Opening the Black Box: Soil Microbial Communities in Field-Based Plant-Soil Feedback Experiments

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OPENING THE BLACK BOX: SOIL MICROBIAL COMMUNITIES IN

FIELD-BASED PLANT-SOIL FEEDBACK EXPERIMENTS

by

Julia Kate Aaronson

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Ecology

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ABSTRACT

Opening the black box: Soil microbial communities in field-based

plant-soil feedback experiments

by

Julia Aaronson, Master of Science

Utah State University, 2023

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Department: Wildland Resources

There is a great potential to use plant-soil feedbacks (PSFs) to manage plant growth, and there is considerable interest in utilizing soil microbes to regulate plant communities in both agricultural and wildland environments. However, the bioassay approaches commonly used to measure PSF treat soils as a 'black box' and do not describe the soil organisms that cause plant growth responses. For example, two recent large PSF field experiments in Minnesota, USA, and Jena, Germany, reported that plants created soils that changed subsequent plant growth by roughly 25% and helped explain diversity-productivity relationships. However, these studies did not describe the soil microbial communities associated with or potentially causing these plant growth responses. In this study, we analyzed the soil microbial communities throughout the four years of these experiments which contributes to the field of plant-soil feedback by untangling the factors affecting the soil microbial community and how this may relate to PSF effects on plant growth. In descending order of variation explained, the microbial community composition differed between the two study sites, among years, between bulk and rhizosphere soils, and among rhizosphere soils cultivated by different plant species.

Microbial community composition in the rhizosphere (as summarized by multivariate axis values) was correlated with the plant biomass on two of ten NMDS axes, suggesting that the organisms defining these axes are likely plant growth promoters or suppressors. Based on previous PSF research, we expected to find one or two ASV's with strong effects on soil types and plant biomass. However, our analyses found that differences among soils cultivated by different plants were caused by whole microbial communities, not individual ASVs, suggesting that communities of soil organisms and not individual ASVs cause PSF. Our findings indicate that future studies should adopt ecologically minded approaches that consider the whole microbial community instead of focusing solely on individual ASVs, and that it may be ubiquitous, generalist microbes that differentially affect plants causing plant growth changes. More broadly, we provided mechanistic links for the different steps of the PSF process: plants changed soil microbial communities and soil microbial communities were correlated with subsequent plant growth.

(55 pages)

PUBLIC ABSTRACT

Opening the black box: Soil microbial communities in field-based

plant-soil feedback experiments

Julia Aaronson

Plant-soil feedback is a process through which plants modify the properties of their

associated soils, affecting their growth. PSF can play a key role in regulating plant

growth and communities including altering plant invasion, rarity, and abundance.

However, our understanding of the soil organisms that drive these plant growth responses

is limited. Most studies treat soils as a 'black box' and do little to reveal which specific

microbes or microbial communities may be responsible. This chapter examines two

recent large PSF field experiments conducted in Minnesota, USA, and Jena, Germany.

These experiments revealed that plants altered their soils, changing subsequent plant

growth by roughly 25%. To unravel the factors influencing soil microbial communities,

we analyzed the microbial communities in these two experiments. The analysis showed

that the microbial communities varied between the two study sites, among years, and

between bulk and rhizosphere soils. The microbial communities differed among

rhizosphere soils cultivated by different plant species, showing that plants can shape

distinct microbial communities. Our analyses revealed that the differences among soils

were due to the influence of the entire microbial community rather than by individual

microbial species. Additionally, rather than one or two plants consistently creating soils

correlated with increased or decreased plant growth, we found that several plant species

from three plant functional groups created soils significantly correlated with changes in

plant growth. These findings indicate that different plant growth responses are likely caused by ubiquitous, generalist microbes that differentially affect plants, rather than specific microbes that associate with only one or two plant species. These results highlight the importance of considering the entire microbial community when adopting soil management strategies, rather than focusing solely on individual soil microbes. This may lead to more effective soil and plant management practices. Additionally, we found that it may be ubiquitous, generalist microbes that differentially affect plants causing plant growth changes rather than plant-specific microbes which could explain why PSF research results are so variable and why repeat experiments often get different results. This could provide guidance to researchers investigating how to leverage soil microbes in managing plants and plant communities.

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CHAPTER 1

INTRODUCTION

Significance of the Research

Plant-soil feedback (PSF) has gained attention in the past few decades due to its potential to shape plant communities in natural and agricultural systems [1 – 3]. While plants can affect soil chemistry and soil structure, interactions with soil microbes are typically assumed to be the co-determinant of PSF because soil microbes interact extensively with plants and influence plant health, productivity, invasiveness, herbivory, and succession [4 – 7].

The Bio-assay Approach

Most studies use a bio-assay approach to study PSFs [7 – 9]. In this type of experiment, target plant species are grown in a common soil (phase 1), removed, then subsequent plant growth on soils with different plant growth histories is measured (phase 2) [1]. This two-phase approach uses plants as a bioassay to measure the net legacy effect of previous plant growth. These PSF experiments are well suited to identifying soil communities that cause changes in plant growth. In contrast to natural-experimental designs (i.e., survey sampling), PSF experiments have two advantages. First, they randomly assign plants to grow on common garden soils so pre-existing soil traits are controlled for. Second, they measure plant growth responses to soils with different plant growth histories, so it is possible to correlate microbial community composition differences with plant growth responses. For example, in two recent large field PSF experiments, plants were reported to create soils that caused moderate changes in plant

aboveground growth [10, 11]. However, this approach treats soils as a 'black box' and does not reveal information about the mechanisms determining PSF [1 – 3].

Statement of the Problem

There are many obstacles to understanding the microbial drivers of PSF, the first of which is the overwhelming diversity of soil biota, with one gram of soil estimated to contain 100,000 – 1,000,000 species [12 – 14]. Additionally, most PSF studies take place over the short term (< 12 months), whereas PSFs may occur on much longer time scales [15]. Also of note, many studies aiming to identify specific microorganisms contributing to plant growth have relied on cultivation-dependent techniques [16]. However, many soil microbes are not recovered by typical culturing techniques, so this methodology greatly reduces the number of microbes available to study. Finally, most PSF studies occur in a greenhouse setting, which has been found to be poorly correlated with PSFs in the field [8]. These obstacles make it difficult to predict which microbes cause positive or negative feedbacks and whether there are patterns for feedbacks at higher organizational levels of plants such as grasses, forbs, and legumes.

Potential Factors Influencing PSF

There are many potential factors that determine microbial community composition. Studies have found that the main factors determining microbial communities are related to soil physiochemical characteristics, especially pH, but also include factors such as soil texture and nutrient and carbon availability [14, 17]. Microbial composition also varies over time, both within and between seasons [15, 18, 19]. Soil communities can also vary widely in small spaces within soils, for example in the rhizosphere vs bulk soils [20]. Finally, plant species themselves can alter microbial

composition both directly and indirectly [21 – 23]. There are few comprehensive studies investigating the patterns of microbial community assembly through large spatial scales or long time scales, so the factors driving these patterns are still poorly understood [17, 19]. There remains a need to improve the understanding of how plants structure microbial communities relative to other drivers [24].

Purpose of the Study

The goal of this study was to describe the microbial communities in two PSF field experiments with previously reported PSF values [10, 11] in the USA and Germany. Because plants demonstrated PSF effects in these experiments, we expected that plant effects on microbial communities would emerge across the four years of the study and be the largest in rhizosphere soils where plants have more immediate effects.

Research Questions

Sampling two experiments on two continents over four years allowed us to compare the magnitude of plant effects on soil microbial communities to the magnitude of site, year, and soil location on soil microbial community composition. Further, because PSF experiments involve soil disturbances before phases 1 and 2, we were also able to examine disturbance effects on soil microbial communities. Our final objective was to use our large datasets to conduct an exploration of whether microbial community composition was correlated with plant biomass. Positive correlations between PSF and plant biomass would suggest the presence and importance of plant growth-promoting soil organisms. Negative correlations would suggest the presence and importance of plant growth suppression soil organisms. We used these PSF values and the microbial community data from these experiments to test for differences in microbial community

composition associated with each plant species and whether microbial community composition is correlated with plant biomass. By analyzing data from four-year field experiments on two continents, we aim to gain a robust understanding of how plants affect soil microbial communities relative to factors such as site, year, and soil location and explored the feasibility of detecting microbial ASVs or microbial communities that might be correlated with plant growth responses.

CHAPTER 2

METHODOLOGY

Study sites

This study describes the soil microbial communities in two fully-factorial, four-year-long PSF experiments performed at the Cedar Creek Ecosystem Science Reserve Long Term Ecological Research Site, East Bethel, Minnesota, USA (45.403290 N, 93.1874411 W) and the Jena Experimental field site, Jena, Germany (50.951276 N, 11.620545 E). Methods, site conditions, and PSF values for those studies are reported elsewhere [10, 11]. Briefly, the Cedar Creek site is located on sandy soils in the Nymore series (mixed, frigid, Typic Udipsamment). During the four years of the study (2015-2018), the mean annual precipitation (MAP) was 723 mm and mean annual temperature (MAT) was 6.5°C, which is consistent with 1963 – 2019 records at the site (769.3mm and 6.6°C). The Jena site is located on alluvial soils (eutric fluvisols). During the four years of the study (2015 – 2018) the MAP was 499 mm and the MAT was 10.4°C, which is slightly warmer and drier than the long-term averages at the site for 2002-2018 (544mm and 9.8°C respectively). The first and final years of the experiment (2015 and 2018) were drier than average with 459mm and 395mm of precipitation, while 2017 was wetter than average (615mm).

Experimental design

At Cedar Creek, 16 plant species were randomly assigned to common garden plots and grown for two years before being killed with herbicide and replanted with the same plant species (Table 1). In Year 1 (2015), $10g/m^2$ of live seed was planted in 170 replicate plots for each species. At the end of the growing season in Year 2, aboveground

and belowground biomass was removed and each of the 16 species was planted on 27-35

replicates of self-soils (variation in replication was due to poor establishment of some

plants). Aboveground plant biomass was clipped, dried, and weighed at the end of the

growing season in Year 4.

Table 1: Plant species, functional groups, and species codes used at Cedar Creek

Species	Functional Group	Code
Amorpha canescens	Legume	AMOCAN
Andropogon gerardii	C4	ANDGER
Achillea millefolium	Forb	ACHMIL
Dalea purpurea	Legume	DALPUR
Elymus canadensis	C3	ELYCAN
Koeleria macrantha	C3	KOEMAC
Liatris aspera	Forb	LIAASP
Lespedeza capitata	Legume	LESCAP
Lupinus perennis	Legume	LUPPER
Monarda fistulosa	Forb	MONFIS
Poa pratensis	C3	POAPRA
Pascopyrum smithii	C3	PASSMI
Panicum virgatum	C4	PANVIR
Sorghastrum nutans	C4	SORNUT
Solidago rigida	Forb	SOLRIG
Schizachyrium scoparium	C4	SCHSCO

At Jena, nine plant species (Table 2) that had been previously grown at the site

were randomly assigned to 139 common garden plots and grown for two years before

being killed with herbicide and replanted with either the same plant species or other plant

species in the experiment. In Year 1, $4g/m^2$ of live seed was planted in 1251 plots. Due to

poor establishment, *Anthriscus sylvestris* and *Geranium pratense* were reseeded in the

fall of Year 1. At the end of the growing season in Year 2, above- and below-ground biomass was removed. Each of the nine species was planted on 14 replicate plots of self-cultivated soils at the beginning of the growing season in Year 3. The aboveground biomass was clipped, dried, and weighed at the end of the growing season in Years 3 and 4.

Table 2: Plant species, functional groups, and species codes used at Jena

Species	Functional Group	Code
Alopecurus pratensis	C3	ALOPRA
Anthirscus sylvestris	Forb	ANTSYL
Arrhenatherum elatius	C3	ARRELA
Dactylis glomerata	C3	DACGLO
Geranium pratense	Forb	GERPRA
Phleum pratense	C3	PHLPRA
Poa trivialis	C3	POATRI
Trifolium pratense	Legume	TRIPRA
Trifolium repense	Legume	TRIREP

Soil sampling

Bulk soil samples were collected from three, self-cultivated plots for each of the four years at each site. In addition, a rhizosphere sample was collected at the end of the experiment when it was possible to destructively harvest plants. This design allowed us to examine microbial community composition between sites, over time, between bulk and rhizosphere samples, and among soils cultivated by different plant species.

At Cedar Creek, a 10 cm x 2.5 cm soil core was taken from three randomly selected plots for each of the self-cultivated soils for microbial analysis. In Jena, each soil

sample represents a composite of three replicate plots. The soil samples were immediately put on ice and placed in -80°C storage within a few hours of sampling. In the final year of the study, rhizosphere soil was collected from three mature plants in each of the three replicate plots for each plant species. Excavated plant roots were placed into a sterile plastic bag and shaken in the bag for two minutes. Soil samples were stored in the -80°C freezer for one week for samples from 2015 – 2017 and for one month for samples from 2018, prior to DNA extraction.

DNA extraction

DNA was extracted from the bulk and rhizosphere soil samples using a PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). DNA concentrations were checked using PicoGreen assay on a Modulus Picoplate reader. Purified DNA was diluted to a maximum concentration of 6.0 ng/ul for bulk soil samples and 50.0 ng/ul for rhizosphere soil samples. All samples were stored at -80°C until sequencing. Argonne National Laboratory conducted PCR on all soil samples using ITS1f-ITS2 (ITS) primer sets and 515F-806R for bacteria [25]. The genes were sequenced on an Illumina MiSeq platform (Novogene Corporation, Beijing China) using the Earth Microbiome Protocol. Sequences were then processed using the QIIME2 pipeline [26]. The DADA2 package was used to denoise samples and remove chimeras [27]. The forward and reverse reads were joined before denoising for the bacterial data. For the fungal data, only the forward reads were used due to the poor quality of the reverse reads (Phred quality score less than 13). For both fungal and bacterial samples, reads with expected errors higher than 1 were removed and truncated when quality was less than 13. Four samples were removed due to low sequence count (<50 sequences).

Amplicon sequence variants (ASVs) were used for the downstream analysis, so no clustering was performed. Taxonomy was assigned using the Silva and UNITE reference databases [28, 29] and a naïve Bayesian classifier [30, 31]. In total, 260 bacterial and 1843 fungal amplicon sequence variants (ASVs) were obtained for both sites.

Statistical analyses

Statistical analyses were conducted using R v4.2.2. [32]. Total sum scaling (TSS) was used on the raw count data obtained from sequencing. Data from July of 2017 at Jena was then removed from the dataset to leave only data from the September sampling, as this matched sampling times from other years. NMDS on the first 3 axes (with a stress between .11 – 1.7 for all plots) based on the Bray-Curtis dissimilarity from the package "vegan" [33] was used to examine the overall patterns in the microbial communities. Detection of site, year, soil location and plant effects on the structure of the microbial communities was conducted using separate PERMANOVAs, also from the vegan package. Significance was evaluated at a p-value of .05 and the intensity of the signal was measured using the R-squared (R^2) value. Envfit from vegan was used to fit ASV vectors onto the ordination, with p-value adjustment using the Benjamini-Hochberg method to control for the false discovery rate. Differential abundance was calculated using ANCOMBC2 [34]. Fungal trophic modes were assigned using the FUNGuild Database [35]. Trophic modes were only able to be obtained for ASVs identified to at least the family level. Plant PSF score was taken from previous publications at the sites [10, 11]. We performed linear regression to assess the effect of microbial community on log-transformed plant biomass. The NMDS score of one plot in 2018 was regressed against the logged biomass of the plant grown on that plot providing the x and y

coordinates for that point respectively. This was conducted for every plot and plant in the Cedar Creek 2018 rhizosphere data set. This was only conducted on Cedar Creek 2018 rhizosphere data as the rhizosphere soil in Jena was sampled from different plots than the plant biomass data, which does not allow for a direct comparison of the microbial community to the plant biomass of the plant grown on that soil.

CHAPTER 3

RESULTS

Microbial Community Composition

Sequencing of the 16S amplicon yielded a total of 4,718,857 high-quality, non-chimeric sequences across all samples (including both sites), with a median of 14,792 sequences per sample (range 1,171 – 31,674 sequences per sample). Sequencing of the ITS2 region yielded 5,294,116 high-quality fungal sequences with a median of 16,390 sequences per sample for both sites (range 2,734 – 32,972 sequences per sample).

There were 260 bacterial and 1843 fungal ASVs identified in the dataset from 24 phyla and 355 families. There were 1370 fungal and 232 bacterial ASVs in Cedar Creek and 959 fungal and 194 ASVs in Jena. The most abundant ASVs (relative abundance > 1%) were found in association with almost every plant species and represent predominantly fungal taxa from Ascomycota, Basidiomycota, Mortierellomycota, and bacterial taxa from Firmicutes, Proteobacteria, and Bacteroidetes for Cedar Creek and Jena respectively. These abundant ASVs represented 79.82% (Cedar Creek) and 75.79% (Jena) of all sequences, numbers typical of other studies [24]. Fungal (18.01%, 15.07%) and bacterial (12.00%, 14.89%) ASVs that could not be assigned to a phylum made up a significant portion of the microbial communities at Cedar Creek and Jena respectively.

Effects of Site, Year, and Soil Location

There was an average of 122 ASVs per sample in Cedar Creek and 162 ASVs per sample in Jena. Ordination revealed that the two sites harbored distinct microbial communities (Figure 1), with site explaining 14.15% of the variance (p = 0.001). While

distinct, the sites also shared 540 ASVs (27.76%) among all years. The relative

abundance of bacterial phyla at both sites were similar, with Firmicutes dominating,

followed by Bacteroidetes and then Proteobacteria. ANCOM revealed that 13 of 19 phyla

differed between the two sites (Figure 2): Mortierellomycota, Rozellomycota,

Zoopagomycota, Kickxellomycota, Olpidiomycota, and Actinobacteria had greater

relative abundance in Jena than in Cedar Creek. The relative abundance of

Glomeromycota, Basidiomycota, Ascomycota, Monoblepharomycota, Mucoromycota,

Proteobacteria, and Tenericutes was greater in Cedar Creek than Jena.
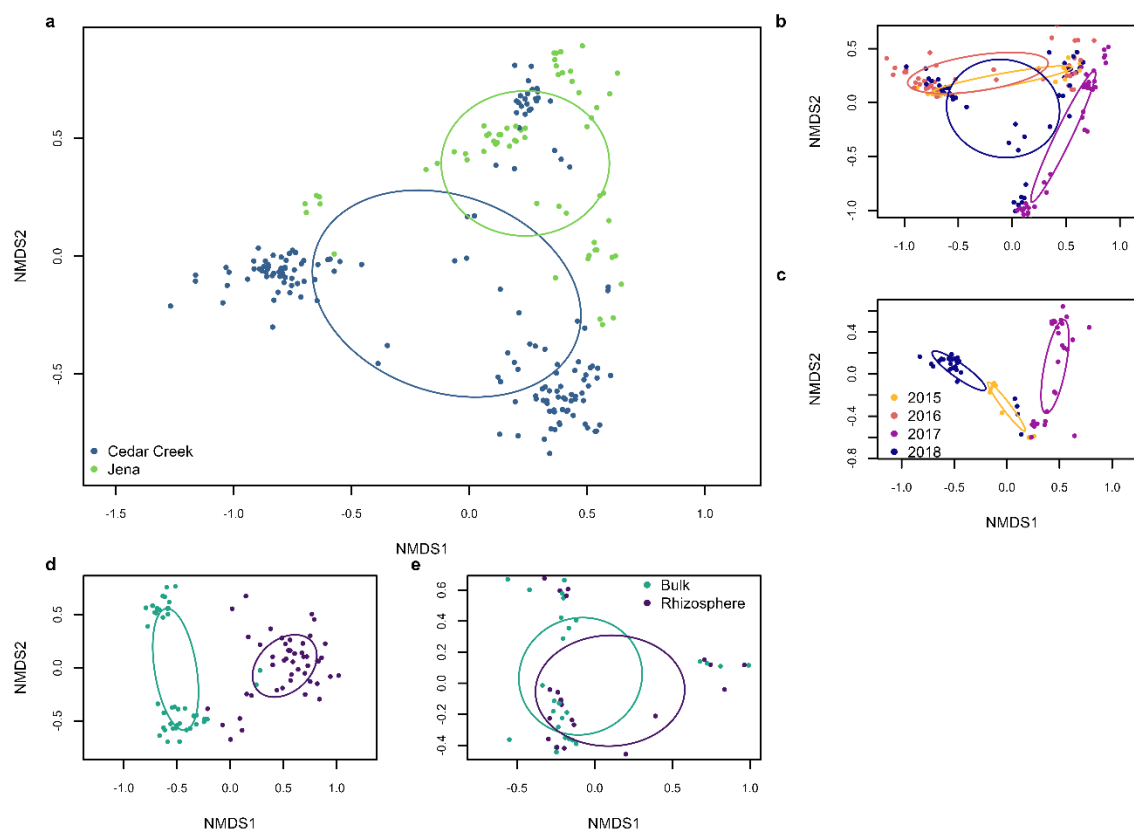
Figure 1: Non-metric multidimensional scaling (NMDS) of microbial communities throughout the study **a)** NMDS of bulk soil microbial communities from all years of the study at both Cedar Creek and Jena **b)** NMDS of bulk soil microbial communities at Cedar Creek for all years of the study **c)** NMDS of the bulk soil microbial communities at Jena for all years of the study **d)** NMDS of the 2018 data including both bulk and rhizosphere data at Cedar Creek **e)** NMDS of the 2018 data including both bulk and rhizosphere data at Jena

Figure 2: The relative abundance of the bulk soil microbes and their functional guilds at both sites **a)** The relative abundance of fungal phyla at both Cedar Creek and Jena **b)** The relative abundance of bacterial phyla at both Cedar Creek and Jena **c)** The relative abundance of the fungal guilds at Cedar Creek and Jena.

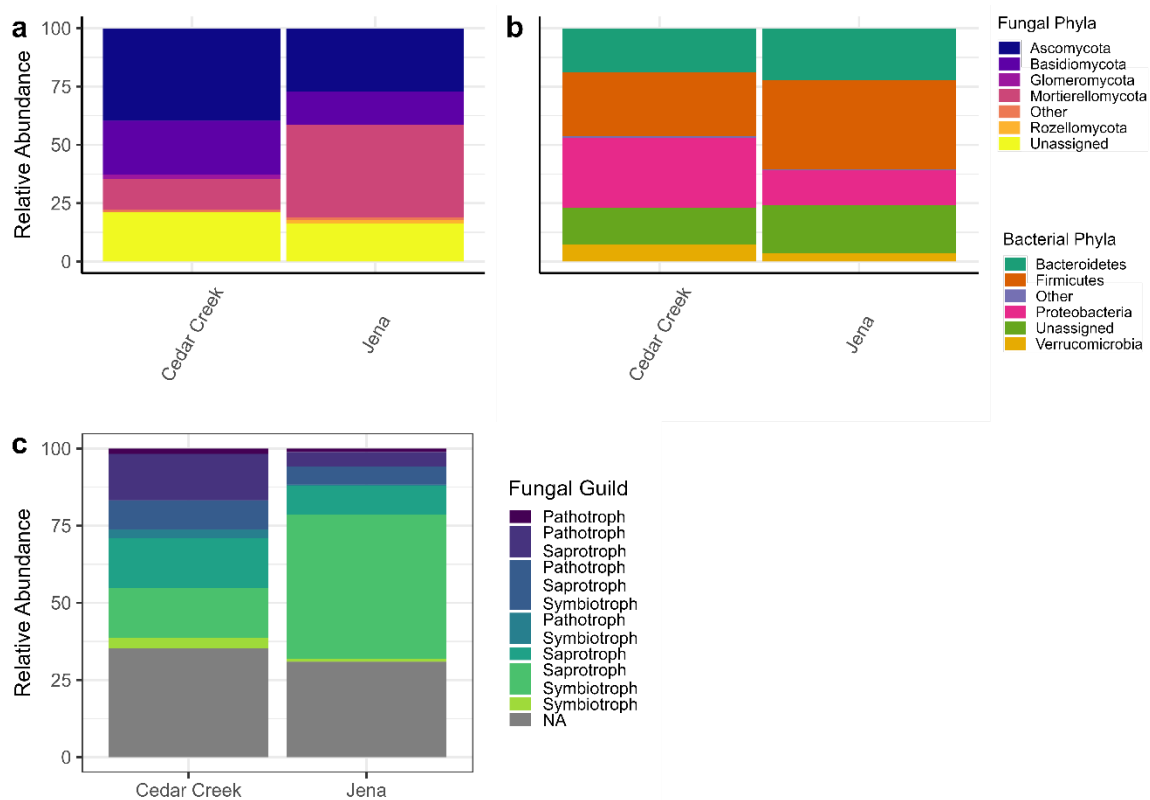Firmicutes and Proteobacteria dominated the bacteria in Cedar Creek, and Firmicutes and Bacteroidetes dominated the bacteria in Jena, with a high relative abundance of Proteobacteria in the first year of the study (Figure 3). Both sites showed a decrease in Bacteroidetes in the second year following disturbance (2016 and 2018). Additionally, Cedar Creek showed an increase in Ascomycota, Glomeromycota, and Proteobacteria and a decrease in Monoblepharomycota and Mortierellomycota in the second year following disturbance. For the fungi assigned fungal guilds, both sites showed an increase in pathotroph-saprotrophs, and pathotroph-saprotoph-symbiotrophs in the second year following disturbance (Figure 3).
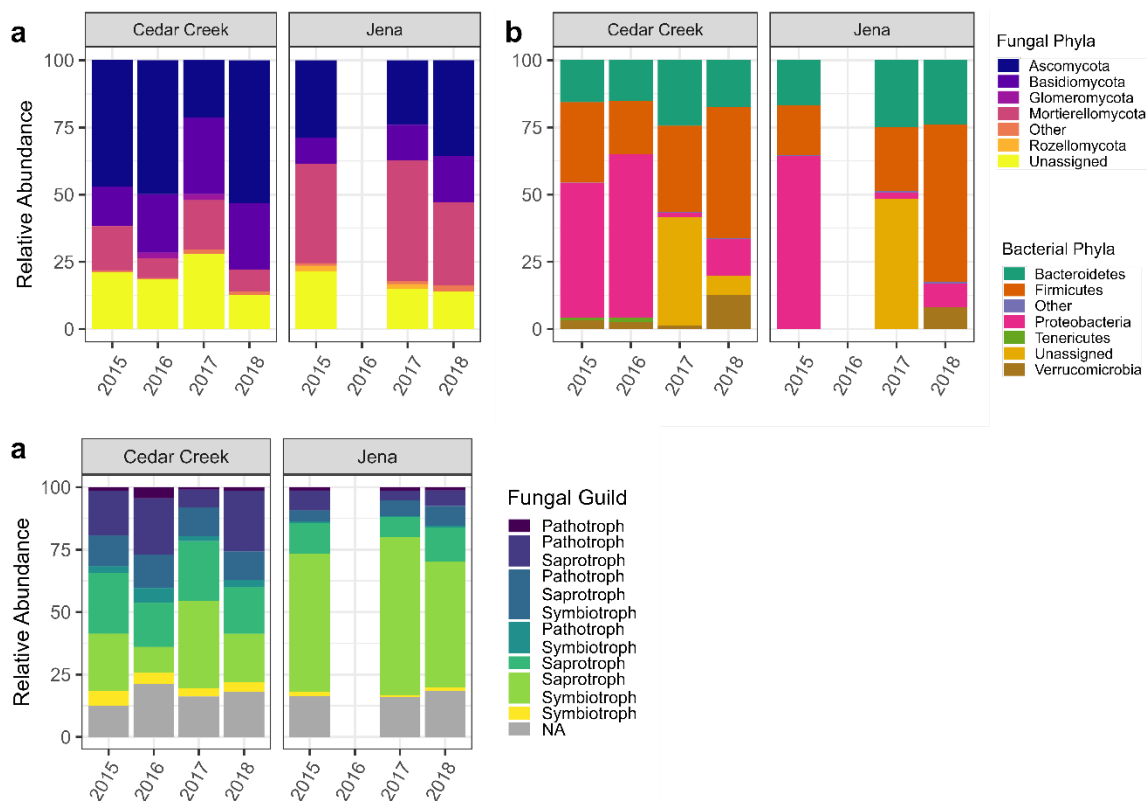
Figure 3: The relative abundance of the bulk soil microbes for each year of the study **a)** The relative abundance of the bulk soil fungal phyla throughout all years of the study at Cedar Creek and Jena **b)** The relative abundance of the bulk soil bacterial phyla throughout all years of the study at Cedar Creek and Jena **c)** The relative abundance of the fungal guilds for each year of the study at Cedar Creek and Jena

Ordination revealed that the 2018 soil samples differed by soil location (i.e., bulk vs rhizosphere soil) (Figure 1). Soil location explained 17.04% (p = 0.001) of the variation in the microbial community at Cedar Creek in 2018, and 4.85% (p = 0.007) of the variation in the community at Jena in 2018. The bacterial and fungal alpha diversity did not vary between soil locations. However, the bacterial alpha diversity of plant

species differed at both sites, but fungal alpha diversity did not vary between plant

species at either Cedar Creek or Jena (Appendix A).

Microbial composition differed by plant species and plant functional groups in

rhizosphere but not in bulk soils (Table 3). The effects of plant functional groups on the

microbial communities were driven by legumes: when legumes were removed from the

dataset, the functional groups of the plants were no longer significant ($R^2 = 0.08$ p =

0.052 for Cedar Creek, $R^2 = 0.07$ p = 0.220 for Jena).

Table 3: Summary of effects of species and functional groups on soil location

		Cedar Creek		Jena	
Independent Variable	Dependent	R^2	p-value	R^2	p-value
Plant species	Bulk soil	0.09	0.531	0.13	0.516
Plant species	Rhizosphere soil	0.40	0.002	0.48	0.004
Plant functional group	Bulk soil	0.02	0.329	0.03	0.430
Plant functional group	Rhizosphere soil	0.11	0.004	0.17	0.007

.
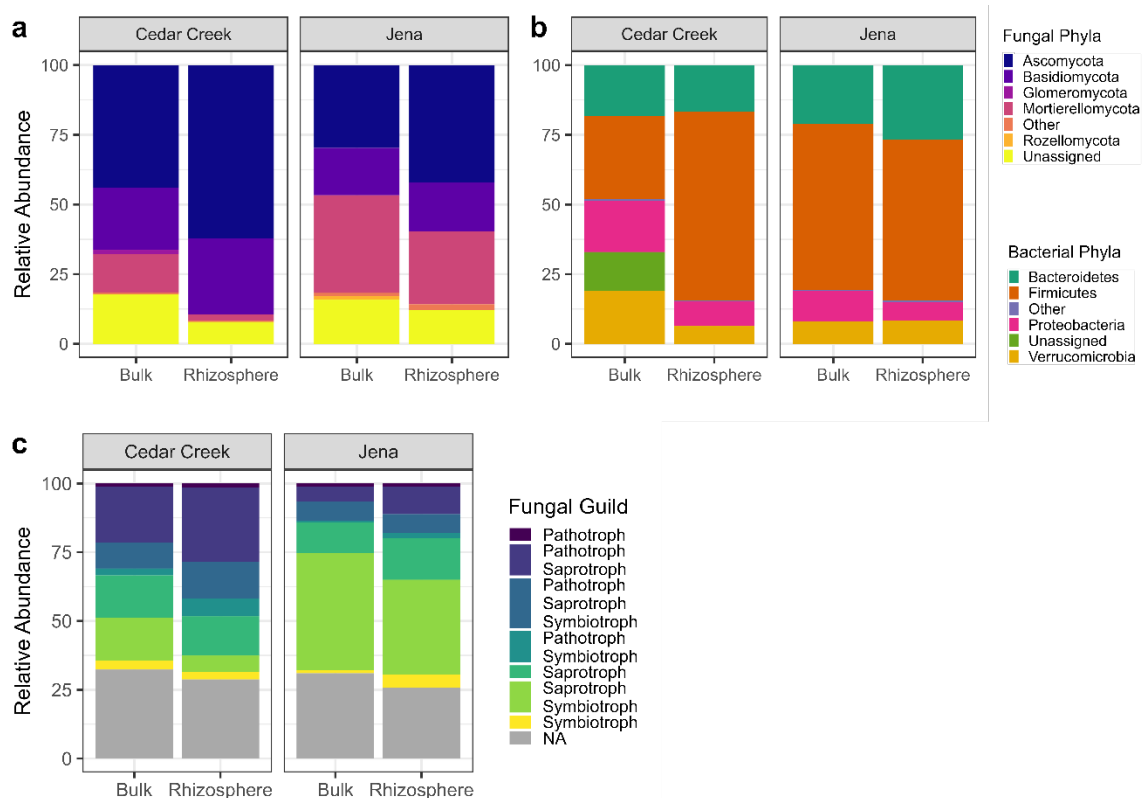
Figure 4: The relative abundance of the bulk and rhizosphere microbes in 2018 **a)** The relative abundance of the fungal phyla for the bulk and rhizosphere soils at Cedar Creek and Jena **b)** The relative abundance of the bacterial phyla for the bulk and rhizosphere soils at Cedar Creek and Jena **c)** The relative abundance of the fungal guilds at Cedar Creek and Jena

At both sites, Ascomycota were more abundant, and Chytridiomycota, Mucoromycota, and Rozellomycota were less abundant in rhizosphere than bulk soils (Figure 4). At Cedar Creek, Firmicutes had greater relative abundance and Glomeromycota, Monoblepharomycota, Mortierellomycota, and Tenericutes, had reduced relative abundance in rhizosphere than bulk soils. At Jena, Kickxellomycota and Proteobacteria were less relatively abundant in the rhizosphere than bulk soils. For fungal

functional groups, pathotroph-symbiotrophs and symbiotroph-pathotroph-saprotrophs were more abundant in the rhizosphere than bulk soils at Cedar Creek.

Relationship between microbial communities and plant biomass

The envfit test was used to determine the significance of each individual ASV in NMDS. We expected this test to find that a few ASVs that had strong effects on soil differences both between plant species and plant locations. However, this test revealed that many microbial ASVs differed between sites, years, and soil locations (Appendix B). For the bulk and rhizosphere soils together, 233 fungal and 128 bacterial ASVs differed between the two sites ($p < 0.05$). For Cedar Creek, 38 fungal and 77 bacterial ASVs differed between years, and 23 fungal and 86 bacterial ASVs ($p < .05$) differed between soil locations. For Jena, 8 fungal and 72 bacterial ASVs differed between years, and 14 fungal and 48 bacterial ASVs ($p < .05$) differed between soil locations.

To explore the dataset to determine if NMDS score captures variation in microbial communities that cause differing plant responses, we regressed the plant biomass against the NMDS score of the soil type that plant was grown on (Figure 5). As this was data exploration, we used the first ten NMDS axes determine if there was a pattern with any NMDS axis and plant biomass and used the Benjamini-Hochberg p-adjustment to account for multiple testing. NMDS axes 2 and 4 had significant relationships with log-transformed plant biomass (Figure 5). The soils associated with both negative NMDS2 and positive NMDS4 (correlated with reduced plant biomass) were from soils created by *Koeleria macrantha, Amorpha canescens,* and *Andropogon gerardii* and the soils associated with positive NMDS2 and negative NMDS4 (correlated with increased plant

biomass) were from *Lespedeza capitata*, *Lupinus perennis*, *Schizachyrium scoparium,* and *Sorghastrum nutans*.
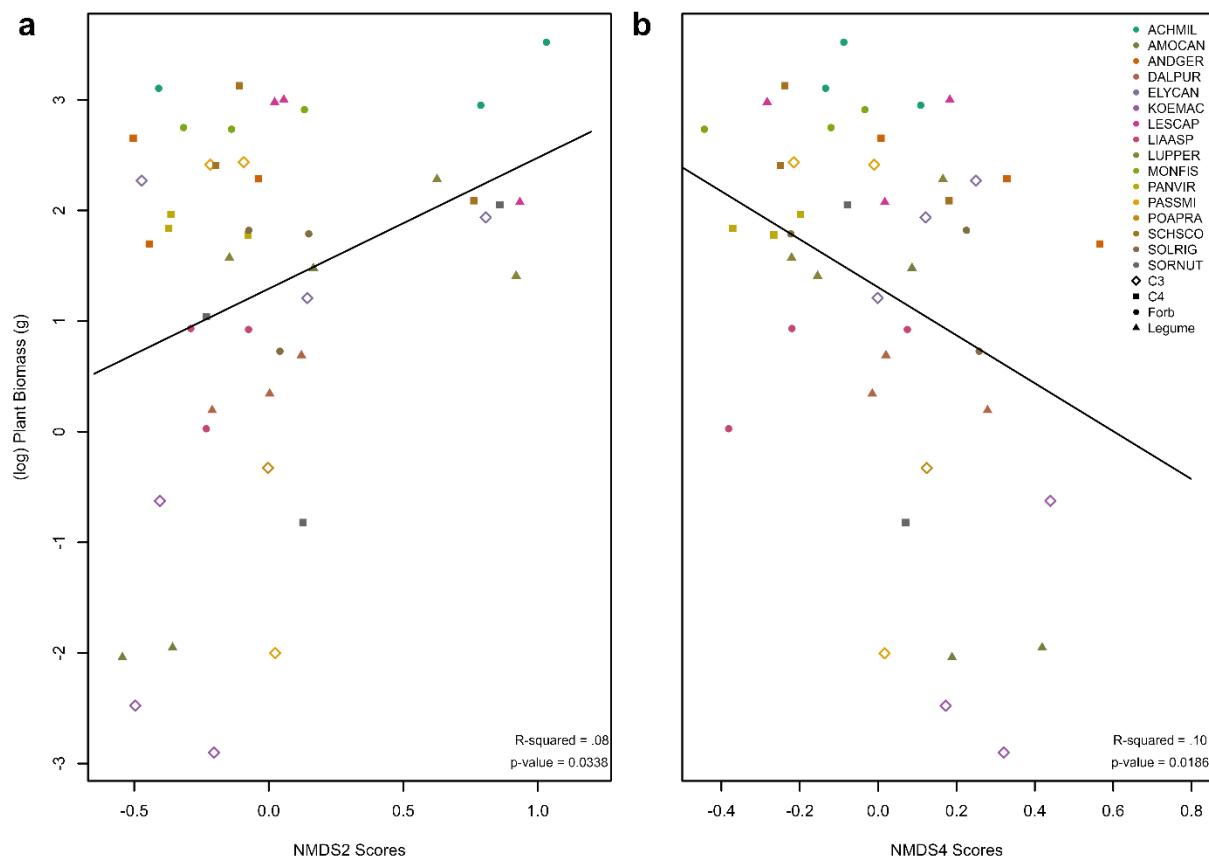

Figure 5: Regression of logged plant biomass against the NMDS score of the soil type that plant grew on. **a)** Regression of logged plant biomass against NMDS2 at Cedar Creek. **b)** Regression of logged plant biomass against NMDS4 at Cedar Creek. The plant biomass of a plot was regressed against the NMDS score for the microbial community of that same plot for 2018 rhizosphere data.

CHAPTER 4

DISCUSSION

Summary of Study

We know from PSF studies that plants create soils that change subsequent plant growth, but we know relatively little about the soil organisms that cause these plant growth responses. To identify the organisms that cause PSF, we described soil microbial communities over four years in field-based PSF experiments performed in Minnesota, USA, and Jena, Germany. In descending order of variation explained, microbial communities differed between sites, among years, between bulk and rhizosphere soils, and among rhizosphere soils cultivated by different plant species. We found a correlation between the microbial community composition, represented by NMDS ordination scores, and plant growth and PSF value. This correlation appeared to be caused by large groups of ubiquitous soil organisms and not by single pathogens or symbionts. With data from large field experiments on two continents, our results provide an unusually comprehensive perspective on microbial community composition in PSF experiments and an explicit link between plant effects on soil microbial community and subsequent correlation between microbial community and plant growth. Results also suggest that it is large communities of common soil organisms and not individual symbionts or pathogens that drive this effect.

The Role of Site, Year, and Soil Location

Study site explained the most variation in microbial community composition. This was not surprising because the two sites are on separate continents with different climatic regimes and soil types. The sites differed in their proportions of fungi, with Jena, the

more nutrient- and carbon-rich site having a higher relative abundance of the saprotrophic phyla Mortierellomycota and Cedar Creek being dominated by Ascomycota which can have multiple trophic modes [35]. The sites, while on different continents with different climatic regimes and soil histories, still shared 28% of their ASVs and the relative abundances of bacteria at both sites were similar; Firmicutes and Proteobacteria dominated the bulk soils. Our results are consistent with previous studies demonstrating an abundance of Firmicutes and Proteobacteria but differ from previous studies in that Actinobacteria were less common in our sites [36]. Plant species effects were not detectable when data from both sites were analyzed together.

Within sites, 'year' explained the most variation in microbial community composition. Both the collection year and the time since disturbance had significant impacts on the microbial community, which overshadowed the effects of plant species. It is reasonable to expect that plants would exert cumulative effects on soil microbial community composition over time. However, differences among years did not appear to be directional in ordination space. This suggests that random climate variation and the effects of experimental manipulations (disturbances due to killing, removing, and reseeding plants in years zero and two of the experiments) and not plant-driven effects explained variation in microbial communities from year to year. The amount of time since disturbance (one year for 2015 and 2017 and two years for 2016 and 2018) and the collection year both impacted the microbial communities. However, we could not separate these effects with our dataset. There were few patterns dictating how phyla differed year to year for both sites, but we did see that Bacteroidetes increased after disturbance (in 2015 and 2017) for both sites. Bacteroidetes are typically pathogen-

suppressing microbes that contribute to phosphorus mobilization in the rhizosphere [37] and likely support plant growth after disturbance. In addition, fungal guild assignments show that there was an increase in pathotroph-saprotrophs with plant growth (in 2016 and 2018) at both sites. The increase in pathotroph-saprotophs with plant growth two years following disturbance aligns with the standard PSF theory that most plants have negative PSF because as they grow, plants accumulate pathogens that negatively affect their growth [1].

After 'site' and 'year', soil location (bulk vs. rhizosphere) explained the most variation in soil microbial communities. ASV richness was lower in rhizosphere soils than in bulk soils, a result supported in other studies and consistent with the idea that root exudation results in high resource availability and competitive suppression among soil organisms in the rhizosphere relative to bulk soils [38, 39]. Alternatively, it is also possible that lower richness reflects plant 'selection' for some organisms and 'suppression' of other organisms. Rhizosphere soils had higher proportions of Ascomycota and decreased relative abundances of Chytridiomycota and Mucoromycota than the bulk soils. This corresponded to an increase in pathotrophs in the rhizosphere compared to bulk soils in Cedar Creek, further supporting the standard PSF theory that most plants have negative PSF because as they grow, plants accumulate pathogens that negatively affect their growth. Though the soil locations were distinct, approximately half of the ASVs were found in both the bulk and rhizosphere soils. This finding is consistent with the idea that the rhizosphere microbiota is a subset of the bulk microbial community as the bulk soils serve as a "seed bank" for rhizosphere microbes, and plants can select beneficial or detrimental microbes from this "seed bank" [16, 40].

As plants use root exudates and litter to select for their associated microbial communities [3, 41], it is commonly believed that the rhizosphere microbial community is more closely tied to plant growth and health [3, 40, 42]. We saw this when comparing the effect of plant species on microbial community in the rhizosphere vs. bulk soils at both sites; plant species effects were only significant in rhizosphere soils and their effect was not detected in the bulk soil samples. This effect of plant species on the microbial community is at the heart of the question behind the mechanism driving PSF and could only be detected once the larger scale variation (i.e., site, year, and soil location) had been removed. The rhizosphere in both sites had an increase in Ascomycota, which are part of all fungal guilds, and a decrease in Chytridiomycota, Mucoromycota, and Rozellomycota [35]. The last three Phyla are mostly undefined in terms of function. However, they may be mainly pathotrophs or pathotroph-saprotrophs, which could indicate that the plants are selecting for microbes that are not pathogens in their rhizospheres.

The Role of the Whole Microbial Community

Many PSF studies have focused on identifying how a few important ASVs, such as pathogens or symbionts, affect plant biomass [43 – 46]. Even more recent studies that use network analysis to investigate the microbial community, still try to identify hub or keystone species that may be the main drivers in determining plant biomass [47 – 50]. Due to this trend in the field, we expected to be able to identify strong effects of a few important ASV's that consistently drove the differences in soil communities created by plant species. However, we observed that differences among sites, years, bulk, and rhizosphere soils and among soils cultivated by different plants were caused by microbial communities, not individual ASVs. In addition to the results of the envfit test identifying

many microbial differences between site, year, and soil location, it is of note that the microbial communities can share roughly half of their members (bulk soils vs rhizosphere soils) and still be statistically different. This provides further support that it is large shifts in microbial communities rather than one or two ASVs that are important in soils. Furthermore, many microbes were correlated with the significant NMDS axes (NMDS2 and NMDS4). Other studies have shown that while specific microbes are able to benefit or harm plants, their effects are greatly influenced by the rest of the microbial community and microbe-microbe interactions may play a large part in PSF [16]. Our results may help explain why single ASV inoculations have often failed to affect microbial community composition or plant growth [51].

In addition to finding that whole microbial communities impact plant growth, our research found that the soils associated with three plant species (KOEMAC, AMOCAN, and ANDGER) from three different functional groups (C3 grasses, C4 grasses, and legume) were correlated with reduced plant biomass and soils associated with four plant species (LESCAP, LUPPER, SCHSCO, and SORNUT) from two functional groups (legumes and C4 grasses) were correlated with increased plant biomass. Our results support several other studies that theorize that it is ubiquitous, generalist microbes that differentially affect plants that lead to changes in their growth [24, 36, 52]. The correlation between soil microbial communities and plant growth across several different plant species and functional groups suggests that it is these ubiquitous microbes, present in soils created by many different plants, that affect plant growth.

While not being able to identify individual ASVs that drive PSF makes it more challenging to manage soil microbial communities to manipulate plant growth, these

results suggest that more ecological approaches may be needed for leveraging microbes in managing plant communities. As entire microbial communities are likely important for plant growth, using approaches that manipulate the entire microbial community such as rotation cropping or whole soil inoculations may be needed [53 – 55]. These results may also help explain why plant-soil feedbacks are so unpredictable, and why repeat experiments often get different results [56].

Future Research

This study can provide some guidelines for future research. First, the effect of plant species may only be detected once larger-scale influences (such as site and year) are removed. Second, it is likely that the microbes affecting plant growth inhabit the rhizosphere soil and not the bulk soils. Another confounding factor in identifying the microbial drivers behind PSF is that the microbial community, and not individual ASVs, affect plant growth. Finally, where microbial communities and not individual ASVs drive PSF, community-level soil manipulations, such as whole soil inoculation, rotation cropping, or planned community planting may be needed.

CHAPTER 5

CONCLUSION

This thesis supports the developing theory that the whole microbial community plays a role in PSF and plant health rather than a few microbes. While unable to pinpoint specific microbes or microbial communities that play the most significant role in plant growth, this study provides some guidelines for future research.

Firstly, the effect of plant species on the soil microbial community can only be detected once larger-scale influences are removed. Site, collection year, year since disturbance, and soil location significantly influenced the soil microbial communities more than the plant species or functional group. Only once these effects were removed were plant species' influences detectable.

Secondly, biologically significant microbes are likely to reside in the rhizosphere soils rather than in bulk soils. While bulk soils serve as a necessary "seed bank" for rhizosphere soils, the rhizosphere microbial community is more specifically tailored to each plant and its needs. While the bulk and rhizosphere soils in both sites had roughly 50% of the same ASVs, the effect of plant species on the microbial community could only be detected in the rhizosphere soils.

This study also found that communities created by many plant species from several functional groups influence plant growth, which supports the idea that ubiquitous microbes are associated with many plant species and functional groups affecting plant growth.

Finally, commonly used multivariate methods were able to reveal that it is whole microbial communities that impact plant growth rather than a few ASVs. While much PSF research is focused on well-known plant pathogens or symbionts, this study supports the growing theory that microbes work in communities responsible for PSF.

These results should direct future research towards ecologically-minded approaches for soil manipulations, such as crop rotations or whole soil inoculations. Research exploring the interactions between soil microbes, such as network analysis, may provide further insight into how the soil microbial community functions to influence plant species differentially. This study also should encourage further research to use long-term field studies for PSF research, as the effects of site, year, and soil location greatly influence the soil microbial community. Identifying the microbial communities and interactions responsible for plant success would be a significant step towards leveraging microbes in managing wildland and cultivated plants and plant communities.

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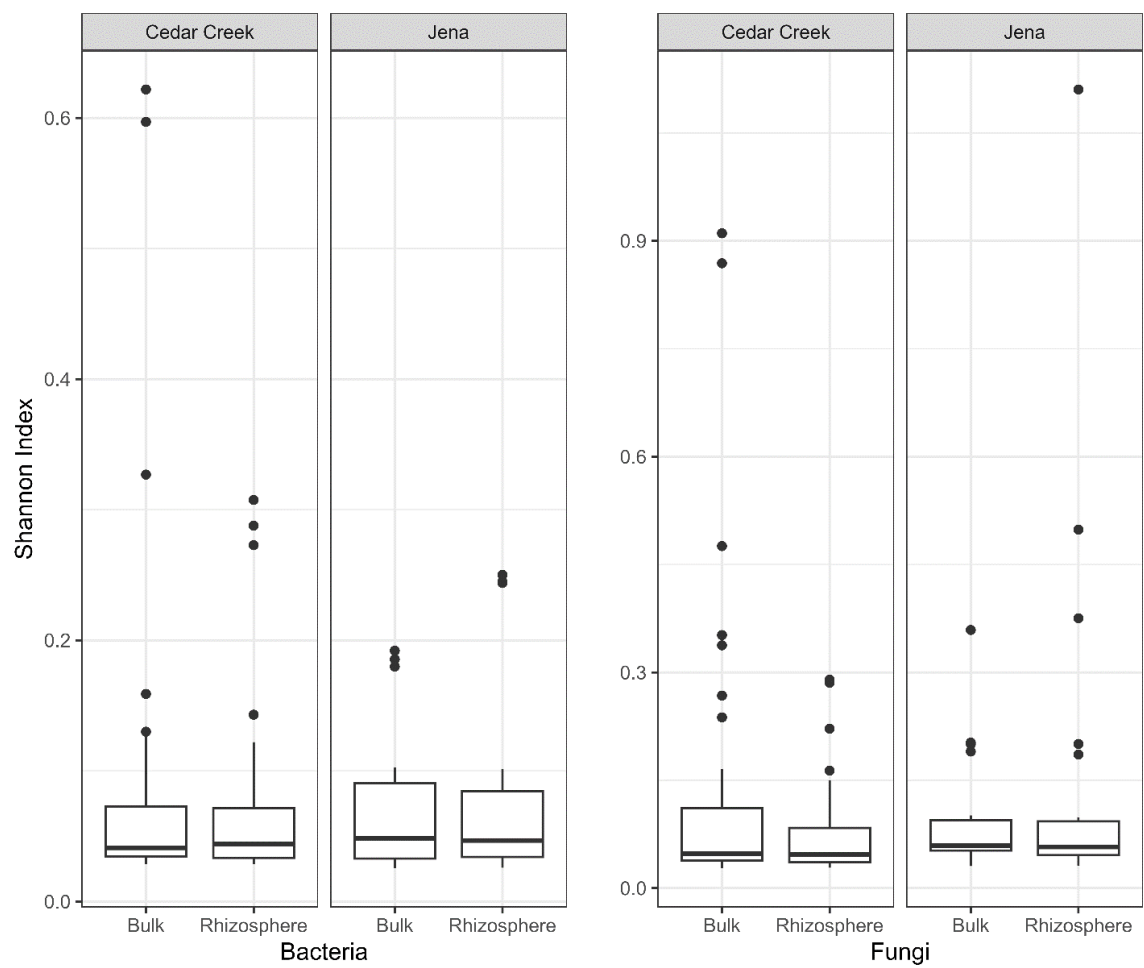
APPENDICES

Appendix A. Alpha diversity of bacteria and fungi

Figure A.1. Alpha diversity of bacteria and fungi at both sites for rhizosphere and bulk soils
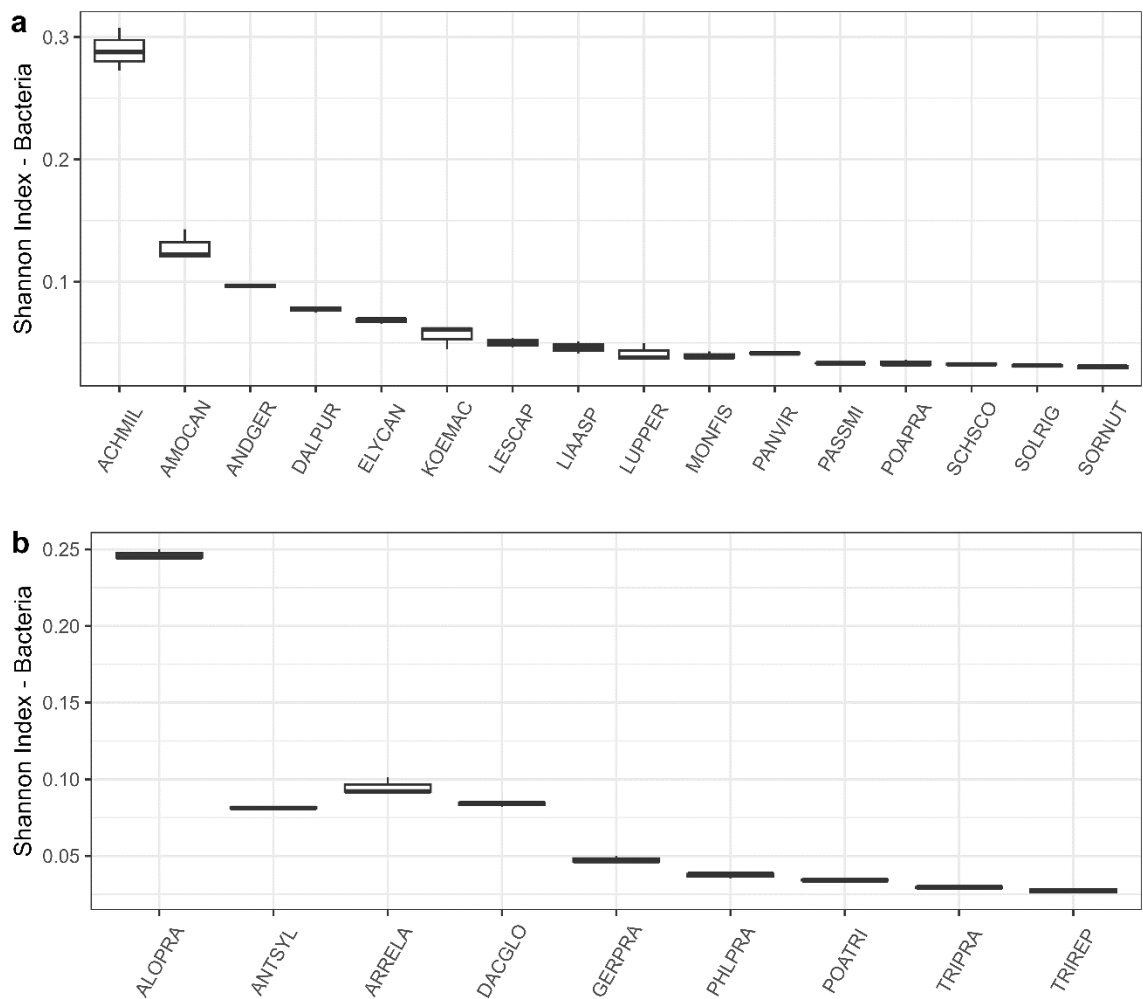
Figure A.2. Shannon index for bacterial diversity for each plant species in the rhizosphere in **a)** Cedar Creek and **b)** Jena
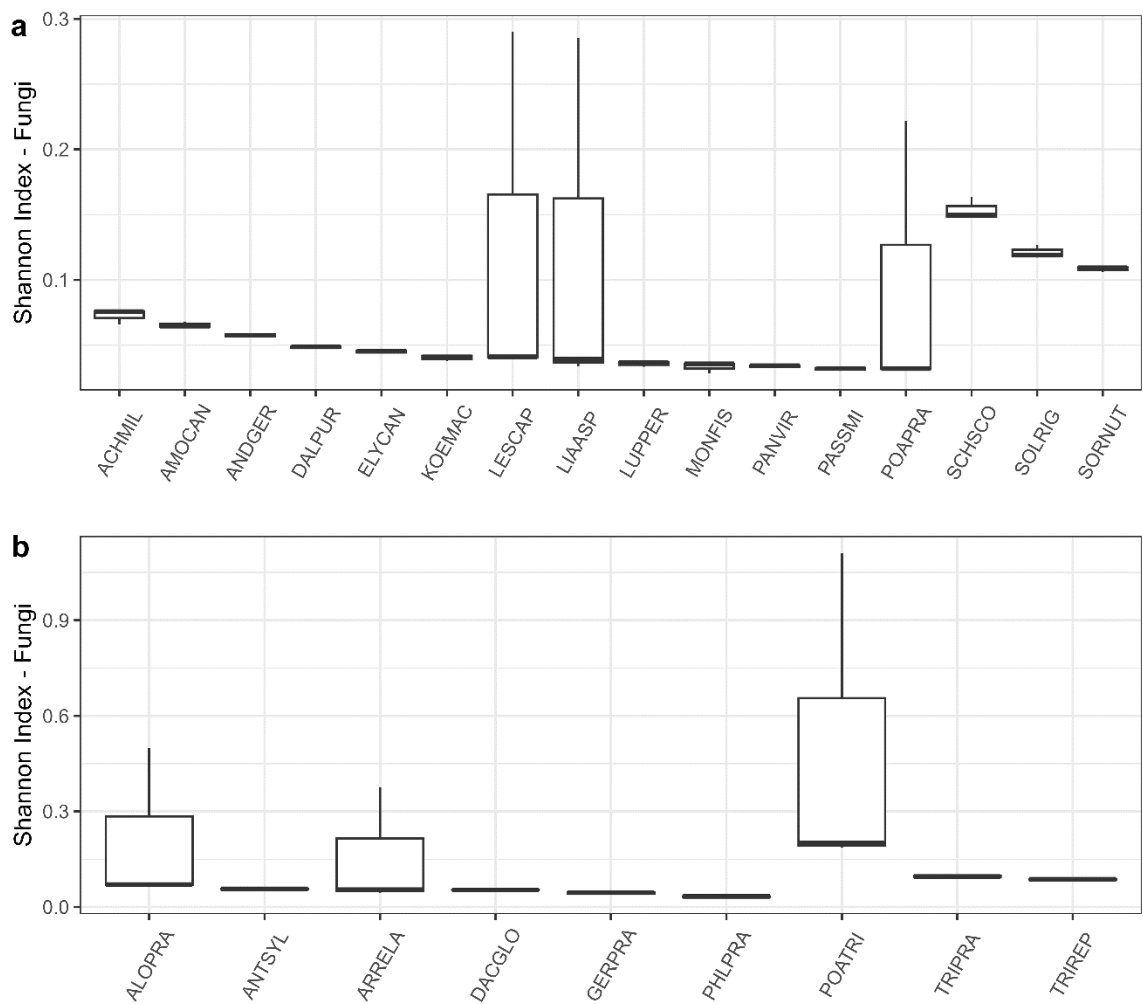
Figure A.3. Shannon index for fungal diversity for each plant species in the rhizosphere in **a)** Cedar Creek and **b)** Jena
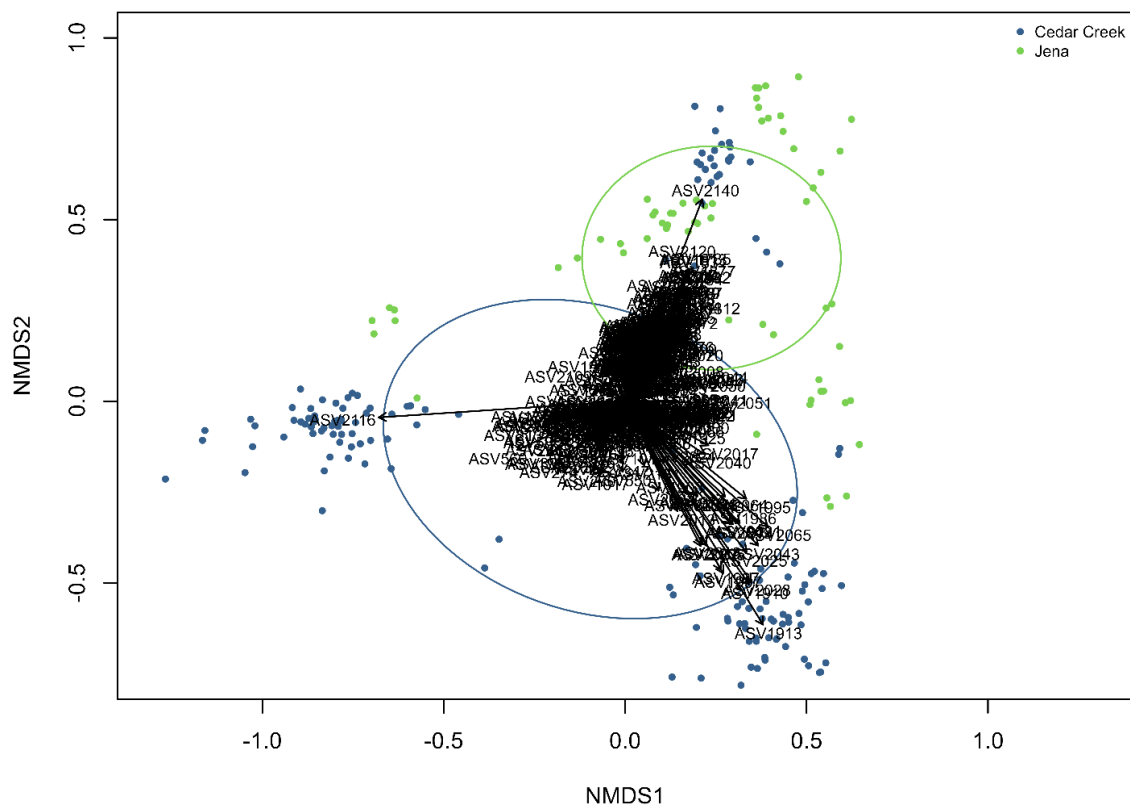
Appendix B. Ordination with ASV vectors as determined by envfit

Figure B.1. NMDS of all years of bulk data from both sites fitted with the significant ASVs (p < 0.01) and their vectors as determined by envfit
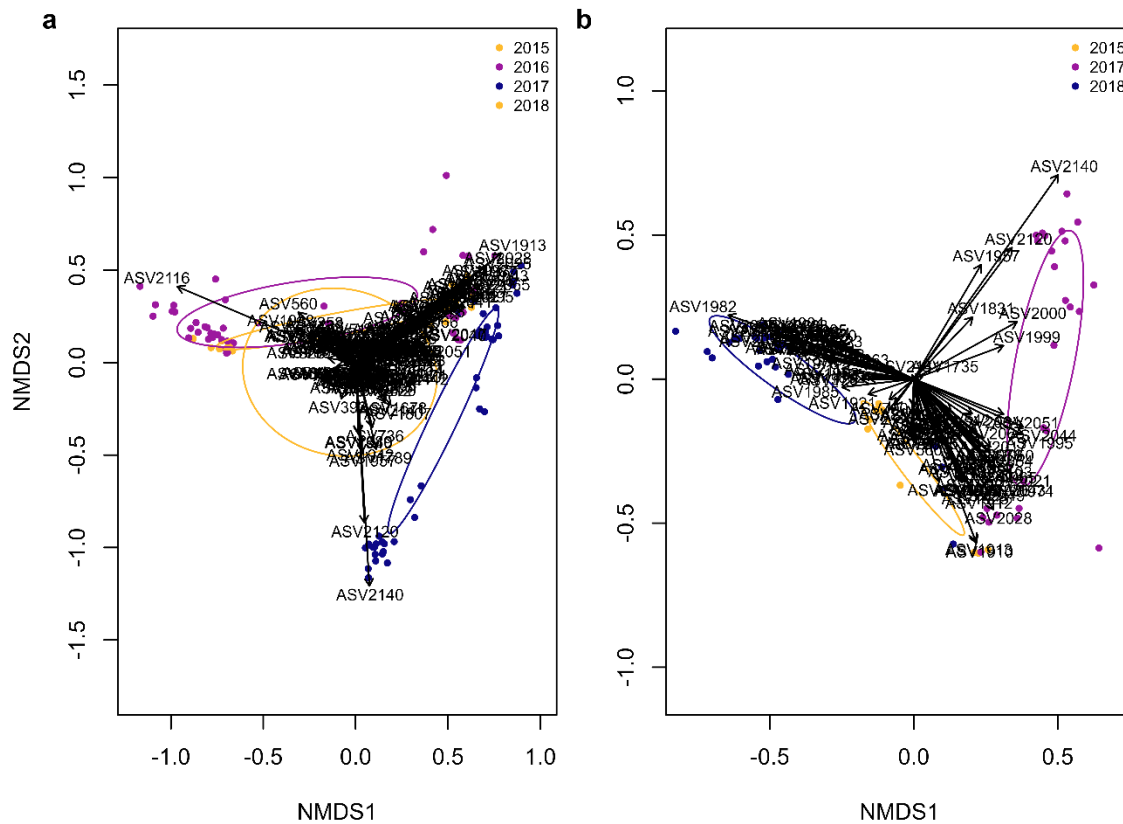
Figure B.2. NMDS of all years of bulk data with the significant ASVs (p < 0.01) and their vectors as determined by envfit for **a) Cedar Creek** and **b)** Jena
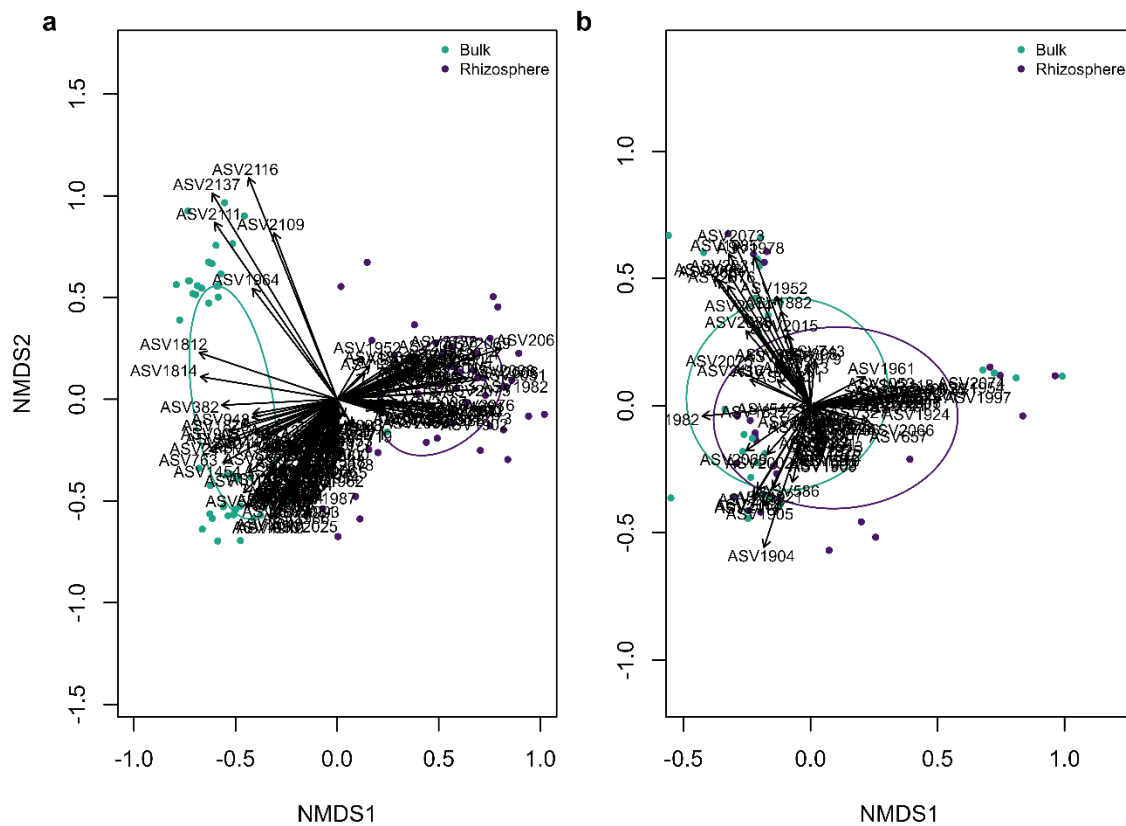
Figure B.3. Ordination of 2018 data with bulk and rhizosophere data with the significant ASVs (p < 0.01) and their vectors as determined by envfit for **a) Cedar Creek** and **b)** Jena

Appendix C. Database and Accession Number of Raw Sequence Data

Database	Accession Number	URL
NCBI	PRJNA683074	www.ncbi.nlm.nih.gov/bioproject/683074
USU Digital Commons	1236212650	digitalcommons.usu.edu/all_datasets/127