVs selected across each set of models (plant pathogens: 30 tillage features, 37 mineral fertilizer features; saprotrophs: 36 tillage features, 42 mineral fertilizer features), so we focused feature ASV analyses for these groups on ASVs that were detected as features in at least 2 seasons of our dataset.

For AM fungi, there were 11 feature ASVs selected across mineral fertilizer models, and 4 for tillage models, most of which were identified to the genus level (Fig. 5). *Entrophospora infrequens* was identified as a feature taxon for both tillage and no mineral fertilizer practices, and *Rhizophagus irregularis* was a feature taxon for tillage and mineral fertilizer practices (Fig. 5).

Fungal ASVs identified as putative plant pathogens and detected as features in at least two seasons for mineral fertilizer and tillage models were taxonomically affiliated with 10 genera across 14 ASVs for tillage models and 13 ASVs for mineral fertilizer models. Notably, 3 of the mineral fertilizer feature ASVs in the plant pathogen dataset were *Gibberella intricans* and 3 of the no-till feature ASVs were identified as *Endophoma elongata* (Fig. 6). There were fewer plant pathogen feature ASVs consistently detected across multiple seasons for the classification of tillage samples, 2 of

which were identified as *Clarireedia bennettii*. The genus *Coniochaeta* was represented by 4 of the ASVs in the plant pathogen features (Fig. 6).

There were 18 feature ASVs from the mineral fertilizer models and 15 features from the tillage models in at least two seasons for the saprotroph dataset, representing 11 genera (Fig. 7). Most of the features that were important in mineral fertilizer models were identified as *Geminibasidium* and *Mortierella* ASVs. There were several feature ASVs identified as *Hormonema viticola*, a saprotrophic yeast known to colonize *Vitis vinifera*, for both mineral fertilizer and no till management practices across all seasons except Winter (Fig. 7). There were only four features that correlated with tillage practices, all from the *Tetracladium* and *Trematosphaeria* genera (Fig. 7).

DISCUSSION

We found that vineyard soil fungal and bacterial composition significantly differs throughout the growing season, across growing regions, and across different agricultural management regimes. These results together suggest that vineyard soil microbes exhibit both biogeographic and seasonal patterns, and that specific management practices outweigh these factors in shaping vineyard microbial community structure. This result reveals the strength of the effects of agricultural management on soil microbial community dynamics across both space and time. Previous studies in the Napa Valley growing region (CA) found similar structuring of vineyard soil bacterial communities, with management practices and geography structuring bacterial composition through their direct influences on soil properties [20,43]. However, these studies were done independently, and the relative roles of management and geography were not explored.

Our results provide a broader understanding of how these factors hierarchically impact soil microbial dynamics by integrating greater spatial scales (all growing regions in OR) and how seasonal differences in vine phenology and climate differentially drive shifts in microbial composition.

The interactive effect that we found between season and management, where management-induced shifts in fungal and bacterial composition predominates seasonal variability in all seasons except winter, implies that sampling time must be taken into consideration when aiming to understand vineyard microbial community dynamics. Winter is the wet season in Oregon, and *Vitis* plants are in a state of metabolic dormancy throughout winter, with no photosynthetic activity or accumulation of biomass. This ecological strategy of entering a state of reversible dormancy when environmental conditions are suboptimal is also common in soil microbes, which can maintain biodiversity and coexistence of competing species [44]. Thus, it is likely that the dormancy of *Vitis* plants in the winter season was reflected in belowground communities, resulting in more similar relative abundances of microbial groups regardless of management. In these conditions, the majority of soil microbes are metabolically inactive due to unfavorable environmental conditions and lack of nutrient inputs from *Vitis* root exudates and photosynthetic activity. This is particularly the case for soil microbial groups that are tightly linked with plant activity, such as AM fungi and plant pathogens, which obligately and facultatively rely on the host plant fixing carbon for nutrient acquisition and survival [45]. Stressful conditions during winter months may also result in soil microbial death, leaving extracellular relic DNA in the environment that our sampling methods would not have disentangled from DNA in intact cells. Indeed, a recent study characterizing soil

microbial composition found greater dissimilarity between samples after removing relic DNA[46]. By this reasoning, dormant and dead microbes may be masking the management-induced shifts in microbial composition in our winter dataset. This is supported by the decline in machine learning model accuracy with the winter dataset as compared to the other seasons, but further investigation is required to determine whether this phenomenon is due to relic versus intact DNA, microbial dormancy, or another unmeasured variable.

The consistently high values for accuracy, precision and recall across the L1-regularized logistic regression models classifying samples into AVAs indicates strong underlying patterns in vineyard soil bacterial and fungal communities across geographic regions that are distinct in their climate, soils and topography. These patterns of regional distinctiveness in microbial communities have been shown in vineyard soils and grape skin microbiota from California[43,47,48], Italy[49,50], and Chile [51,52], and biogeographic patterns in soil microbial communities have been studied extensively in other systems[53-56]. Here we show that vineyard soil microbial communities in Oregon exhibit biogeographic patterns across AVAs, and this can be detected across the major stages of vine phenology despite major seasonal shifts in soil microbial composition.

We also found that specific viticultural management practices – tillage and application of mineral fertilizer – can be predicted from soil fungal and bacterial composition with nearly the same levels of accuracy, precision and recall as AVA. This result confirms our first hypothesis, that management predominates as a significant driver of soil microbial composition across all spatial scales, and reveals the scale at which vineyard soil communities respond to differences in management regimes, implying that the effects of tillage and mineral fertilizer practices may be generalizable across different

regional soil and climate properties. This strongly suggests that management practices in agroecosystems must be addressed when considering the regional distinctiveness of soil microbial communities. Most in-depth studies to date that investigated the effects of vineyard management on soil and vine-associated microbial communities were within a single growing region[15,22,23], making it difficult to draw general conclusions given the notion that soil biota may respond differently to management depending on soil and climate contexts. A few studies in Napa Valley (CA) and Italy examined how vineyard bacterial communities respond differently to management across growing regions, and found that the effects of management on soil bacterial diversity were most pronounced at the scale of sub-growing region or site level [20,49]. Our study revealed major effects of tillage and fertilizer management at a broader spatial scale (all growing regions in OR) through application of statistically robust machine learning algorithms. The fact that our ML models classifying samples into till/no-till and mineral fertilizer categories performed with high accuracy, precision and recall, and consistently identified the underlying fungal and bacterial sequence variants differentiating management practices across growing regions and seasons, implies that the same microbial groups are responding to management practices across space and time.

A notable result from our study was the distribution of bacterial and fungal feature ASVs across the dataset; nearly all of the feature ASVs consistently selected by till/no-till and presence/absence fertilizer models were rare. This finding is important from a methodological perspective in addition to ecological: first, most normalization methods for microbial data impose a prevalence threshold and/or filter out rare taxa prior to ML or other multivariate analyses. This would have removed the features driving

major differences in our dataset, and decreased overall model accuracy when we imposed prevalence thresholds. Second, it revealed the utility of the ML feature selection approach to winnow down large, complex microbiome data to only those distinguishing features in the fungal and bacterial communities that respond to particular management practices. Ecologically rare taxa driving major differences across management practices in vineyard soils across broad spatial scales suggests that the *Vitis* crop may select for a small subset of the soil microbiome, but that those taxa are sensitive to mechanical (tillage) and chemical (inorganic fertilizer) perturbations. A recent study examined the roles of rare fungal taxa in a maize-wheat/barley system, and found that the majority of crop-associated fungi were rare taxa that were mostly structured by the crop plant host rather than environmental variability, which is consistent with our findings [57]. They also found that the rare sub-community contributed to co-occurrence networks and soil enzyme activities, implying a key role of rare taxa in the crop-associated microbiome. Together, these results suggest that the rare subcommunity of soil microbes might play essential roles in crop and agroecosystem functions, necessitating a closer look at the contributions of rare taxa across other managed and unmanaged ecosystems.

The arbuscular mycorrhizal (AM) fungal models were able to classify samples into till/no-till and presence/absence of mineral fertilizer categories, which suggests AM fungal composition varies with these practices which is consistent with a long history of AM fungal research in cropping systems. The AM fungal mutualism depends upon reciprocal exchange of nutrients, and application of mineral fertilizer directly supplies the plant with labile nutrients, which in turn can reduce the plant's need for mycorrhizal nutritive services [58]. Additionally, tillage practices mechanically disturb the soil

environment, destroying AM hyphal networks and selecting for disturbance-tolerant AM fungi [59]. Generally, increasing the intensity of management (through tillage and labile nutrient addition) has been shown to reduce AM fungal functional diversity, with implications for agroecosystem functioning and productivity [6]. Due to the high reliance on AM fungal partners in *Vitis* spp., our results imply that the consequences of more intensive practices on AM fungal composition likely have lasting negative effects on the functioning of the mycorrhizal symbiosis in Oregon viticulture.

We found consistent responses of putative plant pathogenic fungi to mineral fertilizer and tillage practices across all seasons and growing regions; feature ASVs for the plant pathogen dataset revealed directional responses of fungi identified as *Gibberella intricans* and *Endophoma elongata* to mineral fertilizer application and no-till soils, respectively. *G. intricans* has been shown to cause vascular wilt disease on *Vitis vinifera* plants as its anamorph *Fusarium equiseti* in a vineyard in Spain[60], suggesting that mineral fertilizer application may promote survival of this pathogen potentially causing disease in host grapevines. Interestingly, multiple ASVs identifying to *E. elongata* were strongly correlated with no-till soils, but there is little evidence of this taxon causing disease in *Vitis* plants; it is identified as both a putative plant pathogen and undefined saprotroph by FunGUILD, and requires further research to confirm its hypothesized functional roles in agricultural soils. The recently described *Clarireedia bennettii* was assigned to two of the feature ASVs for tilled soils, which is a known pathogenic species occurring primarily on *Festuca rubra* which is also used as a cover crop in Oregon vineyards [61].

The most frequent saprotrophic fungal feature ASV detected in our till/no-till and fertilizer models was *Hormonema viticola*, a saprotrophic yeast closely related to

Aureobasidium spp. that is named for its frequent isolation from host *Vitis vinifera*. This species complex was recently highlighted as an emerging fungus responsible for grapevine trunk disease as it was the most prevalent on diseased grapevines in Portugal [62]. In our study, *H. viticola* correlated with application of mineral fertilizer and no-till systems, but it is not clear whether the fungus is residing as a saprotroph or pathogen causing grapevine trunk disease. Several *Mortierella* spp. were also detected in the saprotroph feature ASVs in correlation with mineral fertilizer application; these fungi are commonly found in agricultural soil inoculants due to mounting evidence of their plant growth promoting properties as root endophytes or indirectly through increasing bioavailable nutrients[60]. There is, however, context dependency in the life history of several saprotroph-endophyte-plant pathogenic fungal taxa, and experimental research across environmental conditions is necessary to further elucidate the roles of these groups under different agricultural contexts. The plant pathogen and saprotroph features identified here provide a robust seasonal and regional basis for future studies examining the effects of agricultural practices on crop pathogen ecology and management.

CONCLUSIONS

Our results reveal the spatial scale at which agricultural management practices can be predicted based on shifts in soil fungal and bacterial composition, and the importance of considering season of sampling when deciphering the effects of geography and management on soil microbial assembly. We also highlight the importance of focusing on specific agricultural practices (i.e. tillage, fertilizer application) rather than farm certification variables, as certifications do not always represent differences in

management across sites. The results presented here support our predictions; first, samples were classified into tillage and fertilizer application practices with nearly the same level of accuracy as geographic region. Second, season was a strong predictor of shifts in microbial composition, and the PERMANOVA analysis revealed that growing region, climate and soil characteristics structure bacterial and fungal communities in Oregon vineyards. Finally, we show that fungal functional groups respond differently to vineyard management regimes, with specific groups of mycorrhizal fungi, plant pathogens and saprotrophs shifting under tillage and fertilizer practices. Markedly, we found that members of the fungal and bacterial communities with the greatest feature importance distinguishing till vs. no-till and presence/absence of mineral fertilizer were relatively rare in the datasets. Our study shows the utility of machine learning methods to distill complex microbial datasets to features meaningful to specific treatments or ecological outcomes. Our feature taxa analyses provide a basis for future studies investigating the interactive effects of agricultural management and regional climate and soil differences across spatial scales.

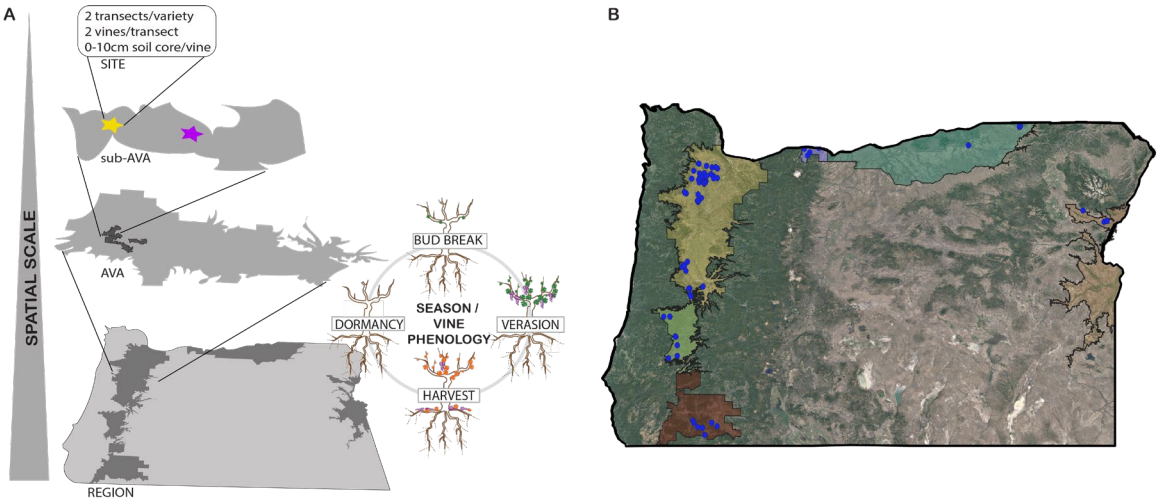

Figure 1. (A) Sampling design of vineyard soil microbial communities across different spatial scales and across seasons. All sites were visited each season, and each season represents a distinct stage in vine phenology: dormancy in winter, bud break in spring, verasion in summer and harvest in fall. (B) Distribution of sampling sites across major growing regions of Oregon; blue dots indicate individual sites and polygons show major AVAs.

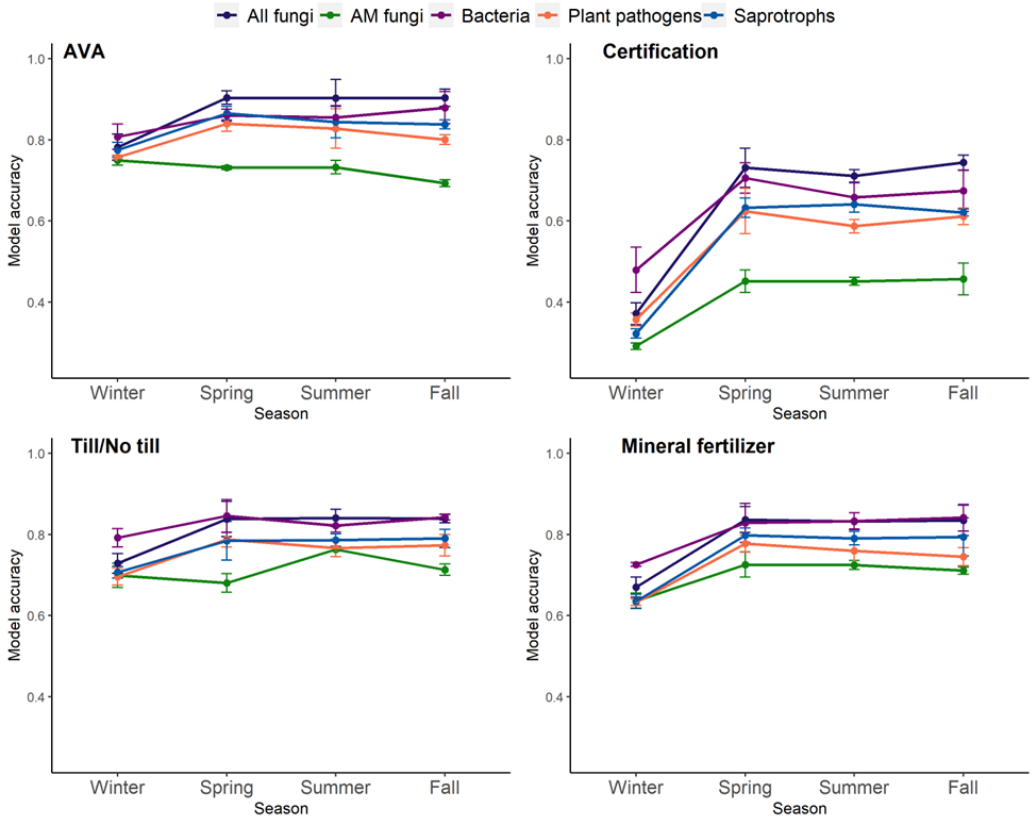

Figure 2. Model accuracy (0-100%) for bootstrapped L1-regularized logistic regression classifiers across the four seasons sampled for region (AVA, top left) and management variables (vineyard certification, tillage practices and presence/absence of mineral fertilizer). Error bars show standard error across three models, and colors indicate the different microbial groups tested.

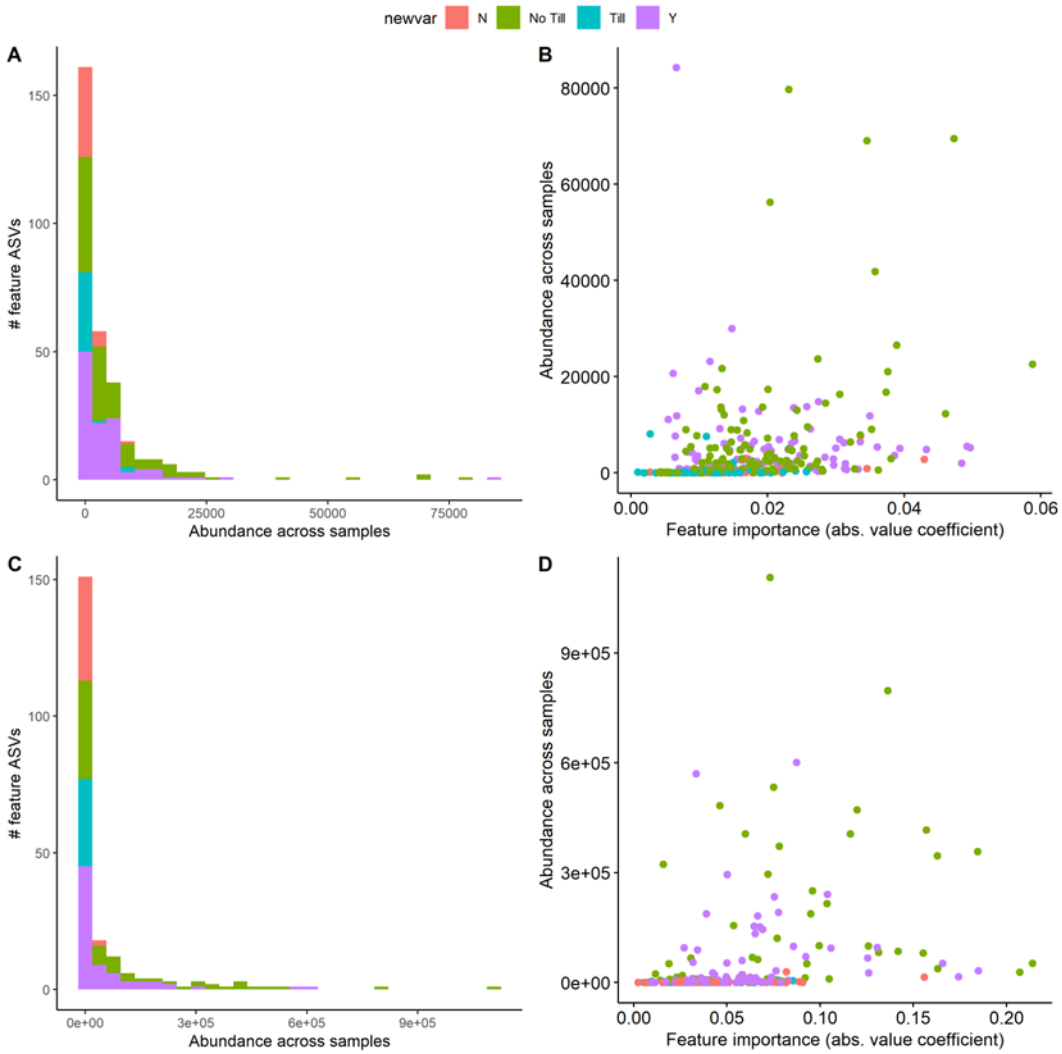

Figure 3. Abundances of feature ASVs across all samples in the bacteria (A, B) and all fungi (C,D) datasets for till/no till and mineral fertilizer variables are relatively rare across all samples (A and C); the few that are prevalent were selected for No till and Mineral fertilizer variables (B and D) ('N' = no mineral fertilizer, 'Y' = mineral fertilizer).

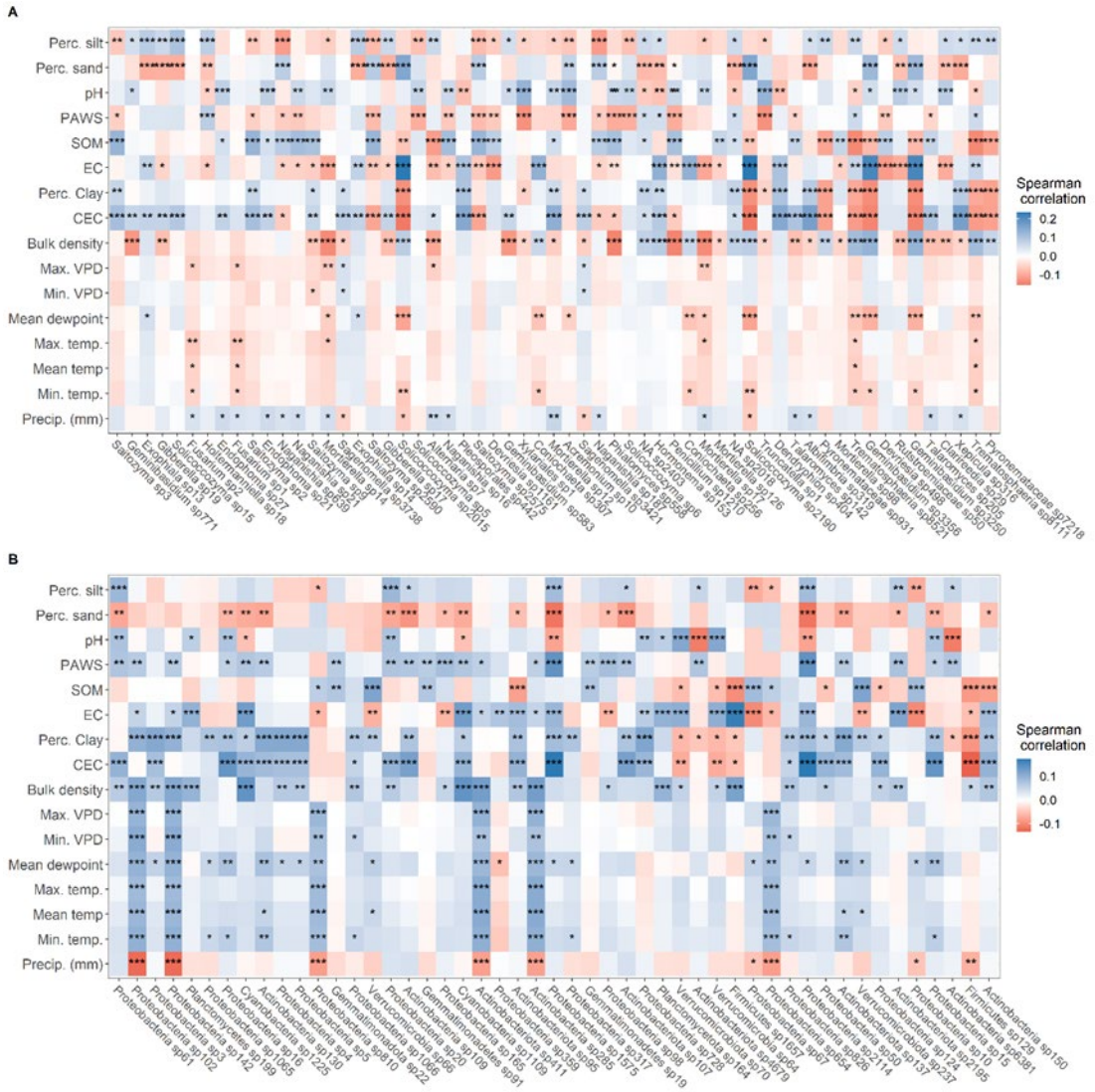

Figure 4. Relative abundances for feature ASVs from the 'all fungi' dataset (A) and bacteria dataset (B) correlate significantly with monthly climate variables and site-level soil properties. Only ASVs detected as feature ASVs for mineral fertilizer and tillage categories in at least 2 seasons included. Taxonomy is given for feature ASVs on X axis, and ordered by increasing feature importance, with the lowest values on the left increasing to the right (range 0.0095 – 0.2138 fungi and 0.0028 – 0.0587 bacteria). Colors indicate the strength of correlation and stars indicate the level of significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

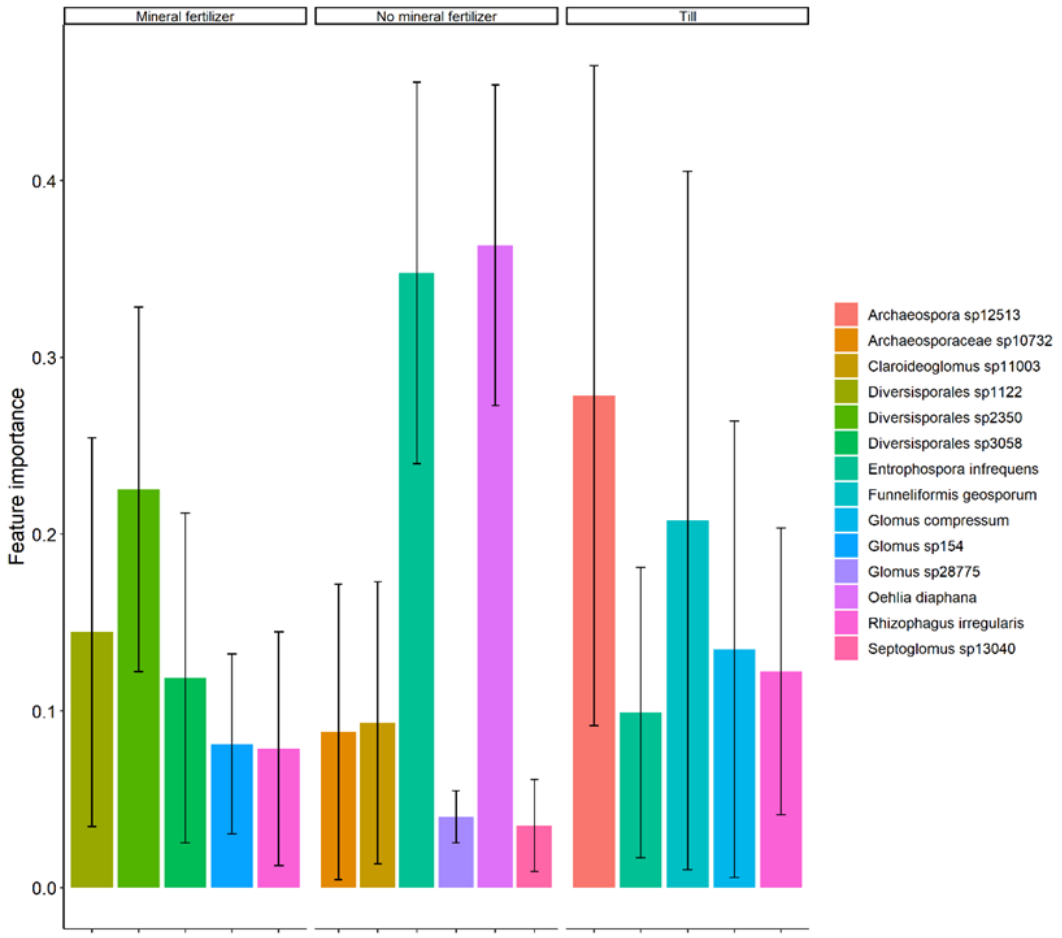

Figure 5. Feature ASVs selected by the tillage and mineral fertilizer models for AM fungal data. Error bars indicate standard deviation of coefficients/feature importance across three bootstrapped models. Panels indicate the variables each feature ASV correlated with (mineral fertilizer, no mineral fertilizer and tillage).

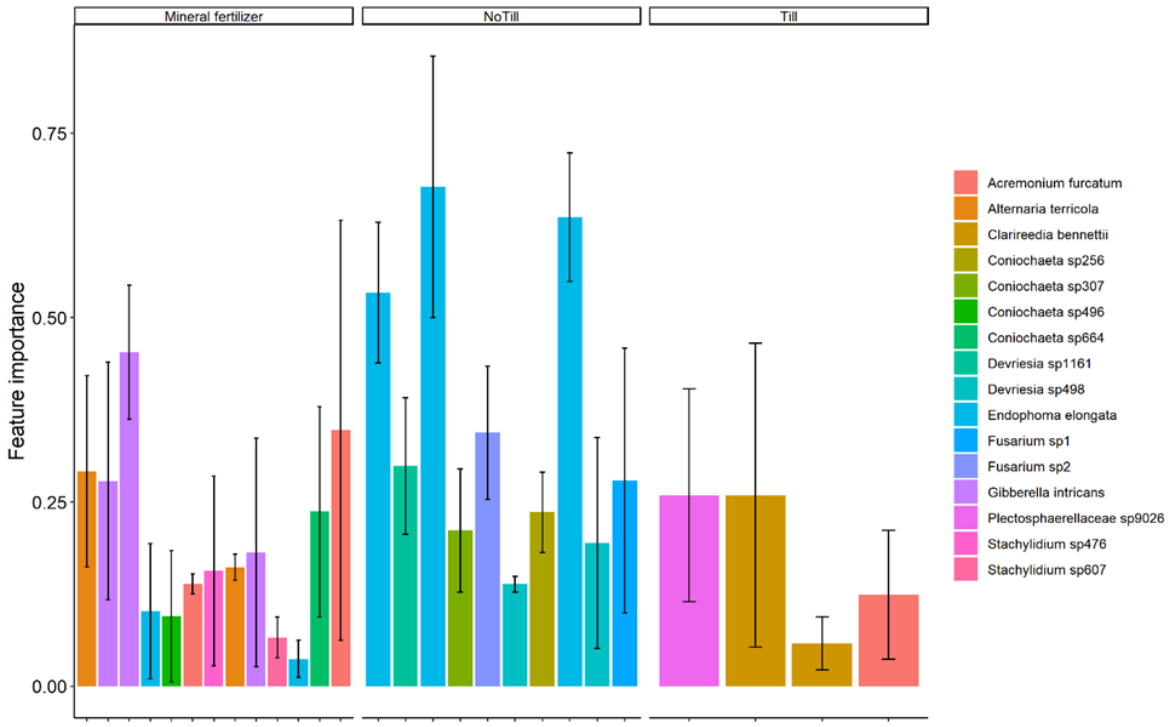

Figure 6. Feature ASVs selected by the tillage and mineral fertilizer models for fungal ASVs assigned as plant pathogens by the FunGUILD algorithm. Error bars indicate the standard deviation of coefficients across three bootstrapped classifier models. Panels indicate the variables that each ASV correlated with (Mineral fertilizer, No Till and Till). Only ASVs detected as feature ASVs for mineral fertilizer and tillage categories in at least 2 seasons included.

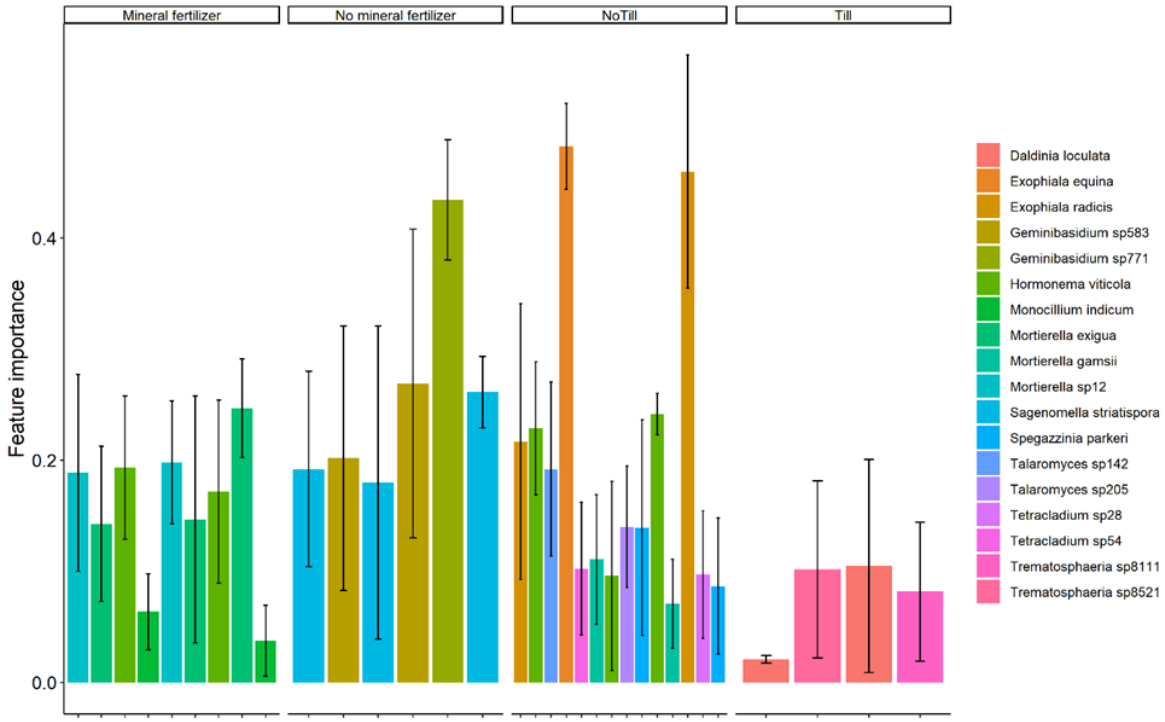

Figure 7. Feature ASVs selected by the tillage and mineral fertilizer models for fungal ASVs labeled as saprotrophs by the FunGUILD algorithm. Error bars indicate the standard deviation of coefficients across bootstrapped classifier models. Panels indicate the variables that each ASV correlated with (Mineral fertilizer, No mineral fertilizer, No till and Till); only ASVs detected as feature ASVs for mineral fertilizer and tillage categories in at least 2 seasons included.

CHAPTER II

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CHAPTER III

COFFEE FARM MANAGEMENT DRIVES SHIFTS IN SOIL ENZYMATIC POTENTIAL AND FUNGAL COMMUNITY COMPOSITION IN CHIAPAS, MEXICO

Contributions

This chapter is submitted to *Agriculture, Ecosystems and Environment* as coauthored material with K. L. Shek, S. M. Philpott, M. R. Gradoville, C. Gillikin, and K. L. McGuire. All coauthors contributed to collecting samples and performing lab work for the dataset, while all analyses and writing are my own with comments and edits from coauthors.

Supplementary material for this chapter found in Appendix B.

INTRODUCTION

Significant declines in biodiversity in areas where land has been converted for agricultural use are tightly linked to the growing concerns regarding food supply for a growing population, particularly in tropical regions (Gibbs et al., 2010). Tropical agriculture's contribution to biodiversity loss largely depends on the intensity and type of agriculture, with highly intensified production causing major losses in local and regional biodiversity (Matson et al., 1997; Foley et al., 2005; Philpott et al., 2008). However, the majority of this research has focused almost exclusively on macroorganisms, with few

studies addressing the impacts of agricultural intensification on microorganisms in the tropics (Waldrop et al., 2000). This lacuna in knowledge stems partly from the limitations in sampling methodologies, as microbial diversity is greater than macroscopic species diversity by several orders of magnitude and is notoriously difficult to assess (Curtis et al., 2006; Lozupone et al., 2007; Peay et al., 2008). However, growing sophistication of molecular techniques has opened up new avenues for studying microbial patterns and processes (Caporaso et al., 2012; Foster et al., 2012), which will be vital indicators of the ecological consequences of agricultural land use in tropical ecosystems. Since soil microbes govern essential ecosystem processes upon which higher trophic levels depend – including nutrient cycling - changes in the composition and/or biochemical activities of soil microbial communities due to land use intensification may have cascading effects on the overall functionality of the ecosystem (Johansson et al., 2004; Bever et al., 2012; Bender et al., 2016). Specifically, soil fungi act as valuable indicators of soil fertility, structure and suitability for crop production due to their functional roles as decomposers, pathogens and mutualists in the rhizosphere of crop plants (Frąc et al., 2018).

In Central and South America, Arabica coffee agriculture (*Coffea arabica* L. Rubiaceae) is a salient concern for conservation biologists, as it accounts for 12% of arable land (FAO, 2020) and ranks as the second most traded commodity in the world behind petroleum (Donald, 2004; Ricketts et al., 2004; Tscharntke et al., 2011). Traditionally, *C. arabica* is grown as an understory shrub in tropical agroforestry systems consisting of diverse assemblages of trees similar to unmanaged forests (Perfecto and Vandermeer, 2002). However, some coffee farmers have intensified management of coffee plantations by removing overstory vegetation and implementing use of chemical fertilizers and

pesticides in order to increase productivity of the coffee plants and overall yield per harvest (Moguel and Toledo, 1999). Thus, the significant variation in the intensity and scale by which coffee is cultivated and managed has profound impacts on the associated biodiversity of the agroecosystem (Perfecto and Vandermeer, 2002; Philpott et al., 2008). For instance, farms managed traditionally with more organic practices, maintaining a canopy of diverse and dense shade trees, include leguminous trees that form symbiotic relationships with nitrogen-fixing bacteria, thereby reducing the need for synthetic nitrogen fertilizers. On the other end of the continuum, coffee is grown as a high-density monoculture and is often correlated with heavy use of external chemical inputs with little to no overstory vegetation. Understanding how these disparate management practices affect soil fungal communities, soil functional potential, and soil physicochemical properties has important theoretical and practical applications. For example, quantifying microbially-mediated nutrient cycling rates can inform decisions about how much compost or fertilizer needs to be applied to the soil for maximal crop productivity (Elliot et al., 1997; Doran and Zeiss, 2000). Similarly, evaluating soil fungal composition and functional potential across agroecosystems under different management regimes will aid in predicting the resilience and stability of these systems under further disturbances such as drought and fire.

Previous studies in coffee plantations have found that increasing management intensification affects soil variables such as earthworm abundance and soil stability (Perfecto and Vandermeer, 2002; Philpott et al., 2008). While few studies have examined the relationships between coffee farm characteristics, soil physicochemical properties, and soil microbial composition, the effects of coffee management on soil functional

potential are not as well characterized (Duong et al., 2020). It is likely that microbial composition and nutrient cycling functions will be affected by differences in agricultural management, as the biotic and abiotic factors that change with increasing management intensity such as soil porosity, pH, and plant community composition, are factors that structure soil microbial communities in unmanaged ecosystems (Fierer et al., 2009; Hartmann et al., 2015). Additionally, intensive management practices in non-coffee agricultural systems can alter soil microbial composition and function (Waldrop et al., 2000; Johnsen et al., 2001). However, microbial responses to variation in agricultural management are context-dependent, with shifts in composition and function depending on soil type, climate, and the particular agricultural system being examined (Bossio et al., 1998), which makes extrapolating general agroecological responses challenging. An additional complicating factor in making belowground predictions is that the relationship between increasing management intensity and decreasing biodiversity is not always linear. Non-linear responses, or threshold effects, have been demonstrated in a number of habitats (With and King, 1999; Swift and Hannon, 2010), and if these effects extend to the belowground soil community, then at some threshold level of biodiversity loss, soil microbes may be vulnerable to drastic shifts in composition whereby the entire system is functionally altered. Since coffee production occurs along a management intensity continuum, these agroecosystems are ideal systems in which to test structure-function relationships and threshold effects.

In this study, we examined how increasing management intensity of coffee plantations influenced soil fungal composition and emergent nutrient cycling functions. We focused on soil fungal communities, as they are the dominant decomposers of plant

material (Boer et al., 2005; Boddy, 2008), form important mycorrhizal symbioses with tropical plants, including coffee, and can act as major agricultural pests and pathogens affecting coffee plant health and productivity.

We tested the hypotheses that differences in coffee farm management would shift soil fungal composition and emergent nutrient cycling function, with the traditional high shade coffee farms harboring fungal communities and functional potential most similar to forest soils compared to monoculture coffee farms. Specifically, the declines in aboveground biodiversity in monoculture coffee farms would be reflected belowground, due to the lack of diverse forms of nutrient inputs via management differences which promote fungal diversity through formation of microhabitats for different fungi. We predicted that these patterns would be detectable in the composition and diversity of both fungal communities as a whole, as well as within four major functional groups of fungi: decomposers, plant pathogens, endophytes and mycorrhizal fungal communities. Further, we predicted that the functional potential of soil communities would reflect shifts in fungal composition and diversity. To test these hypotheses, soils were collected from replicate plots in three coffee management types (monoculture/low shade, traditional polyculture/high shade, and commercial polyculture/medium shade), as well as from an intact rainforest fragment in Chiapas, Mexico. Fungal DNA was sequenced from soils across the management intensity gradient and microbial functional potential was assessed using extracellular enzyme assays for several plant-derived, organic substrates prevalent in surface soils. Microbial biomass was measured using phospholipid fatty acid analysis (PLFA) and coffee farm soil micro- and macronutrients were quantified.

METHODS

Site description and field sampling

This study was conducted in Chiapas, Mexico near the city of Tapachula (Fig. 1). The region has a mean annual temperature of approximately 25^{o}C and mean annual precipitation of ≥4000 mm. The sites examined in this study exhibit a gradient of four agricultural management intensities. The most intensively managed site was a shade monoculture farm, which grows coffee as a near-monoculture, with very few interspersed shade trees (hereafter, 'low shade'). Crops at this site were treated with a variety of fertilizers and pesticides, including potassium nitrate, glyphosate, endosulfate, and leaf fertilizers containing magnesium, boron, and zinc. The least intensively managed site was a traditional polyculture, or 'high shade' coffee farm, where coffee has been grown organically without the use of chemicals for more than 100 years. This farm is vegetatively complex, and includes an assemblage of overstory trees, which are also common in the intact rainforest. We also sampled a commercial polyculture coffee farm, which contains more shade trees than the monoculture farm but fewer than the traditional polyculture farm (hereafter, 'medium shade'). Finally, we sampled a nearby rainforest fragment, which served as an unmanaged control. All four sites were at least one-kilometer away from each other (Fig. 1) (Perfecto and Vandermeer, 2002). Coffee has been continuously grown on all plantations for over 100 years, though the specific management practices have varied over time (W. Peters, personal communication). The forest site was included in the study, as the ability of agroecosystems to maintain

biodiversity is typically gauged by comparing species assemblages of the managed systems to nearby, intact habitats as reference points.

Soil was collected from six randomly selected plots (10 m X 10 m) within each of the four sites during the month of September 2009. Ten soil cores (1.5 cm diameter by 10 cm in depth) were taken from each plot and pooled together. Pooled soil samples (approximately 300 g) were immediately frozen and shipped to Barnard College, Columbia University, where they were stored at -20° C until processing.

Figure 1. Map of coffee farm and rainforest fragment sites in Chiapas, Mexico sampled in this study. Colors indicate site type.

Soil and microbial analyses

Each soil sample was individually homogenized with a UV-sterilized 2 mm sieve so that subsamples taken for downstream analyses would be representative of the entire sample. Ten grams of air-dried soil from each sample was used to analyze pH using a 1:2

water ratio and a glass electrode. Electrical conductivity was measured using an Oaklon Conductivity meter (EuTech Instruments, Singapore). An additional 10 g subsample of air-dried soil from each sample was sent to the Auburn University Soil Testing Laboratory (Al, USA) to analyze a suite of macro and micronutrients (Ca, K, Mg, P, Al, B, Cu, Fe, Mn, Na, Ni, Pb, Zn). Inductively coupled plasma atomic emission spectroscopy (ICP-MS) was used to evaluate soil cations and trace metals. Loss on ignition was used to quantify total C and N.

To quantify microbial biomass and bacterial to fungal ratios, we performed phospholipid fatty acid (PLFA) analysis on 5 g lyophilized soil from each sample. A chloroform, methanol, and citrate buffer single-phase mixture was used to extract lipids, which were subsequently transesterified to fatty acid methyl esters (Bligh and Dyer, 1959; Frostegard et al., 1991) and quantified on an Agilent 6980N capillary gas chromatography system (Agilent Technologies, Santa Clara, CA, USA). We used identified standards to quantify fungal and bacterial biomarkers (Matreya, LLC, Pleasant Gap, PA, USA). Total microbial biomass was estimated from the sum of the following PLFAs: 10Me16:0, 10Me17:0, 11:0, 12:0, 14:0, 15:0, 16:0, 16:1,9, 16:1w6c, 16:1w7t, 17:0, 17:0 delta, 18:0, 18:1w7c, 8:1w7c/9t, delta 20:0, 2-OH 10:0, 2-OH 12:0, 2-OH 14:0, 2-OH 16:0, 3-OH 12:0, 3-OH 14:0, a15:0, i14:0, i15:0, i16:0, i17:0, i17:1w8c. Fungal to bacterial ratios were estimated using the 18:2ω6,9 biomarker for fungi and the sum of 10Me16:0, 10Me17:0, i15:0, a15:0, 15:0, i17:0, 17:0, cis17:0, cis19:0, 16:1ω7t, and 18:1ω7c for bacteria (Frostegard et al., 2011).

Fungal community characterization

To assess fungal community composition, DNA was extracted from three 0.25 g subsamples of soil from each of the 24 study plots, using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) following modified protocols previously described (Fierer et al., 2008; Lauber et al., 2009). Triplicate DNA extractions from each sample were pooled to better represent fungal composition from each plot (Feinstein et al., 2009). Modified primers were used for the amplification of fungal DNA from the 18S rRNA region (Borneman and Hartin, 2000; Rousk et al., 2010; McGuire et al., 2012). PCR amplifications were performed using 3 μL of DNA template, 0.25 ul of each primer (30 mM), and 22.5 μL Platinum PCR SuperMix (Invitrogen, Carlsbad, CA). A Roche 454 Gene Sequencer using Titanium chemistry was used to sequence fungal amplicons at the Environmental Genomics Core Facility at the University of South Carolina (Columbia, SC).

The fungal sequence data generated by pyrosequencing were passed through the Quantitative Insights Into Microbial Ecology (QIIME) bioinformatics pipeline using the default settings (Caporaso et al., 2010). In QIIME, sequences were quality checked, aligned, and grouped into operational taxonomic units (OTUs) at a 97% sequence similarity cutoff (Price et al., 2009). Representative OTUs were aligned against a sequence database of near full length, high-quality 18S sequences, which were selected and compiled from the Silva database (Pruesse et al., 2007). The compiled database sequences (~4000 total) contained representative genera from all major fungal clades. Abundant fungal taxa that were not identified with the database were manually identified in Genbank using the BLAST algorithm (Altschul et al., 1997). Sequence data for each sample were rarefied to 1000 sequences per sample prior to downstream analyses. The

resulting OTU count table was used with the FUNGuild program to identify putative functional groups of fungi (Nguyen et al., 2016).

Soil enzymatic activity

Microbial functional potential was analyzed with extracellular enzyme assays (Saiya-Cork et al., 2002). The activity level of each soil microbial community was measured by incubating soil sub-samples with six different substrates commonly found in natural plant litter (cellulose, hemicellulose, chitin, nucleotides, and amino acids). Commercially manufactured substrates linked to mullibelliferone (MUB) were used for the assays, so that when fluorescent MUB was released by the breakdown of the substrate, there was a measurable proxy for enzymatic activity. We evaluated six ubiquitous enzymes: acid phosphatase (AP), β-1,4-glucosidase (BG), cellobiohydrolase (CBH), β-1,4-N-acetylglucosaminidase (NAG), L-leucine aminopeptidase (LAP), and β-1,4-xylosidase (BX), described in Table 1. Soil homogenates were prepared by blending 125 mL 50 mM sodium acetate buffer (pH = 5.0) with 1-2 g of wet soil. Fifty µL of 1000 µM substrate was added to 200 µL soil homogenate and incubated in a 96 well microtiter plate. Controls were used alongside the experimental assays to correct for auto-fluorescence of buffer and homogenate occurring in the absence of MUB-linked substrates and quenching of fluorescence due to the presence of soil particles. Each plate included control wells of 250 µL buffer only, 200 µL buffer + 50 µL 10 µM MUB, and 200 µL buffer + 50 µL substrate. Eight analytical replicates of each well were run on each plate to account for variability in quenching. Each well contained a final volume of 250 µL. β-glucosidase, cellobiohydrolase, and β-xylosidase assays were incubated for 2

hours; N-acetylglucosaminidase and acid phosphatase assays were incubated for 0.5 hours. Each reaction was incubated on the laboratory bench at room temperature (approximately 22° C). Assays of all soil samples were run simultaneously for each enzyme. Reactions were terminated through the addition of 10 µL 1 M NaOH to each well. Plates were read at 365 nm excitation and 450 nm emission using a Tecan Infinite M200 plate reader within one minute of reaction termination. The above procedure was also used to analyze leucine aminopeptidase activity, with the substitution of 7-Leucine-7-amido-4-methylcoumarin hydrochloride (AMC) for MUB. For this assay, the substrate (leucine amino acids) had been linked to AMC instead of MUB, which also emits fluorescence when cleaved from the substrate. The leucine aminopeptidase assay was incubated statically for approximately 21 hours at room temperature. All extracellular enzyme activity levels are expressed as µmol productg^{-1} oven-dried soil h^{-1}.

Table 1. Description of enzymes tested in this study and results of enzyme assays across land use types. Data are means ±1 SE of 6 replicates. Units for all enzyme activities are umol h-1 g-1 soil.

Enzyme	**Nutrient cycling function**	**Forest**	**High shade**	**Medium shade**	**Low shade**
β-1,4-glucosidase	Cellulose degradation (hydrolysis of beta-1,4-glycosidic bonds in cellobiose to glucose)	18.25 ± 2.44	17.19 ± 1.57	24.74 ± 2.32	22.80 ± 2.72
cellobiohydrolase	Cellulose degradation (release cellobiose/glucose)	3.17 ± 0.25	3.48 ± 0.26	3.50 ± 0.63	2.09 ± 0.27
acid phosphatase	P mineralization from SOM (hydrolysis of ester-phosphate bonds)	44.07 ± 3.91	33.51 ± 2.89	60.75 ± 5.31	32.05 ± 2.67
β -1,4-N-acetylglucosaminidase	Chitin degradation	7.35 ± 0.61	12.51 ± 0.82	9.44 ± 0.66	5.81 ± 1.07
L-leucine aminopeptidase	Polypeptide degradation	0.47 ± 0.07	0.72 ± 0.06	1.40 ± 0.19	0.71 ± 0.06
β -1,4-xylosidase	Hemicellulose degradation (degrades xylosidase)	3.68 ± 0.41	2.08 ± 0.28	3.17 ± 0.75	4.78 ± 0.44

Statistical analyses

All statistical analyses were performed in R version 4.0.3 (R Development Core Team, 2021). Principal component analysis was performed on the Euclidean distance matrix for all soil physicochemical and nutrient data, followed by a k-means clustering analysis (with 4 clusters across 25 configurations) to determine whether soil abiotic properties partition by site type in the *stats* package (R Development Core Team, 2021). All multivariate analyses, richness estimates and ordinations for fungal community and enzyme data were performed using the *phyloseq* and *vegan* packages (Dixon, 2003; McMurdie and Holmes, 2013). Specifically, PERMANOVA analyses were performed using the vegan function 'adonis', and NMDS ordinations were generated using the vegan functions 'metaMDS' with vectors fitted using the 'envfit' function. Correlation analyses were performed using the 'rcorr' function in the *Hmisc* package (Harrell, 2004). The core microbiome analysis was performed using the microbiome package with a fairly conservative prevalence threshold (90%) and a relative abundance detection threshold of >0.0001 to account for limited taxonomic resolution with pyrosequencing datasets (Lahti, 2012-2019). We repeated the core microbiome analyses with fungal data grouped in two different ways: comparing all farms grouped together versus forest, and sun coffee versus shade coffee farms. All data visualization were generated using ggplot2 (Wickham, 2009).

Data availability

Sequence data are publicly available in the NCBI Sequence Read Archive, BioProject accession number PRJNA765687. Enzyme expression data are available in the supplementary data.

RESULTS

Soil physicochemistry and microbial biomass

Initial data analyses identified soil nutrient data for two samples as having measurement or data entry errors for Cd and B; these samples were removed from further analyses that involve soil nutrient data. The first two axes of a principal component analysis for all other samples including all soil nutrients and physicochemical variables (pH, texture, moisture, electrical conductivity) explained 48.7% of variation in soil abiotic environment across farm types (Fig. S2A). While PC1 (27.7%) is mostly an axis representing % silt and various micronutrients, PC2 (21.0%) runs from lower pH and electrical conductivity to higher % clay, C, N and P. The k-means cluster analysis revealed that these abiotic soil variables do not significantly vary by site type (Fig. S2B). Mean quantities +/- standard error for all physicochemical and micronutrient variables across site type listed in Table S1.

Total microbial biomass was significantly lower in high shade coffee soils than all other farm types (ANOVA $p < 0.01$, $F = 5.081$); however, this pattern was driven by significantly lower bacterial biomass in the high shade coffee soils, and thus marginally significantly greater fungal to bacterial ratios in high shade coffee than other farm types (ANOVA $p = 0.02$, $F = 4.115$).

Fungal DNA sequence data and functional guild assignments

Pyrosequencing of fungal DNA generated 24,535 sequences, averaging 1022 sequences per plot. Of the 2,827 fungal taxa in the rarefied OTU table, 1,521 fungal OTUs were identified as saprotrophs, 369 as plant pathogens, 301 as endophytes and 43 as AM fungi by FUNGuild. Subsequent analyses were performed separately for all of these functional groups in addition to the whole fungal community dataset (hereafter, 'all fungi').

The relative abundance of fungal functional groups varied across farm type (Fig. 2), with endophytes having significantly greater relative abundance in forest soils (Fig. 2a, ANOVA $p \leq 0.01$, $F=7.232$), saprotrophs more abundant in medium shade coffee soils (Fig. 2c, ANOVA $p < 0.05$, $F=4.259$) and plant pathogens with moderately significantly greater relative abundance in high shade coffee soils (Fig. 2d, ANOVA $p < 0.05$, $F=3.411$). AM fungal relative abundance did not significantly shift with farm type (Fig. 2b).

There were no significant differences in fungal alpha diversity when considering farm type categories globally (two-way ANOVA $p>>0.05$) and in pairwise comparisons (Wilcoxon tests $p > 0.05$) (Fig. S1). There were also no significant differences in alpha diversity within three major fungal functional groups: plant pathogens, saprotrophs, and endophytes (Fig. S1). Alpha diversity of AM fungi significantly differed across land use (two-way ANOVA $p < 0.001$, $F=9.719$) with the greatest AM fungal diversity in low shade coffee soils and lowest in the rainforest soils (Tukey's test $p < 0.01$ for all farm types versus forest).

Fungal composition across farm type

There were significant shifts in fungal composition across forest and coffee farm management categories (Fig. 3, PERMANOVA F model=1.55, R^2=0.19, $p \leq 0.001$). A pairwise-PERMANOVA analysis revealed that these differences were driven by significant shifts in fungal composition between all coffee soils versus forest soils, and low versus medium shade coffee soils (Table S2, corrected $p < 0.05$). Environmental vectors for external continuous variables measured (soil nutrients and physicochemical properties, microbial biomass, enzyme activities and geographic variables) were fit to the ordination, and six variables were detected as marginally significantly correlated ($p \leq 0.05$) with the NMDS configuration; rapid change in variables representing the distance of a site to the forest (R^2=0.33), soil aluminum (R^2=0.26), soil moisture (R^2=0.26), fungal biomass (R^2=0.27), the relative abundance of endophytic fungi (R^2=0.26) and the activity of BG (R^2=0.34) varied with the whole fungal community ordination (Fig. 3a).

When considered separately, the community composition of endophyte, saprotroph and plant pathogen fungal communities varied across farm type (Fig. 3b-d, PERMANOVA $p \leq 0.03$ for all groups). Saprotroph communities were most distinct across coffee farms, particularly between low shade farm soils and forest soils. Further, the community composition of all fungi and saprotrophs were highly positively correlated (Mantel test statistic r=0.92, p=0.001). All pairwise PERMANOVA comparisons of fungal pathogen community composition revealed nonsignificant corrected P values, and only marginal significance for shifts in endophytic fungal community composition (Table S2). AM fungal communities were not significantly different across farm types ($p \geq 0.05$).

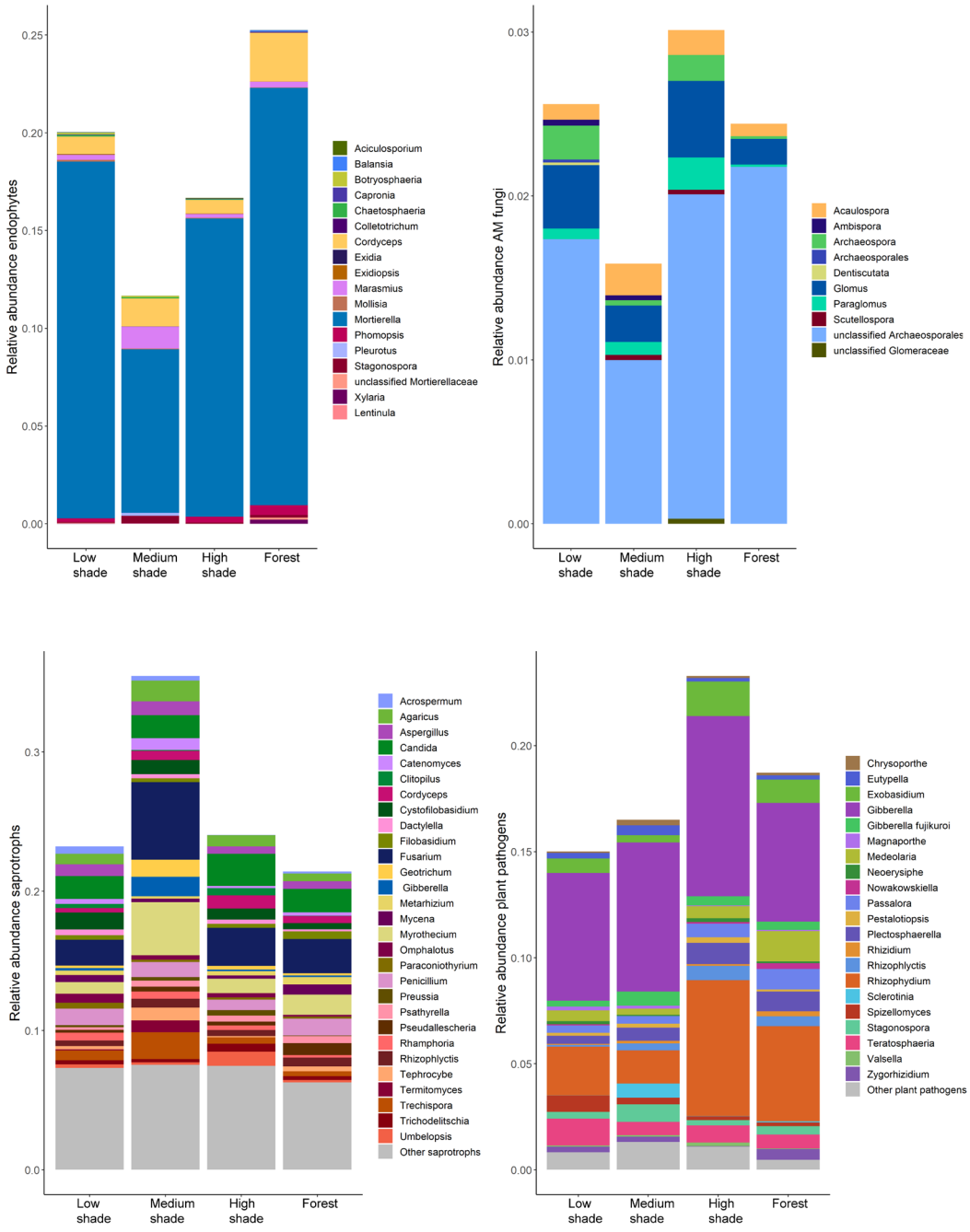

Figure 2. Relative abundance of fungal genera by functional group assigned by the FunGUILD program across different site types. Clockwise from top left panel: endophytes, AM fungi, saprotrophs, plant pathogens.

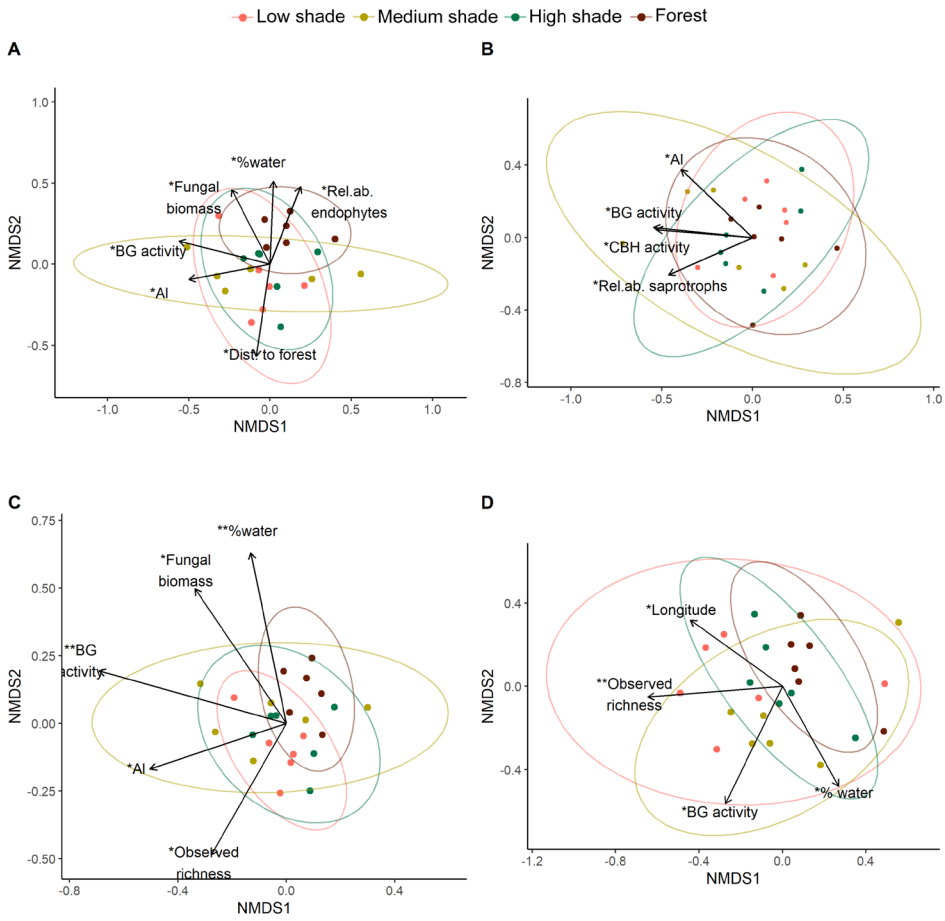

Figure 3. NMDS plots depicting shifts in fungal community composition for all fungi (A), endophytes (B), saprotrophs (C) and plant pathogens (D) using the Bray-Curtis dissimilarity index. Fungal composition varied with external environmental and biotic variables (fitted as vectors); only significant variables ($p<0.05$) retained for plotting. The magnitude and direction of arrows indicate the direction and strength of correlation for variables in ordination space. Asterisks indicate significance of correlations (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

Soil enzyme activity

Four out of the six enzymes assayed significantly varied in activity across farm types: acid phosphatase (AP), β-1,4-N-acetylglucosaminidase (NAG), L-leucine aminopeptidase (LAP), and β-1,4-xylosidase (BX) (Fig. 4). Cellobiohydrolase (CBH) and

β-1,4-glucosidase (BG) activities did not vary across farm types. Data for mean enzyme activities by farm type ± 1 SE across six replicate samples presented in Table 1. Enzyme activity profiles for each sample varied significantly by coffee farm type (PERMANOVA $F=9.397$, $R^2=0.59$, $p \leq 0.001$) and correlated significantly with farm type in ordination space (Fig. 5, goodness of fit $R^2=0.65$, $p \leq 0.001$). These PERMANOVA tests revealed a greater percentage of variance in soil functional potential (enzyme activity profiles) than fungal composition that was explained by farm type (all fungi farm type PERMANOVA $F=1.55$, $R^2=0.19$). Additionally, several external variables were significantly correlated with enzyme activity profiles, shown as fitted vectors in the ordination (Fig. 5). There was marginally significant correlation between shifts in the relative abundances of endophytes and pathogens and shifts in enzyme activity profiles in forest and high shade coffee soils, respectively ($R^2=0.30$ and $P < 0.05$ for both). Change in elevation ($R^2=0.52$, $p \leq 0.001$) and longitude ($R^2=0.38$, $p \leq 0.01$) of coffee farm sites were correlated with shifts in enzyme profiles across samples. Finally, soil Na was slightly correlated with enzyme activities in medium and high shade coffee soils ($R^2=0.29$, $p \leq 0.05$).

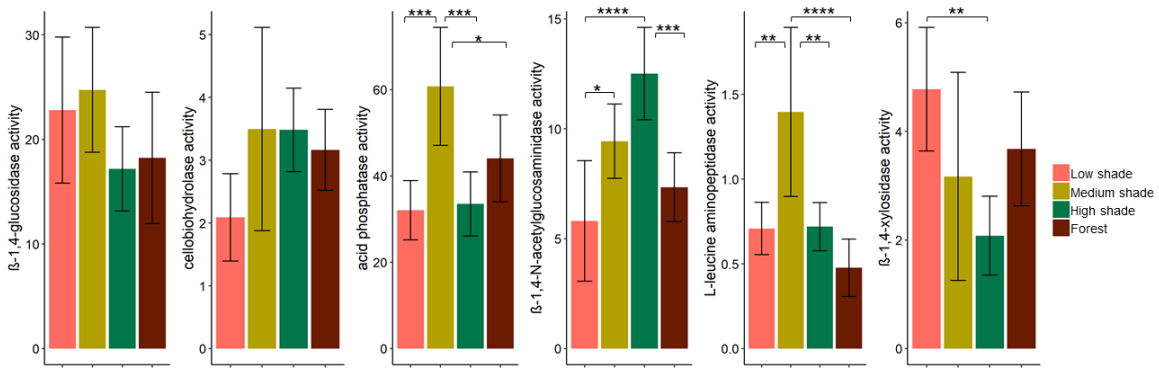

Figure 4. Soil enzyme activities in umol h^{-1} g^{-1} soil for the six enzymes tested. Data are means ±95% confidence intervals with n=6 replicate samples measured per land use category. Asterisks denote significant differences from a Tukey post-hoc multiple comparisons test among pairs of site types (* p<0.05; ** p<0.01; *** p<0.001; **** p < 0.0001).

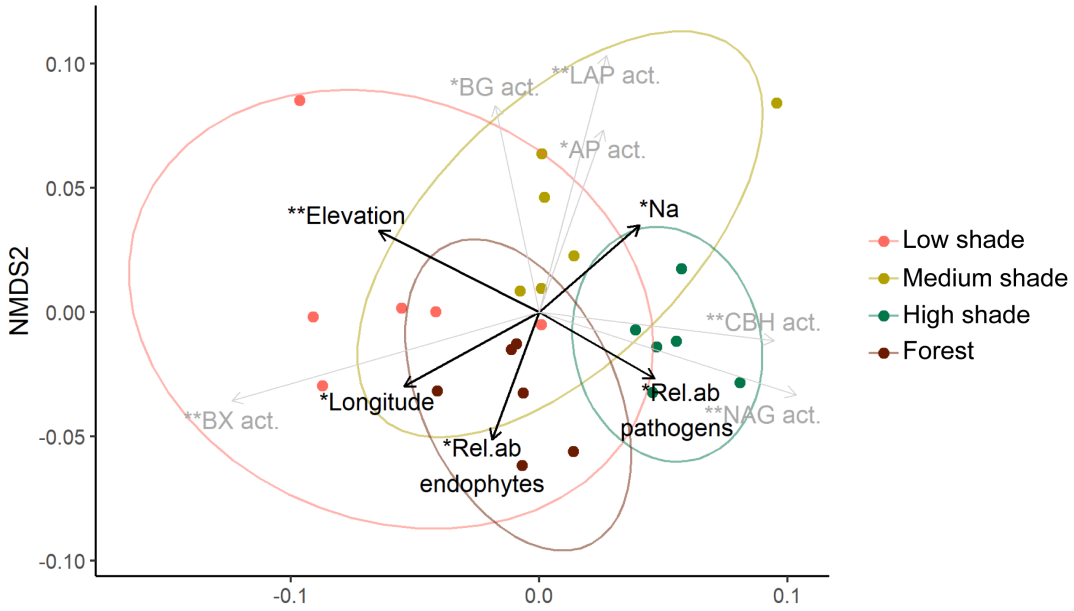

Figure 5. Nonmetric multidimensional scaling plot depicting enzyme activity profiles measured across samples with vectors fitted for correlations with environmental and fungal variables. Asterisks indicate significance level (* p≤0.05; ** p≤0.01). Enzyme expression profiles varied significantly across farm type (PERMANOVA F=9.397, R^2=0.585, p≤0.001).

Enzyme activity correlation with fungal OTUs

Individual enzyme activities correlated significantly with the relative abundances of specific fungal OTUs within all site types (Fig. 6). Most OTUs that correlated with enzyme activity were unique to different farm types, with the exception of four OTUs: *Hypocrea* sp10 and *Gibberella* sp19 were present in both forest and medium shade coffee soils, and *Candida* sp14 and *Rhizopogon* sp42 shared between medium and high shade coffee soils. However, these common OTUs correlated with different enzymes across farm type. *Mortierella* spp. were positively correlated with enzyme activity in all coffee farm soils but not in forest soils.

Core microbiome analysis

The core microbiome analyses were performed separately for four groupings of samples and compared to see which core OTUs were the same and which were unique across groups (forest vs. all farms together; low vs. high shade farms only). This analysis selected 62 and 69 total OTUs (respectively) in the two comparisons, detected in at least 90% of samples in each category at >0.01% relative abundance. There were 25 core OTUs shared across all farms and forest soils, 4 core OTUs in coffee farm soils not in forest soils, and 33 core OTUs unique to forest soils (Fig. 7). The four OTUs that were unique to the coffee farms were *Cryptococcus* sp. 9, *Gibberella* sp. 37, *Fomes* sp. 67 and *Hypocrea* sp. 84. For the low versus high shade coffee core OTU comparison, 34 core OTUs were common to both farm types, but low and high shade coffee soils had 21 and 14 (respectively) OTUs unique to each farm type. The majority of core fungal OTUs

were assigned to the *Mortierella*, *Gibberella* and *Cryptococcus* genera, which are listed as endophytes, plant pathogens and saprotrophs (respectively) by FUNGuild (Fig. 7).

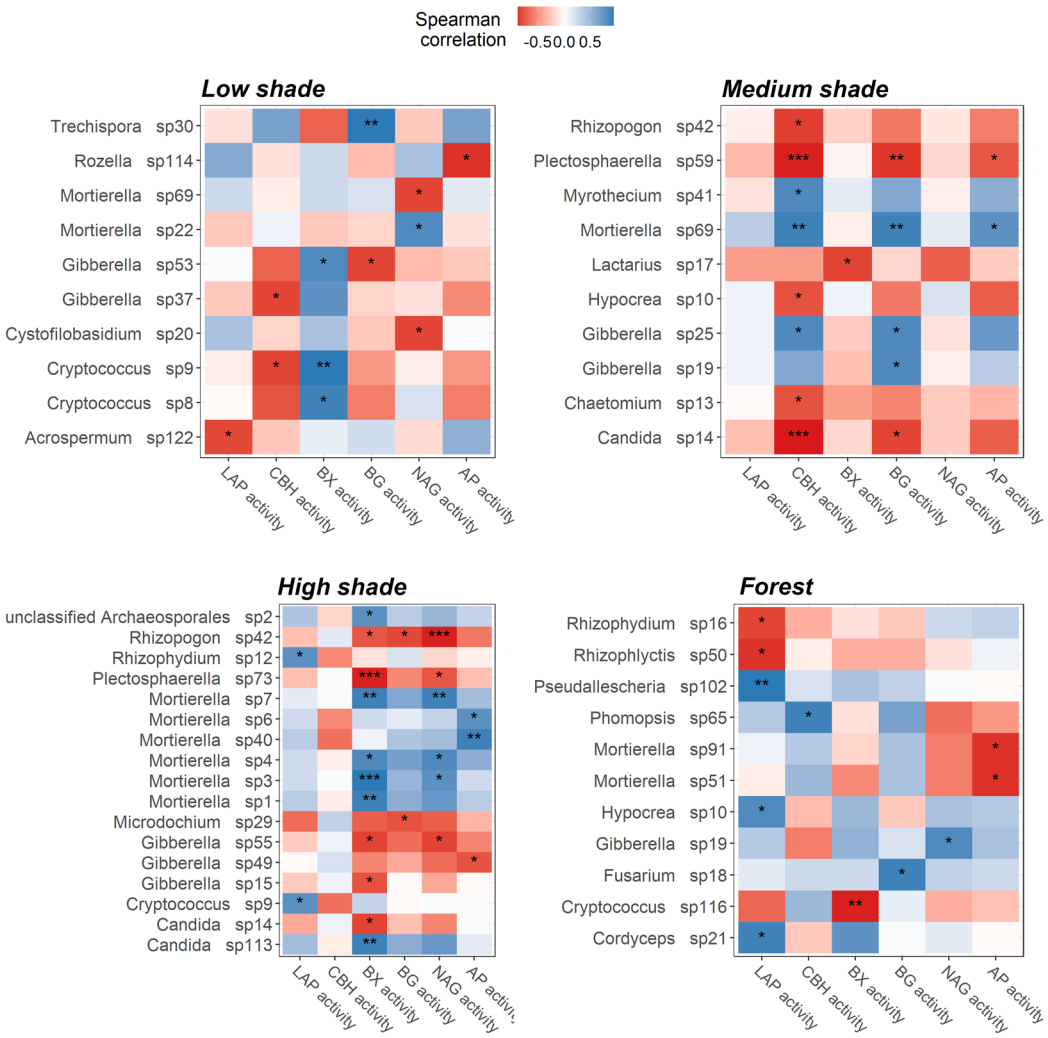

Figure 6. Spearman correlations of fungal OUT relative abundances with individual enzyme activities within each site type. Only taxa that significantly correlated with at least one of the six enzymes within each category are shown ($p<0.05$). Hue of colors indicates strength of correlation; asterisks indicate significance level (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

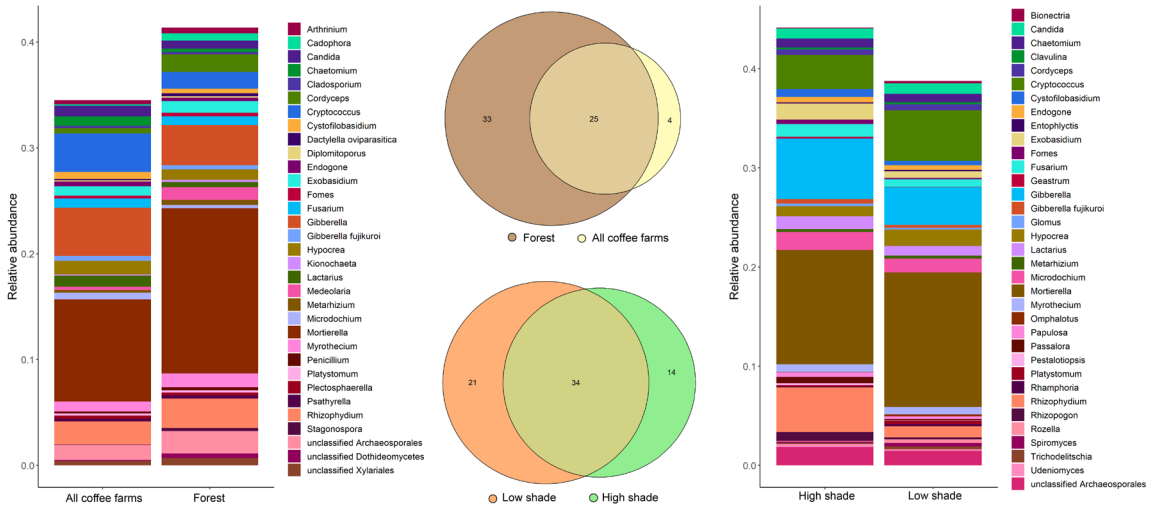

Figure 7. Stacked bar charts depicting the relative abundances of core fungal OTUs in all coffee farms versus forest soils (left panel), and high versus low shade coffee farm soils (right panel). Euler diagrams in the center panel show the proportion of core OTUs shared between groups. Prevalence threshold for detection of core OTUs was 90% of sample in each site type.

Variation partitioning

Approximately 15% of fungal compositional variance was explained by microbial biotic variables (total microbial biomass, Shannon diversity and relative abundance of functional groups), enzyme activities and farm type in a variance partitioning analysis (Fig. S3A). When considering saprotrophic fungal composition separately, the same combinations of variables explained 16% of variance (Fig. S3B).

DISCUSSION

Coffee agroecosystems have a wide range of management intensities and we found that these differences in management have significant impacts on microbial composition and function. Specifically, we found that low, medium and high shade

coffee farms had compositionally distinct communities of saprotrophic fungi, fungal endophytes, and fungal pathogens, all of which have important functions in agroecosystem productivity. Our PCA and k-means clustering analyses revealed that the soil abiotic variables did not significantly vary across site types (forest and all three coffee farm management categories), implying that differences in physicochemical characteristics across sites are not major contributors to the management-correlated shifts in fungal composition or functional potential. Surprisingly, we did not find differences in mycorrhizal fungal composition across management, although this may be due to the majority of AM fungal sequences in this dataset's taxonomic assignments at coarse resolution (unclassified beyond Order or Family). We also identified core fungal OTUs that are unique to coffee farm soils, and show that these taxa are contributing to shifts in soil functional potential through significant correlations with enzyme activity.

Farm type influences functional potential more than fungal community structure and diversity

Our finding that land use intensity had a stronger influence on soil enzymatic potential than soil fungal community structure implies divergent responses of biodiversity-ecosystem function this pattern has been observed in other ecosystems (Li et al., 2015; Yuan et al., 2021), a small number of studies to date examine both biodiversity and ecosystem functions simultaneously, making general patterns of belowground biodiversity-ecosystem function relationships difficult to characterize (Guerra et al., 2020). The different farm types in our study represented different levels of aboveground diversity, and the absence of shifts in fungal diversity across farm types for all groups of

fungi analyzed may be due to aboveground responses to shifts in environmental conditions acting at different spatial and temporal scales than belowground organisms. The only major shift in alpha diversity we detected was a significant decline in AM fungal diversity in forest soils compared to all coffee farms. The source of this variation could be a result of high dependency by *Coffea* plants on AM fungi relative to other plants, a characteristic of coffee systems that has been extensively studied (Andrade et al., 2009). Despite fungal diversity remaining consistent across sites, enzyme activity profiles were distinct. We interpret this result as a potential outcome of differences in management strategies (i.e. nutrient additions, pesticide use) selecting for fungal communities with different functional capabilities. For instance, application of the common herbicide glyphosate has been shown to significantly impact soil microbial respiration, but depending on soil type only has limited influence on soil community structure (Lane, 2011). Additionally, modeling of soil multifunctionality across agricultural management regimes indicated that management differences can drive nutrient cycling functions, and shifts in soil activity varies with soil composition nonlinearly (Nazaries et al., 2021). Minor shifts in composition relating to larger shifts in functional potential may indicate plasticity in fungal functions across coffee farm soils as in other agricultural systems (Bowles et al., 2014), and/or functional redundancy among fungal groups as found in undisturbed tropical soils in Costa Rica (Kivlin and Hawkes, 2020).

Fungal saprotrophs drive management-induced shifts in fungal composition

We found that composition of fungal saprotrophs in coffee farm and forest soils tightly and positively correlated with the whole fungal community compositional shifts, despite saprotrophs only comprising between 16-60% of the whole fungal communities across samples. This finding suggests that soil organic nutrient economies vary across coffee farm soils, likely due to differences in recalcitrance of nutrient additions for *Coffea* plant crops and diverse litter inputs in polyculture farms selecting for different types of decomposer fungi (Landesman et al., 2014). While these saprotroph-driven shifts in composition varied across farm type, enzyme activity profiles only explained 3.4% of variation in saprotroph composition, which conflicts with a previous finding in Californian *Pinus* forest stands where there were strong correlations between saprotroph community composition and the activity of some of the same enzymes tested here (Talbot et al., 2013). This discrepancy is most likely due to the vast differences in edaphic, climatic and disturbance histories between North American temperate pine forest, and tropical agricultural/rainforest soils. Further, numerous fungal OTUs in this study that were delineated as endophytes or plant pathogens by FUNGuild assignments also likely function as saprotrophs depending on environmental context; in fact, most of the fungal OTUs with significant, positive correlations with individual enzyme activities were classified by FUNGuild as saprotrophs, but others were classified as endophytes that also function as saprobic in soils (i.e. *Mortierella* spp.).

Notably, *Mortierella* spp. were dominant across all samples in our study. Species in this large genus have been applied as biofertilizers across various agricultural systems for their potential functions as plant growth-promoting saprotrophs and endophytes suppressing phytopathogens (Eroshin and Dedyukhina, 2002; Al-Shammari et al., 2013).

Mortierella species are cosmopolitan in nature, detected in soils and plant tissues across various biomes, and seem to be selected for in agricultural fields under organic amendments (Ozimek and Hanaka, 2021). Here, we found that *Mortierella* species are not only present at high relative abundance across coffee farm and forest soils, but also significantly positively correlated with various enzyme activities. In the medium and high shade coffee soils, there were seven OTUs identified as *Mortierella* that positively correlated with enzymes involved in hemicellulose and chitin degradation, as well as P mineralization from soil organic matter. This is consistent with previous studies that found *Mortierella* species in bulk soil and on root surfaces readily decompose a variety of labile and recalcitrant compounds, including all of those tested in the enzyme assays of this study (Jackson, 1965; Gawas-Sakhalkar and Singh, 2011; De Tender et al., 2019). Additionally, the positive correlations we detected between *Mortierella* OTUs and acid phosphatase activity support previous evidence that *Mortierella* species can be used in soil inocula as phosphate solubilizing microorganisms (Ozimek and Hanaka, 2021). The P-solubilizing functions provided by *Mortierella* species may be in synergy with AM fungal services to *Coffea* plants, as previous studies have shown (Osorio and Habte, 2001; Zhang et al., 2011). These patterns warrant further examination of the ecological mechanisms governing these emergent outcomes of multiple plant growth-promoting fungal groups acting in synergy.

Most fungal OTUs correlated with enzyme activity are in the coffee farm core mycobiome

Nearly all of the fungi that significantly correlated with individual enzyme activities emerged as core fungal OTUs in our core microbiome analyses, suggesting that

shifts in relative abundance of the most prevalent OTUs across samples drive functional differences within each farm type. There is much debate surrounding the notion of whether microbial controls over agroecosystem multifunctionality are driven by relatively more abundant, prevalent taxa versus rare taxa (Jiao and Lu, 2020; Chen and Sinsabaugh, 2021). For instance, a recent study investigated whether rare versus abundant fungi contribute to nutrient cycling enzyme activities, and concluded that the composition of rare, less prevalent fungal taxa significantly correlated with enzyme activity more than the widespread, abundant fungi in a maize-wheat-barley cropping system; their core mycobiome consisted of abundant taxa and none of the enzyme-related rare taxa (Xiong et al., 2021). On the other hand, a core microbiome is typically defined as members of the community that are prevalent across all sites meeting the conditions under examination, and thus the consistent ecological selection for core taxa across environmental conditions implies some critical functional importance for the plant host or habitat conditions (Shade and Handelsman, 2012).

Our study was limited to somewhat similar environmental and edaphic conditions across coffee farms, so further research investigating the impacts of management regimes on *Coffea* mycobiome composition and function is necessary. For instance, trade-offs in soil fungal community traits may favor fungal growth yield or stress responses over extracellular enzyme production depending on the spatiotemporal context and resource availability (Malik et al., 2019; Alster et al., 2021). These trade-offs may complicate our understanding of relationships between fungal taxonomic composition and function with extracellular enzyme assay data, requiring further research simultaneously accounting for

soil community stress responses, growth yield, and enzymatic potential in order to more explicitly link soil fungal composition with function across agricultural landscapes.

CONCLUSIONS

Our study began to elucidate structure-function relationships in the soil fungal communities associated with different intensities of coffee (*Coffea arabica* L.) cultivation in Chiapas, Mexico. We identified the ecological outcomes of differences in coffee farm management in terms of soil functional potential in relation to the composition of soil fungal communities across sites. The synergistic shifts in enzyme expression profiles and fungal saprotroph community composition in our study demonstrates the central role soil fungi play in emergent functions of the soil environment across coffee farms. Additionally, identification of core fungal taxa that correlated with enzyme function the rhizosphere of *Coffea* plants presents opportunities for enhancing productivity while maintaining conservation efforts.

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CHAPTER III

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CHAPTER IV

LOCAL AND REGIONAL SCALE MYCORRHIZAL NETWORK ASSEMBLY IN AN EXPERIMENTAL PRAIRIE-PASTURE SYSTEM

Contributions

This chapter is coauthored with K. L. Shek, H. R. Dawson, P. Reed, S. Bridgham, L. Silva as part of a large collaborative project in an experimental grassland system established by coauthors. For the data presented herein, field and labwork was primarily performed by me with assistance from coauthors; all writing is mine with edits from coauthors.

Supplementary material for this chapter found in Appendix C.

INTRODUCTION

The arbuscular mycorrhizal (AM) mutualism is a symbiotic relationship between soil-dwelling fungi and >80% of all plants, and is essential to the functioning of terrestrial ecosystems. In these partnerships, communities of mycorrhizal fungi colonize the root systems of plants, and reciprocal exchange of nutrients and other resources occur in the rhizosphere [1]. The essential ecosystem services provided by these plant-fungal symbioses include increased resilience of communities to biotic and abiotic pressures, below-ground resource partitioning, and alteration of competitive dynamics in plant communities through equalizing fitness differences and expanding niche space. AM fungi

are obligately bound to their plant hosts, and serve nutritional functions through uptake and transfer of limiting nutrients such as N, P and K, in addition to providing increased resistance to stressors such as drought [1]. This symbiosis exists on a continuum; plants vary in their dependency on AM fungi, and individual plants can form associations with numerous AM fungal taxa in their roots. Likewise, an individual AM fungus, or multiple conspecific AM fungal individuals, can form associations with multiple host plants, forming mycelial connections between neighboring root systems, called common mycorrhizal networks (CMNs)[2]. These networks can connect the rhizospheres of the same or different species of plants, thus influencing plant community dynamics, primary productivity and the response of ecosystems to drought pressures[3]. Despite several studies investigating the impacts of CMNs on plant composition and behavior, the form and function of arbuscular CMNs in natural systems remain poorly characterized. For instance, differential allocation of resources among coexisting plants by the CMN has been shown in flax and sorghum plants[4], but there is also evidence for lack of discrimination between high- and low-quality partners by the CMN[5]. One relatively unexplored factor that may drive differences in CMN behavior across these experimental systems is the composition of AM fungi present, and whether different groups of AM fungal taxa vary in their contributions to CMN functions.

Identifying AM fungi forming CMNs, and characterizing the emergent outcomes CMN-mediated functions in plant communities thus requires a better understanding of the factors that shape plant-fungal co-occurrences in nature. For instance, AM fungi are widely regarded as host generalists[6], as there are currently only 384 estimated species of AM fungi known to form associations with a vast diversity of host plants[7,8]. However,

recent evidence suggests a role of preferential partner selection in the AM symbiosis, with some studies suggesting trait-based correlations between AM partners[9] and others suggesting matching life histories[10], but these patterns may depend on the identity of neighboring plants in the community[11]. Together these findings highlight the need for further investigation of how plant community diversity structures AM fungal composition and diversity, which has been shown to feedback to maintain plant diversity and ecosystem productivity[12]. Further, the species pool of AM fungal taxa available to colonize host plants in a given environment is largely driven by abiotic factors such as soil properties, climate, latitude and disturbance history[13], adding environmental context and disturbance intensity in addition to host plant identity as determinants of AM fungal-plant associations across different systems[14].

A common approach that has been used to decipher interaction patterns driving plant-AM fungal cooccurrences in nature is ecological network theory[15]. This theory posits that mutualistic interaction networks almost always show patterns of nestedness, where specialist taxa interact with a subset of the partners that generalists interact with[16,17]. Indeed, several studies to date have shown AM fungal-plant interaction networks with significantly higher nestedness than expected randomly[18-20]. The opposite of nestedness in plant-fungal assembly patterns would show interaction networks with high modularity, in which interacting species form distinct groups and preferential partner selection plays a major role determining the composition of AM fungi colonizing individual plant species[9,21]. Measuring network theory metrics such as these can help elucidate the relative roles of environment and host filtering on AM fungal communities. These network analyses are not to be confused with determining CMN structure and

function; while network theory is generally applied at larger spatial scales to tease apart plant-fungal assembly patterns, CMNs occur at the local or plot-level spatial scale and require more refined analyses of fungal-mediated resource sharing between plants under varying environmental conditions.

Pastures are prevalent agricultural systems that are governed by AM connections, where AM fungi play essential roles in plant nutrition and productivity under variable environments [22,23]. Pastures in the Pacific Northwest of the United States (PNW) are ideal systems to investigate the roles of AM fungal composition and plant resource acquisition and sharing via CMNs, as these systems are N-limited and maintenance of plant diversity and productivity likely rely on AM fungal functions. The PNW is a Mediterranean climate zone that was historically dominated by diverse prairies, but decades of land management resulted in conversion of prairies to less diverse pastures that are dominated by invasive grasses[24]. Additionally, extreme drought events have increased in frequency and intensity in the region, and caused measurable declines in PNW plant cover, especially for annual forbs and exotic annual grasses[25]. PNW prairie and pasture systems therefore provide the opportunity to simultaneously investigate the relative roles of host plant diversity, identity and drought pressures on plant-AM fungal coassembly patterns and the form and function of CMNs.

Here, we first characterized how differences in environmental conditions, plant diversity and drought influence AM fungal composition and plant-fungal interaction patterns at broad spatial scales. We then utilized ^{13}C and ^{15}N stable isotopes at the plot-level to illustrate CMN assembly and nutritional function across plant diversity and drought treatments. Specifically, we aimed to address two major questions regarding

plant-AM fungal assembly and CMN structure in PNW prairie/pasture ecosystems: (1) what biotic and abiotic factors drive shifts in the cooccurrences of AM fungi-plant partnerships in pasture/prairie plants; and (2) do CMNs form and facilitate nutrient transfer in high- vs. low-diversity plant communities under drought? We hypothesized that AM fungal communities exhibit hierarchical spatial structuring, where shifts in latitude, climate and soil properties structure which AM fungi are present across sites/at large spatial scales, and biotic factors such as plant host diversity and identity are major determinants of AM composition at the local scale[13]. We predicted plant-fungal interaction networks would exhibit nestedness, with relatively greater nestedness in low diversity plots, and greater levels of preferential partner selection in high diversity plots due to more host filtering for specific fungal partners across plant functional groups in high diversity plant communities. Finally, we hypothesized that CMNs are formed by widespread generalist AM fungal taxa that associate with nearly all plants in a plot, while more specialist AM fungal-plant interactions do not correlate with resources shared. To address our aims, we characterized AM fungal composition in the roots of plants in experimentally manipulated plots representing high and low plant diversity treatments and drought versus control treatments, all spanning three sites along a 520km latitudinal gradient in the PNW.

METHODS

Description of sites and experimental treatments

This study was conducted across three sites spanning a 520km gradient of increasing Mediterranean drought intensity from the southernmost site in southwestern

Oregon, the central site in central-western Oregon, and the northernmost site in central-western Washington. Within each site, plots are divided into two plant diversity treatments: a high-diversity state and a low-diversity pasture state. The high-diversity plots were planted using an identical mix of 29 native grasses and forbs seeded in 2012/2013 (more details in references 26-29[26-29]), while the low-diversity pasture plots were not manipulated are dominated by one to a few species of invasive perennial grasses (i.e. *Schedonorous arundinaceus*, *Agrostis capillaris, Poa compresa*). Briefly, the high diversity plots were mowed and sprayed with herbicide at initial planting, then annually seeded with 14 native grasses and forbs between 2015-2017[27]. Over the course of the experiment, non-native species have also made it into these plots, but overall they have maintained higher plant diversity than the unmanipulated (hereafter, low diversity) plots which were adjacent and not experimentally manipulated. The experimental plots used in this study consisted of 20 plots per site under 2 climate treatments: control and -40% of precipitation, with 5 replicate plots for each combination of diversity and climate treatments (Fig. 1). The drought plots had rain-out shelters installed in February 2016 that covered 40% of the plot area with clear acrylic plastic (MultiCraft Plastics, Eugene, OR, USA), which absorbed ≤8% of light transmittance[30].

Stable isotope labelling and plant harvest

Baseline samples were collected before labelling, between April 15 and May 20, 2019. To avoid destructive sampling of plants in the plots, we collected soil cores 0-10cm depth in triplicate for each plot. Root fragments were picked from soil core samples for DNA extraction and encapsulation for stable isotope analyses.

We selected plants located roughly in the center of each plot that were healthy individuals of *Sidalcea malviflora* in high diversity plots, and *Alopecurus*, *Schedonorus* or *Agrostis* individuals in low diversity plots as 'donor' individuals. The donor plants each received isotopically labeled $^{13}CO_2$ and $^{15}NH_3$ gas within a clear plastic cylinder at 20-minute intervals; labeled CO_2 was pulsed three times enriched at 98 atm % ^{13}C, and NH_3 was pulsed twice at 98 atm % ^{15}N. All labeling took place between 11am and 3pm on dates selected based on peak NDVI for each site, between April 22 and May 25, 2019. We selected ≥5 (average 10) additional plants within each plot as putative 'receivers' at variable distances from donor plants, representing both con- and heterospecific plants. Leaf samples were collected from all donor and receiver plants at the time of labelling, and again at 4- and 10-days post-labelling. At 21 days post-label, we harvested the entire plants and rhizosphere soils for all donor and receiver individuals into sterile WhirlPak bags, and transported them on ice until storage at -20ºC in the laboratory.

We processed all plants as soon as possible after collection. Plant processing consisted of shaking off rhizosphere soils into sterile WhirlPak bags for storage at -80ºC, followed by tracing above- and belowground plant parts to ensure root samples were from target individuals. We discarded roots that could not be traced to aboveground biomass of each individual before separating above and belowground biomass samples. Approximately 10x ~3cm fine root samples were picked for DNA extraction, and another subset of roots were oven dried before encapsulating for stable isotope analysis at the UC Davis Stable Isotope Facility (Davis, CA, USA). Leaves were also oven dried before encapsulating for stable isotope analysis. The remaining belowground plant tissue was stored at -20ºC until further analysis.

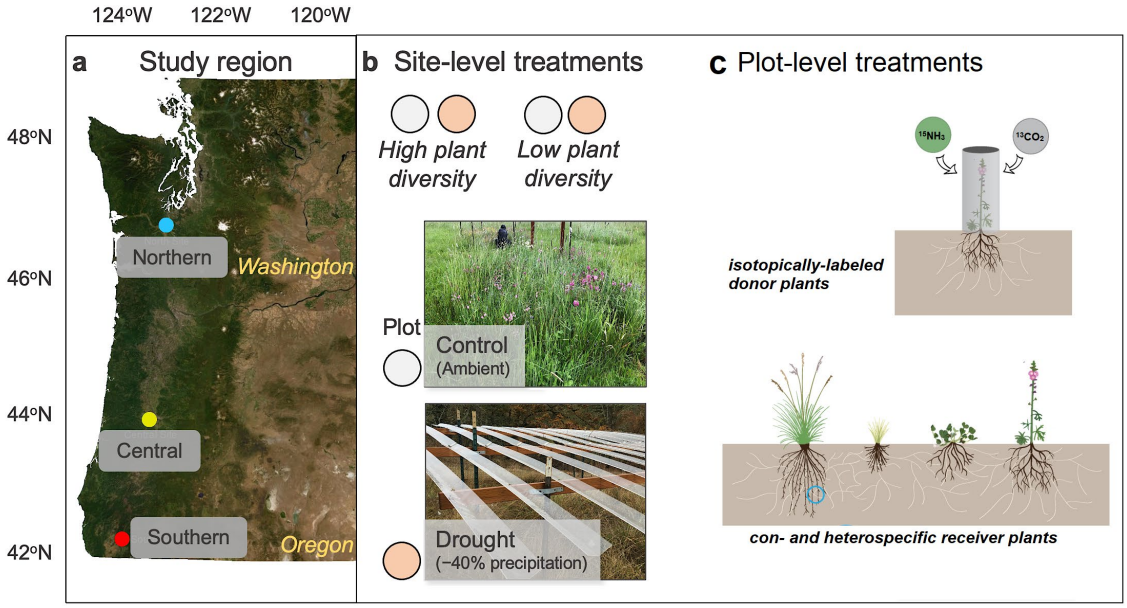

Figure 1. Experimental design for the study. Sites along 520km latitudinal gradient (a) from southern Oregon (dry/warmest site, 'Southern'), central-western Oregon (middle site, 'Central') and central-western Washington (cool/wettest site, 'Northern'). Within each site, 5 plots of each treatment combination were sampled (high and low diversity, control and drought) for 20 plots per site (b). Within each plot, we sampled donor plants (labeled with ^{13}C and ^{15}N) and >6 other plants of the same and different species per plot (c). Figure adapted from Bomfim et al. *in prep*.

AM fungal community composition

DNA was extracted from root samples representing 450 individual plants using Qiagen DNeasy Powersoil HTP kits (Qiagen, Hilden, Germany) following the manufacturer's protocol. To characterize AM fungal composition in each sample, we adapted a novel two-step PCR protocol for preparation of Illumina libraries to amplify a ~550bp fragment of the SSU rRNA gene of AM fungal genomes, which is currently the most well-supported region of AM fungal rRNA for taxonomic resolution[31] and

amplicons can be queried against a well-curated database for taxonomic assignment [7]. The primers we used for this region are WANDA (5'- CAGCCGCGGTAATTCCAGCT-3') and AML2 (5'- GAACCCAAACACTTTGGTTTCC-3')[32,33]. The first round of PCR amplified the target region using WANDA and AML2 primers modified to include heterogeneity spacers and Illumina TruSeq 'stubs' in 20uL reactions consisting of: 2uL genomic DNA template, 10uL GoTaq Green Master Mix (Promega Corp., Madison, WI, USA), 6.9uL nuclease-free water, 10uM of each primer and 0.1uL BSA. PCR1s were run on a BioRad T100 thermal cycler (BioRad, Hercules, CA, USA) for the following protocol: initial denaturation 94ºC for 3min, followed by 30 cycles of 94ºC for 45s, 50ºC for 60s, 72ºC for 90s, final extension 72ºC for 10min. Amplification success and amplicon length were confirmed for all PCR1 products on 1% agarose gels before proceeding with PCR2. PCR2s were carried out in 25uL reactions containing 3uL undiluted PCR1 product as template, 1uL of each forward and reverse PCR2 primers, 10uL GoTaq Green Master Mix, 0.1uL BSA and 9.9uL nuclease free water. These reactions were run on the same thermal cyclers with the following conditions: initial denaturation at 94ºC for 3min followed by 12 cycles of 94ºC for 45s, 52ºC for 60s, 72ºC for 90s with a final extension at 72ºC for 10min. PCR2 primers bind to the Illumina TruSeq 'stubs' on PCR1 products, and add Illumina adapters and indices for multiplexing several samples on a single sequencing lane. Our PCR2 primers have unique indices on both forward and reverse primers, permitting multiplexing several projects on a single run. Further details regarding primer modifications and PCR protocols provided in the Supplementary Information (Note S1).

Successful PCR2 amplicons were quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Waltham, MA, USA) on a SpectraMax M5E Microplate Reader (Molecular Devices, San Jose, CA, USA). Quantification data were used to pool amplicons in equimolar concentrations, before PCR purification with QIAquick PCR Purification kits (Qiagen). Purified pools were sequenced on the Illumina MiSeq platform (paired-end 300bp, Illumina Inc., San Diego, CA, USA) at the University of Oregon Genomics and Cell Chracterization Core Facility (Eugene, OR, USA).

Raw sequence processing and taxonomic assignment

All sequence data were demultiplexed and deduplicated using an in-house bioinformatics pipeline that handles the different aspects of our novel PCR protocol. Briefly, reads are re-oriented by querying each individual sequence for the biological primers before demultiplexing sequences into their respective samples with generous tolerance and quality thresholds, since downstream processing (see next steps, DADA2) performs sequence quality filtering. Orienting and demultiplexing scripts are in-house python scripts. Once sequence reads are assigned sample IDs, our pipeline utilizes the heterogeneity spacers in PCR1 primers as unique molecular identifiers (UMIs) that can parse apart duplicated sequences due to PCR bias as opposed to true biological multiplicity. The deduplication step aligns reads to the taxonomic reference database (MaarjAM[7]) using bowtie2[34] and assigns UMI tags to each sequence using samtools[35]. UMItools is then used to filter out duplicate reads from PCR duplication (those with the same UMI), and retains reads with the highest alignment score to the reference database.

Deduplicated sequences were used as input to the DADA2 pipeline for quality filtering and assembling into amplicon sequence variants (ASVs) with standard parameters[36], which does not cluster sequences at a simialrity threshold like traditional OTU approaches, maintaining strain-level diversity in DNA sequences in our target region[37]. Taxonomy was assigned using the MaarjAM database with the 'assignTaxonomy' function in DADA[27]. Any ASVs that were not assigned taxonomy by DADA2 were searched using the MaarjAM BLAST+ feature in the gDAT bioinformatics pipeline[8]; we manually added these taxonomic assignments to our resulting ASV table if they met the following BLAST criteria: ≥95% alignment, a BLAST e-value <1e-50, and ≥90% sequence similarity. The MaarjAM database classifies AM fungal sequences into 'virtual taxa' (VT) as proxies for AM fungal species; here we perform analyses both at the ASV and VT levels.

To normalize differences in ASV counts across samples, we performed variance stabilization in the Deseq2 R package[38]. This method utilizes a Bayesian mixture model that scales ASV counts within and across samples, avoiding taxon abundance biases introduced by traditional rarefying methods[39]. We also retained the unnormalized dataset for some of the analyses (see below).

Plant community composition and soil nutrient data

Plant community composition in the experimental plots have been surveyed and studied extensively since initial seeding; for fine details for seasonal plant surveys see Reed et al.[27]. Point-intercept methods were used to characterize plant composition at the plot-level, and these count data were used to calculate plant species diversity for each

plot. Specifically, we calculated observed richness (number of plant species in each plot), Faith's phylogenetic diversity and Rao's phylogenetic diversity using a phylogenetic tree pruned from a preexisting megatree using the 'phylo.maker' function in V.PhyloMaker [40]. Soil nutrient data were collected using buried anion and cation probes (ug $10cm^{-2}$ burial period $^{-1}$,Plant Root Simulator Probes, Western Ag, Saskatoon, SK, Canada) as described in Reed et al. [27].

Statistical analyses

All statistical analyses were performed in R version 4.0.3, and all plots were generated using ggplot2 unless otherwise indicated[41].

AM fungal community diversity and composition were analyzed using the *phyloseq* package[42]. To address our first hypothesis, we tested whether site, plant diversity treatment and drought treatment significantly correlated with shifts in AM fungal composition using PERMANOVA models and distance-based redundancy analysis (dbRDA) with vegan functions 'adonis2' and 'capscale', respectively[43].
We calculated two different metrics for nestedness, as each metric represents the compositional nestedness of plant-AM fungal networks differently. First, we used the 'nestedtemp' function in the vegan package to calculate the temperature of presence-absence interaction matrices for high- and low-diversity plots separately[43]. Matrix temperature is a normalized value describing how far interaction data diverge from being perfectly nested; that is, how many interactions exist outside of the hypothetical presences and absences in a perfectly nested matrix[44]. A perfectly nested matrix would have a temperature of 0, while a nestedness temperature of 100 would be considered anti-

nested. The other nestedness metric we measured was NODF (nestedness based on matrix overlap and decreasing fill) which is another quantitative measurement of nestedness that accounts for plants and fungi (rows and columns in interaction matrices, respectively) separately and standardizes differences in total interactions; NODF values are the opposite of temperature, where a NODF value of 100 is a perfectly nested matrix[45]. All nestedness calculations were repeated with a randomized version of the community matrix using 'quasiswap' with 'oecosimu' in *vegan* with 500 simulations; this function tests the null hypothesis that the nestedness statistic is significantly different from a series of simulated random communities while retaining row and column sums of community matrices[43].

We used the *bipartite* package[46] to calculate network-level specialization (H2')[47] in addition to network modularity with the Beckett algorithm[48]. All of these analyses were performed on presence-absence data that were aggregated by plot type then plant species. Modularity was calculated for plant species-AM fungal interaction networks separately for high and low diversity plots at both the ASV and VT level. Values for each network were calculated using the 'metaComputeModules' function with 100 simulations, and tested for significance against null models with 100 simulations of randomized networks with the 'r2dtable' function, which keeps row and column sums constant but randomly changes cell values in an interaction matrix[49]. We compared observed modularity scores (Q_{obs}) relative to the null distribution (Q_{null}) using z-scores: $(Q_{obs} - \text{mean}(Q_{null})) / \text{standarddev}(Q_{null})$.

Threshold indicator taxa analysis (TITAN) to identify specific AM fungal ASVs that change in occurrence, frequency of occurrence and direction of change with leaf ^{15}N

enrichment values for receiver plants only[50]. Briefly, the 'TITAN2' function calculates indicator taxa values from traditional indicator species analyses with a continuous environmental variable and identifies change points along the environmental gradient at which individual ASVs change in abundance or frequency of occurrence. These calculations were bootstrapped and standardized to z-scores, maintaining magnitude and direction of the ASV responses to environmental variables – in this case, leaf ^{15}N enrichment. Fungal ASV table was transformed to presence-absence before TITAN analysis. Indicators visualized using the 'plotTaxaRidges' function in TITAN2 [50].

RESULTS

After removing low-coverage samples (samples with fewer than 100 reads) we obtained 1,352 AM fungal ASVs belonging to seven families, eight genera and 95 virtual taxa in 429 samples. Of the ASVs detected in our dataset, 1,026 were in high diversity plots and 745 in low diversity plots. There were 10 ASVs that did not have taxonomy assigned in DADA2, and did not meet our BLAST+ criteria (<90% sequence similarity with taxonomy in MaarjAM database). Although our samples were not equally distributed across all host plant species, we observed saturation of accumulation curves for ASV richness for all plant species in both high and low diversity plots (Fig. S1).

AM fungal diversity depends on latitude and plant diversity, but not drought treatment

We first examined whether AM fungal richness varied with plant functional group, lifespan, species, and plot-level diversity metrics. For plant species and functional groups, we detected significantly higher AM fungal ASV richness in perennial grasses

compared to annual grasses in both high and low diversity plots (Fig. S2B, Wilcoxon $p \leq 0.015$). Overall, there were no differences in AM fungal richness across plant species, but a pairwise post-hoc analysis revealed significantly lower fungal richness in annual *Vulpia* spp. and *Bromus hordaceus* compared to perennial *Sidalcea malviflora* and *Schedonorous* spp. (Fig. S2A, Tukey's Honest Significant difference corrected $p \leq 0.01$). We did not detect any differences in AM fungal richness in drought versus control plots.

When we grouped ASV data at the plot level, observed richness was significantly greater in high versus low diversity plots (Wilcoxon $p \leq 0.0001$), and correlated with plant community phylogenetic diversity across plots (Fig. 2A-C, $R^2=0.18$, $p < 0.001$).

Shifts in AM fungal composition as measured by Bray-Curtis dissimilarities in our variance-stabilized data were significantly distinct across sites at both the ASV and VT levels of AM fungal classification (Table 1, PERMANOVA $p \leq 0.001$). This pattern remained significant when considering all plots, within each plot type, with an interaction between site and plot type (high vs low diversity treatments; Table 1, PERMANOVA $p \leq 0.001$). We found no evidence for an effect of drought treatment on AM fungal composition, except for weakly significant correlations at the VT level for the whole dataset and within pasture plots (Table 1, PERMANOVA $p \leq 0.01$). Host plant species, plant functional group (grass vs. forb) and plant life span (perennial vs. annual) were all significant factors driving shifts in AM fungal composition, except for in the high diversity plots, where functional group was only moderately (ASV level) or not significant (VT level, Table 1).

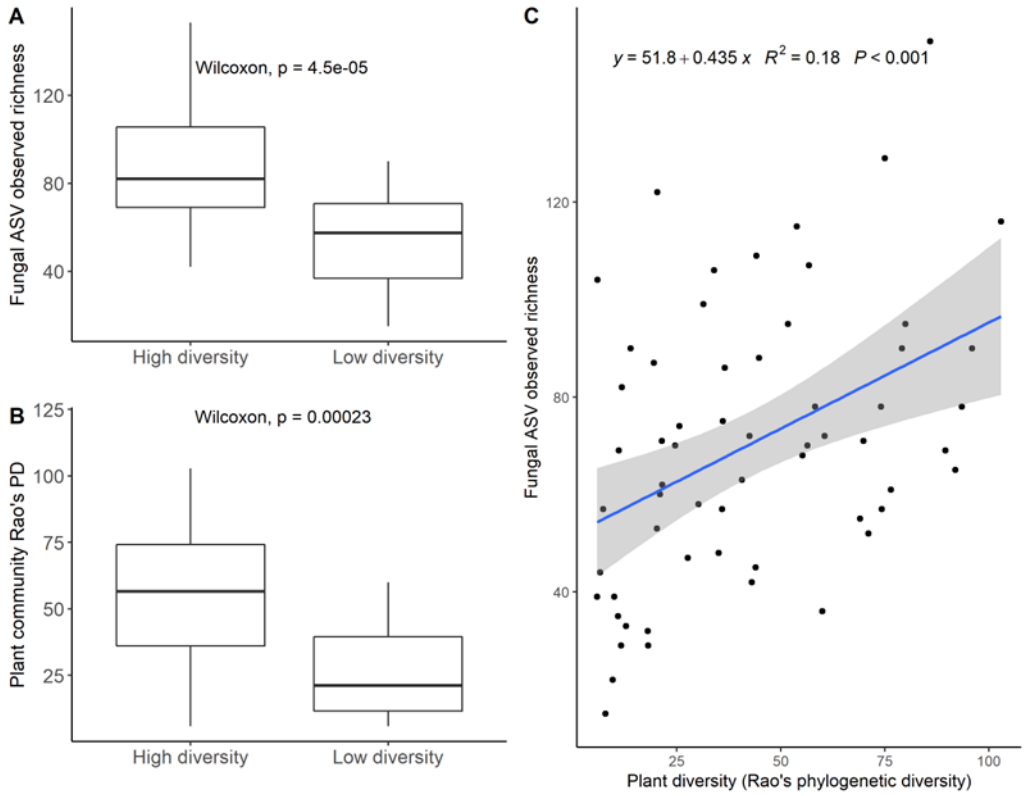

Figure 2. AM fungal alpha diversity (observed richness ASVs) reflected aboveground phylogenetic diversity patterns at the plot level. High diversity treatment plots had greater ASV richness (A) and phylogenetic diversity in the plant communities (B). The correlation between ASV richness and plant phylogenetic diversity was significant (C).

To further examine the specific factors driving shifts in AM fungal composition across sites and plot types, we used a constrained ordination method (distance-based redundancy analysis, or dbRDA), where specific soil properties and plot-level plant species diversity variables were modeled against the Bray-Curtis dissimilarity matrix for AM fungal composition; the model included plant identity, soil nutrient data and plant species richness (Fig. 3). The overall model was significant (adjusted $R^2 = 0.14$, $p \leq 0.0001$) and revealed that several soil nutrient properties (total N, Ca, Mg, K, B, Fe, Mn,

Cu, S, Al and Cd) as well as plant species richness were significant predictors of shifts in AM fungal composition (Fig. 3).

Table 1. Results of permutational analysis of variance (PERMANOVA) models run on the Bray-Curtis dissimilarity matrices for normalized AM fungal sequence data at the level of ASV and VT. All models were run for each variable independently with 999 permutations except where an interaction is indicated. Non-significant relationships in grey.

		ASV-level					VT-level		
		df	p	pseudoF	R^2		p	pseudoF	R^2
all samples	Site	2	0.001	8.59	0.038		0.001	50.34	0.196
	Plot type (high vs low diversity)	1	0.001	2.79	0.006		0.001	6.34	0.012
	Site * plot type	2	0.001	1.91	0.009		0.001	5.70	0.022
	Host plant identity	10	0.001	2.30	0.056		0.001	9.38	0.194
	Plant functional group	1	0.005	1.57	0.004		0.006	2.67	0.006
	Plant lifespan	1	0.001	2.46	0.006		0.001	8.72	0.021
	Func. group * life span	1	0.001	1.84	0.005		0.001	6.21	0.015
	Climate treatment	1	0.593	0.95	0.002		0.003	2.79	0.006
high diversity	Site	2	0.001	5.97	0.459		0.001	33.71	0.215
	Host plant identity	7	0.001	1.3437	0.03622		0.001	6.5914	0.16069
	Plant functional group	1	0.045	1.3772	0.00551		0.162	1.402	0.00544
	Plant lifespan	1	0.002	1.959	0.00784		0.001	6.5913	0.02558
	Func group * life span	1	0.02	1.4852	0.00595		0.001	4.67	0.01812
	Climate treatment	1	0.771	0.857	0.0033		0.118	1.58	0.00504
low diversity	Site	2	0.001	3.842	0.04948		0.001	21.5018	0.22413
	Host plant identity	5	0.001	2.7078	0.06948		0.001	10.847	0.27358
	Plant lifespan	1	0.003	1.6235	0.01045		0.001	5.8983	0.03833
	Climate treatment	1	0.382	1.0459	0.00671		0.008	2.5411	0.01324

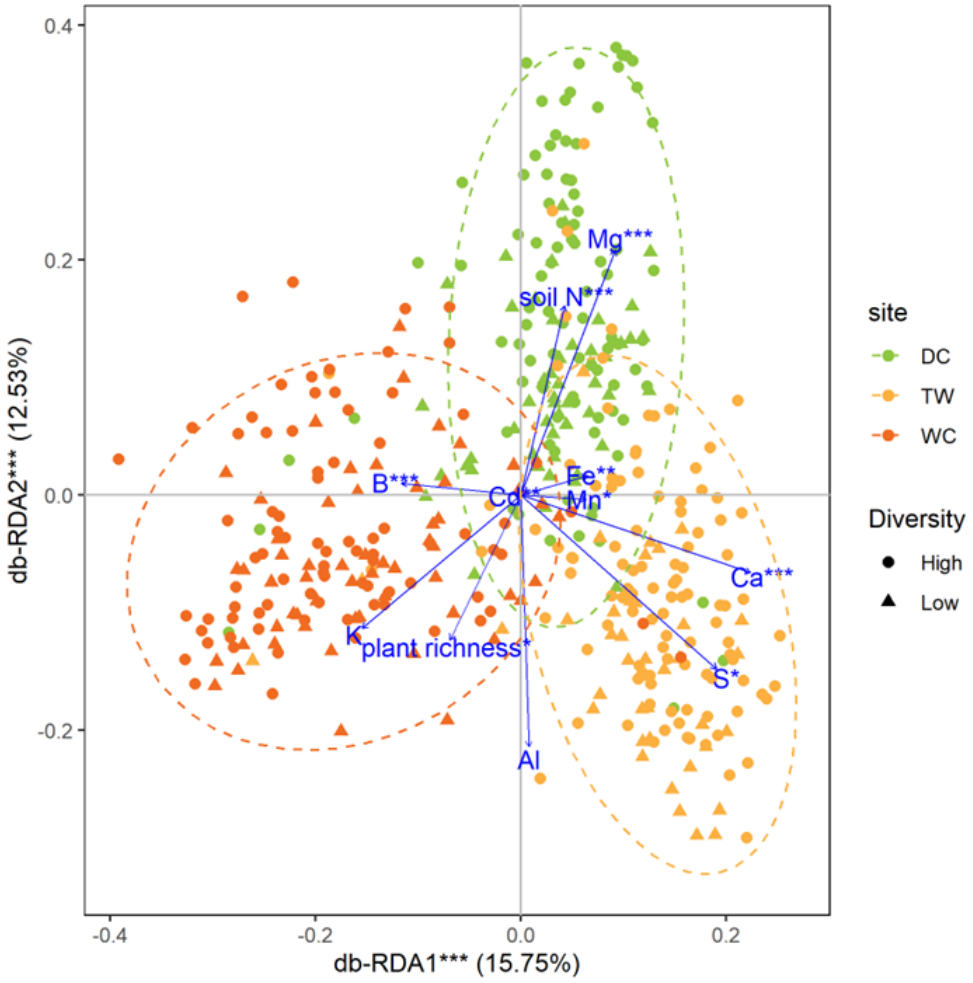

Figure 3. Constrained ordination analysis of Bray-Curtis dissimilarities for AM fungal communities at the ASV level across sites (colors) and plant diversity treatments (shapes). dbRDA model is significant ($F = 1.5793$, $p \leq 0.0001$). Plot shows the main soil nutrient and plant richness variables correlating with shifts in AM composition (with 9999 permutations, * = $0.01 \leq P < 0.05$; ** = $0.001 \leq P < 0.01$; *** = $P < 0.001$; no asterisk = $0.05 \leq P < 0.1$). Dotted ellipses delineate 95% confidence intervals for grouping samples by site. DC = southern site, TW = northern site, WC = middle site.

AM fungal-plant interaction network topology

We addressed our second hypothesis by examining the nestedness, specialization and modularity metrics for AM fungal-plant interaction networks separately for high- and low-diversity plots. Nestedness temperature for high diversity plots was significant

relative to null models under 500 simulations (Fig. 4A; T=29.556, $p \leq 0.002$), but not significant when using the NODF metric (NODF = 21.441, p=0.07). Low diversity plots had similar nestedness values, but did not significantly differ from null models for both temperature (Fig. 4B; T= 37.248, p=0.7126) and NODF (27.379, p=0.066) metrics. To examine whether the anti-nestedness in plant-AM fungal interaction networks was due to greater specialization in partner interactions, overall network specialization (H2', Bluthgen et al. 2006) was calculated. Interestingly, overall network specialization was high, with slightly higher in low diversity plots across sites, but when comparing high versus low diversity plots' network specialization, the difference was not significant (p = 0.056, Fig. S4).

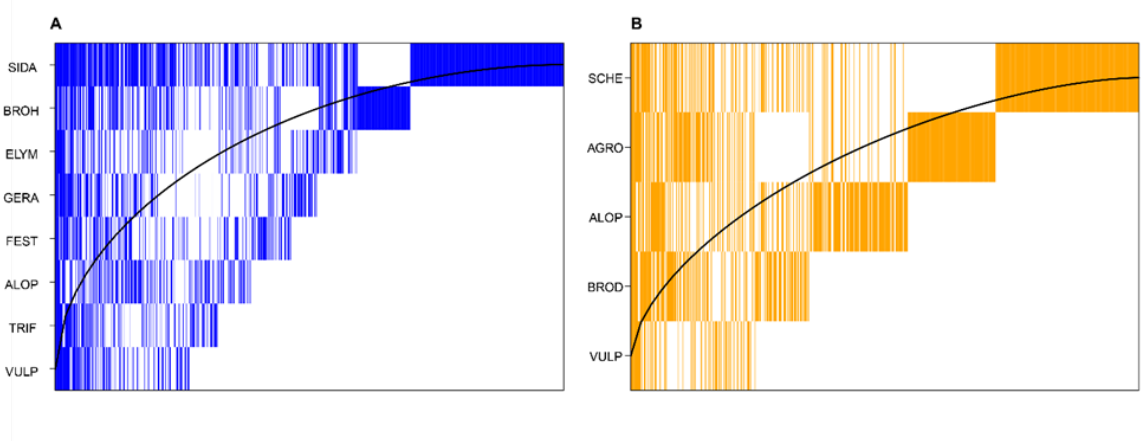

Figure 4. Nestedness of AM fungal ASVs (vertical lines) and their associations with host plant species (rows) for high diversity (A) and low diversity (B) plots across sites. High diversity plots had lower nestedness temperature (T=29.556) relative to the null models ($p \leq 0.002$) but not when considering the nestedness based on overlap and decreasing fill of interaction matrices (NODF = 21.441, p=0.07). Low diversity plots were not significantly nested relative to null models. Curved lines show theoretical isocline where all interactions in a perfectly nested matrix would appear above the line (T=100 or NODF=0).

The AM-fungal-plant interaction networks for both high and low diversity treatments were significantly modular relative to null models, both at the ASV and VT levels (Table 2, Fig. 5). All z-scores for observed modularity values relative to the null distributions revealed observed modularity in networks ≥9 standard deviations more modular than random networks generated with the same marginal totals of interacting species (Table 2). ASV-level networks had significantly higher modularity values than the VT networks; due to the large number of ASVs in the ASV-level networks (>700), we present VT-level networks for visualization and more detailed analyses (Fig. 5). Low diversity networks were more modular than high diversity networks (Q_{low}=0.2519, Q_{high}=0.1453 for VT-level), and species interactions separated into three distinct modules for low diversity versus four modules in high diversity networks. Each distinct module consists of the plant-fungal interactions which occur significantly more frequently within the modules they belong to than with the taxa outside of the modules (Fig. 5). For each individual taxon's membership in a module, among-module connectivity (c values) and within-module degree (z values) can be examined to better understand each taxon's contribution to network topology[49,51,52]. Taxa with high values of both c and z are both major within- and between-module connectors and thus be considered major connectors in an interaction network[51]. We calculated c and z values for all AM fungi in both networks, to compare with subsequent local CMN and isotope enrichment analyses.

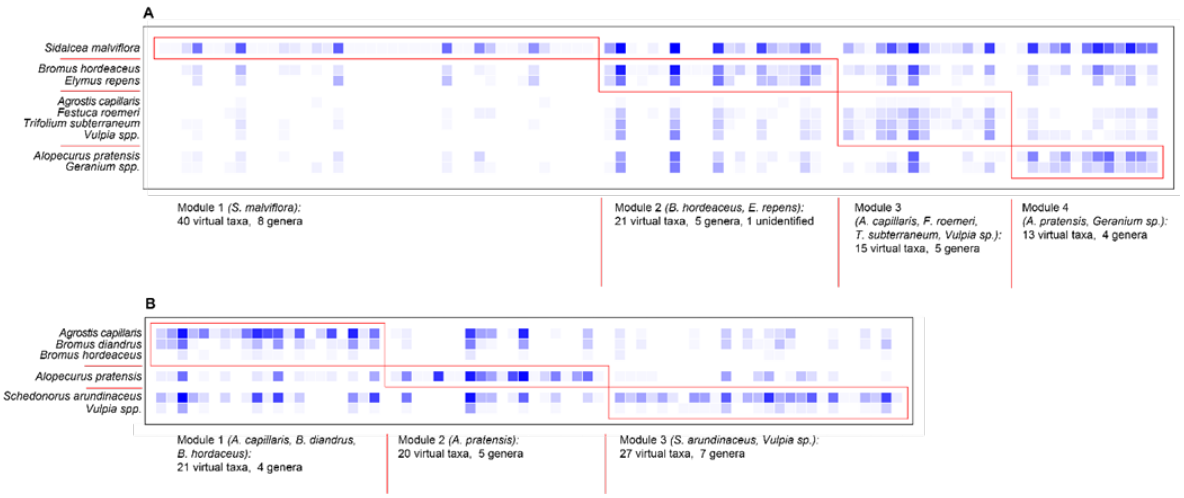

Figure 5. Plant-AM fungal interaction matrices with AM fungal VTs in columns and plant species in rows for high diversity (A) and low diversity (B) plots, ordered to show modularity and module membership of each taxon. Both matrices are significantly modular (high diversity $Q=0.1453$, $p \leq 0.0001$; low diversity $Q=0.2519$, $p \leq 0.0001$). Number of interactions for each plant-AM fungal VT combination indicated by color of tiles, ranging from 0 interactions (white) to 77 in high diversity and 39 in low diversity (brightest blue). Fungal VTs belonging to each module are listed in Tables S1 and S2.

Plot-level network analysis and stable isotope enrichment

There was no relationship between how many fungal ASVs are shared between plants within a plot and leaf ^{15}N enrichment (linear mixed effects models $p > 0.1$). When we analyzed plant-fungal networks at the plot-level, there were significantly more fungal ASVs shared between at least 2 plants (i.e. symbiont range of individual plants within a plot) in high versus low diversity plots (Wilcoxon $p \leq 0.0001$). Additionally, we found significantly negative correlations between the first dbRDA axis and the number of shared fungal ASVs between plants in a plot, where the first axis largely discriminates between sites (Fig. S5, Fig. 3).

Threshold indicator taxa analysis (TITAN) detected 7 ASVs as significant indicators of increasing ^{15}N enrichment in leaf samples (Fig. 6). This analysis calculates

the classic IndVal index for indicator species analysis along a continuous environmental variable over several permutations and bootstraps, returning a standardized z-score representing the magnitude of IndVal scores for each fungal ASV that significantly increased (Fig. 6) or decreased (Fig. S6) with change in the leaf 15N enrichment variable.

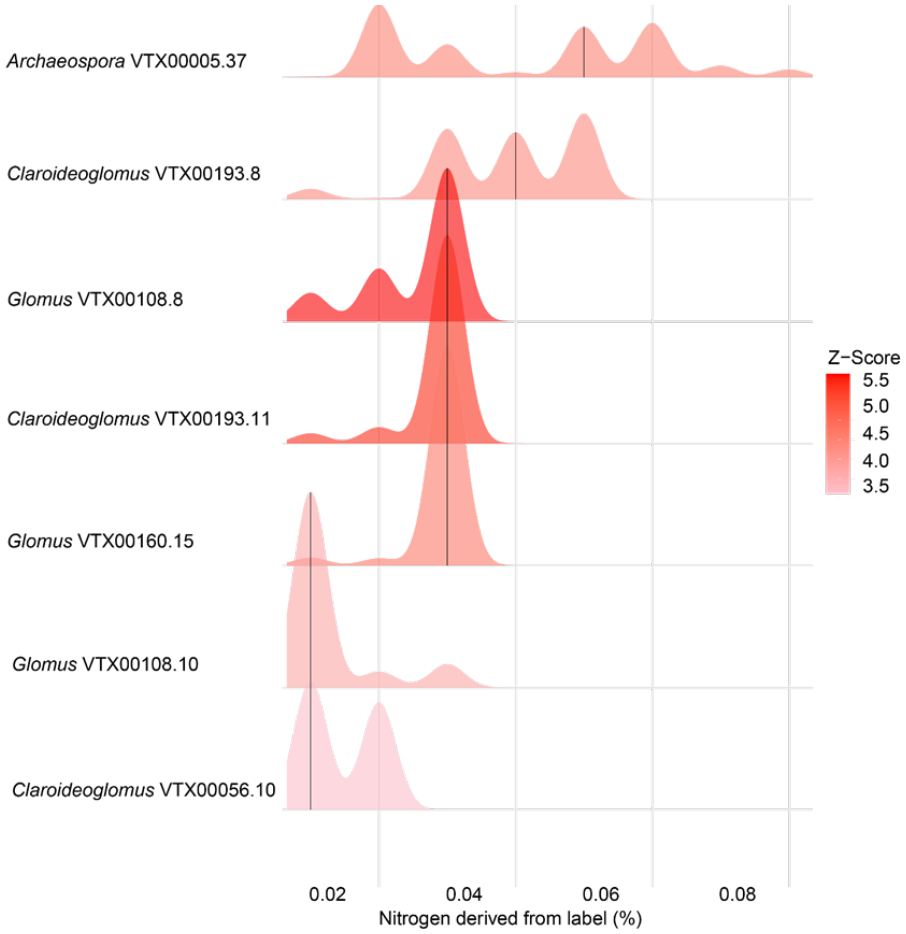

Figure 6. Results of the indicator taxa analysis (TITAN) showing taxonomy for the AM fungal ASVs that increased in frequency of occurrences with increasing leaf 15N enrichment in host plants. Height of peaks indicate the magnitude of z-scores representing standardized IndVal scores (Dufrene and Legendre, 1997) bootstrapped 500 times with 250 random permutations.

DISCUSSION

Theory suggests that AM fungi play essential roles in plant species coexistence, particularly in grasslands[53]. One major way in which AM fungi are thought to maintain plant coexistence in grassland ecosystems is through transfer of limiting resources such as N via CMNs[54], but evidence for these behaviors in nature remains limited, and we still lack an understanding of how CMNs vary in structure and function across environments. Our finding that plant-AM fungal interaction networks in high and low diversity pasture systems display significant patterns of modularity, and not nestedness, implies greater levels of preferential partner selection than is expected in mutualistic networks. This suggests that some partners are selected for by specific hosts, and may not provide beneficial services for other host plants. We also provide evidence for hierarchical spatial structuring in plant-AM fungal assembly patterns, in which AM fungal composition colonizing roots of the same communities of host species across sites was primarily driven by environmental variables at larger spatial scales (across sites), and that plant host identity and local plant diversity shapes AM fungal assemblages at a more local scale (within sites). This suggests that future studies should incorporate larger spatial scales in sampling efforts to capture the full diversity of plant-AM fungal interactions. The absence of a significant relationship between number of shared AM fungal partners and quantity of resources shared between plants at the plot-level suggests that there are more nuanced patterns of CMN-mediated resource transfer than frequency of interactions alone.

Mycorrhizal fungal diversity increases with plant community diversity

We observed positive correlations between AM fungal richness and plant community diversity, both at the coarse level of plant diversity plot treatments, and within-plot phylogenetic diversity in plant communities. This finding is consistent with previous research and theoretical patterns in mycorrhizal diversity, in which aboveground diversity facilitates greater diversity in plant-associated microbial communities (reviewed in ref. 55 [55]), including the abundance and diversity of mycorrhizal fungi [56]. Specifically, different functional groups of plants have been shown to associate with distinct mycorrhizal fungal communities[57,58], facilitating a consistent, positive relationship between plant and AM fungal alpha and beta diversity across biomes[59]. We found no evidence of an effect of long-term drought pressure on AM fungal community diversity across plant diversity treatments and sites. This finding was surprising, since AM fungi have been shown to respond strongly to drought in similar experimental grassland systems[60], and generally provide plant community resilience under drought conditions[61,62]. This discrepancy in our study may be due to changes in plant-AM fungal behaviors, but not composition, under drought (i.e. plasticity of responses by both partners[63]), or differences in the severity of drought studied in other systems relative to ours.

Mycorrhizal network assembly is shaped by environment and plant diversity

It is well-established that AM fungal composition is sensitive to differences in climate, soil properties and nutrient availability[64], but few studies have addressed these patterns in nature at the community-level including multiple trophic levels. Our study

analyzed how these factors shape both plant and fungal cooccurrences in both broad-scale interaction networks and local-scale common mycorrhizal networks. Unexpectedly, we found no evidence of nestedness in plant-fungal interaction networks, which is contrary to what is expected in mutualistic networks and shown in previous studies if plant-AM fungal systems[16,18,20]. This result may be due to the taxonomic resolution at which we analyzed nestedness in interaction networks; the fungal ASVs in our study are considered distinct individuals with a single nucleotide difference in the small subunit (SSU) marker region of AM fungal genomes, while previous studies reporting significant nestedness in plant-AM fungal networks have examined patterns at coarser taxonomic scales (i.e. clustered sequences or genera). Nonetheless, the high modularity in our interaction networks suggest non-random interaction patterns between AM fungi and host plants in nature, which is supported by previous studies examining host specificity in AM fungal communities[9,59]. Most of this preexisting research has been in relatively simple experimental systems either in the greenhouse or with single or few species consortia of mycorrhizal fungal taxa; our study provides one of the first characterizations of plant-AM fungal assembly patterns with naturally-occurring AM fungal communities, under field experimental conditions and including a plant diversity manipulation. Our findings provide promising evidence that preferential partner selection in the AM symbiosis extends beyond simple experimental systems, and that even with the vast diversity of AM fungal taxa in natural soils (here, >1000 sequence variants and >90 virtual taxa), some level of specificity is still apparent.

Nutrient sharing via local common mycorrhizal networks

A major gap in our knowledge regarding CMN form and function concerns whether the number of shared AM fungal taxa on roots of neighboring plants correlates with quantity of resources shared through common AM fungal symbionts. We tested this using stable isotopes and fine-scale AM fungal symbiont characterization (strain-level variation/ASVs) and found no evidence for this quantity of partners/quantity of resources relationship. To our knowledge, this has not been previously tested in complex, multi-species natural communities using current stable isotope methods. To determine whether resource sharing in CMNs is via higher quality partners rather than quantity of partners, we performed threshold indicator taxa analysis to get a general look at whether any AM fungal ASVs on plant roots served as indicators of stable isotope enrichment. The indicator ASVs selected by this algorithm were *Glomus*, *Claroideoglomus* and *Archaeospora* species, and the virtual taxa were all detected as 'connectors' (high c values) in our modularity analysis. This is theoretically interesting, as 'connectors' of modules in larger scale interaction networks implies that those taxa are linking distinct sets of interacting species, suggesting that resource sharing indicator taxa are connecting distinct groups of plants. Ecologically, this result may imply that loss of these species would cause a decline in CMN functioning in prairie/pasture systems in the PNW. Further research is necessary implementing stable isotope probing in fungal DNA extracts, to confirm whether these indicator AM fungal taxa are actively transporting isotope-enriched nutrients, or if they are simply indirectly responding to another factor that is driving CMN resource transfer functions.

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CHAPTER IV

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CHAPTER V

CONCLUSION

Land use for agricultural purposes is one of the most intense disturbances to terrestrial ecosystems, resulting in low-diversity ecological systems that are sensitive to climate change disturbances such as variable precipitation and prolonged drought. Soil microbes that control ecosystem processes essential to the functioning of agricultural systems such as nutrient cycling through decomposition and storage of organic matter, or pathogens and mutualists that colonize plants, are also sensitive to disturbances, particularly those imposed by agricultural management. To account for this, sustainable practices aim to harness endogenous soil microbial functions to enhance agroecosystem resiliency to perturbations; however, the impacts of specific management regimes on soil microbial structure and function are not well-characterized, and require investigations spanning spatial scales (from local site characteristics to regional differences), climate zones (temperate Mediterranean to tropics) and crop types (perennial crops, agroforestry, grasslands) to decipher general patterns. With a better understanding of general patterns of agricultural soil microbial responses to management regimes, we may more accurately predict the stability and behavior of food systems under climate change pressures. My dissertation aimed to elucidate how plant-associated and soil microbial responses to management varied across agroecological contexts.

Chapter II of my dissertation examined assembly patterns in vineyard soil bacterial and fungal communities throughout grapevine phenological stages across sites

spanning spatial scales. This study showed that soil microbial communities in vineyards are hierarchically spatially structured by season and region, with local-scale climate and soil properties nested within these factors as determinants of microbial composition. Notably, we found that specific management practices can outweigh these factors, such that vineyard soil microbial composition can respond to management at the same scales as regional and seasonal differences. These broad scale impacts of agricultural practices on crop-associated microbiomes suggest that there are generalizable patterns of management-induced shifts on soil composition across environmental contexts.

In the third chapter of my dissertation, I present an examination of the ecological consequences of coffee farm management intensity by characterizing soil fungal composition and enzyme activity profiles from coffee plantations under various management regimes in addition to nearby undisturbed forests. We found that soil functional potential responds more strongly to coffee farm management than fungal composition, and that saprotrophic fungi drive differences in community composition across coffee farm soils. Results from this chapter have implications to inform functional consequences of management regime shifts in coffee agroecosystems, one of the most traded commodities in the world. This study also draws linkages between specific fungal groups and their putative roles in soil functional potential, that may be generalizable across regions and different cropping systems.

Chapter IV aimed to characterize whether common mycorrhizal networks exist in pasture/prairie systems, and if so whether plant diversity mediates shifts in AM fungal composition and function. We examined plant-AM fungal coassembly patterns across sites varying in their natural climate, in addition to the effects of experimental drought on

the composition and function in AM fungal communities. While most mutualistic networks show nested assembly patterns, we found evidence for preferential partner selection in AM fungal-plant host interaction networks for PNW prairie and pasture species. We also identified AM fungal taxa putatively important in CMN function between plants of different functional groups and lifespans, linking individuals across diversity and climate contexts and facilitating sharing of nutrients via common mycorrhizal networks at the plot-level.

Altogether, these studies link patterns in soil and plant-associated microbial assembly from local- to regional- scales in agricultural systems varying in management intensity. Future directions of this research would quantify more agroecosystem functions to better understand and predict specific functional responses of agricultural soils under disturbance regimes and climate change pressures.

APPENDIX A

SUPPLEMENTAL INFORMATION FOR CHAPTER II

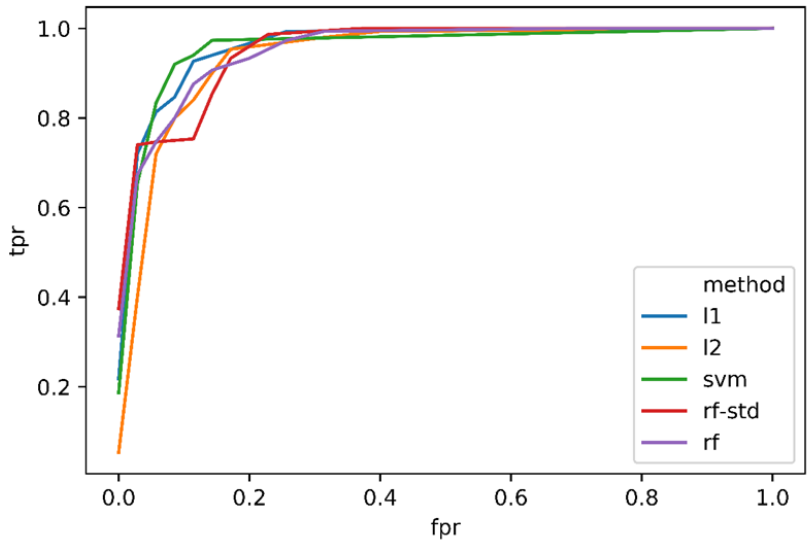

Figure S1. Receiver operating characteristic (ROC) curves for models built with L1-regularized logistic regression (l1), L2-regularized logistic regression (l2), support vector machine (svm), and random forest (rf-std and rf) methods for the 'all fungi' Fall season dataset predicting till/no till management practices. Y axis shows true positive rate, x axis shows false positive rate. Area under the curve (AUC) values for each type of model: L1-reg 0.973, L2-reg 0.94,SVM 0.974, RF 0.95.

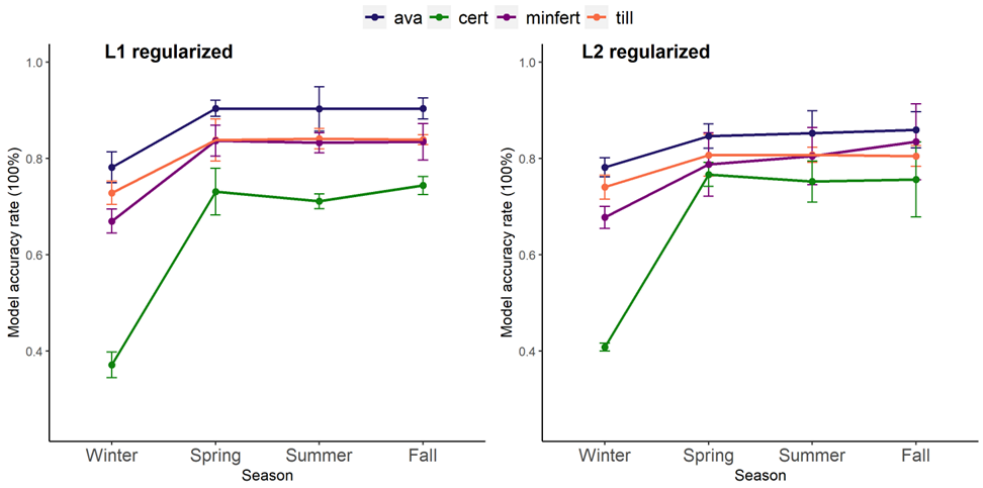

Figure S2. Average model accuracy rates for bootstrapped logistic regression solvers with two different regularization methods (L1 and L2) show nearly identical results. Models trained and tested on 'all fungi' dataset across seasons; error bars show standard deviation across three models. Colors indicate which categorical variable models were classifying samples into (ava = growing region, cert = vineyard certification, minfert = application of mineral fertilizer, till = till vs. no till soils).

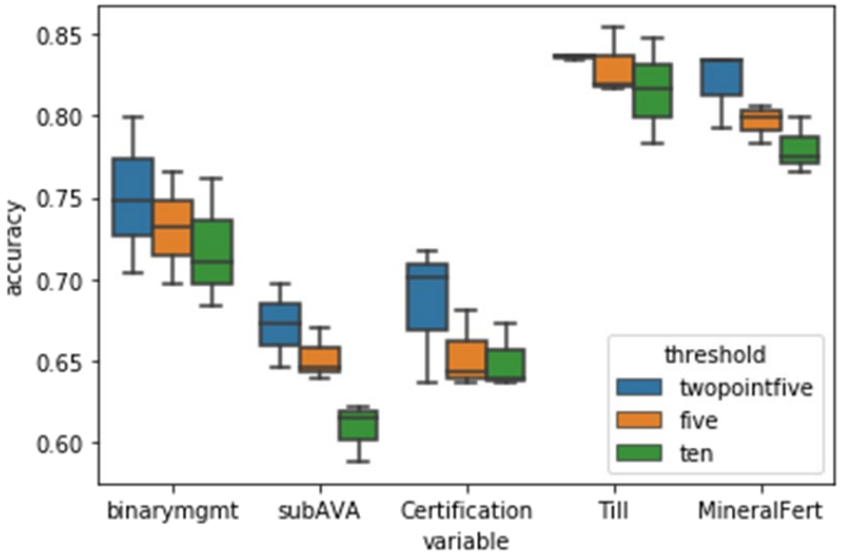

Figure S3. Model accuracy declines across all variables when imposing a prevalence threshold for ASVs in the 'all fungi' Fall season dataset. Threshold indicates the percentage of samples ASVs had to be present in for including in model training and test datasets; no threshold n=75,454 ASVs, 2.5% threshold n=4,734 ASVs, 5% threshold n=2,515 ASVs and 10% threshold n=1,326 ASVs.

Table S1. Average accuracy, precision and recall values across three bootstrapped models for all L1-regularized classifiers across the datasets tested. Model accuracy = # correct predictions / total # predicitons; precision = # true positives / # true positives + # false positives; recall = # true positive / # true positives + # false negatives.

		AVA			Vineyard certification			Till			Mineral Fertilizer		
		Accuracy	Precision	Recall	Avg Accuracy	Avg Precision	Avg Recall	Accuracy	Precision	Recall	Accuracy	Precision	Recall
Winter	all fungi	0.7818	0.6886	0.7818	0.3713	0.3735	0.3713	0.7286	0.6715	0.7286	0.6699	0.6423	0.6699
	AMF	0.7492	0.6342	0.7492	0.2910	0.3039	0.2910	0.6992	0.6142	0.6992	0.6363	0.5876	0.6363
	pathogens	0.7568	0.6783	0.7568	0.3572	0.3476	0.3572	0.6960	0.6229	0.6960	0.6330	0.5857	0.6330
	saps	0.7752	0.6851	0.7752	0.3225	0.3120	0.3225	0.7068	0.6558	0.7068	0.6352	0.6010	0.6352
	bacteria	0.8077	0.7286	0.8077	0.4792	0.4614	0.4792	0.7917	0.7695	0.7917	0.7260	0.7196	0.7260
Spring	all fungi	0.9040	0.8963	0.9040	0.7311	0.7460	0.7311	0.8381	0.8511	0.8381	0.8368	0.8500	0.8368
	AMF	0.7311	0.6326	0.7311	0.4513	0.4603	0.4513	0.6804	0.6327	0.6804	0.7257	0.6799	0.7257
	pathogens	0.8395	0.8225	0.8395	0.6241	0.6369	0.6241	0.7874	0.7866	0.7874	0.7778	0.7795	0.7778
	saps	0.8656	0.8618	0.8656	0.6324	0.6359	0.6324	0.7846	0.7821	0.7846	0.7984	0.7929	0.7984
	bacteria	0.8603	0.8310	0.8603	0.7058	0.7622	0.7058	0.8454	0.8570	0.8454	0.8287	0.8384	0.8287
Summer	all fungi	0.9028	0.9114	0.9028	0.7111	0.7355	0.7111	0.8407	0.8525	0.8407	0.8324	0.8419	0.8324
	AMF	0.7324	0.7285	0.7324	0.4509	0.4880	0.4509	0.7630	0.7446	0.7630	0.7250	0.7078	0.7250
	pathogens	0.8278	0.8325	0.8278	0.5870	0.6065	0.5870	0.7667	0.7548	0.7667	0.7593	0.7466	0.7593
	saps	0.8435	0.8540	0.8435	0.6407	0.6686	0.6407	0.7861	0.7751	0.7861	0.7907	0.7950	0.7907
	bacteria	0.8548	0.8643	0.8548	0.6578	0.6945	0.6578	0.8220	0.8343	0.8220	0.8333	0.8413	0.8333
Fall	all fungi	0.9036	0.9123	0.9036	0.7438	0.7707	0.7438	0.8390	0.8547	0.8390	0.8345	0.8479	0.8345
	AMF	0.6927	0.6751	0.6927	0.4569	0.4816	0.4569	0.7132	0.6645	0.7132	0.7109	0.7024	0.7109
	pathogens	0.8005	0.8006	0.8005	0.6111	0.6230	0.6111	0.7732	0.7592	0.7732	0.7449	0.7478	0.7449
	saps	0.8379	0.8545	0.8379	0.6202	0.6402	0.6202	0.7902	0.7835	0.7902	0.7937	0.7935	0.7937
	bacteria	0.8792	0.8932	0.8792	0.6739	0.7135	0.6739	0.8428	0.8550	0.8428	0.8413	0.8487	0.8413

Table S2. PERMANOVA model results for bacteria and all fungi datasets across seasons.

	Bacteria						Fungi					
	DF	SS	R2	adjusted R	Pseudo-F	Pr (>F)	DF	SS	R2	adjusted R	Pseudo-F	Pr (>F)
All seasons												
Season	3	16.7	0.03549	0.033796	31.1312	0.001	3	18.15	0.0326	0.030987	28.0359	0.001
AVA	5	23.98	0.05095	0.048168	26.8188	0.001	5	27.72	0.04978	0.047136	25.6866	0.001
Certification	3	4	0.00849	0.006748	7.4508	0.001	3	6.42	0.01154	0.009892	9.9227	0.001
Till/No till	1	1.43	0.00303	0.002447	7.9788	0.001	1	0.96	0.00172	0.001166	4.4308	0.001
Mineral fertilizer	1	1.71	0.00364	0.003057	9.5861	0.001	1	1.68	0.00302	0.002466	7.7805	0.001
Grape variety	8	6.19	0.01316	0.008524	4.3278	0.001	8	5.97	0.01073	0.006319	3.4591	0.001
RS	8	5.68	0.01207	0.007429	3.9715	0.001	8	7.14	0.01283	0.008428	4.1484	0.001
Season:AVA:Mana	319	167.21	0.35529	0.207544	2.9313	0.001	319	174.93	0.3142	0.166681	2.5411	0.001
Residual	1363	243.73	0.51788	0.682285			1454	313.77	0.56359	0.726926		
Winter												
AVA	4	5.18	0.04736	0.038156	5.7676	0.001	4	2.321	0.01756	0.008923	2.275	0.001
Certification	3	2.321	0.02122	0.014144	3.4456	0.001	3	1.69	0.01279	0.006295	2.2083	0.001
Till/No till	1	0.537	0.00491	0.002524	2.3907	0.002	1	0.391	0.00296	0.000783	1.5337	0.031
Mineral fertilizer	1	0.696	0.00637	0.003987	3.1018	0.001	1	0.589	0.00446	0.002286	2.3103	0.001
Grape variety	8	3.254	0.02976	0.010828	1.812	0.001	8	3.342	0.02529	0.008	1.6379	0.001
RS	7	2.55	0.02332	0.006686	1.6225	0.001	7	3.599	0.02723	0.012165	2.0154	0.001
AVA:Certification	60	19.841	0.18142	0.044228	1.4729	0.001	60	24.587	0.18602	0.063617	1.6065	0.001
Residual	334	74.988	0.68565	0.879447			375	95.655	0.7237	0.89793		
Spring												
AVA	5	10.446	0.1182	0.105567	13.4026	0.001	5	11.737	0.11367	0.101291	12.1552	0.001
Certification	3	2.786	0.03153	0.023252	5.9583	0.001	3	3.525	0.03414	0.026091	6.0846	0.001
Till/No till	1	0.637	0.0072	0.004388	4.0839	0.001	1	0.779	0.00755	0.004808	4.0345	0.001
Mineral fertilizer	1	1.425	0.01613	0.013343	9.1448	0.001	1	1.304	0.01263	0.009902	6.7507	0.001
Grape variety	7	4.298	0.04864	0.029448	3.939	0.001	7	4.084	0.03955	0.020665	3.0212	0.001
RS	7	4.158	0.04706	0.027836	3.8112	0.001	7	4.294	0.04158	0.022735	3.1762	0.001
AVA:Certification	50	20.974	0.23735	0.111914	2.6912	0.001	50	21.721	0.21036	0.084219	2.2495	0.001
Residual	280	43.645	0.49389	0.684252			289	55.811	0.54052	0.730288		
Summer												
AVA	5	17.7	0.1221	0.113408	19.6771	0.001	5	17.666	0.10383	0.095423	16.3708	0.001
Certification	3	4.166	0.02874	0.022993	7.7192	0.001	3	5.024	0.02953	0.024088	7.7592	0.001
Till/No till	1	0.923	0.00637	0.004418	5.1322	0.001	1	1.192	0.007	0.005151	5.5219	0.001
Mineral fertilizer	1	1.56	0.01076	0.008817	8.6699	0.001	1	1.113	0.00654	0.00469	5.1549	0.001
Grape variety	8	4.349	0.03	0.014542	3.0221	0.001	8	4.687	0.02755	0.012872	2.7149	0.001
RS	8	4.648	0.03207	0.016645	3.2298	0.001	8	5.123	0.03011	0.01547	2.9673	0.001
AVA:Certification	77	38.4	0.26489	0.134166	2.7721	0.001	77	41.455	0.24365	0.117318	2.4945	0.001
Residual	407	73.22	0.50508	0.685012			435	93.883	0.55179	0.724988		
Fall												
AVA	5	17.618	0.15866	0.148668	23.2289	0.001	5	18.215	0.13728	0.127341	18.9009	0.001
Certification	3	2.456	0.02212	0.015185	5.3977	0.001	3	3.831	0.02888	0.022198	6.6261	0.001
Till/No till	1	1.18	0.01063	0.008302	7.7794	0.001	1	0.751	0.00566	0.00339	3.8938	0.001
Mineral fertilizer	1	1.165	0.01049	0.008162	7.6822	0.001	1	1.373	0.01035	0.008091	7.1223	0.001
Grape variety	8	4.712	0.04243	0.024103	3.8829	0.001	8	5.053	0.03809	0.020236	3.2774	0.001
RS	8	4.707	0.04239	0.024063	3.8785	0.001	8	4.776	0.036	0.018107	3.0976	0.001
AVA:Certification	58	27.324	0.24608	0.127256	3.1058	0.001	58	30.265	0.22809	0.110581	2.7073	0.001
Residual	342	51.877	0.4672	0.644262			355	68.423	0.51567	0.690057		

APPENDIX B

SUPPLEMENTARY INFORMATION FOR CHAPTER III

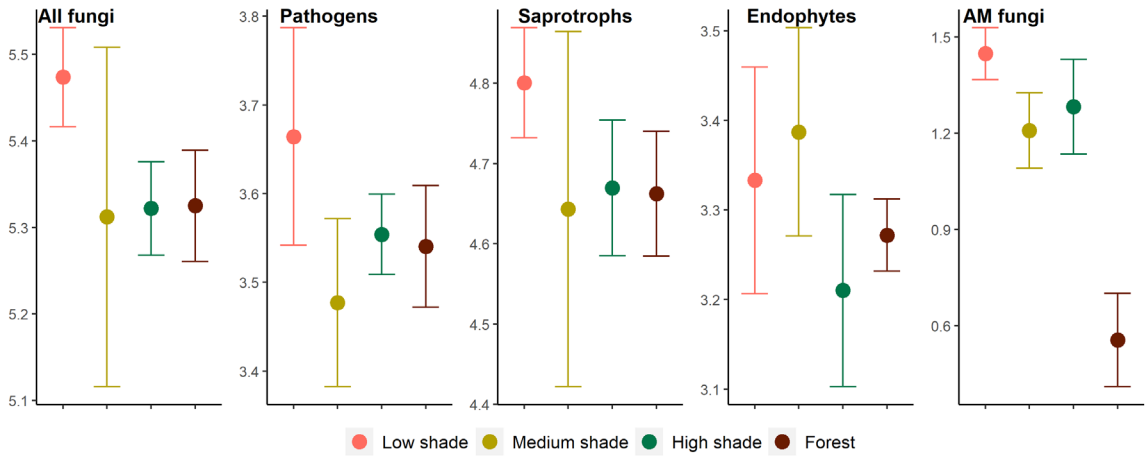

Figure S1. Shannon's alpha diversity across all land uses. From left to right: the whole fungal community, fungi identified as putative pathogens, saprotrophs, endophytes and AM fungi. All comparisons are not significant (ANOVA $p>0.05$) except for AM fungi (ANOVA $p<0.001$, $F=9.719$; Tukey's test $p<0.01$ for all coffee farm types compared to forest.

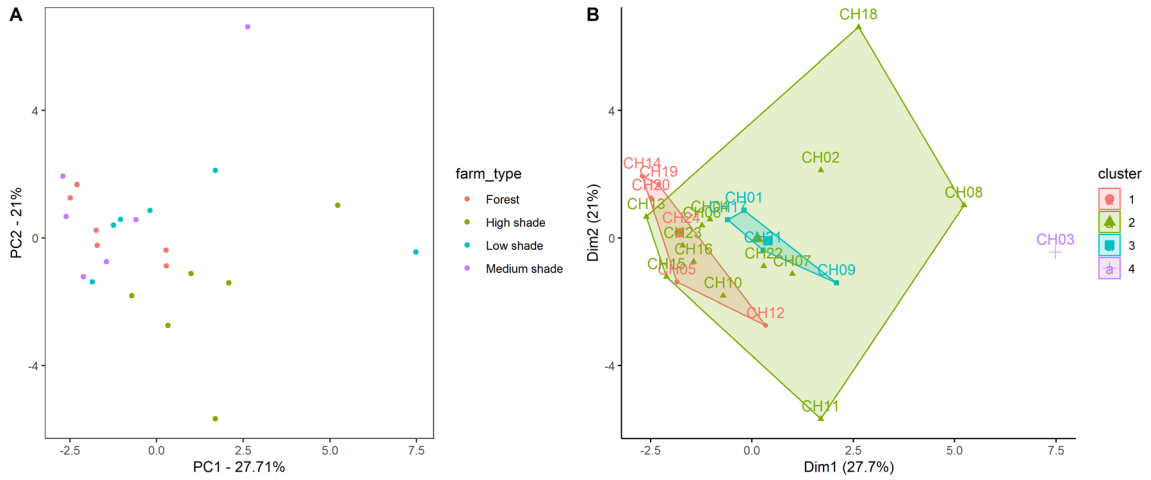

Figure S2. (A) Principal component analysis of all soil nutrient and physicochemical variables (pH, electrical conductivity and texture). PC1 separates % silt and various nutrients (B, Cu, K, Zn, Fe, Al, Mg, Na and P). PC2 separates % clay, Ni, Cd, and Cr from %N, %C, Na, P, pH and % silt. (B) k-means clustering analysis of the same data.

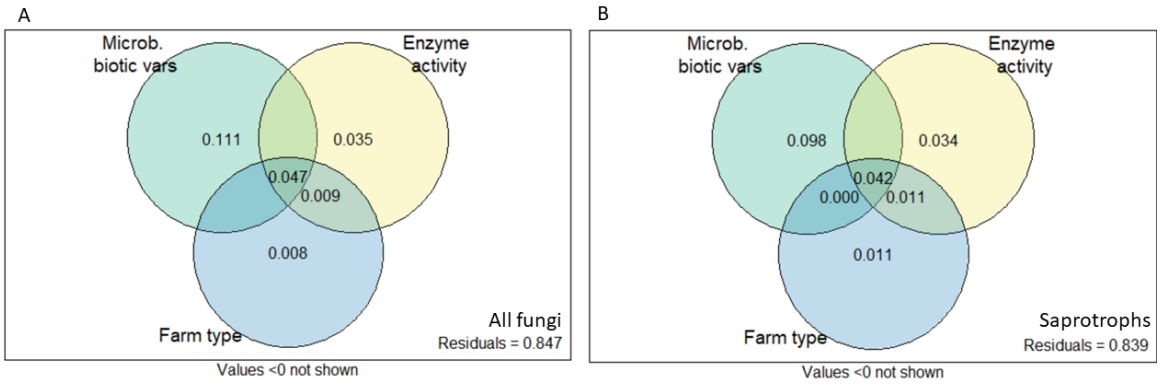

Figure S3. Variance partitioning of microbial biotic variables (bacterial and fungal biomass, Shannon diversity and relative abundance of functional groups), enzyme activities and site type on the Bray-Curtis dissimilarity matrices for all fungi (A) and saprotrophs (B).

Table S1. Summary statistics for all soil nutrient and physicochemical variables measured. Data are presented as means within farm type ± 1 SE.

Soil parameter	units	Forest	High shade	Medium shade	Low shade
pH	1:2 H2O	5.93±0.1	5.89±0.12	5.94±0.08	5.73±0.07
moisture	% H2O	39.37±1.05	32.81±1.72	40.2±1.07	36.07±1.32
electrical conductivity	dS/cm	241.83±8.8	184.77±1.46	215.97±6.06	185.78±6.34
N	%	0.68±0.05	0.47±0.03	0.84±0.09	0.72±0.04
C	%	7.54±0.65	5.14±0.46	9.68±1.31	7.63±0.51
C:N	ratio	11.12±0.43	10.83±0.34	11.25±0.49	10.66±0.08
Ca	%	0.45±0.07	0.36±0.05	0.38±0.07	0.32±0.03
K	%	0.2±0.03	0.31±0.12	0.27±0.11	0.27±0.09
Mg	%	0.57±0.02	0.57±0.08	0.39±0.03	0.49±0.06
P	%	0.06±0	0.06±0.01	0.07±0.01	0.1±0.01
Al	ppm	31045.5±3674.22	37878.17±1832.33	39197.5±3635.22	47771.33±8008.07
B	ppm	72.17±14.83	142.5±39.04	160.17±71.66	164.5±61.51
Cd	ppm	3±0.26	4.67±1.12	3±0.26	3.5±0.72
Cr	ppm	13.17±1.19	17.6±2.04	8.83±0.6	11±1.24
Cu	ppm	59±7.36	118.17±23.45	91.67±30.29	134.33±22.23
Fe	ppm	26098.5±2571.11	33380.33±3668.32	23678.5±1216.75	28316±4046.18
Mn	ppm	1101.83±110.31	892±67.72	569.17±132.2	832.5±147.11
Na	ppm	696.17±86.49	1335.83±470.39	1585.5±700.81	1250±487.22
Ni	ppm	6.33±0.42	27.67±18.52	4.67±0.21	5.67±0.95
Pb	ppm	11.5±0.85	19.67±5.08	13.5±1.18	17±3.43
Zn	ppm	75.33±5.28	72.83±5.86	69.17±12.35	86.5±16.67
Total microbial biomass	nmol g-1	224.11±12.96	153.39±18.5	243.96±13.49	212.41±22.43
Fungal biomass	nmol g-1	11.94±0.59	8.52±1.34	10.46±1.29	8.08±1.19
Bacterial biomass	nmol g-1	73.58±6.55	43.66±3.51	77.83±3.93	65.35±6.61
Fungal to bacterial ratios	ratio	0.17±0.01	0.19±0.02	0.14±0.02	0.12±0.01
Clay	%	24.2±2.87	30.24±3.2	27.37±4.84	25.34±3.58
Silt	%	30.19±5.2	20.01±4.67	31.41±3.44	28.57±6.11
Sand	%	45.62±2.95	49.75±6.26	41.22±2.98	46.09±2.57

Table S2. Results of PERMANOVA statistical tests on the Bray-Curtis dissimilarity matrices for all groups of fungal sequence data across farm type. Only statistically significant ($p < 0.05$) results shown.

Global PERMANOVA				**Pairwise PERMANOVA**				
Variables	**F model**	**R^2**	**P**	**comparison**	**F model**	**R^2**	**P value**	**Corrected P value**
allfungi ~farm_type	1.55	0.189	0.001	Low shade-Medium shade	1.46	0.127	0.018	0.031
				Low shade-Forest	1.79	0.152	0.006	0.018
				Medium shade-High shade	1.53	0.133	0.026	0.031
				Medium shade-Forest	1.84	0.155	0.006	0.018
				High shade-Forest	1.44	0.126	0.023	0.031
saprotrophs~farm_type	1.57	0.191	0.001	Low shade-Medium shade	1.71	0.146	0.007	0.018
				Low shade-Forest	2.04	0.17	0.009	0.018
				Medium shade-Forest	1.86	0.157	0.004	0.018
endophytes~farm_type	1.98	0.229	0.003	Low shade-Medium shade	2.63	0.208	0.008	0.024
				Low shade-Forest	1.72	0.146	0.031	0.046
				Medium shade-High shade	2.07	0.172	0.025	0.046
				Medium shade-Forest	3.33	0.25	0.008	0.024
pathogens~farm_type	1.47	0.18	0.026	all comparisons n.s. after p.val correction				

APPENDIX C

SUPPLEMENTARY INFORMATION FOR CHAPTER IV

Note S1. Description of two-step PCR protocol.

Our two-step PCR protocol contains primer modifications and bioinformatics processing steps that account for some of the most pernicious issues that compromise modern high-throughput amplicon sequencing datasets, particularly those with longer amplicon lengths. The major factors of Illumina sequencing that our protocol accounts for include: poor reverse sequence read quality, which affects the merging of paired-end reads during bioinformatics processing of sequences; low base-call diversity in amplicon-generated datasets due to conserved regions of marker genes; PCR amplification biases that often result in inaccurate estimates of sequence abundances and primer interactions with Illumina adapters and indices.

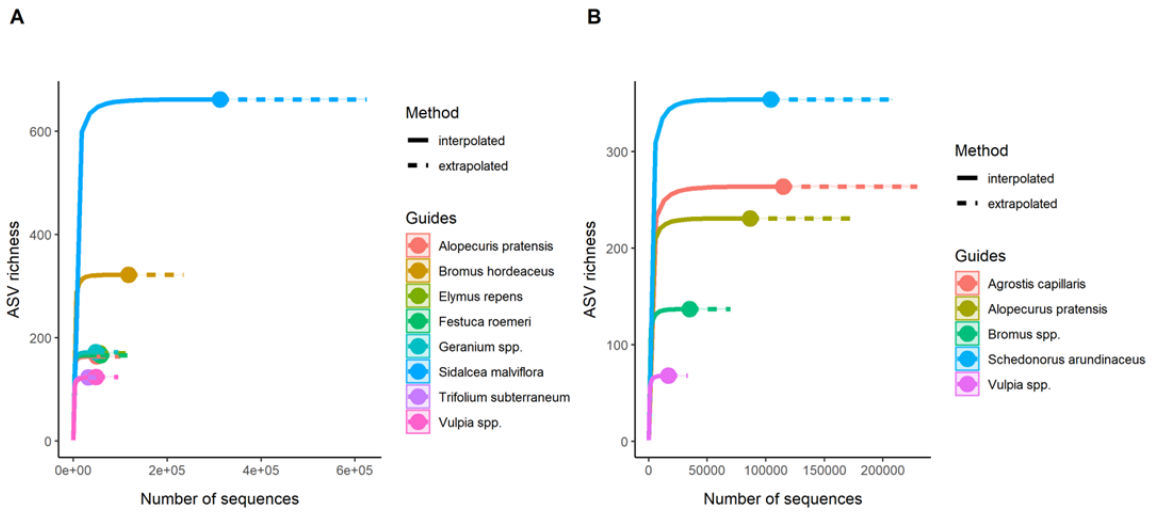

Figure S1. ASV accumulation curves by host plant species for high (A) and low (B) diversity plots across all sites. Curves were generated based on unnormalized ASV counts detected in roots of each host plant species (excluding plant species with <6 samples across sites). Rarefaction curves with extrapolation (solid and dashed lines, respectively) calculated with 200 replicate bootstraps for high- and low-diversity plots separately.

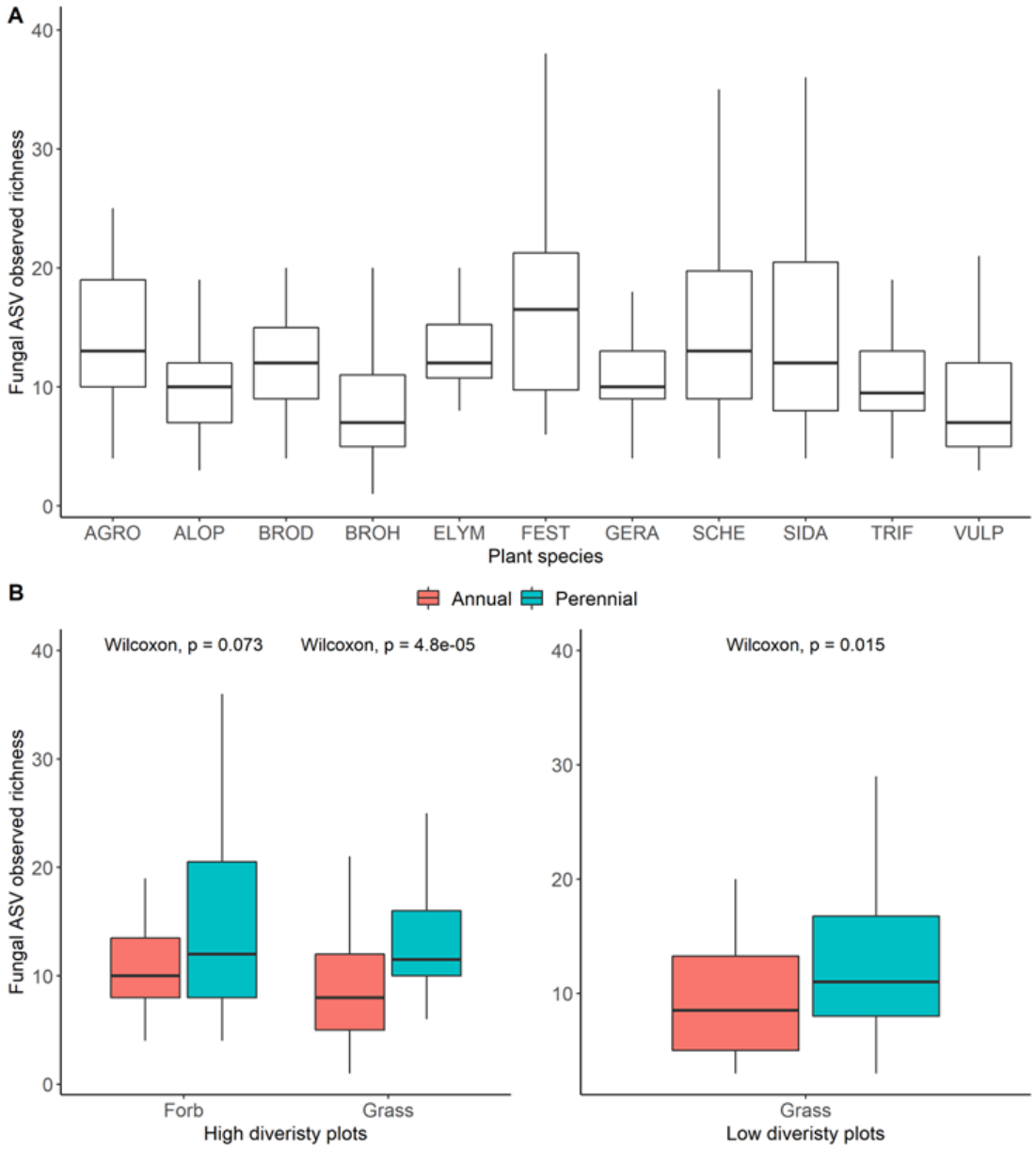

Figure S2. Boxplots depicting observed richness values for AM fungal ASVs detected across plant species (A) and plant functional groups in the high versus low diversity plots (B).

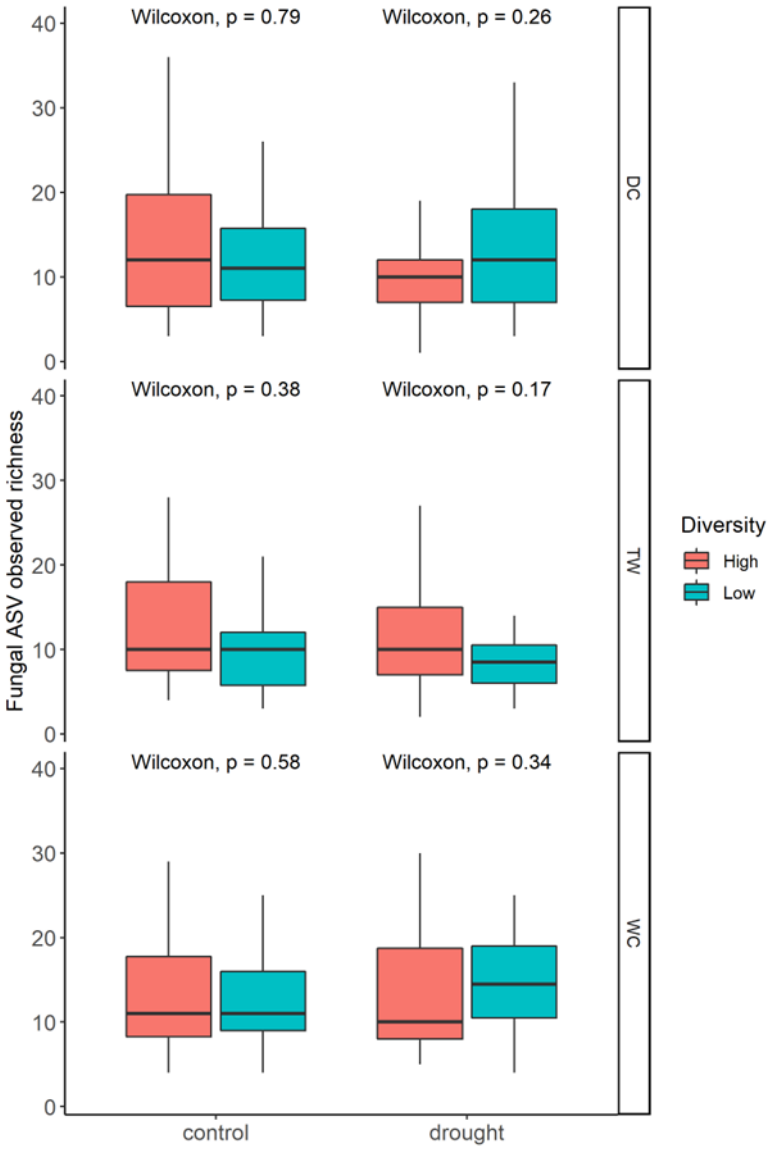

Figure S3. Boxplots depicting no effect of drought on the richness of AM fungal ASVs. No comparisons are significant.

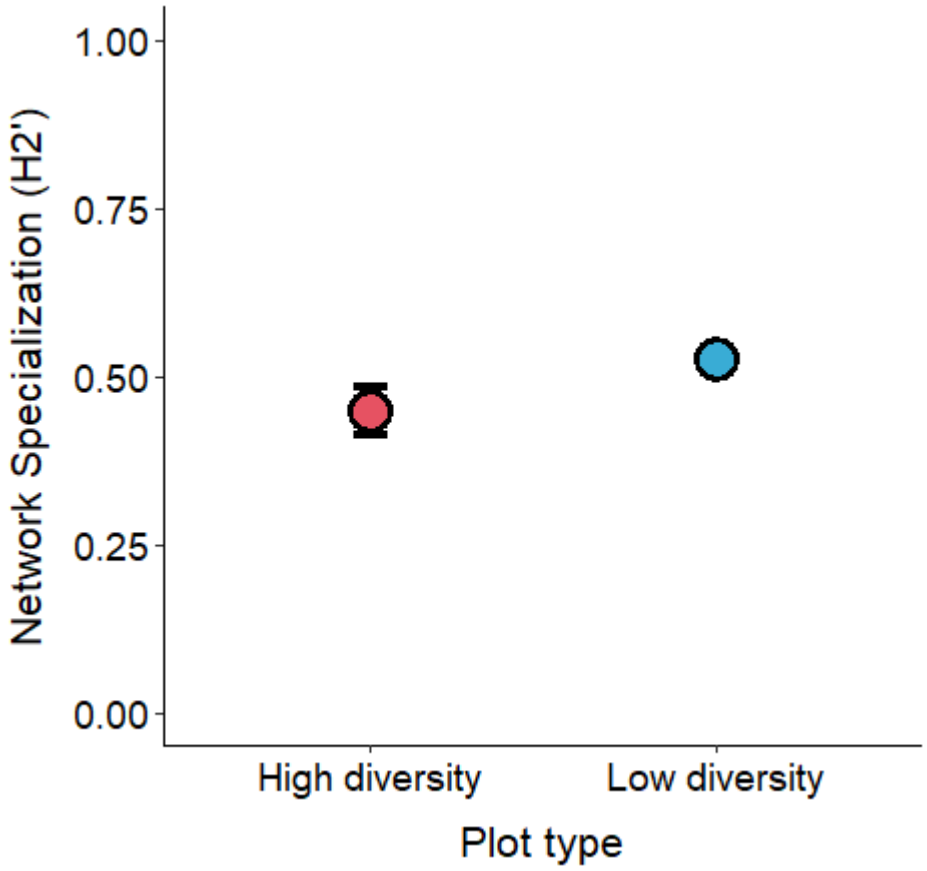

Figure S4. Overall network specialization (H2') calculated for high and low diversity plots at each site revealed slightly more specialized plant-AM fungal networks for low diversity plots at the ASV level, but the relationship was not significant (Welch's two sample t-test p=0.056, Bluthgen et al. 2006).

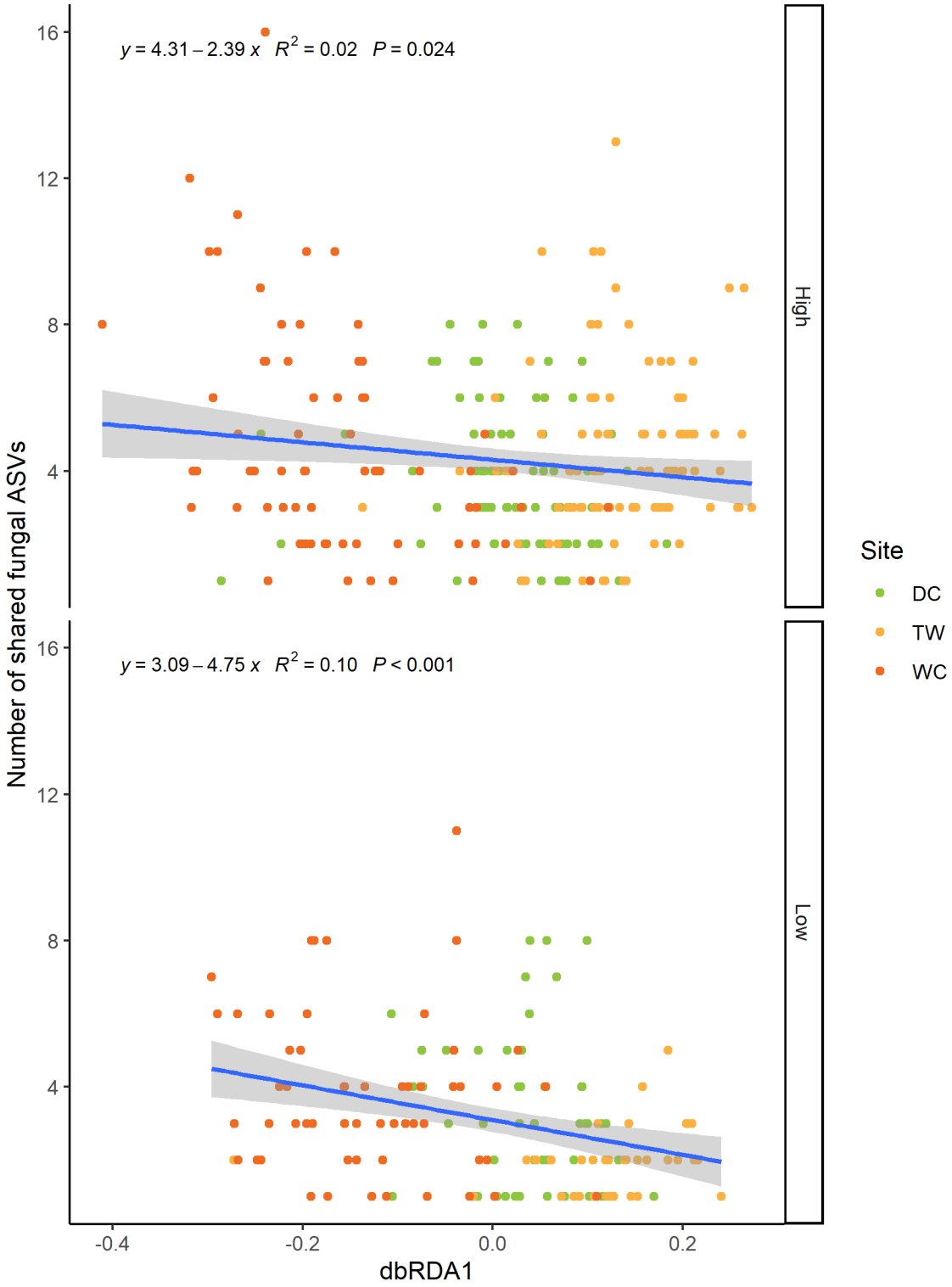

Figure S5. Significant declines in number of shared AM fungal ASVs between multiple plants within a plot and the first environmental axis from the dbRDA1 generated using soil nutrient and plant richness data.

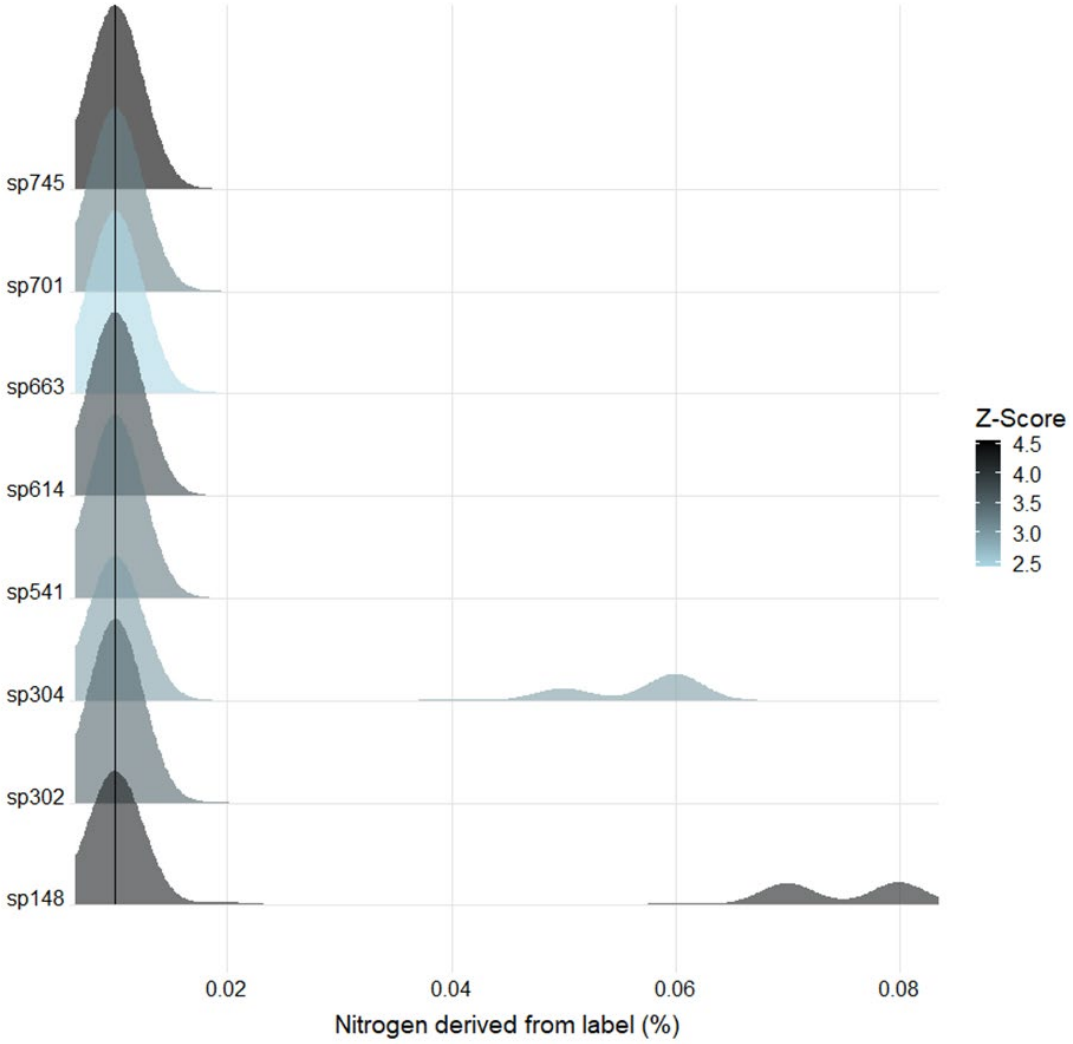

Figure S6. Results of threshold indicator taxa analysis (TITAN) for AM fungal ASVs that were indicators for declines in leaf 15N enrichment across all host plants.

Table S1. Module membership for AM fungal taxa in the high diversity plant-AM fungal interaction networks. C and z values are provided as the among-module connectivity (c) and within-module degree (z) columns.

Family	Genus	Species	Module	Among-module connectivity	Within-module degree
unidentified	unidentified	unidentified	2	0.652871844	-0.136671164
Paraglomeraceae	*Paraglomus*	VTX00001	1	0	-0.526130062
Archaeosporaceae	*Archaeospora*	VTX00004	4	0.674001256	-1.391756482
Archaeosporaceae	*Archaeospora*	VTX00005	4	0.710389499	0.605931665
Archaeosporaceae	*Archaeospora*	VTX00008	1	0	-0.526130062
Archaeosporaceae	*Archaeospora*	VTX00009	1	0.734234117	0.054427248
Acaulosporaceae	*Acaulospora*	VTX00021	1	0.71933866	2.231517158
Acaulosporaceae	*Acaulospora*	VTX00023	4	0.708362525	-1.140550188
Acaulosporaceae	*Acaulospora*	VTX00026	1	0	-0.526130062
Acaulosporaceae	*Acaulospora*	VTX00027	1	0	-0.526130062
Acaulosporaceae	*Acaulospora*	VTX00028	1	0.624277719	-0.235851407
Acaulosporaceae	*Acaulospora*	VTX00030	1	0.601657861	2.812074468
Gigasporaceae	*Gigaspora*	VTX00039	1	0	-0.526130062
Diversisporaceae	*Diversispora*	VTX00040	1	0	-0.526130062
Acaulosporaceae	*Acaulospora*	VTX00047	1	0	-0.526130062
Gigasporaceae	*Scutellospora*	VTX00049	1	0.655632472	-0.09071208
Gigasporaceae	*Scutellospora*	VTX00052	3	0.336293294	-0.041603399
Glomeraceae	*Glomus*	VTX00053	1	0.469556418	-0.235851407
Diversisporaceae	*Diversispora*	VTX00054	1	0	-0.526130062
Claroideoglomeraceae	*Claroideoglomus*	VTX00056	2	0.69350312	2.435463161
Claroideoglomeraceae	*Claroideoglomus*	VTX00057	1	0.365420382	0.054427248
Diversisporaceae	*Diversispora*	VTX00060	2	0.40937163	-0.707124717
Diversisporaceae	*Glomus*	VTX00061	1	0	-0.235851407
Glomeraceae	*Glomus*	VTX00064	2	0	-0.640911358
Glomeraceae	*Glomus*	VTX00067	1	0.698498721	2.66693514
Glomeraceae	*Glomus*	VTX00070	1	0	-0.526130062
Glomeraceae	*Glomus*	VTX00072	2	0.595346684	-0.663831367
Glomeraceae	*Glomus*	VTX00074	4	0.577485165	-0.15765572

Glomeraceae	*Glomus*	VTX00080	1	0	-0.526130062
Glomeraceae	*Glomus*	VTX00086	2	0	-0.707124717
Glomeraceae	*Glomus*	VTX00093	1	0	-0.526130062
Glomeraceae	*Glomus*	VTX00096	1	0	-0.526130062
Glomeraceae	*Glomus*	VTX00101	1	0	-0.526130062
Glomeraceae	*Glomus*	VTX00108	2	0.708430649	2.522049861
Glomeraceae	*Glomus*	VTX00113	4	0.556780934	0.440454503
Glomeraceae	*Glomus*	VTX00114	4	0.198186757	-1.391756482
Glomeraceae	*Glomus*	VTX00115	4	0.562587334	0.066635613
Glomeraceae	*Glomus*	VTX00125	3	0.47280272	-0.762489889
Glomeraceae	*Glomus*	VTX00126	1	0	-0.526130062
Glomeraceae	*Glomus*	VTX00135	2	0	-0.707124717
Glomeraceae	*Glomus*	VTX00137	2	0	-0.707124717
Glomeraceae	*Glomus*	VTX00142	3	0.502451908	-0.873671387
Glomeraceae	*Glomus*	VTX00143	4	0.706011089	0.472353715
Glomeraceae	*Glomus*	VTX00155	2	0	-0.707124717
Glomeraceae	*Glomus*	VTX00159	3	0.328287594	0.723858161
Glomeraceae	*Glomus*	VTX00160	3	0.533551558	0.955187407
Glomeraceae	*Glomus*	VTX00166	3	0.295153396	0.205608918
Glomeraceae	*Glomus*	VTX00180	1	0	-0.526130062
Glomeraceae	*Glomus*	VTX00183	1	0	-0.526130062
Glomeraceae	*Glomus*	VTX00191	1	0	-0.526130062
Claroideoglomeraceae	*Claroideoglomus*	VTX00193	2	0.675421507	1.353129411
Glomeraceae	*Glomus*	VTX00197	2	0.570360047	-0.289471223
Glomeraceae	*Glomus*	VTX00199	1	0.672949237	2.376656486
Glomeraceae	*Glomus*	VTX00204	1	0	-0.526130062
Glomeraceae	*Glomus*	VTX00209	1	0	-0.526130062
Glomeraceae	*Glomus*	VTX00212	2	0.708617471	-0.447364617
Glomeraceae	*Glomus*	VTX00214	2	0.40937163	-0.707124717
Glomeraceae	*Glomus*	VTX00216	4	0.48466135	2.107188326
Glomeraceae	*Glomus*	VTX00219	3	0.660753413	2.11004039
Glomeraceae	*Glomus*	VTX00222	3	0.329867931	0.659301162
Glomeraceae	*Glomus*	VTX00223	3	0.3548493	-0.809114388
Claroideoglomeraceae	*Claroideoglomus*	VTX00225	1	0.664613732	1.796099176

Acaulosporaceae	*Acaulospora*	VTX00228	3	0.209554787	-0.873671387
Acaulosporaceae	*Acaulospora*	VTX00230	3	0.226745091	-0.237323825
Acaulosporaceae	*Acaulospora*	VTX00231	1	0.70203254	0.48984523
Claroideoglomeraceae	*Claroideoglomus*	VTX00237	2	0.678553507	0.365022363
Archaeosporaceae	*Archaeospora*	VTX00245	4	0.723937932	-0.707917126
Glomeraceae	*Glomus*	VTX00256	1	0	-0.526130062
Claroideoglomeraceae	*Claroideoglomus*	VTX00276	3	0.663991082	-0.753523639
Claroideoglomeraceae	*Claroideoglomus*	VTX00278	2	0.542422969	-0.024617787
Claroideoglomeraceae	*Claroideoglomus*	VTX00279	3	0.38369548	-0.873671387
Paraglomeraceae	*Paraglomus*	VTX00281	4	0.720865849	0.798323787
Glomeraceae	*Glomus*	VTX00295	1	0	-0.526130062
Glomeraceae	*Glomus*	VTX00296	1	0.484662773	-0.235851407
Diversisporaceae	*Diversispora*	VTX00306	1	0.525038004	1.650959849
Glomeraceae	*Glomus*	VTX00309	4	0.479857889	0.589982059
Glomeraceae	*Glomus*	VTX00315	3	0.434473087	1.573858648
Archaeosporaceae	*Archaeospora*	VTX00338	2	0.464733254	-0.37860459
Claroideoglomeraceae	*Claroideoglomus*	VTX00340	1	0.244777575	0.054427248
Diversisporaceae	*Diversispora*	VTX00380	2	0.483181707	-0.312391232
Glomeraceae	*Glomus*	VTX00399	1	0	-0.526130062
Claroideoglomeraceae	*Claroideoglomus*	VTX00402	2	0.670443149	0.871809225
Glomeraceae	*Glomus*	VTX00410	1	0	-0.526130062
Glomeraceae	*Glomus*	VTX00423	4	0.667746638	-0.29123367
Glomeraceae	*Glomus*	VTX00426	1	0	-0.526130062
Paraglomeraceae	*Paraglomus*	VTX00444	2	0.61279728	0.296262336
Glomeraceae	*Glomus*	VTX00445	2	0	-0.707124717
Paraglomeraceae	*Paraglomus*	VTX00446	1	0	-0.526130062
Archaeosporaceae	*Archaeospora*	VTX00456	3	0.532298644	-1.002785385

Table S2. Module membership for AM fungal taxa in the low diversity plant-AM fungal interaction networks. C and z values are provided as the among-module connectivity (c) and within-module degree (z) columns.

Family	Genus	Species	Module	Among-module connectivity	Within-module degree
unidentified	unidentified	unidentified	3	0.389423452	-0.077389947
Archaeosporaceae	*Archaeospora*	VTX00004	2	0.413911778	-0.774747707
Archaeosporaceae	*Archaeospora*	VTX00005	2	0.623868615	0.258249236
Archaeosporaceae	*Archaeospora*	VTX00009	3	0.327538423	-0.700245592
Acaulosporaceae	*Acaulospora*	VTX00021	3	0.478847723	-0.01974778
Acaulosporaceae	*Acaulospora*	VTX00023	2	0	-0.860830785
Acaulosporaceae	*Acaulospora*	VTX00028	2	0	-0.860830785
Acaulosporaceae	*Acaulospora*	VTX00030	1	0.554111376	-0.385016805
Gigasporaceae	*Scutellospora*	VTX00049	3	0.403705812	-0.815529927
Gigasporaceae	*Scutellospora*	VTX00052	1	0.258397448	-0.158711366
Glomeraceae	*Glomus*	VTX00053	3	0	-0.077389947
Claroideoglomeraceae	*Claroideoglomus*	VTX00056	1	0.560221621	2.738331695
Claroideoglomeraceae	*Claroideoglomus*	VTX00057	1	0.480353059	-0.68985361
Diversisporaceae	*Diversispora*	VTX00060	3	0	-0.873172095
Glomeraceae	*Glomus*	VTX00064	3	0	-0.653811624
Glomeraceae	*Glomus*	VTX00067	3	0.306756952	0.037894388
Glomeraceae	*Glomus*	VTX00072	1	0.368115343	-0.070198485
Glomeraceae	*Glomus*	VTX00074	2	0	1.463412335
Glomeraceae	*Glomus*	VTX00079	2	0	-0.860830785
Glomeraceae	*Glomus*	VTX00086	1	0	-0.916159049
Glomeraceae	*Glomus*	VTX00089	2	0	-0.860830785
Glomeraceae	*Glomus*	VTX00108	2	0.617557776	1.807744649
Glomeraceae	*Glomus*	VTX00113	2	0.652854882	0.430415393
Glomeraceae	*Glomus*	VTX00115	2	0.665607992	1.31E-16
Glomeraceae	*Glomus*	VTX00125	1	0.54763503	-0.689009187
Glomeraceae	*Glomus*	VTX00135	3	0	-0.01974778
Glomeraceae	*Glomus*	VTX00137	3	0	-0.596169456
Glomeraceae	*Glomus*	VTX00142	1	0	-0.866056601
Glomeraceae	*Glomus*	VTX00143	3	0.570322171	2.402824432
Glomeraceae	*Glomus*	VTX00151	3	0	-0.98845643
Glomeraceae	*Glomus*	VTX00159	1	0.071237245	0.182847025
Glomeraceae	*Glomus*	VTX00160	1	0.414982508	1.408798066
Glomeraceae	*Glomus*	VTX00166	1	0.118803903	0.181158178
Glomeraceae	*Glomus*	VTX00191	2	0	-0.774747707
Claroideoglomeraceae	*Claroideoglomus*	VTX00193	1	0.543114488	0.992086002

Glomeraceae	*Glomus*	VTX00197	3	0.472236274	-0.527319089
Glomeraceae	*Glomus*	VTX00199	3	0.560880307	-0.192674283
Glomeraceae	*Glomus*	VTX00212	3	0.612848292	1.352456044
Glomeraceae	*Glomus*	VTX00214	1	0.326338185	-0.891107825
Glomeraceae	*Glomus*	VTX00216	2	0.16330027	0.946913864
Glomeraceae	*Glomus*	VTX00219	2	0.590034132	2.324243121
Glomeraceae	*Glomus*	VTX00222	1	0.339440793	0.409996888
Glomeraceae	*Glomus*	VTX00223	2	0	-0.860830785
Claroideoglomeraceae	*Claroideoglomus*	VTX00225	2	0.201446281	-0.516498471
Acaulosporaceae	*Acaulospora*	VTX00228	1	0.319408382	-0.814265307
Acaulosporaceae	*Acaulospora*	VTX00230	1	0.313641547	-0.841005377
Acaulosporaceae	*Acaulospora*	VTX00231	1	0.098896689	0.257156274
Claroideoglomeraceae	*Claroideoglomus*	VTX00237	3	0.498908079	1.491757949
Archaeosporaceae	*Archaeospora*	VTX00245	3	0.531696771	0.095536556
Claroideoglomeraceae	*Claroideoglomus*	VTX00276	3	0.430584065	0.314897027
Claroideoglomeraceae	*Claroideoglomus*	VTX00278	3	0.26807423	0.833676536
Claroideoglomeraceae	*Claroideoglomus*	VTX00279	1	0	-0.814265307
Paraglomeraceae	*Paraglomus*	VTX00281	2	0.493931562	0.344332314
Glomeraceae	*Glomus*	VTX00292	2	0	-0.860830785
Glomeraceae	*Glomus*	VTX00296	3	0	-0.98845643
Diversisporaceae	*Diversispora*	VTX00306	3	0.408906352	0.7183922
Glomeraceae	*Glomus*	VTX00309	2	0	1.31E-16
Glomeraceae	*Glomus*	VTX00310	3	0	-0.98845643
Glomeraceae	*Glomus*	VTX00315	1	0.368015671	1.737841671
Paraglomeraceae	*Paraglomus*	VTX00335	3	0	-0.98845643
Archaeosporaceae	*Archaeospora*	VTX00338	3	0.475126429	-0.135032115
Claroideoglomeraceae	*Claroideoglomus*	VTX00340	1	0.420003393	-0.81510973
Diversisporaceae	*Diversispora*	VTX00380	3	0	-0.307958618
Claroideoglomeraceae	*Claroideoglomus*	VTX00402	1	0.612270242	0.042542849
Glomeraceae	*Glomus*	VTX00423	2	0.514675627	0.516498471
Paraglomeraceae	*Paraglomus*	VTX00444	3	0.502483716	2.633393103
Glomeraceae	*Glomus*	VTX00445	3	0	-0.930814263
Archaeosporaceae	*Archaeospora*	VTX00456	2	0.476074219	-0.860830785