EFFECTS OF SOIL TEMPERATURE ON FUNGAL DIVERSITY AND COMPOSITION

By

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

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the of CHELESA A. HARRIS find it satisfactory and recommend that it be accepted.

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EFFECTS OF SOIL TEMPERATURE ON FUNGAL DIVERSITY AND COMPOSITION

Abstract

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Washington State University
May 2018

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Cryptic species, such as soil fungi, represent a highly diverse and undiscovered group. These organisms are responsible for important ecological processes and ecosystem services that regulate the environment and provide economic value. Their composition and diversity directly impact these processes (Hunt et al., 2002, Wagg et al., 2013) Examining how different environmental factors drive their diversity and composition has been difficult in the past, but due to advances in molecular techniques we are now able to explore them further. Experimental studies suggest that pH, root exudates, concentrations nitrate and ammonium, C:N, soil moisture, and latitude all play a role in fungal composition; however, we lack an observational study isolating long- term temperature effects on soil fungal composition. Short term soil warming experiments have found significant changes in fungal composition and no changes in diversity. Due to the timescale of these experiments, ecological processes, such as immigration and emigration, do not have time to occur. This is necessary for stable shifts in community structure and adaptations to occur. Our aim is to determine if long term increases in soil temperature result in distinct communities and increased fungal diversity. We conducted a field study of four sites in Idaho (USA) surrounding hot springs to assess differences in fungal community composition between thermal (soils near the hot spring) and control (soils away from the hot spring)
treatments at each site. We used next generation sequencing to determine fungal taxa abundance. We collected soil cores from the A horizon of plant associated soils at thermal (22.8-26.8°C) and control (16.8-17.9°C) treatments and quantified soil properties including: C:N, nitrate, ammonium, pH, and temperature. Based on a Permutational Multivariate Analysis of Variance we found a significant effect of treatment on fungal composition across sites (p<0.001). Fungal diversity, based on measures of species richness and Shannon-Weiner index, did not differ between treatments in three of the four sites. However, Weir creek hot spring, with the highest soil temperatures, had significantly higher diversity in thermal soils.
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Dedication

For Debra Goulding, Mary Harris, Donna Gardner, and Dr. Paula Jackson.

I would not be here without you.
CHAPTER ONE: INTRODUCTION

A primary question in ecology is what controls species composition and diversity. Highly diverse, cryptic groups, such as soil fungi, remain relatively undescribed and answering this question has been challenging. Based on estimates only ~8% of fungal species have been described (Hawksworth, 1991; Blackwell, 2011). However, recent advancements in molecular techniques, such as environmental metagenomics, have allowed researchers to start understanding these communities better. These organisms play important roles in ecological processes and provide numerous ecosystem services. Soil fungi directly impact primary productivity, rates of decomposition, nutrient cycling, food web structure, soil structure and fertility, and succession (Dighton, 1997; Botha, 2011; Kao-Kniffin, 2014; Azcón-Aguilar et al., 2015). Their composition and diversity can influence how ecological processes occur based on proportions or presence of different functional groups (i.e. mycorrhizal, saprotrophic, or parasitic/pathogenic fungi). Wagg et al. (2013) found that with reduced mycorrhizal colonization and fungal richness organic phosphorus leaching and N₂O emissions increased and nitrogen turnover and litter decomposition decreased. Hunt et al (2002) modelled the effects of deleting different functional soil groups from a grassland ecosystem and found that deletion of saprotrophic fungi reduced net primary productivity by 85% and nitrogen mineralization by 60%. Identifying environmental factors that influence soil fungal composition and diversity is important for understanding soil ecosystem functioning and potential responses to environmental change (Bardgett et al., 2014).

Environmental factors found to impact fungal diversity and composition include: pH, root exudates, phosphorus content, concentrations nitrate and ammonium, C:N, soil moisture, and latitude (Broekling et al., 2008; Kivlin et al., 2014; He et al. 2016; Zhang et al., 2016).
According to Zhang et al. (2016) pH played a significant role in controlling fungal composition. Kivlin et al. (2014) found that soil fungal community composition varied significantly with nitrate, C:N, nitrogen concentration and soil moisture interactions, nitrate and ammonium concentration interactions, and total carbon and latitude interactions. While the effects of most soil properties on fungal communities have been studied, the long-term effects of temperature difference remain unknown. He et al. (2016) found that fungal composition varied more significantly due to phosphorus addition to the soil than to nitrogen addition to the soil. Root exudates created by plants can regulate composition of the fungal community by maintaining native fungi and not non-native fungi (Broekling et al. 2008).

Prior studies of temperature effects on fungi have aimed to determine impacts of climate change on soil temperature and its effects on diversity and composition of soil fungi, using manipulated field experiments with short-term soil warming. Allison et al. (2008) found that significant shifts in fungal community structure when soils warmed by 0.5°C over three years, whereby relative abundance of a dominant Thelephoroid fungus decreased and relative abundance of Ascomycetes and Zygomycetes decreased. Fujimura et al. (2007) found no significant changes in composition, diversity, or evenness after 5 years of soil warming in a tundra ecosystem. Solly et al. (2017) found significant shifts in fungal community composition and no changes in diversity after five years of soil warming (~3.6°C) during snow free periods. In a 20-year warming study, DeAngelis et al. (2015) found that fungal abundance was marginally reduced by a 5°C increase in soil temperature. Zogg et al. (1997) took soil samples from the environment and heated them in a lab and found significant shift in fungal composition when comparing before and after heating. Studies of soil warming effects on fungal composition over short time scales show conflicting results. Experiments performed in the field may not ahev
occurred over a time frame that allowed for long-term ecological processes to occur, such as adaptation, and studies performed on an isolated sample in the lab did not allow for immigration and emigration resulting in gene flow between natural populations. These processes all contribute to distinct compositional changes over time. Our aim is to determine if long term increases in soil temperature, in a system that allows for movement and gene flow between populations results in distinct communities of soil fungi.

We used a hot spring as a study system because it has persistent increased soil temperatures over a time frame that allows for immigration and emigration leading to distinct compositional changes. We expect that fungal composition of thermal sites will be significantly different from that of control sites due to persistent increased temperature that soils near hot springs provide. We also expect that fungal communities in thermal treatment areas will exhibit higher levels of diversity due to higher and less variable soil temperature, based on global patterns of species diversity (Fischer, 1960). Although ectomycorrhizal fungi exhibit the opposite pattern of global diversity (higher diversity in temperate regions than in tropical ones), we expect that other fungal groups such as saprotrophs and pathogens will not (Tedersoo et al., 2009). The main goal of our study is to determine if long-term exposure to increased soil temperatures leads to distinct fungal communities. The objectives of our study are 1) To assess the magnitude of difference of fungal community composition between control and thermal treatments and 2) To determine if fungal diversity increases with higher soil temperatures.
CHAPTER TWO: METHODOLOGY

Sites & Sampling

We chose four sites across central Idaho within the Nez Perce Clearwater National Forest and the Boise National Forest (Figure 1). We chose sites based on the presence of publicly accessible hot springs and contained similar vegetation and lithology across sites. The two sites in Nez Perce Clearwater National Forest (Jerry Johnson and Weir Creek hot springs) are spruce-fir dominated but also contain pine, cedar, and larch species. The two sites in Boise national forest (Molly’s Tubs and Rocky Canyon hot springs) are pine-spruce dominated. All sites are in the Idaho batholith ecoregion and are characterized by a granitic parent material. These areas receive an annual average rainfall of 380-550 mm and snowfall of 1000-2000mm. Elevation of all sites are between 1000-1500m. Mean annual daily maximum temperatures are 12-14°C and minimums are -2-1°C year around. We collected all samples in late July and early August of 2017 at approximately the same time of day to account for soil temperature fluctuations due to shading, radiation, and changes in ambient temperatures.

Figure 1A and 1B: A) A map of central Idaho where each labeled pin denotes the site of a site and B) a map of the United States with site pins.
At each site we defined treatment areas around the hot spring as thermal or control based on a survey of surface soil temperature. The thermal zone includes a radius of ~20m around the hot spring at each site. Each hot spring was located adjacent to a stream. Control areas were across the stream and a similar distance away from the stream as the hot spring. We determine which tree species to sample near based on the most abundant tree species present while also attempting to select species that have no known host specificity with the fungal community. Grand fir (*Abies grandis*) was selected as the host tree for Jerry Johnson and Weir Creek, Ponderosa Pine (*Pinus ponderosa*) for Rocky Canyon, and Douglas Fir (*Pseudotsuga menziesii*) for Molly’s Tubs. Ectomycorrhizal fungi associated with Douglas fir (*Pseudotsuga menziesii*) and Bishop pine (*Pinus muricata*) show no host-specificity (Horton & Brims, 1998). Saprobic fungi have been found to be less host restricted than fungi living on plants, such as pathogens and mycorrhizae (Lindblad, 2000). Once we defined our treatment areas and which tree to sample near we began collecting soil samples. We collected soil samples (~1000 cm$^2$ of soil) using a manual soil auger from the A horizon of plant-associated soils within the thermal and
control areas using a soil auger (Figure 2). We took two soil cores per tree then pooled them, for a total of twelve samples per treatment per site (n=96 soil samples).

**Figure 2:** Aerial map of Jerry Johnson hot spring site where sample sites are approximately labeled to show orientation to hot spring (circled in red), stream, and each other. Other sites where sampled similarly. Yellow stars = thermal samples and blue stars = control samples

![Aerial map of Jerry Johnson hot spring site](image)

**Soil Analyses**

For each soil sample, we measured percent carbon and nitrogen, nitrate, ammonium, and pH. All soil analyses were performed in the Soil Plant Waste Analytical Lab (SPWAL) of WSU Department of Crop and Soil Sciences. Using a digital soil thermometer and soil pH meter, we measured soil temperature(°C) and pH of the exact site where the soil core was taken (Table A1 in appendix). All soil samples were air dried for two days then sifted through a 2 mm sieve. Percent carbon and nitrogen ratio was measured using a dry combustion carbon and nitrogen analyzer (TrueSpec Micro CN, St. Joseph, MI, USA). These values were used to determine
carbon to nitrogen ratio. Potassium chloride was used to extract nitrate and ammonium from soil samples. Filtered extract was analyzed using a Lachat 8500 Series 2 Flow Injection Analysis System (Hach Company, Loveland, CO, USA).

**Environmental Metagenomics**

From the initial soil sample, we subsampled soil for the fungal community analysis. Soil was air dried for two days then put through a 2mm sieve. DNA was extracted from 0.25 g of soil using the Powersoil DNA Isolation kit (MO BIO, Carlsbad, CA, USA) for all 96 samples following the manufacturer’s protocol with the extra wash step. ITS1 rDNA was amplified with fungal-specific primers ITS1f (Gardes & Bruns, 1993) and ITS2 (White et al., 1990) using eight i5 (forward) and 12 i7 (reverse) TruSeq barcoded adapters (Illumina, San Diego, CA, USA). PCR conditions were: denaturation at 94°C (1 min) followed by 30 cycles at 94°C (30 sec), 52°C (30 sec), 68°C (30 sec) and final extension at 68°C (7 min), using 5–10 ng DNA and the Phusion High-Fidelity PCR Mix (New England Biolabs, Ipswich, MA, USA). Amplicons were verified on 1.5% agarose gels and normalized at equimolar concentration with the SequalPrep Normalization Plate Kit (ThermoFisher Scientific, Waltham, MA, USA). The library was purified with the Agencourt AMPure XP kit (Beckman Coulter, Brea, CA, USA) to remove primer dimers before sequencing with a MiSeq 300 bp paired-end protocol (Illumina, San Diego, CA, USA) at the Interdisciplinary Center for Biotechnology of University of Florida. Quality filtering was performed with the AMPtk software pipeline (Palmer et al., 2017) by setting sequence size to 150 bp. Sequences were clustered into operational taxonomic units (OTUs) based on 97% similarity and the OTU table was subsequently filtered with an estimated bleeding index of 0.005 % after normalizing the number of sequences per samples (Palmer et al., 2017). Denoising and clustering parameters were calibrated based on a
20-species mock community with DNA extracts from fruiting bodies. We used negative and positive controls as suggested in Nguyen et al. (2015) and OTUs with sequence count <0.05% of the total per sample were removed from the dataset. Taxonomy assignment was performed against the “species hypothesis” UNITE fungal database (Koljalg et al., 2005) and the trophic mode for each OTU was assigned using FUNGuild (Nguyen et al., 2016) as implemented in the AMPtk software pipeline (Palmer et al., 2017).

**Statistical Analyses**

We calculated Shannon-Wiener diversity index ($H'$) for each soil sample and performed a Welch two sample t-test to compare differences in OTU richness between thermal and control treatments among sites (n=4). We compared OTU richness across treatments with a Welch two sample t-test. OTU abundance values were log transformed to correct for normality and homogeneity of variance across treatments within sites. We performed a Welch two sample t-test to determine differences in soil property means across treatments. We transformed nitrate, ammonium, and C:N ratio via a Box Cox transformation to correct non-normal distribution. All statistical analyses were conducted using the software R (R Core team, 2016).

We used Principle coordinate analysis (PCA) to visualize and interpret species composition differences among samples. We use Sørensen (Bray–Curtis) distances. We quantified the effects of treatment on differences in taxa composition, based on abundances of taxa within the community (one soil sample), using Permutational Multivariate Analysis of Variance (PerMANOVA). The PerMANOVA is a nonparametric, multivariate analysis for testing differences in mean within-group distances. The null hypothesis is that there are no differences between groups based on distance between centroids and/or spread. The PerMANOVA was performed with treatment (thermal and control) as the grouping variable.
blocked by site (n=4). We used 999 randomized runs and Sørensen distances. We ran a canonical correspondence analysis which explains the relationship between species assemblages and measured environmental variables that correlate to composition or abundances. We then calculated a Spearman’s correlation coefficient for any variables that strongly explained changes in species composition between our treatment samples. The Spearman’s coefficient determine how strongly two variables are correlated based on a monotonic relationship, the farther they stray from the monotonic relationship the smaller the coefficient and the less correlated they are.
CHAPTER THREE: ANALYSIS

Soil Properties

Based on a Welch Two Sample t-test mean soil NH$_4^+$ and C:N were not significantly different across treatments within each site, one exception being NH$_4^+$ was significant at Molly’s Tubs site. Temperature and NO$_3^-$ were significantly different across treatments (p<0.001). Temperature in thermal treatments was significantly higher than control and NO$_3^-$ concentration was significantly lower in thermal treatment than in control (Table 1).

Table 1: Results of Welch Two sample t-test comparing control and thermal treatment for each soil properties at each site.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Temperature</th>
<th>pH</th>
<th>NO3</th>
<th>NH4</th>
<th>C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weir Creek</td>
<td>3.86E-19</td>
<td>0.1424</td>
<td>6.51E-08</td>
<td>0.1132</td>
<td>0.1387</td>
</tr>
<tr>
<td>Jerry Johnson</td>
<td>3.21E-14</td>
<td>0.0898</td>
<td>0.01372</td>
<td>0.5766</td>
<td>0.1101</td>
</tr>
<tr>
<td>Molly's Tubs</td>
<td>3.86E-14</td>
<td>0.1682</td>
<td>2.05E-07</td>
<td>4.96E-11</td>
<td>0.1077</td>
</tr>
<tr>
<td>Rocky Canyon</td>
<td>1.30E-13</td>
<td>0.8683</td>
<td>0.02119</td>
<td>0.09643</td>
<td>0.7959</td>
</tr>
<tr>
<td>All Sites</td>
<td>5.38E-43</td>
<td>0.2271</td>
<td>2.6E-09</td>
<td>0.1908</td>
<td>0.2538</td>
</tr>
</tbody>
</table>
Fungal Diversity

We had total of 4 372 832 sequences for the final analysis ranging from 15 059 to 68 527 sequences per sample (mean = 45 550). The mean number of sequences per sample (with standard deviations) for each treatment within site was: 44 505 (± 9460) in Weir creek control samples, 49 573 (± 6750) in Jerry Johnson control samples, 51 441 (± 10 459) in Molly’s tubs control samples, 47 601 (± 13 185) in Rocky canyon control samples, 39 562 (± 10 083) in Weir creek thermal samples, 47 555 (± 10 466) in Jerry Johnson thermal samples, 40 189 (± 7615) in Molly’s tubs thermal samples, 44 058 (± 7529) in Rocky canyon thermal samples. We removed all OTUs that were non-fungal. After filtering, out dataset was comprised of 3159 OTUs.

OTU richness was similar across seven sampling areas, ranging from an average of 104 to 132 OTUs per sample. One sampling area, Weir creek thermal, had significantly higher OTU richness, with an average of 180 OTUs per sample. Raw OTU richness is plotted on a box-and-whisker plot (Figure 3). Based on the Welch two sample t-test, richness was not significantly different between treatments within all sites except Weir creek (p<0.001, Table 2).
**Figure 3:** Box-and-whisker plot showing species richness for each treatment within site and total species richness combined across all sites.

**Table 2:** Welch Two Sample t-test for comparing species richness across control and thermal treatments at each site.

<table>
<thead>
<tr>
<th>Site</th>
<th>T</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weir Creek</td>
<td>-5.377</td>
<td>19.466</td>
<td>3.18E-05</td>
</tr>
<tr>
<td>Jerry Johnson</td>
<td>0.39402</td>
<td>21.671</td>
<td>0.6974</td>
</tr>
<tr>
<td>Molly’s Tubs</td>
<td>-0.6769</td>
<td>21.405</td>
<td>0.5057</td>
</tr>
<tr>
<td>Rocky Canyon</td>
<td>-0.3927</td>
<td>17.807</td>
<td>0.6992</td>
</tr>
</tbody>
</table>
Diversity, based on the Shannon-Weiner diversity index (H’), shows similar patterns to OTU richness (Figure 4). The H’ value was similar for seven of the eight sampling areas ranging between 2.9 and 3.4. Weir creek thermal had a H’ value of 4.1.

**Figure 4:** Shannon-Weiner Index calculated for each sample (n=12) and averaged to represent each treatment within site with one standard deviation.

![Shannon-Weiner Diversity Index](image)

**Fungal Community Composition**

We had total of 4 129 732 sequences for the final analysis ranging from 12 406 to 68 310 sequences per sample (mean = 43018). The mean number of sequences per sample (with standard deviations) for each treatment within site was: 41 431 (± 8452) in Weir creek control samples, 42 147 (± 8377) in Jerry Johnson control samples, 48 429 (± 11 911) in Molly’s tubs...
control samples, 46 547 (± 13 558) in Rocky canyon control samples, 38 296 (± 9993) in Weir
creek treatment samples, 43 160 (± 11 437) in Jerry Johnson treatment samples, 38 543 (± 8680)
in Molly’s tubs treatment samples, 43 133 (± 6749) in Rocky canyon treatment samples. We
removed all OTUs that comprised less than 2% of all sequence reads and non-fungal OTUs.
After filtering, out dataset was comprised of 1634 OTUs. Species accumulation curves for soil
fungi indicated that each treatment within site and each site was equitably sampled and based on
the Chao1 estimator we sampled between 36% and 51% of the diversity within each site (Chao,
1984, Figure A1 in appendix).

The PerMANOVA results show that thermal treatment and control treatment
communities have significantly different composition (p<0.001, Table 3). This was detected
across treatments within each site and when each treatment across sites were combined.
Consistent with these results, PCA for each site shows distinct separation of thermal and control
communities along first principle component axis (Figure 5). Sampling sites only had a weak
effect on compositional differences (Figure 6).

The canonical correspondence analysis (CCA) shows aggregation of species scores (open
tan dots) with thermal samples (solid red dots) and control samples (solid blue dots).
Temperature is correlated with the CCA1 axis and NH₄⁺ is correlated with the CCA2 axis
because they are parallel with those axes. Due to temperature and NH₄⁺ being orthogonal they
are considered independent from one another in the analysis. Eigenvalues for the CCA1 and
CCA2 axes are .03 and .019. Due to the NO₃⁻ arrow being orientation between temperature and
NH₄⁺, there may be a correlation between NO₃⁻ and those two variables. Therefore, we calculated
a Spearman’s correlation coefficient to determine the magnitude of their correlations. NO₃⁻
correlated moderately with temperature (p=0.549) and weakly with NH₄⁺ (p=0.18).
Table 3: Results of the PerMANOVA examining effects of treatment on community composition of all fungal OTUs.

<table>
<thead>
<tr>
<th>Site</th>
<th>Df</th>
<th>Sum of Squares</th>
<th>Mean Squares</th>
<th>F Model</th>
<th>$R^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>2.584</td>
<td>2.5845</td>
<td>7.0924</td>
<td>0.07016</td>
<td>0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>94</td>
<td>34.254</td>
<td>0.3644</td>
<td>0.92984</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>95</td>
<td>36.838</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jerry Johnson</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>1.3394</td>
<td>1.33842</td>
<td>3.988</td>
<td>0.15345</td>
<td>0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>22</td>
<td>7.3835</td>
<td>0.33561</td>
<td>0.84655</td>
<td>1</td>
<td></td>
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<tr>
<td>Total</td>
<td>23</td>
<td>8.722</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weir Creek</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>2.1418</td>
<td>2.14177</td>
<td>10.45</td>
<td>0.32202</td>
<td>0.001</td>
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<tr>
<td>Residuals</td>
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<td>4.5092</td>
<td>0.20496</td>
<td>0.67798</td>
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<tr>
<td>Total</td>
<td>23</td>
<td>6.651</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molly's Tubs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>2.0952</td>
<td>2.09517</td>
<td>7.8466</td>
<td>0.25437</td>
<td>0.001</td>
</tr>
<tr>
<td>Residuals</td>
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<td>6.1414</td>
<td>0.26702</td>
<td>0.74563</td>
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<td>Total</td>
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<td>8.2366</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rocky Canyon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>1.4707</td>
<td>1.47067</td>
<td>5.2082</td>
<td>0.19873</td>
<td>0.001</td>
</tr>
<tr>
<td>Residuals</td>
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<td>5.9298</td>
<td>0.28237</td>
<td>0.80127</td>
<td>1</td>
<td></td>
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<tr>
<td>Total</td>
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<td>7.4005</td>
<td></td>
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</tbody>
</table>
**Figure 5:** The first four PCAs show community composition differences based on the Sørensen (Bray–Curtis) distances where each dot represents a fungal community. The “Treatment” PCA shows differences for all thermal and control communities combined across all sites. Red dots=thermal communities and black dots=control communities.
**Figure 6:** PCA shows community differences for all communities for each site.

![PCA plot](image)

**Figure 7:** Canonical correspondence analysis for species composition within thermal and control samples with soil properties correlating with axes that explain the variation in species composition.

![CCA plot](image)
CHAPTER THREE: CONCLUSION

Increased soil temperature has been implicated as a driver of diversity and composition of fungal communities in several studies (Allison et al., 2008; Solly et al., 2017) while other studies have found no evidence of this (Fujimura et al., 2007; DeAngelis et al., 2015). We hypothesized that due to the persistence and time scale of soil warming in our study, we would find distinct communities of fungi in thermal soils. We inferred that with long-term warming, emigration and immigration as well as adaptation would have time to occur to produce these distinct communities. Of the soil properties measured, only temperature and NO$_3^-$ varied significantly between thermal and control treatment across all sites. Our results indicated that thermal soils harbored compositionally distinct communities of fungi when compared to soils with similar properties but lower temperatures. The PERMANOVA analyses indicated that treatment significantly influenced fungal composition and that communities in thermal soils are more similar to one another than they are to control soils. Based on the CCA, we determined that temperature explains most of the variation in species composition among treatments. Eigenvalues indicate that 3% of the variation is explained by temperature and 1.9% is explained by NH$_4^+$. The low explanatory power of this analysis is expected due to the size of the dataset as well the extensive variation in species abundances across samples.

Fungal diversity, measured by species richness and Shannon-Weiner index, across treatments in three of the four sites did not differ. However, in one site, Weir creek hot spring, fungal diversity was significantly higher in the thermal treatment. This site had the highest temperature soil of all the sites (26.8°C). The study done by Pietikainen et al. (2009) determined the optimal temperature range for fungal growth and found that it was highest between 25-30°C.
Weir creek hot spring having soil temperatures well within optimal growth ranges (26.8°C±1.1) for fungi may help explain why fungal diversity was higher only in this site. However, further research is needed to determine any direct relationship between optimal growth ranges and diversity.

Soil temperature may correlate with changes in composition and diversity indirectly by regulating soil moisture. Soil moisture, although similar across treatments during the summer, likely differs significantly in the winter due to snow melt dynamics. Snow will melt more quickly on thermal soils, therefore changing soil moisture during the winter months compared to control soils. If the source of increased soil temperature is due to changes in air temperature, vegetative cover, or radiation increased soil drying can occur which may suppress decomposition rates (Verburg et al., 1999). Therefore, the mechanism of increased temperature should be considered due to how it interacts with soil moisture when assessing fungal composition and diversity. Higher nitrate concentration corresponded with thermal treatments and nitrate correlated with temperature moderately but is likely a result of increased nitrogen mineralization and nitrification due to increased microbial biomass since these soils are within optimal temperature growth ranges for both fungi and bacteria (Kaiser, 1994; Pietikainen et al., 2004).

Our study found that fungal communities in soils that have been persistently warmed have distinct composition when compared to soils with similar lithology and site but higher temperatures. Studies that did not find compositional changes due to warming may have not persisted long enough to allow for ecological processes such as migration and adaptation to occur. Correlations between temperature, nitrate, and ammonium and changes in fungal diversity support prior studies that have found similar results. Through the use of relatively new molecular
techniques and a novel study system we are able to add a more temporal aspect to prior studies, showing that over long time-frames, we still see distinct changes in fungal composition. Because there are species that are present in thermal samples that are not present in control samples, and visa-versa, we can consider whether their presence or lack thereof translates to physical changes in the environment. We can then begin to create a more complete picture of how environmental variables affect composition and how that relates to changing ecosystem function. Future research focusing on long-term experiments is needed to further assess how fungal communities are shaped by temperature and determine the relationship between soil moisture, temperature, and soil properties such as nitrate and ammonium.
REFERENCES

Allison SD, Treseder KK. 2008. Warming and drying suppress microbial activity and carbon

science and plant nutrition* **15**: 362-396.


Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco JM. 2008. Root exudates
regulate soil fungal community composition and diversity. *Applied and environmental
microbiology* **74**: 738-744.


SD. 2015. Long-term forest soil warming alters microbial communities in temperate forest soils.
*Frontiers in microbiology* **6**: 104.


Horton TR, Bruns TD. 1998. Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed stand of Douglas fir (Pseudotsuga menziesii) and bishop pine (Pinus muricata). *The New Phytologist* **139**: 331-339.


APPENDIX

Table A1: Averages and standard deviation of soil properties at each treatment within each site.

<table>
<thead>
<tr>
<th>Site</th>
<th>Treatment</th>
<th>C:N</th>
<th>NH4 (ppm)</th>
<th>NO3 (ppm)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weir Creek</td>
<td>Control</td>
<td>19.19±1.98</td>
<td>5.35±1.29</td>
<td>1.79±1.22</td>
<td>16.8±1.1</td>
</tr>
<tr>
<td></td>
<td>Thermal</td>
<td>20.77±2.94</td>
<td>10.34±7.55</td>
<td>9.82±3.91</td>
<td>26.9±0.4</td>
</tr>
<tr>
<td>Jerry Johnson</td>
<td>Control</td>
<td>22.43±5.96</td>
<td>6.49±2.67</td>
<td>0.10±0.02</td>
<td>17.0±1.0</td>
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<tr>
<td></td>
<td>Thermal</td>
<td>18.48±6.83</td>
<td>8.39±6.15</td>
<td>2.28±4.45</td>
<td>22.8±0.6</td>
</tr>
<tr>
<td>Molly’s Tubs</td>
<td>Control</td>
<td>15.30±1.87</td>
<td>6.79±1.46</td>
<td>0.19±0.48</td>
<td>17.8±0.7</td>
</tr>
<tr>
<td></td>
<td>Thermal</td>
<td>13.72±3.04</td>
<td>1.83±0.7</td>
<td>6.87±4.95</td>
<td>23.9±1.0</td>
</tr>
<tr>
<td>Rocky Canyon</td>
<td>Control</td>
<td>18.32±7.51</td>
<td>3.92±4.29</td>
<td>0.08±0.07</td>
<td>17.8±0.9</td>
</tr>
<tr>
<td></td>
<td>Thermal</td>
<td>17.47±6.33</td>
<td>2.07±1.69</td>
<td>1.95±4.46</td>
<td>24.2±1.1</td>
</tr>
</tbody>
</table>

Figure A1: Species accumulation curves for each site and treatment