THESIS

COMBINED EFFECTS OF WARMING AND DRYING ON A TEMPERATE-TO-BOREAL FOREST ECOTONE EXERT ADDITIVE CHANGES ON SOIL MICROBIOME STRUCTURE AND DIVERSITY

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ABSTRACT

COMBINED EFFECTS OF WARMING AND DRYING ON A TEMPERATE-TO-BOREAL FOREST ECOTONE EXERT ADDITIVE CHANGES ON SOIL MICROBIOME STRUCTURE AND DIVERSITY

The soil microbial community is an important mediator of many ecosystem functions, so understanding dynamics under climate change. These responses could be more robust in transitional zones such as the temperate-to-boreal forest ecotones, which are poised to experience substantial changes under projected climate change over the next century and beyond. Because these systems are projected to move towards a warmer, drier climate, it is important to understand how the soil microbiome's structure and interactions shift under such conditions. Here, we examined the response of microbial communities to simulated warming and drought conditions using the B4WarmED (Boreal Forest Warming in an Ecotone in Danger) experiment in Minnesota, USA. B4WarmED is a fully factorial blocking experiment which uses *in situ* experimental 3.4°C warming and precipitation reduction to simulate the projected regional late-21st century climate. Using Shannon-Weaver Diversity and Canonical Analysis of Principled Coordinates, we found that combined warming and drying effects exerted significant effects on the diversity and structure of microbial communities after 8 years of warming, and 5 of drought treatments. Specifically, warming and drying effects appeared to combine additively, rather than exhibiting nonlinear interactive effects, at the community level. Per-taxon linear models revealed a sizeable portion of individual microbes exhibit a significant abundance response to one or both of warming and drying effects. However, co-occurrence network analysis and Dufrene-Legrende Indicator Value characterization revealed a smaller portion of bacterial sub-communities with persistent taxonomical makeup and response profiles across treatments. Within the microbial communities our analysis identified three types of taxon-specific responses to climate change

stressors: resistant, opportunistic, and sensitive, with most taxa being resistant to warming and drying effects. However, our results provide strong evidence that combined warming and drought influences will impact soil microbial communities of temperate-to-boreal ecotone forests ("boreal ecotone" hereafter), with potential implications for ecosystem functioning.

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CHAPTER 1

Introduction

Over the next century and beyond, the climate is projected to see changes including warmer mean temperatures in many regions, shifts in precipitation, and increased frequency of events like drought and extreme weather (IPCC 2013). One concern in predicting the ecological implications of these changes is that many climate driven trends, such as warming and drying, are interlaced. Thus, individual studies that test a single climate driven trend cannot fully explain the potential impacts of anthropogenic climate change and necessitates large multifactorial experimental designs to empirically understand likely shifts in ecosystem function and stability (Steinweg et al. 2013; Rich et al. 2015). Moreover, the potential for positive feedback effects and other nonlinear responses to greenhouse gas inputs introduces additional uncertainty to the task of modeling, and preparing for, climate change effects (IPCC 2013). This dynamic is of special concern for *ecotones*, or regions transitioning between two ecosystem types, because of the strong potential for nonlinear feedback as dissimilar successional communities take hold in altered environments (Evans and Brown 2017). Studies have linked increases in temperatures and decrease in precipitation to changes in the abundance, distribution, phenology, and community composition of tree species at ecotones, as well as compositional and functional changes in soil invertebrate food networks (e.g. Rich et al. 2015; Schwartzeberg et al. 2014; Schwarz et al 2018). These changes are postulated to influence belowground microbial communities that play a critical role in major ecosystem processes (Singh et al. 2010; Jansson and Hofmockel 2019; Hutchins et al. 2019). However, the interacting effects of climate change drivers on the soil microbiome at ecotones is relatively unstudied. This leaves a critical knowledge gap in characterizing and anticipating the response of the soil microbiome to warming and drying stressors and as a result, it can be difficult to identify the degree to which climate change will impact ecosystem functioning and forest health in ecotones.

The boreal ecotone is a critical habitat which is predicted to experience overall increase in the pace of change of future climate, including higher global temperature and longer periods of extreme drought than lower latitude biomes (Crowther et al. 2016). Because these transitional communities comprise tree species both at the upper and lower limits of their respective ranges, there is a risk of community breakdown as the southern range of more warming-sensitive species contracts (Reich et al 2015), and keystone species may become separated by differential migration rates or warming tolerance (Evans and Brown 2017). Warming also induces physiological changes in many tree species in this region, including reduced photosynthetic activity (Reich et al 2018). Significant functional changes have already been documented, like documented forest range contraction or migration (Evans and Brown 2017), and the seasonal transition of a northern Swedish boreal forest from a carbon sink to a carbon source due to changes in cold-weather dynamics (Hadden and Grelle 2016). These large functional shifts coincide with, and to varying extents are driven by, more granular changes like reorganization of the soil food network (Schwarz et al. 2017) or weakening of the symbiotic relationship between trees and Ectomycorrhizal fungi as a consequence of reduced tree photosynthetic capacity (Fernandez et al. 2017). A recent study reported significant shifts in the soil microbiome diversity and associated functions in response to warming and disturbances in a northern soil ecotone (Van Nuland et al. 2020). Various ecological functions including soil C storage (Trivedi et al. 2016) and greenhouse gas emissions (Martins et al. 2017) are influenced by multiple direct and indirect interactions of microbes with climate change stressors. Therefore, changes in microbial functions and feedback mechanisms will play important roles in determining the climate sensitivity and future climatic state of ecotones (Clemmensen et al. 2013, Martins et al 2017). This context makes it critical to further our understanding of how multiple global change drivers modify the structure of the soil microbiome as a whole, and of key taxa that modulate alterations in microbial interactions.

Although it is now well established that soil microbial communities are crucial for ecosystem functioning, we lack a general framework for predicting their response to climate change (de vries and Griffiths 2018; Jansson and Hofmockel 2019; Hutchins et al. 2019). In recent years, significant efforts have been made to study the impacts of climate change on soil microorganisms in different climate-sensitive soil ecosystems (e.g. Bardgett and Caruso 2020, Llado et al 2017, van Nuland et al 2020, Dubey et al 2019, Bradford et al 2016). Overall the composition and structure of microbial communities is sensitive to climate change however, the strength and direction varies with the stressor(s) (Drigo et al. 2017), their intensity (van Nuland et al. 2020; DeAngelis et al. 2015), their duration (de Vries et al. 2018; Acosta-Martinez et al. 2014), and the ecosystem in question (Evans and Wallenstein 2013; Ladau et al. 2018). Soil microbes have different strategies to cope with changing environmental conditions and therefore vary significantly in their sensitivity to climate change stressors (Der Voort et al 2016; Bardgett and Caruso 2020,). In general, the soil fungal community is often less impacted by climate change as compared to bacteria (Yuste et al. 2011; de Vries et al. 2012; 2018; Acosta-Martinez et al 2014). Different members within a particular microbial group can respond sensitively, tolerant, and opportunistically to climate change stressors (Evans and Wallenstein 2014; Crowther et al. 2014; Drigo et al. 2017; Meisner et al. 2018). The degree to which taxa are able to maintain relative dominance in the community using resistance or resilience strategies, especially under sustained exposure, could have important implications for community structure in the long term. While a majority (~80% per Oliviero et al. 2017) of bacterial taxa appear not to show a consistently significant response to warming (whether negative or positive), community-level shifts in alpha and beta diversity in this range are frequently observed (e.g. De Angelis et al. 201; van Nuland et al. 2020). This suggests that associations between microbes, independent of direct physiological responses to warming or drying, may play an important organizing role in shaping a new community order under climate change stressors.

Climate change mediated alterations in microbial community structure can alter interactions among species (Jansson and Hofmockel 2020). However, we have a very limited understanding about how microbial networks shift in response to climate extremes and other disturbances (de Vries et al. 2018). It is postulated that environmental change influences a multitude of direct and indirect interactions that occur between the networks of coexisting microbial communities resulting in shifts in resilience, stability, and ecosystem functioning (Jansson and Hofmockel 2020). For instance, a high degree of positive interactions in a community are generally held to amplify perturbance effects (potentially leading to a cascade effect in highly mutualistic systems), while negative interactions dampen these disturbances and tend toward stability of the existing order (Coyte et al. 2015; Landi et al. 2018). Interactions between members of soil microbial communities affect their growth and metabolism resulting in altered patterns of species abundance across space and time. This information is particularly valuable in evaluating the impacts of environmental changes on microbial communities.

To address how climate change-related warming and drying effect soil microbial communities across a boreal forest ecotone, both independently and in conjunction, we used soil samples from the long-term ecological research from the B4WarmED (Boreal Forest Warming in an Ecotone in Danger) experiment. Beyond being located in a boreal-to-temperate ecotone of interest, this experimental system accounts for both direct and interactive effects of predicted late-21st century warming and drying in a full-factorial blocking experimental design. The B4WarmED system has also hosted a wealth of research into subjects ranging from warming effects on leaf respiration (e.g. Wei et al 2016), soil food web dynamics (Thakur et al 2018, Schwarz et al 2017), and differential success of undergrowth species under warming regimes (e.g. Thakur et al 2014). Most relevantly, van Nuland et al (2020) studied the soil microbiome in the B4WarmED system from 2009 to 2011, providing a valuable picture of early successional dynamics under warming, but predating the implementation of a summer drought treatment, precluding the study of interactive effects. For the present study, we focused on three

primary research directions: First, we measured soil bacterial and fungal community structure to explore compositional changes under separate or combined sustained warming and drying stressors, especially whether these effects appear to drive divergence between the control community and those subjected to one or both treatments. Second, we use a range of treatment response indices (correlation between abundance and treatments, direct changes in abundance, and Dufrene-Legendre indicator value scoring) to evaluate the relative proportion of taxa showing a significant abundance response to these pressures, and the prevalence of positive, or negative, effects on abundance. Third, we use bacterial and fungal co-occurrence networks to detect persistent clusters of positively-associated taxa across treatment conditions, and characterize their taxonomic composition and indicator value response profiles to warming and drying stresses (sensitive, or decreasing in abundance, opportunistic, or increasing in abundance, or resistant, with no significant changes). Together, these approaches will give us insight into how the taxonomic composition of this Temperate to Boreal Ecotone forest system soil microbiome may develop under predicted climate trends.

Materials and Methods

Site Description:

We made use of the B4WarmED open-air climate system to explore the effects of paired Warming and Drying stressors on the temperate boreal ecotone microbiome in a controlled experiment. This northern Minnesota experiment was established in in spring 2008, and comprises two sites about 150 km apart, located near the Cloquet Forestry Center (46°40′46”, 92°31′12″ W), and the Hubachek Wilderness Research Center (47°56’42” N,91°45’29” W). Both sites have a similar endemic tree species composition of 40-60-year aspen-birch-fir forest, with 11 native and naturalized species being planted as saplings for the experiment in 2008 (Rich et al. 2015). Local climates are broadly similar (Supplementary Table 1), with Ely being somewhat colder and drier on a mean annual basis, although this is not uniform overall, with Cloquet

experiencing cooler summers and warmer winters (Rich et al. 2015). Both sites' experimental plots have a mean pH of ~5.3, with no substantial observed treatment-associated changes as of 2013 (Martins et al. 2016). Local soil is primarily comprised of sandy loam, with roughly 10% clay at each site; per USDA soil taxonomy, most CFC soil is classified as Inceptisols, and HWRC as Entisols (Eddy 2015; Martins et al. 2016). B4WarmED features on-site logging of precipitation, above- and below-ground temperatures, and other pertinent environmental traits, including volumetric soil moisture ($\frac{cm^3\ H_2O}{cm^3\ soil}$), hourly records for ambient above- and belowground temperature, and rainfall (Eddy 2015; Rich et al. 2015). These sensor systems, as well as regular soil collection and other measurements, provide a wealth of data for isolating experimentally induced changes in the system.

In this study, we used two overlapping treatment factors of the B4WarmED experimental design: a Warming and a Drying treatment. The Warming treatment condition is 3.4 °C above ambient, and was implemented at the outset of the experiment (along with a clearing of upper-canopy vegetation), and maintained during a field season bracketed by the first, and last, time that mean daily temperatures reached at least 1°C for at least 5 days, using combined above- and belowground heating elements with real-time feedback (full details in Rich et al. 2015). The Drying (more specifically, summer drought) treatment was added in 2012, and uses event-based canvas canopies to intercept ~45% of precipitation from June through September (Rich et al. 2015). Taken together, these overlapping treatments create four primary conditions: an unmodified control condition with no temperature elevation and ambient precipitation (Designated hereafter as CO), a warming condition elevated by 3.4 °C with ambient precipitation (WO), a summer drought condition 45% reduction of precipitation from June-September with no warming (DO), and a combined stressor treatment combing the summer drought and 3.4 °C warming conditions (WD).

This representation of the B4WarmED experimental model omits an intermediate 1.8 °C warming level, as we chose to focus on the higher warming treatment due to lower site- and seasonal dependence (e.g. Eddy 2015, Martins et al. 2016). Similarly, we excluded an undisturbed "Closed" canopy condition, in which the summer drought treatment was not implemented. The experimental system as represented in our study therefore comprises 8 study plots (7 m^2 circles) distributed across three larger experimental blocks per site (24 plots across both sites). There is one study plot in each block with a given combination of Warming and Drying treatment conditions (CO, WO, DO, and WD) giving three experimental replicates per site. In addition, we analyzed samples from June, August, and October of 2017, making a total of 36 samples.This simplified experimental design set the stage for sequencing-based analysis of the soil microbiome.

Soil Sampling and Analysis

To gather data on the microbial composition of B4WarmED soil communities, we collected bulk soil cores (5 cm diameter, 8 cm deep) from randomized locations within each 7 m^2 plot within a 1-2-day span during June, August, and October of 2017, which we accounted for as a fixed factor in models for taxon response (diversity, abundance, etc.). These cores were stored in a cooler in the field before being shipped to Ft. Collins, CO on dry ice and stored at -80°C thereafter, with subsamples being thawed as needed for subsequent analysis. Bulk soil was also collected at the outset of the experiment at 0-5, 5-10, and 10-20 cm depths, and dried and sieved to obtain soil textures in terms of sand, silt, and clay (Rich et al. 2015). Soil CO_2 efflux was measured *in situ* using a LI-COR 6400 Infrared gas analyzer (LI-COR Biosciences Inc, Lincoln, NE USA) with an attached soil respiration chamber and three 10.2-cm PVC respiration collars (installed in 2008) per plot, with two extending 20 cm, and one extending 40 cm, into the soil, to measure total and heterotrophic respiration, respectively (Eddy 2015).

Measurements (n=2) were collected at two-to-three-week intervals during the growing season (June-October) (Eddy 2015; Rich et al. 2015).

DNA Extraction and Sequencing Preparation:

We performed DNA extraction on soil core subsamples using Qiagen's DNEasy® Powersoil kit, following standard protocol, with the exception of increasing initial soil weight from 0.25 g to 0.50 g, and using nuclease-free water in place of Qiagen's proprietary elution buffer (Qiagen 2016). Extracted DNA was tested for quality (repeating extracting with a 260/280 absorbance ratio below 1.70) and concentration (ng/μL DNA) on a spectrophotometer (Thermo Fisher Scientific NanoDrop 2000C; Waltham, MA 02451). DNA was stored at -80 °C, with aliquots subsequently diluted to a target concentration of 25 ng/μL and transferred to 96-well plates in preparation for shipping to Argonne National Laboratory's Environmental Sample Preparation & Sequencing Facility (ESPSF), where 16S (region v4; 515f-806r) and ITS (ITS1f-ITS2) paired-end 250-read sequencing was performed via MiSeq® Illumina instrument, to observe bacterial and fungal-associated sequences, respectively (Caporaso et al. 2012). The resulting data was returned to our lab as multiplexed FASTQ files for downstream analysis.

Sequencing Analysis and Bioinformatics:

Sequences were received from Argonne National Laboratory in the FASTQ format and processed using CUTADAPT to remove adapters from the sequences in conjunction with the USEARCH v.11 pipeline for demultiplexing, denoising (UNOISE; Edgar 2016), quality filtering (UCHIME; Edgar 2011), and 97% Operational Taxonomic Unit (OTU) generation (UPARSE; Edgar 2013). We also performed taxonomic assignment with USEARCH and UCLUST against the SILVA (Quast et al. 2013) database for 16S sequences, and UNITE (Nilsson et al. 2018) for ITS, also removing sequences matching mitochondrial or chloroplast samples, using standard protocols per Edgar (2016). After taxonomic identification and generation of abundance data,

we used sample metadata to filter for low-quality reads and rare or unevenly-distributed taxa. This entailed excluding samples containing <5000 total reads from analysis, along with OTUs with <500 total reads, or present in <20% (bacterial) or <10% (fungal) of samples, bringing the total to 4576 16S and 216 ITS OTUs; in addition, 72 samples (24 each for August, September, and October 2017) were used from a larger 2010-2017 sample set. These final pre-filtering steps allowed us to focus on representative taxa with sufficient abundance and ubiquity for downstream statistical analysis.

The resulting feature and taxonomic tables were resolved to the operational taxonomic unit (OTU) level and passed to R v 4.0.2 for follow-up analysis (R Core Team 2019). OTU abundances, taxonomic identity tables, and metadata were passed to the Phyloseq system of functions for easier manipulation (McMurdie and Holmes 2013). Here and elsewhere, the tidyverse family of functions were used extensively in data import and transformation (Wickham et al. 2019). To normalize for read abundance, OTU tables were rarified to 3500 reads using the vegan package's rrarefy function (Okasen et al. 2019). From this point, we moved to characterize the general structure of the community using alpha and beta diversity metrics.

Community Alpha and Beta Diversity Analysis

To characterize community alpha diversity, or characteristics of taxonomic diversity within specific sites, we analyzed rarefied OTU abundance using the alpha function from the microbiome package (Lahti et al. 2019) to calculate Shannon-Weaver Diversity Index scores for each sample. To study the influence of warming and drying treatment effects on these metrics, as well as any nonlinear interactions, we used a negative binomial linear model with warming, drying, and a warming-by-drying interaction as fixed effects, and Shannon-Weaver Diversity as the response variable. In these models, warming and drying were represented as a binary value with 0 for the control condition (0°C or AMB, respectively), and 1 for the treatment (3.4°C or DRY). We performed an Analysis of Variance (ANOVA) on this linear model using the car

function Anova to assess the model-level significance of the three equation terms with type II Sums of Squares (Fox and Weisberg 2019). Given that only two samples were available, we elected to analyze Cloquet and Ely separately throughout, rather than as experimental replicates. To complement the testing of treatment effects and interactions, we used a Wilcoxon signed-rank test performed using the ggplot2 extension ggsignif (Ahlmann-Eltze 2019) to formally evaluate the significance of differences in the treatment-associated diversity observations themselves. To account for experimental replication and seasonal variation for overall treatment diversity levels, we also performed a 95% confidence interval least square means estimate using the same model to account and the function lsmeans from the eponymous lsmeans package (Length 2016).

To explore how diversity varied between sites, we first converted rarefied OTU abundances per site into a Bray-Curtis distance matrix using the base R distance function (R Core Team 2020). The significance of treatment effects on these distances was tested using permutational analysis of variance (PERMANOVA; Anderson 2001) with the vegan function *adonis* function (with a default 999 permutations), using the model:

$$Y_{wd} \sim W_w + D_d + W{:}D_{wd} + \varepsilon_{wd}$$

where Y is the Bray-Curtis distance as the response variable, W is the warming treatment, D is the drying treatment, and ε is the error term. In addition to testing treatment effects, we used a Canonical Analysis of Principal Coordinates (CAP) analysis with the same formula to provide a constrained ordination primarily representing the variation in our treatments of interest. This analysis was performed using the phyloseq ordinate function, after splitting corresponding phyloseq objects to site-level objects (McMurdie and Holmes 2013); the resulting objects were converted to plots using the phyloseq function plot_ordination, unclassed, and modified using ggplot2 (Wickham 2019).

OTU-level Treatment Effect Calculations

To assess the strength of correlations between warming and drying treatment effects at the OTU level, we performed a fixed-effect negative binomial generalized linear model (GLM) again using warming, drying, and warming-by-drying fixed effects as performed for the PERMANOVA using and rarefied taxon abundance as the explanatory variable. This was accomplished using the MASS glm.nb function (Venables and Ripley 2002),with model significance assessed using a Type II ANOVA function from the car package (Fox and Weisberg 2019), with the default Pillai test statistics, used to assess model significance. To assess the range of specific relative abundance trends associated with treatments, abundance of each OTU was z-scaled, using the base R scale function, to obtain standard deviations (R Core Team 2020). Interaction trend groups were classified by centering scaled relative abundance to the Control level (arbitrarily set to 0.0), and measuring the standard deviation-scaled changes associated with each treatment group in the order CO, DO, WO, and WD. Changes in relative abundance were binned into those above or below 0.5 standard deviations (i.e. 1 standard deviation centered on the Control intercept), and negative or positive changes, giving 3 possible values (< -0.5 standard deviations, $<|0.5|$ standard deviations, or > 0.5 standard deviations) for each treatment, and a total of 26 possible outcomes. These steps gave us a sense of OTU-level treatment effects, but not of the relative centrality of these OTUs in the community, and the strength of interactions between taxa. For this information, we moved on to network analysis.

Co-occurrence Network Calculation and Analysis

To evaluate the nature and extent of associations between OTUs in the B4WarmED community, we generated parallel co-occurrence networks for bacterial and fungal taxa. This was accomplished by using the graph.edgelist function from the igraph package (Csardi and Nepusz 2006) to convert rarefied abundance-based Spearman Rank correlations generated using the stats package cor.test function (R Core Team 2020) into undirected association

networks. To exclude weak or nonsignificant interactions, as well as negative correlations, we limited network membership to OTU pairs with a significant ($p \leq 0.05$) interaction and positive Spearman correlation value *rho* at or above 0.65 for bacterial OTUs, and 0.60 for fungal OTUs, to limit comparisons to implied cooperative, or at least convergent, interactions (after de Vries et al. 2017). To identify the strength and character of associations between OTUs and experimental treatments, we calculated Dufrene-Legrende Indicator Values (Dufrene and Legrende 1997) for warming and drying treatment effects in parallel (e.g. only considering 0°C and 3.4°C for the warming indicator value) as implemented in the labsdv indval function with a default 1000 permutations (Roberts 2019). Under this metric, an ideal indicator species with respect to e.g. warming would occur in all warmed (3.4°C_AMB and 3.4°C_DRY) samples, and exclusively within this group. OTUs were designated as indicators of either the Control (0°C or AMB) or treatment (3.4°C or DRY) state for a warming and drying, with significant ($p \leq 0.05$) OTUs being referred to as "**Sensitive**" or "**Opportunistic**" for that treatment, respectively. Taxa not exhibiting a significant indicator value ($p \leq 0.05$) for a treatment were designated "**Resistant**" in that respect. To visualize this metric, we wanted to include trends in indicator values beyond what is conveyed using a strict $p \leq 0.05$ threshold, so we opted to use a two-axis color gradient, translating *p* values for warming or drying indicator values for control (sensitive) or treatment (opportunistic) conditions into a derived [0,1] index for each treatment effect, with resistant taxa falling at the midpoint. "Warming" was represented as a cyan-to-red gradient, while "drying" was represented as a blue-to-yellow gradient, with neutral grey (resistant) at the center of both; to prevent skewing from extremely-significant edge cases (e.g. $p < 0.0001$), we imposed an upper threshold of $p = 0.05$ for visualization. Further details are given in Supplemental Section 1. Color assignments were performed using the colorspace HSV and mixcolor functions (Hornik and Murrell 2009). While ultimately an aesthetic interpretation of the Indval scores, this derived metric does entail some compression of the data and carries some representative assumptions.

Enrichment Testing:

To explore the relationship between taxonomy or treatment response and OTU membership in positively associated clusters in our Spearman correlation networks, we used the hypergeometric distribution to obtain a probability that the number of taxa in a group of interest observed in a group was over-, or under-represented relative to the overall distribution of taxa within a particular subnetwork. To characterize network subnetworks, we used the igraph convenience tool components to access the membership attribute, and generated OTU counts per taxon or indicator value group (Csardi and Nepusz 2006). For future reference, these groups were named with the convention "C/E.n" , for Cloquet or Ely, where *n* is the rank of the subnetwork by membership (e.g. the largest Cloquet subnetwork is designated *C.1*). We tested these groups for enrichment of OTUs belonging to taxonomic or treatment response groups using the phyper function from the base R stats package (R Core Team 2020) at each site using the formula *phyper(x, m, n, k, lower.tail = FALSE)*; in the scenario of testing network subnetworks for enrichment of a given phylum, *x* is one fewer than the number of OTUs falling both the phylum and subnetwork of interest, *m* is the number of taxa falling in the phylum of interest across the entire site, *n* is the number of taxa not in the phylum across the entire site, and *k* is the number of taxa occurring in the subnetwork. We also tested for depletion, or under-representation, of taxonomic groups within subnetworks using the above formula, but with the *lower.tail* parameter set to TRUE.

Results

Sequencing Analysis and Bioinformatics:

Our full B4WarmED dataset, containing samples collected from 2010-2017, initially included 1,871,571 and 4,044,902 16S and ITS amplicons detected across 1,128 samples (3 sample runs were ultimately combined to account for poor amplification). After initial quality

control and removal of chimeric sequences, a total of unique 41,130 bacterial and 8,864 fungal amplicons were retained for further analysis. For this study, the full dataset was subset to 126 samples collected across the 36 open-canopy sites in June, August, and October of 2017 in order to address the most recent state of the soil microbiome, and to integrate seasonal differences. The output from the USEARCH pipeline was imported into R (v. 4.0.2) for further quality control (R Core Team 2020). To ensure taxa met a minimum threshold of abundance and occupancy (presence/absence as a percentage of samples) for statistical analysis, we used quality control steps in USEARCH and retained 4,576 of 41,130 and 576 of 8,665 bacterial and fungal taxa, respectively. Of these retained taxa, 91.8% bacterial and 81.3% of fungal OTUs were identified at least to the level of taxonomic order using the SILVA and UNITE databases, respectively. While 28 identified and presumptive bacterial phyla were detected, the majority (~90%) belonged to Proteobacteria, Acidobacteria, Verrucomicrobia, Bacteroidetes, or Actinobacteria phyla (**Figure S1A**). Identified fungal taxa were dominated by Basidiomycota, Mortierellomycota, and Ascoymcota, with these taxa collectively accounting for >90% of OTUs, and all other phyla individually accounting for <5% (**Figure S1B**). While the phylum-level composition of the bacterial community was remarkably similar across sites (generally differing by <5% for any given phylum), there were some noteworthy differences. For example, Cloquet had a greater relative abundance of Bacteroidetes (15.7% to Ely's 12.4%) and Actinobacteria and (8.45% to 5.47%) while Ely had higher relative abundance of Verrucomicrobia (13.9% vs 16%) and Gemmatimonadetes (1.15% vs 2.27%). The fungal community had more dramatic compositional differences (Figure S1B), notably a near-doubling of Mortierellomycota in Cloquet (40.5% abundance) compared to Ely (22%), with most of the difference being made up by increased Basidiomycota and Mucoromycota abundance in Ely, with these phyla advancing to 32.7% (from Cloquet's 24.1%) and 8.67% (from Cloquet's 1.65%), respectively. Ascomycota was a relative constant, with a large presence in both sites, at 33.4% relative abundance in Cloquet, and 36.5% in Ely.

Community Structure and Characteristics

Our first set of results concerned soil bacterial and fungal community structure; this allows us to investigate compositional changes under separate or combined sustained warming and drying stressors, and consider these effects appear to drive divergence between the control community and those subjected to one or both treatments. These methods comprised alpha and beta diversity, concerning sample-specific assemblages of taxa, and the relationships between community structures, respectively.

Alpha diversity

We compared Alpha diversity (measured as Shannon-Weaver index) under Warming and Drying regimens to better understand the relative influence of treatment effects at the community level. Bacterial diversity least square mean (lsm) estimates ranged from 6.36 – 6.63 and 6.44– 6.69 for Ely and Cloquet, respectively. In both sites, the bacterial diversity of CO samples was significantly higher ($p < 0.001$; Wilcoxon signed rank test) than the WD treatment (lsm estimates of 6.63 vs 6.36, and 6.69 vs 6.44 for Ely and Cloquet, respectively). In Cloquet, the bacterial diversity of CO was additionally higher than WO ($p < 0.05$) while there was no significant difference between CO and DO treatment. On the other hand, we observed significant ($p < 0.05$) differences between CO (lsm 6.63) and DO (6.51) in Ely while there was no difference between the bacterial diversity of CO and DO treatments. Fungal diversity least square mean estimates ranged from 2.93 (WD) – 3.49 (WO) and 3.21 (DO) – 3.39 (CO) for Ely and Coquet, respectively. We observed no significant differences in the fungal diversity within different treatments at Cloquet. At Ely, the fungal diversity of CO (3.39) samples was significantly higher than DO (3.21; $p < 0.001$) and WD (3.14; $p < 0.05$).

Beta Diversity

Beta-diversity was analyzed by canonical analysis of principal coordinates (CAP), with Bray-Curtis distance as a response variable, and warming, drying, and the warming-by-drying interaction as fixed effects (Figure 2). In addition, permutational analysis of variance (PERMANOVA) was performed to determine significant differences in the beta diversity patterns between different treatments. For bacterial beta diversity, the first two principal components of CAP Ordination explained between 10.4% and 10.9% of observed variance for Cloquet and Ely, respectively. Both sites also saw a minor overlap between CO and DO treatments (limited to several samples). We observed full separation, as well as a larger Bray-Curtis distance, between these groups and the WO and WD treatments (Figure 2A and B). The PERMANOVA revealed that the bacterial communities in both sites had significant treatment effects for warming (Cloquet $p = 0.002$, Ely $p = 0.001$) and drying (Cloquet $p = 0.007$, Ely $p = 0.006$), with no significant interaction effects. The fungal communities (Figure 2C and D) showed slightly more site dependency; while Ely behaved like its bacterial counterpart with significant warming ($p = 0.001$) and drying ($p = 0.006$) effects, Cloquet had no significant drying treatment effect ($p = 0.093$), instead having a warming effect ($p = 0.022$) and a unique nonlinear warming-by-drying interaction effect ($p = 0.01$). This interactive effect might reflect a buffering response under WD, as the WO treatment showed greater separation from CO than WD, as might be expected with only a warming primary effect

Taxon-Level Treatment Response Characterization

Our next major route of inquiry was to looks beyond aggregate community-level responses, to individual taxon-level responses to treatments, using a range of treatment response indices (correlation between abundance and treatments, direct changes in abundance, and Dufrene-Legendre indicator value scoring) to gain insight into the range of responses observed at this level. We also explored the relationship between the richness, and

abundance, of species as classified by treatment response—this gives context for whether a given response group is more, or less, represented in the community than would be expected from the number of species alone.

Linear Correlations in Relative Abundance by Treatment, and Analysis of Variance

Earlier studies have reported differential responses among individual taxa within microbial communities in response to climate change drivers (Evans and Wallenstein 2014; Drigo et al. 2017). Here we used two parallel methods to evaluate these taxon-level changes; first, we found the overall fraction of taxa showing a response to a given combination of treatment effects using ANOVA on a negative binomial linear model to evaluate the strength of correlation between taxon abundance and treatment factors (Figure 3). We complemented this broad picture of treatment sensitivity with interaction plots of actual (z-scaled) taxon abundance under the CO, DO, WO, and WD treatments to see how general sensitivity translated into negative or positive change under a given exact treatment (Figures 4 and 5).

Roughly half (45% Cloquet, 49.5% Ely) of bacterial OTUs (Figure 3) showed a significant correlation between abundance and one or more of warming, drying, or a non-additive warming-by-drying interaction (shaded groups in heatmaps; designated W--, D--, and –D:W, respectively in Figure 3). Of this broadly-defined “reactive” group (all excluding nonreactive in Figure 3), the majority (71-73% of reactive taxa, or 33-35% of total taxa) saw abundance correlated with a single treatment effect (both warming and drying), or the warming-by-drying interaction alone, with roughly equal proportions of each. A much smaller fraction (<5% combined) saw correlations between abundance and either a single primary treatment and the interaction (*W- D:W, - D D:W*), or—most infrequently—all three effects (*W D W:D*). Response group proportions were similar across sites. Fungal abundance was more frequently tied to treatment effects (Figure 3B, 3D), with over 70% of OTUs showing a significant correlation. Sensitivity to both Warming and Drying was also more common, at 15-20% of all OTUs, as was sensitivity to the

Warming-by-Drying interaction (~16%), with both groups outnumbering OTUs correlated with any single treatment effect (~12%). The relative proportions of fungal OTUs in these groups showed more variation by site than was the case for bacteria, with shifts of up to ~5% relative richness (Figure 3; e.g. group *W – W:D*). As with bacteria, taxa that significantly correlated with a single primary effect with an interaction, or with all three factors, were rare (<5% of OTUs). Studying the share of OTUs accounted for by each treatment response group gives some sense of their relative contributions to the community, but considering agreement between the number of taxa in each category and their abundance gives further context into how taxonomic richness translates into community structure in this system.

Relative Abundance and Richness of ANOVA-based Response Groups

Interestingly, ANOVA response groups showing high taxon richness had a proportional level of relative abundance in the community, indicating that by this metric, there were few cases of outsize presence by a given response group (Table S2). This was measured by comparing the share of OTUs within each ANOVA response group (*W D W:D* through nonreactive) and its corresponding total relative abundance, we found that this ratio was largely similar in bacterial taxa. By contrast, relative abundances of fungal response groups differed markedly both across sites and from relative richness (Table S3). This difference becomes apparent even when considering the ratio of reactive to non-reactive taxa, with this group having a relative abundance of 35.4% in Cloquet, and 39.4% in Ely, compared to relative richness of 25.3% and 23.1%, respectively. Moreover, differences in proportional abundance from richness within the reactive groups of fungi are not merely proportional “compression,” but show noteworthy increases as well as decreases. Across both sites, there was a similar abundance increase for the primary Drying-correlated group, and underrepresentation of the Warming-correlated group; this, in addition to Ely’s higher presence of taxa reactive to both Warming and Drying, could help address Ely’s Drying sensitivity at the community level, rather than Cloquet’s

warming response. While it did not appear to influence community diversity, the high abundance of taxa sensitive to Warming and Drying at Cloquet is consistent with the significant interaction effect detected in CAP ordination. There were also some appreciable site-dependent shifts; for instance, the Warming-by-Drying interaction correlation group was somewhat overrepresented in Cloquet, with 20.4% relative abundance from 15.5% richness, but shrank dramatically in Ely, with 3.8% relative abundance from 12.8% richness; along similar lines, the group reactive to both Warming and Drying had an appreciable richness in each site (18.9% in Cloquet, 19.1% in Ely), but showed site dependence in abundance, with just 4.2% relative abundance in Cloquet to Ely's 10.4%.

Taxonomic Composition of ANOVA-based Response Groups

We were also interested in the taxonomic makeup of negative binomial model correlation-based response groups, a question we explored using the hypergeometric test for over-representation (enrichment) or under-representation (depletion) (Figure 3, Table S4). The bacterial community saw a large number of significant phylum-level enrichments and depletions within any ANOVA significance group (15 with $p < 0.05$ in Cloquet, and 22 in Ely), but very little in the way of overlap between sites. Shared enrichment was limited to an overrepresentation of Actinobacteria (Cloquet $p = 0.030$, Ely $p = 0.001$) and Planctomycetes (Cloquet $p = 0.028$, Ely $p = 0.005$) in the group correlated with both warming and drying. Interestingly, some phyla had near-opposing trends—e.g. in Ely, Bacteroidetes was enriched ($p > 0.001$) in the group correlated to both primary treatment effects and the interaction, while in Cloquet, it was depleted ($p = 0.001$) for the group correlated to both primary effect. Fungal communities only showed significant over- or underrepresentation in phylum Basidiomycota, which was enriched in Cloquet's all-responsive group (*W D W:D;* $p < 0.05$) and depleted in Ely's non-responsive group ($p = 0.024$) respectively (Table S4). On the whole, the bacterial community saw more pronounced differences in taxonomic enrichment by site than did the fungal community, giving

something of a counterpoint to the more-dramatic disparity in alpha and beta responses in fungi, suggesting the limitations of extrapolating purely from the sum of individual OTU responses; indeed, the microbiome is not merely the sum of its constituent OTUs, but an emergent system arising from associations and interactions between these taxa. To this end, we next used co-occurrence networks to identify groups of taxa with strong positive interactions consistent across treatments and collection dates, as well as indicator value scoring to quantify the association between taxon abundance and Warming and Drying treatment factor levels.

Taxon Response Classification by Z-Scaled Abundance

In addition to categorizing by correlation significance, OTUs also showed a wide range of more granular patterns based on z-scaled abundance under each treatment condition (Figure 4), with the 26 possible outcomes of our scaled-change classification system containing at least 0.01% of total taxa, although most taxa were centralized in a smaller subset of response patterns. The distribution of these interaction groups was quite even between sites, with ≤3% difference in relative proportions of any given group. In Bacteria, the most common interaction groups (*b* and *c* at a combined 19% of OTUs in Cloquet) saw an increase in relative abundance with Drying, and decrease with Warming (Figure 4). The next most-common trend, and largest single interaction group (group *a*) saw the inverse pattern, with a relative increase under Drying, and decrease with Warming. In fungal taxa (Figure 5), the largest interaction response group (a; 22% in Cloquet) showed a relative decrease for all non-control treatments, followed by groups (*b, c, d*) showing increased abundance under to a single treatment (WD, WO, and DO, in order of OTU count), with 9-13% abundance in each case depending on site. These results indicate a wide range of responses to warming or drying effects, although with some predominant trends by taxonomic kingdom which largely persisted across sites.

In addition to categorizing by correlation significance, OTUs also showed a wide range of patterns based on z-scaled abundance under each treatment condition, with wide distribution

across the 26 possible outcomes for both bacteria and fungi (Figures 4, 5). The distribution of these interaction groups was quite even between sites, with ≤3% difference in relative proportions of any given group. The most common interaction groups among bacteria (*b*, *c,* and *e*—at a combined 25% of OTUs in Cloquet) were Drying opportunists, which saw an increase in relative abundance with DO and decrease with WO, and variable responses under WD (Figure 4). The next most-common trend interaction group (groups *a, k, o* at a combined 19% in Cloquet) were saw a relative decrease under DO and increase under WO, again with varied WD responses. In fungal taxa (Figure 5), the largest interaction response group (a; 22% in Cloquet) showed a relative decrease in all drying and warming treatments. This was followed by several groups of opportunist fungal taxa (*b, c, d*) that showed increase in abundance under to a single stress treatment (WD, WO, and DO, in order of OTU count). These results indicate a wide range of responses to warming or drying effects, although with some predominant trends by taxonomic kingdom that largely persisted between sites. Indeed, the microbiome is not merely the sum of its constituent OTUs, but an emergent system arising from associations and interactions between these taxa. To this end, we next used co-occurrence networks to identify groups of taxa with strong positive interactions consistent across treatments and collection dates, as well as indicator value scoring to quantify the association between taxon abundance and Warming and Drying treatment factor levels.

Co-occurrence Networks Reveal Clusters of Stable, Sensitive, and Opportunistic Microbial Taxa

While the interaction plots provide a description of individual OTU reactions across the experimental treatments, there is still a considerable level of intermediate variation among the OTUs that cannot be described via either alpha/beta diversity or OTU-level GLMs. Thus, we performed a correlational network analysis to identify and describe groups or clusters of OTUs that respond similarly to each other across the warming and drying treatments (Fig. 3). Using the non-parametric Spearman's Rank-Order coefficient (ρ) between pairs of OTUs and a

conservative, positive edge threshold of 0.65 for bacteria (0.60 for fungi), we found 224 bacterial taxa meeting these parameters in Cloquet, and 227 in Ely, with substantially smaller detected fungal assemblies of 99 and 42 taxa, respectively. We identified 3 large (>10-member) bacterial subnetworks in Cloquet, and 4 in Ely, along with many smaller networks, the largest being two subnetworks of n=8 in Cloquet, and one of n=5 in Ely (Figure 7). Fungal networks, even at a relaxed correlation threshold (Spearman's $\rho > 0.6$) were considerably smaller and fewer in number; we identified only 2 large ($n \geq 10$) subnetworks (C.1, n = 19, C.2, n = 13), and one 8-member subnetwork (C.3), out of the 24 in Cloquet soils, and one 9-member subnetwork (E.1) out of the 15 in Ely soils; in most cases, subnetworks had 2-3 members (Figure 7). From this basis, we moved to characterize the notable subnetworks (focusing principally on the bacterial networks on account of size) in terms of Dufrene-Legrende indicator values and taxonomic composition.

Network Integration of Dufrene-Legrende Indicator Values and Enrichment

To characterize the warming or drying response of network taxa, we used the Dufrene-Legrende Indicator Value (*D*) across all taxa, the majority were non-significant, or "Stable", with respect to warming and/or drying stressors, to a greater degree than observed with GLM-based metrics (Figure 6A). In addition, both bacteria and fungi had larger proportions of sensitive than opportunistic indicators (see Supplemental Section 2 for full details). This method allowed us to qualitatively identify a variety of sub-networks in both Cloquet and Ely.

Spearman Subnetworks and Enrichment for Indicator Taxa

From this foundation, we were able to enumerate and characterize the major subnetworks observable in our Spearman networks, as well as to empirically measure over- or under-representation (enrichment or depletion, respectively) of these traits using the hypergeometric distribution. The largest subnetwork at Cloquet (C.1; n=70) and second-largest

at Ely (E.2;n=37) primarily consisted of taxa commonly found among all treatments and are not representative of any specific experimental condition. Ely contained an additional subnetwork (E.5, n = 22) enriched only for drying-resistant taxa (p = 0.33) This proportion of taxa in these subnetworks likely represent the stable core soil microbiome that is tolerant to warming and drying perturbations. Both sites also contained a large subnetwork (C.2, n = 32; E.4, n = 22) not significantly enriched for any indicator value group, suggesting proportional representation of reactive and nonreactive taxa. This suggests that sizeable portions of the taxa showing strong correlations in relative abundance across the B4WarmED experimental system showed no significant indicator value.

Warming- or Drying-Opportunistic Subnetworks

Additionally, both Cloquet and Ely contained a large subnetwork of closely-correlated taxa which are 'indicators' of either warming, drying, or both. For example, Cloquet's subnetwork (C.3, n = 29) was enriched for warming opportunists ($p < 0.001$), and depleted for warming- and drying- resistant taxa (p = 0.045), while Ely's subnetwork (E.1, n = 39) was more specifically drying-opportunistic (enrichment $p < 0.001$), being somewhat surprisingly enriched (p = 0 .024) for warming-resistant taxa. This suggests that these subnetworks represent opportunistic microbial taxa that can take advantage of newly available niches opening due to the environmental treatment. The differential enrichment of warming-opportunistic taxa in Cloquet, and drying-opportunistic taxa in Ely, may additionally be a reflection of, or partial mechanism for, the corresponding pattern observed in warming- or drying- sensitivity in alpha diversity.

Warming- or Drying-Sensitive Subnetworks

Lastly, both sites showed subnetworks that seem to represent individual taxa that are indicators of the control/unstressed treatment—i.e. occur less frequently in warmed, or dried,

conditions. This Cloquet network fell below the sample threshold for a formal hypergeometric enrichment test, (C.5, n = 8) but was comprised entirely of warming-sensitive indicators, while Ely's sensitive network (E.3, n = 30) was significantly enriched for warming- ($p < 0.001$) and drying-sensitive ($p = 0.005$) taxa. This suggests that these are taxa sensitive or vulnerable to one or both of warming and drying perturbations. Interestingly, the size of this sensitive Ely subnetworks is considerably larger than the analogous network in Cloquet, potentially suggesting Ely soils are more vulnerable to microbial species loss than Cloquet. The remaining 35 subnetworks with >10 members demonstrated a variety of different patterns that are difficult to parse. However, this clearly demonstrates that while many microbes will be resistant to climate-change related abiotic stresses, other subnetworks of taxa will be diminished, or promoted, under warming and drying stressors in the boreal forest microbiome.

Summary of Indicator Values in Spearman Subnetworks

The differential enrichment of warming-opportunistic taxa in Cloquet, and drying-opportunistic taxa in Ely, may additionally reflect, or partial mechanism for, the corresponding pattern observed in warming- or drying- sensitivity in alpha diversity. Lastly, both sites showed subnetworks that seem to represent individual taxa that are indicators of the control/unstressed treatment; the Cloquet network fell below the sample threshold for a formal hypergeometric enrichment test, (C.5, n = 8) but was comprised entirely of warming-sensitive indicators, while Ely's sensitive network (E.3, n = 30) was significantly enriched for warming- ($p < 0.001$) and drying-sensitive ($p = 0.005$) taxa. This suggests that these are taxa sensitive or vulnerable to one or both of warming and drying perturbations. Interestingly, the size of this sensitive Ely subnetworks is considerably larger than the analogous network in Cloquet, potentially suggesting Ely soils are more vulnerable to microbial species loss than Cloquet. The remaining subnetworks >10 members demonstrated a variety of different patterns that are difficult to parse qualitatively. However, this clearly demonstrates that while many microbes will be resistant to

climate-change related abiotic stresses, other subnetworks of taxa will be diminished, or promoted, under warming and drying stressors in the boreal forest microbiome.

Taxonomic Characteristics of Bacterial Spearman Subnetworks

Interestingly, these similarly responding taxa respond in a common phylogenic pattern (Figure 6B). As an alternative to labeling sub-networks based on their indicator value, we can also assess subnetworks based on their general phylogenic membership (Fig. 3.2A, 3.2B). In both Cloquet and Ely, the large stable subnetworks (C.1 and E.2) were enriched for Sphingobacteria (Cloquet $p < 0.001$ and Ely $p < 0.001$) and Betaproteobacteria (Cloquet $p=0.027$ and Ely $p < 0.001$). Alternatively, opportunistic subnetworks were primarily enriched for Acidobacteria (C.3, $p < 0.001$; E.1, $p < 0.001$)—class Acidobacteriia in C.1 and E.2—and Verrucomicrobia (C.3, $p < 0.001$; E.1, $p < 0.001$), as well as Gemmatimondetes in Ely ($p = 0.0036$). Lastly, large subnetworks containing microbial taxa sensitive to either warming or drying showed enrichment for Verrucomicrobia (C.5, $p < 0.001$; E.3, $p > 0.001$). There were even some commonalities among mixed-response subnetworks with no significant divergence from background taxonomic distribution; for instance, Subnetworks C.2 and E.3, which had no significant over- or underrepresentation of stress responses, were enriched for Alphaproteobacteria ($p < 0.001$ at both sites), with site differences including enriched Thermoleophilia ($p = 0.007$) at C.2, and Betaproteobacteria, ($p = 0.035$), Bacteroidetes ($p < 0.001$, among other taxa at E.3. These results demonstrate fairly consistent taxonomic associations relating to climate stress response between sites, suggesting a high degree of continuity in community organization dynamics for bacteria. Fungal networks were considerably sparser (Figure 7), which is likely a function of fewer OTUs found as well as the high presence/absence nature of ITS marker detection (Figure 7). Even with a more relaxed edge threshold (Spearman's $\rho > 0.6$), we only identified 2 large ($n \geq 10$) subnetworks (C.1, $n = 19$, C.2,

n = 13), and one 8-member subnetwork (C.3), out of the 24 in Cloquet soils, and one 9-member subnetwork (E.1) out of the 15 in Ely soils; in most cases, subnetworks had 2-3 members.

Taxonomic and Indicator Characteristics of Fungal Spearman Subnetworks

At the whole-network level in Cloquet, fungal taxa were almost exclusively resistant with respect to drying (with 6 drying-sensitive taxa, and 2 drying-opportunistic), while roughly a fifth of taxa had a warming response (16 warming opportunistic, 3 sensitive). Ely was even more dramatic, with 2 of 44 taxa showing a warming response (both opportunistic), and 7 a drying response (3 opportunistic, 4 sensitive). At Cloquet, the two largest subnetworks (C.1 and C.2) were almost exclusively Resistant with respect to the water and warming treatments with few exceptions (Fig. 8A). The next-largest, 8-member subnetwork (C.3) had mixed membership with respect to the stress treatments consisting of 2 warming opportunists and 6 drying opportunists. Lastly, the next largest 6-member network (C.4) demonstrated a cluster of strong warming opportunist taxa, with two smaller networks (n = 6 and n = 4) following suit. On the whole, these results paint a picture of fewer correlated fungal taxa with Cloquet containing larger clusters of similar functioning taxa.

Fungal taxa membership also clustered within a subnetwork similar to bacterial taxa. Of the 99 OTUs in Cloquet's correlation network, the most-represented phyla were Ascomycota (n = 58)—especially Sordariomycetes (n = 20) and Leotiomycetes (n = 18)—and Basidiomycota (n = 27), largely of class Agaricomycetes (n = 18). Similarly, Ascomycota (n = 18) and Basidiomycota (n = 14) were the largest phyla among the Ely network's 42 OTUs; classes Leotiomycetes (n = 6), Sordariomycetes (n = 5) and Agaricomycetes (n = 9) were again relatively well-represented. Likewise, none of Cloquet's >10-member subnetworks showed significant enrichment or depletion of taxa relative to the network. Ely's largest subnetwork (E.1, n=9) was primarily comprised of 7 Ascomycota OTUs (thee of class Leotiomycetes) and two of the network's three OTUs of Mortierellinycetes. The considerably higher resistance of highly-

connected fungal taxa to warming and drying stressors therefore gives context for the generally weaker effect of warming and drying stressors on community alpha- and beta-diversity characteristics. Given the broadly non-reactive nature of these taxa, the dramatic difference in network sizes at the same significant and ρ threshold between sites (99 OTUs in Cloquet to Ely's 44) may additionally give context for Cloquet's greater resistance to treatment effects in terms of alpha and beta diversity.

Discussion

Warming and Drying Demonstrate Site Dependent Changes in Alpha and Beta Diversity

Our investigation of warming and drying effects on community structure revealed significant effects, albeit with some site-specific differences. Both warming and drying appear to significantly affect microbial community membership and diversity. We observed that bacterial diversity and community structure changed significantly in the combined warming and drying treatment as compared to control, although without a statistically significant interaction between the primary treatments. Interestingly, we didn't observe a strong effect of drought only treatment on both the diversity and community composition of soil bacterial community at both the sites. Only a few *in situ* studies have explored the interactive effects of warming and drying on soil microbial communities (Sheik et al 2012; Zhang et al. 2016). By providing context in a wetter (and in one case cooler) climate (Sheik et al 2012 performed their study in central Oklahoma grassland, while Zhang et al collected soil samples from a range of localities surrounding Yining city, Xinjiang, China), our results complement these studies, which reported that decreased precipitation alone had a slight impactwhile a combination of warming and altered precipitation significantly alters the structure of soil bacterial community (Sheik et al 2012; Zhang et al. 2016). The compatibility of these findings with our system may be debatable (in that small differences in soil moisture in a wetter system are less likely to induce physiological stress than in the above arid or semi-arid conditions)m but slight reduction in bacterial alpha diversity, and restructuring

of the community structure, under sustained warming is also consistent with patterns observed in warming studies performed under much more similar conditions (northern temperate or boreal ecotone forests), including a 2020 soil microbiome investigation also performed in the B4WarmED study system (De Angelis et al 2015; Van Nuland et al. 2020). Although we observed significant differences in the bacterial community structure of warming, drought, and warming + drought combined treatment, the absence of a significant Warming-by-Drying interaction in either site does not provide evidence for a noteworthy nonlinear interaction between the primary treatments (Figure 1).

Fungal alpha diversity showed greater site dependence than bacteria, with no significant responses at Cloquet, while Ely mirrored the bacterial treatment response, with a significant reduction in alpha diversity under DO and WD treatments; There was also no significant ANOVA effects for alpha diversity in Cloquet's fungal community, while Ely saw a primary Warming response. Similarly, beta diversity had pronounced site dependency, with the Ely fungal community again reflecting (albeit with smaller proportional Bray-Curtis distances) its bacterial counterpart in having significant primary warming and drought responses, and the Cloquet community showed only a nonlinear warming-by-drying interaction—the fact that the warming treatment showed a greater distance from the control condition than combined suggests this interactive effect may counteract warming trends when combined with drying. Zhang et al. (2016) reported no significant effect of warming and drought on soil fungal communities in an alpine grassland ecosystem. While de Vries et al. (2018) observed increased fungal alpha diversity under acute drought, this quickly returned to the control baseline after the treatment ended, suggesting that the relative warming- or drought-insensitivity observed in our system may reflect a stable adaption, rather than a short-term community response of the type observed under the intermediate disturbance hypothesis (van der Voort 2016). This somewhat reflects patterns observed during a natural drought event at a prairie warming experiment

(Acosta-Martinez 2014) where previous warming somewhat mitigated community restructuring under drought by priming a community adapted to both stressors.

Since fungal communities are important contributor to soil carbon dynamics in boreal forests (Fernandez at al 2017, Clemmensen et al 2013), the site dependency in the B4WarmED system warrants some attention, with potential abiotic and biotic factors. On the abiotic front, Ely is drier than Cloquet both historically and during the collection period (ambient treatment 2017 average of 0.184 ± 0.030 cm^3 H_2O cm^{-3} soil to Cloquet's 0.223 ± 0.045l). While Cloquet's mean annual temperature is warmer than Ely's, this is not constant over the year, with Ely being warmer from April through October by a difference of, on average, 0.40°C – 1.37°C, while warmer Cloquet temperatures during late Fall and Winter account for the average difference. The fact that Ely undergoes a wider temperature difference over the course of a year may go some way towards explaining the weaker alpha diversity effect under warming, although the range experiences at either site considerably exceeds the 3.4°C difference studied in this investigation..

It was somewhat surprising to see Cloquet exhibit a non-significant drying response, given that soil moisture tends to be higher than that seen in (Fig. S2); in this case, the fact that warming reduced Cloquet's soil moisture to a greater extent than the direct drying treatment may provide a partial explanation, with warming, as well as the control, effectively contributing to combined warming and drying stress. This would be consistent with a study of energy flux in B4WarmED soil food networks (Schwarz et al. 2017). In contrast to higher trophic levels, which saw increased metabolic activity under warming at ambient precipitation, warming-induced drying at the soil surface was enough to dampen this response in the microbial component of the food network (broadly defined by respiration measurements). The larger variation in taxonomic composition of the fungal communities (notably Cloquet's greater abundance of Mortierellomycota, and Ely's corresponding increases in Basidiomycota and Mucoromycota)

could explain some of this variation, although literature and our metrics for temperature and drought sensitivity point to a relative functional equivalence of these groups.

OTU-level Treatment Effect Correlations

The soil microbial community is highly diverse where members differ widely in their physiological traits and dispersal ability. Therefore, it is unlikely that the entire microbial community will respond in a similar fashion with climate change (Evans and Wallenstein 2014; Classen et al. 2015; Drigo et al. 2017). In line with this, our analysis (using both ANOVA-based significance of linear correlations and relative abundance-based approaches) on the responses of individual OTUs to warming or drying effects (singly or in conjunction) revealed a wide range of response traits, with similar percentages of response groups between sites. A substantial percentage of taxa showed a significant correlation between abundance and at least one treatment effect (~50% of bacteria, ~70% of fungi), but the relative contributions of warming and drying sensitivity varied between bacteria and fungi. Meinser et al. (2018) have reported greater abundance changes in fungi (25%) as compared to bacteria (8%) in response to drought in sandy Rhine River Delta soil. Bacteria showed a near-total separation between treatment response groups, with <10% of taxa showing a response to both primary treatments (with or without the interaction term). However, e.g. the total portion of bacteria correlated to Warming (disregarding Water effects) (21.8% in Cloquet, 26.5% in Ely) was quite close to the ~20% fraction of soil bacteria with a consistent warming response observed by a global survey of soil carbon (Oliverio et al 2012). Fungi showed more overlap in treatment effect response, with roughly a quarter of taxa showing correlations to both primary treatment effects (either alone or with an interaction effect).

The higher overlap between warming and drying correlation in fungi might be taken to suggest that physiological and habitat traits (e.g. soil niche selection) influencing drought tolerance in fungi are more likely to overlap with warming tolerance than is the case for bacteria.

This is somewhat in contrast to findings by e.g. Sheik et al (2012) pointing to a convergence in community structure between bacterial communities under warming, and combined warming and drought, pressures. Alternatively, it is possible that fungal responses to unfavorable temperature and soil moisture (e.g. endospores, niche selection, cell walls) are more generically applicable to both conditions than bacterial mechanisms, which could include active physiological tolerance (e.g. high cell water content, thick cell walls, niche selection), dormant life stages, or rapid reactivation and reproduction under favorable conditions (Schimel 2020; van der Voort 2016). Similarly, the extent of taxonomic richness agreement for each correlation response group between sites points to a similar underlying “palette” of possible responses in both communities.

Overall, we observed that the response groups are spread across different phyla (Fig 3). It is possible climate change exerts a legacy effect on the soil microbial community through the selective enrichment/depletion of response groups with broad phylogenetic origins, with possible long-term consequences on ecosystem functioning (Meisner et al. 2018). Microbial groups belonging to phylum Actinobacteria and Planctomycetes showed consistent enrichment with warming, drying and combined warming and drying treatment. Members of both phyla are reported to respond positively to a range of climate change stressors (Sheik et al. 2012; Drigo et al. 2017; Meisner et al. 2018; Ochoa-Hueso et al. 2018). Interestingly, in lines with other studies (Evans and Wallenstein 2014; Meisner et al. 2018) we observed variable effect of climate change stressors on the response of bacterial phylum Bacteroidetes. This suggests that the adaptation traits of members of Bacteroidetes might have evolved independently leading to the differential selection in their ability to respond to climate change stressors.

While ANOVA-based approach of classifying taxa based on linear correlations with treatment effects did not distinguish between positive and negative coefficients, our more-granular categorization of taxa based on relative abundance in each treatment group showed a similar degree of accord between sites, suggesting that (albeit with only two sites), some degree

of large-scale similarity in community response might be expected in the wider boreal ecotone region—this would be good grounds for future exploration. Bacterial interaction groups had similar richness between sites in all cases, with the largest single disparity being group *b*, which showed increased abundance under Warming, and decreased abundance under Drying—this accounted for 9% of richness and Cloquet, but 12% of richness at Ely. Fungal taxa showed a somewhat higher degree of variation where most responsive fungal taxa showed a decrease in relative abundance in response to either treatment, but several fungal groups could take advantage of a single treatment. For bacteria, the largest fraction of taxa, with variable behavior under the Combined treatment (*b* and *c* at a cumulative 20% in Cloquet, 16% in Ely) see an increase under Drying, and a decrease under Warming, followed by the reverse trend, plus decreased abundance under the Combined treatment (*a*, at 14% in each site). These trends suggests that, especially in bacteria, the number of species showing a given climate driver response pattern correspond fairly closely to relative abundance (e.g. bacterial species which opportunistically increasing under warming are not disproportionally rare or abundant in the community); by contrast, temperature- or drying-responsive fungal species did tend to see lower relative abundance in the community, on average.

The extent of similarity in the proportion of response groups between sites, as well as the large presence treatment-reactive taxa, might appear to contradict the site-dependency seen in both bacterial and fungal community responses, as well as the generally lower sensitivity of fungi to treatment effects (especially at Cloquet). However, the taxonomic richness of a group (taxonomic or functional) does not necessarily predict either abundance or relative influence on community structure (Lupatini et al. 2014). We will address network topology in the next section, but comparing measured abundance to response group richness gives some context for this apparent incongruity. Bacterial relative abundance hewed fairly close to taxonomic richness, as might be expected given the broadly similar alpha and especially beta diversity responses, while fungi differed markedly both across sites and between richness and

relative abundance. Both trends were evident at the level of reactive or non-reactive taxa, with both sites' relative abundance of non-reactive taxa exceeding relative taxonomic richness, and Ely almost doubling from 23.1% of OTUs to 39.4% relative abundance, compared to Cloquet's 35.4% relative abundance and 25.3% richness. Within the reactive fraction, one potentially explanatory site difference is the considerably higher abundance of taxa correlated to the warming-by-drying interaction in Ely than in Cloquet (10.4% to 3.9%, respectively), which would agree with Cloquet's significant warming-by-drying interaction effect on beta diversity. In addition to baseline taxonomic differences, this disparity in detected abundances could help explain the site-dependent community responses seen in fungi, as well as the diminished overall sensitivity to treatment effect.

Co-Occurrence Subnetworks Suggests Anaerobic to Aerobic Transition

Community structure and function is generally accepted to be shaped and driven by keystone taxa, which exert an outsize influence for their abundance, with even low-abundance taxa showing dense interconnectivity in association networks (van der Heijden and Hartmann 2016; Banerjee et al. 2018). While composite metrics of community diversity and GLMs of individual taxa provide estimations of how soil microbial communities are acting at the macro- and micro-scale respectively, co-occurrence network analysis provides an intermediate view where we can find groups, or sub-networks, of taxa that are responding similarly across the warming and drying treatments. Some discretion is needed in interpreting results, however; while co-occurrence metrics are useful for evaluating similar patterns in community dynamics, they are unable to strictly distinguish true cooperative relationships from merely similar responses to environmental stimuli. Nonetheless, inter-taxon correlations can point to functional attributes and adaptation strategies of microbial communities in their ecosystems (Barberán et al. 2013). Many of the subnetworks are highly enriched in particular phylogenic classes, but the treatment indicator value (*D*) of each OTU indicates that many of these subnetworks of

phylogenetically-related taxa also respond similarly across the treatments. For instance, networks C.1 and E.2 were enriched for bacteria in the class Sphingobacteria (Bacteroidetes) and □-proteobacteria and are largely stable in response to warming and drying levels in the scope of projected 21st-century climate change effects. These taxa likely make up a stable core of the boreal forest soil microbiome and may not suffer drastically from climate change induced warming or drying in the short-term. However, subnetworks sensitive to warming/drying pressure (C.5, E.3) showed common enrichment for Verrucomicrobia, along with Bacteroidetes of the class Saprospirae (Fig. 5; Table S4). This suggests that these anaerobic eubacterial taxa either have a relatively narrow abiotic stress tolerance or at least suffer from the loss of a competitive advantage in slightly drier soils. In contrast, bacteria of the putative Verrucomycrobia of the candidate class Spartobacteria and members of the class Solibacteres (Acidobacteria) appear to take advantage of the drying and/or warming treatments and increase their relative abundance upon warming or drying (Fig. 7; Table S4). As many members of these eubacteria classes are largely characterized as aerobes, this may suggest a shift whereby members of subnetworks C.3 and E.1 enjoy a relative advantage in formerly more water-saturated niches initially occupied by subnetwork C.5 and E.3, resulting in taxa diminished competitive advantage for species with anaerobic traits. A possible explanation for this shift from anaerobic to aerobic respiration is the reduction in soil moisture content for both the drying and warming treatments (Fig. S3) since excess soil moisture often fills microscopic soil cavities, limits soil oxygen flow, and can reduce local soil respiration regardless of available carbon (Hillel 2003). While these trends appear interesting, a more detailed study of the intraspecific variation of these sensitive and opportunistic taxa under a variety of soil conditions is necessary to fully explore these potential trends and to explore the potential soil functions lost/gained during warming/drought.

Conclusion

The boreal ecotone stands to be altered significantly by projected regional shifts towards a warmer, drier climate, with potentially deleterious effects including reduced carbon storage capacity. The soil microbiomeplays an important mediating role in this relationship between a warming, drying, local climate and altered carbon storage capacity, among other ecosystem features. Warming and drying are, individually and in conjunction, known to exert influence such as reduced community diversity and shifting community structure, but the interactive effects of the two in conjunction is less studied. Our investigation of boreal ecotone microbial communities in an experimental system featuring a blocked factorial experiment for warming and drying effects suggests that these stressors appear to combine additively, rather than exhibiting major nonlinear interactions. Changes associated with primary warming and drying, as well as the combination, were associated with turnover in microbial communities, with a considerable portion of taxa showing a significant positive or negative correlation between abundance and at least one treatment effect. Bacterial communities also exhibited multiple spatially and temporally persistent assemblies of co-occurring organisms with common taxonomic makeup and treatment response patterns. While some groups (dominated by *Betaporteobacteria* and *Sphingobacteria*) showed no significant treatment response, and will likely provide a stable core community under near-term climate changes, other groups appear to be vulnerable to, or benefit from, these climate change agents, pointing toward turnover in key community assemblies, rather than purely stochastic variation. These results provide additional context for the interactive effects of warming and drought on boreal ecotone forest floor microbial community networks, as well as gives a better sense of how such system may change in the future.

Figures

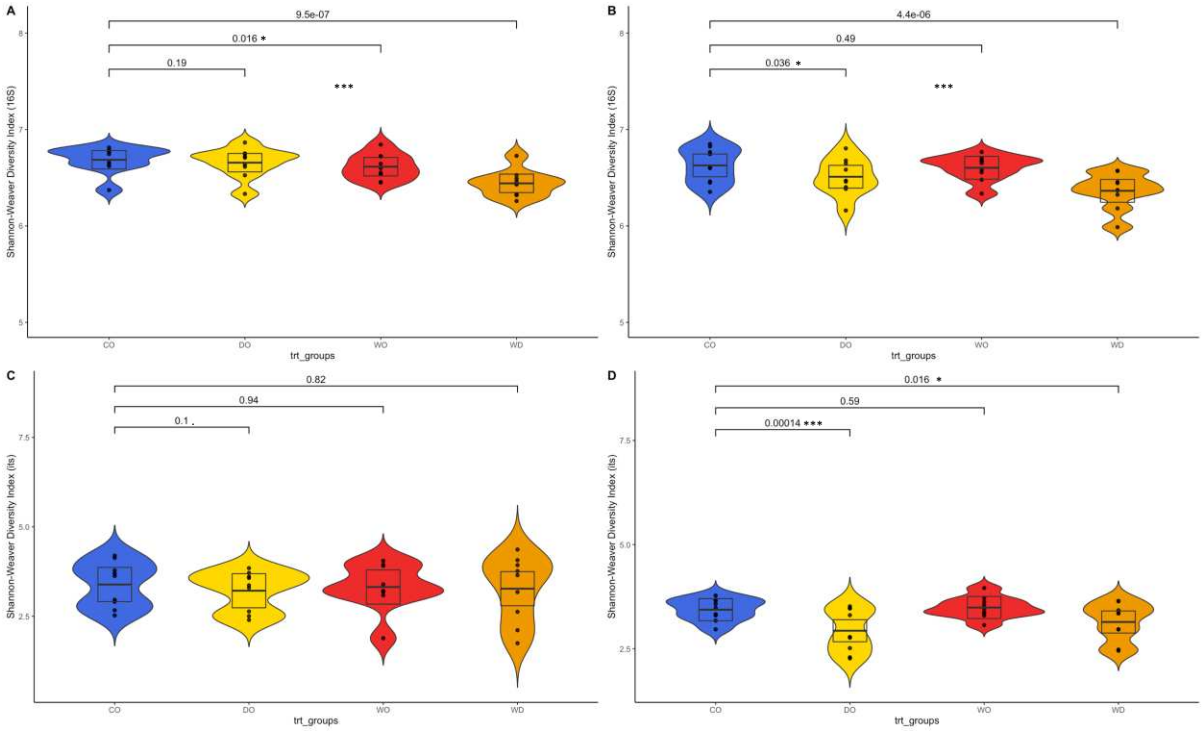

Figure 1. Distribution of Shannon-Weaver Diversity communities (rarefied) across control (CO, blue), drying (DO, yellow), warming (WO, red), and combined warming and drought (WD, orange) treatments for Cloquet's bacterial community (A), Ely's bacterial community (B), Cloquet's fungal community (C), and Ely's fungal community (D). Violin plots represent the distribution of sample community diversity based on average abundance by treatment. The overlaid box plot represents the Least Square Means (LSM) estimate of the same data, corrected for monthly variance using the formula (<Shannon diversity> ~ warming + drying + warming: drying r); the center line shows the direct estimate, while upper and lower bars represent the upper and lower boundaries of the 95% confidence interval. Inset tables show ANOVA p values across sites, collection dates, and experimental replicates (n = 9 for each treatment). Comparison bars above violin plots show difference (Wilcoxon signed-rank test) from Control condition at each site/community type, with values summarized with "***" for p ≤ 0.001, "**" for p ≤ 0.01, "*" for p ≤ 0.05, "." For p ≤ 0.1, and " " for p > 0.1.

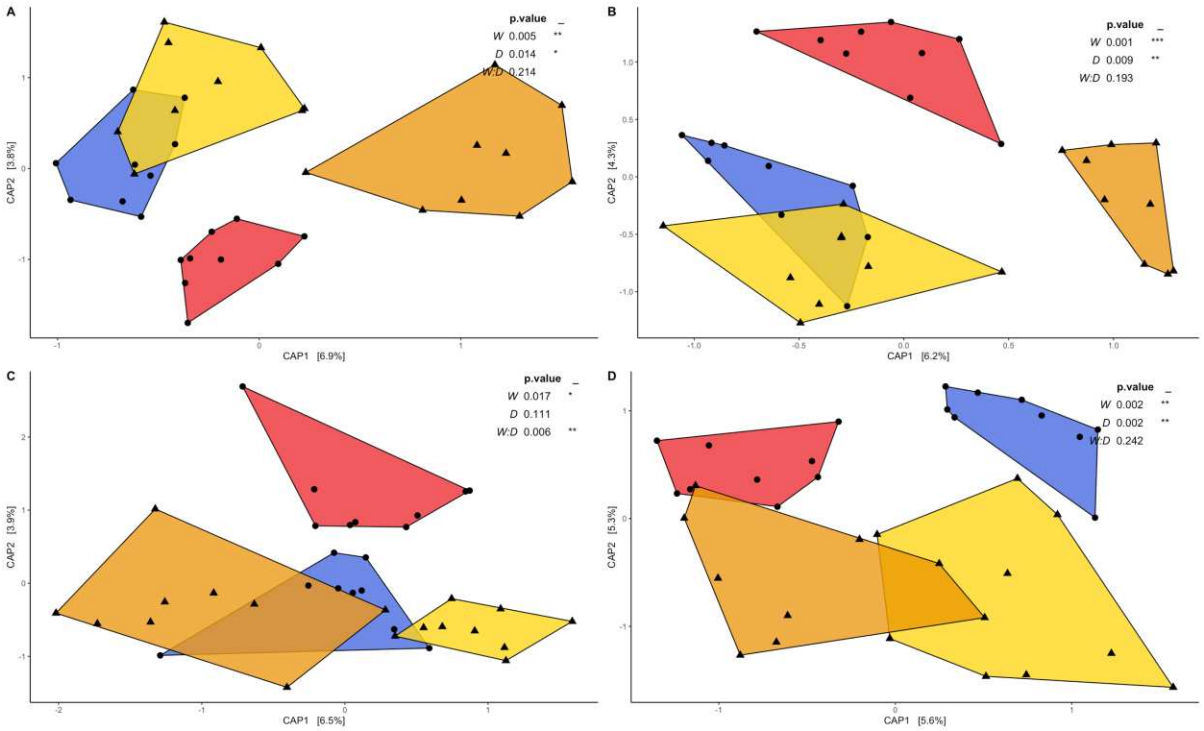

Figure 2: Canonical Analysis of Principal coordinates (CAP) ordination of Bray-Curtis similarities across control (blue), drying (yellow), warming (red), and combined warming and drought (orange) treatments**.** The formula used was (Bray Curtis Distance ~ Warming + Drying + Warming:Drying). Polygons represent contiguous regions of same-treatment communities in the coordinate space of the two primary principal coordinates, which account for between 9.4% (Ely ITS) and 10.9% (Cloquet ITS) of observed variance. Inset tables show PERMOANOVA significance of treatment effects based on Bray-Curtis distances.

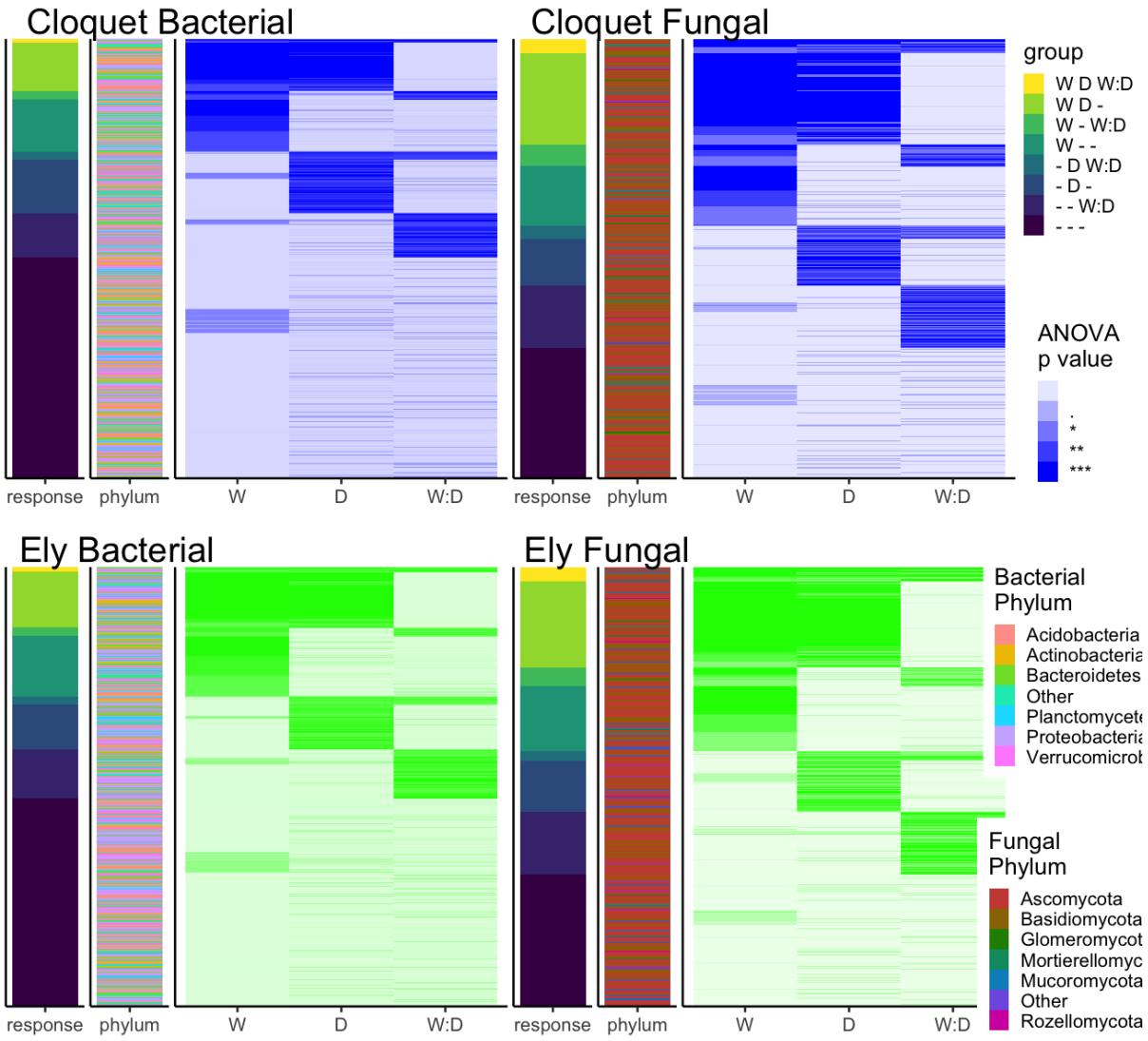

Figure 3: Heatmaps by site and community type representing ANOVA p values associated with the fixed-effect linear model [Abundance ~ Warming + Drying + Warming:Drying], abbreviated W + D + W:D above. The outermost explanatory column represents groups of OTUs significant for shared patterns of terms [e.g. Group 'W D W:D' is significant for Warming (W), Drying (D), and the Warming-by-Drying (W:D) interaction), followed by a column representing membership in major bacterial (A, B) or fungal (C, D) phyla. The remaining three columns represent the significance of treatment or interaction effects for each OTU in the Cloquet (A, C) or Ely (B, D) site. Significance levels are, as in figure 1, summarized with the levels "***" for $p \leq 0.001$, "**" for $p \leq 0.01$, "*" for $p \leq 0.05$, "." For $p \leq 0.1$, and "NS" for $p > 0.1$.

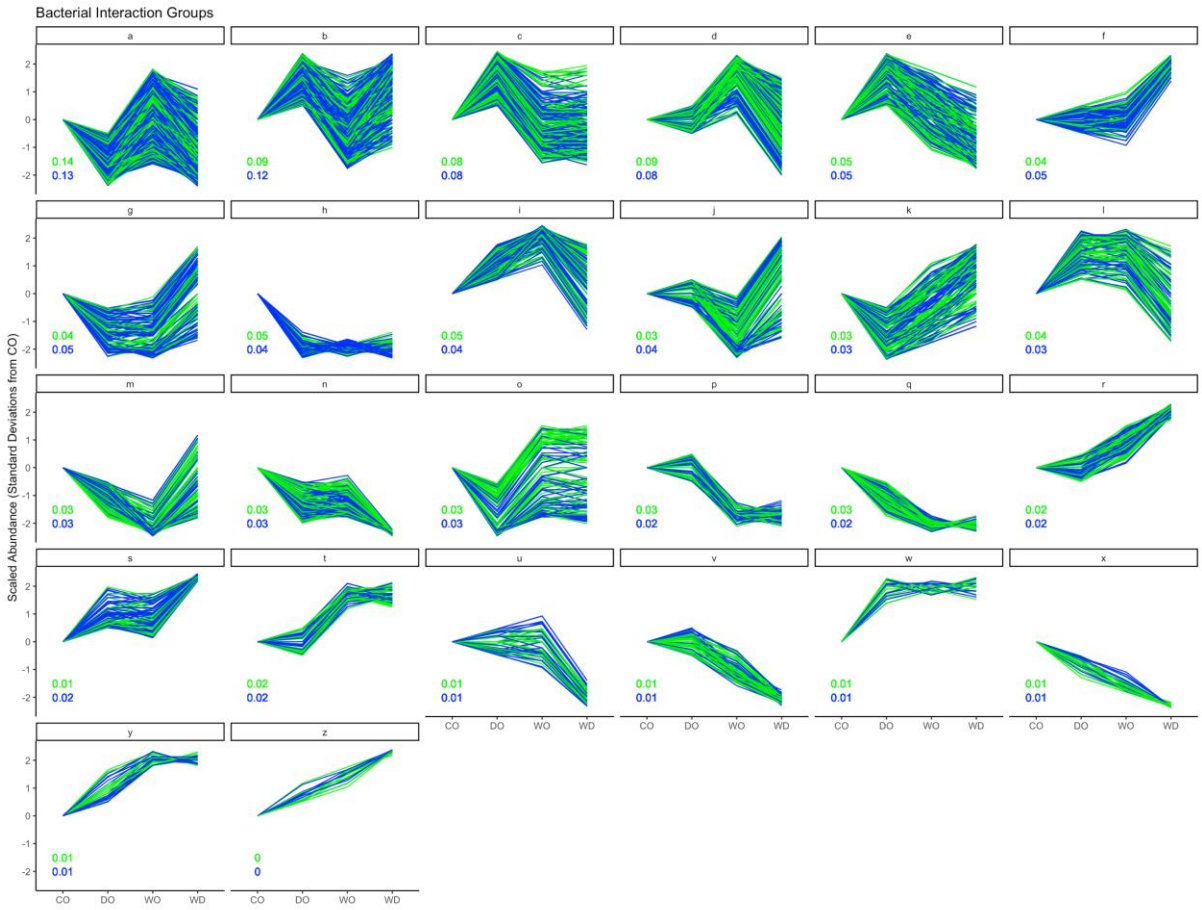

Figure 4: Interaction plot representing bacterial response patters in OTU abundance under Control (CO), Warming (WO), Drying (DO), and Combined treatments (WD) (positions 1 – 4 on the X axis). Abundance values (Y axis) are z-scaled and centered at 0 on the Control treatment abundance. Groups are separated based on negative or positive relative change in abundance with each treatment, using a threshold of | Δ| >= 0.5 SD to differentiate thee changes from minor fluctuation. Line color represents site (blue = Cloquet, green = Ely), and inset numbers (in corresponding color) represent the proportion of OTUs represented by the response group, compared to the entire site. Response groups are organized by total OTU count, and assigned arbitrary alphabetical label in this order.

Figure 5: Interaction plot representing bacterial response patters in OTU abundance under Control (CO), Warming (WO), Drying (DO), and Combined treatments (WD) (positions 1 – 4 on the X axis). Abundance values (Y axis) are z-scaled and centered at 0 on the Control treatment abundance. Groups are separated based on negative or positive relative change in abundance with each treatment, using a threshold of |Δ| >= 0.5 SD to differentiate thee changes from minor fluctuation. Line color represents site (blue = Cloquet, green = Ely), and inset numbers (in corresponding color) represent the proportion of OTUs represented by the response group, compared to the entire site. Response groups are organized by total OTU count, and assigned arbitrary alphabetical label in this order.

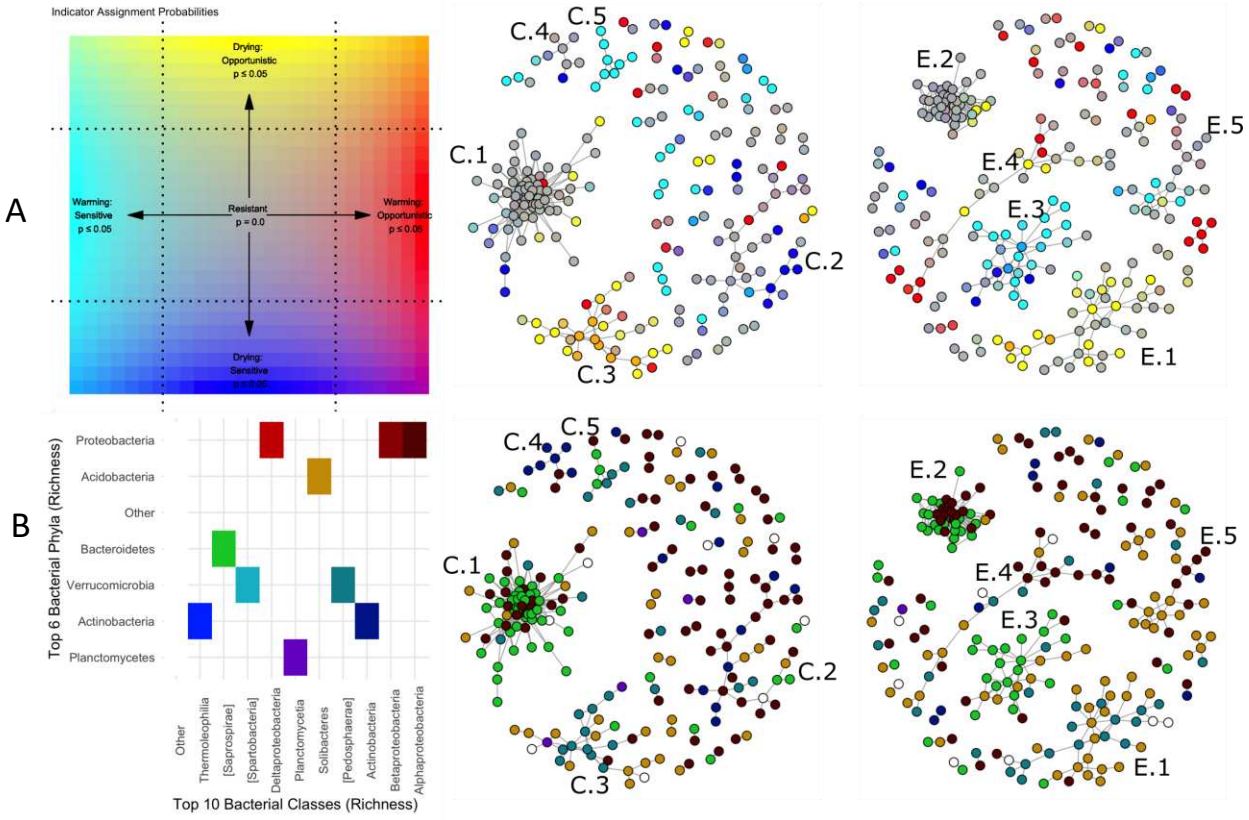

Figure 6: Color representation of major phyla and classes present in Bacterial Spearman Rank Correlation-based networks. The top row (A) shows responses to Warming and Drying treatment effects; the axis is derived from Indval score p values as described in Methods and shown visually in the key. Colors in this coordinate system are assigned to represent positive, negative, or neutral responses to Warming (red – positive through cyan – negative) and Drying (yellow – positive through blue – negative), with mixes of these axes representing taxa significant for more than one response, and grey representing non-significance. In section (B), the top 6 most abundant phyla, and 10 most-abundant classes across both networks are assigned unique colors as shown in the key, while rare phyla and classes aggregated into an "Other" category.

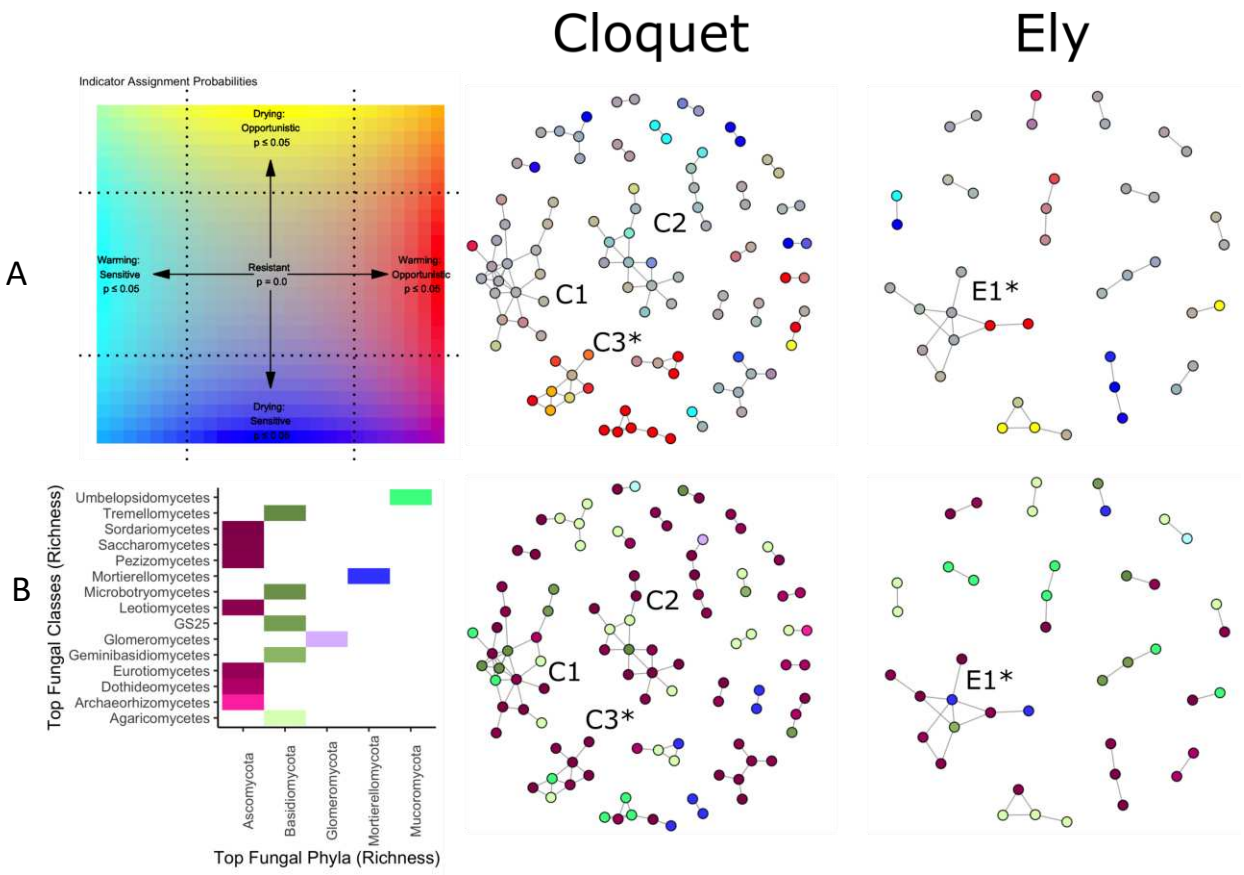

Figure 7: Color representation of major phyla and classes present in Fungal Spearman Rank Correlation-based networks. The top row (A) shows responses to Warming and Drying treatment effects; the axis is derived from Indval score p values as described in Methods and shown visually in the key. Colors in this coordinate system are assigned to represent positive, negative, or neutral responses to Warming (red – positive through cyan – negative) and Drying (yellow – positive through blue – negative), with mixes of these axes representing taxa significant for more than one response, and grey representing non-significance. In section (B), the top 6 most abundant phyla, and 10 most-abundant classes across both networks are assigned unique colors as shown in the key, while rare phyla and classes aggregated into an "Other" category.

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APPENDIX

Supplemental Figures

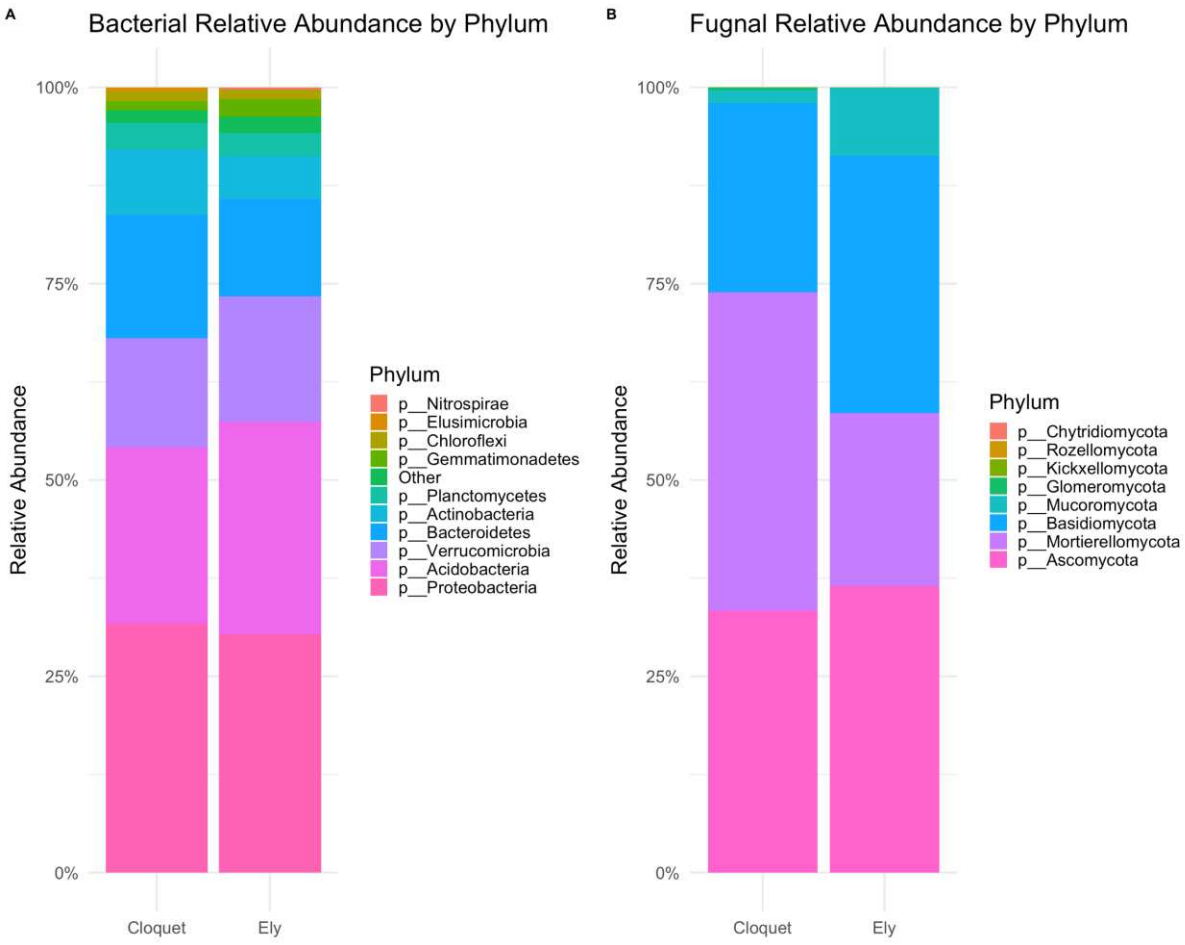

Figure S1: Stacked Bar Plots representing the overall taxonomic makeup of the Bacterial and Fungal communities at Cloquet, and at Ely. The axis represents the relative abundance of a given phylum (with bacterial taxa outside the top 10-most common phyla aggregated into "Other").

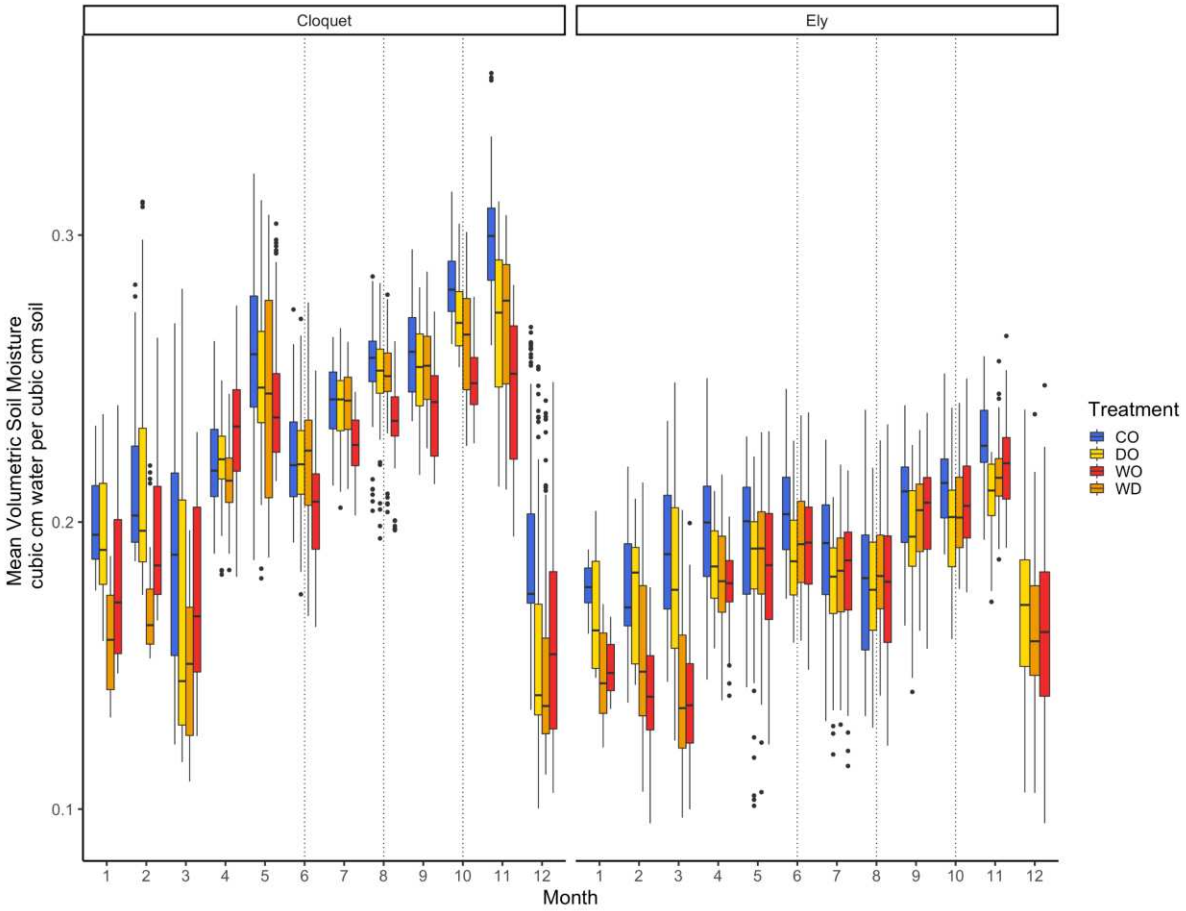

Figure S2: 2017 Field records of daily mean volumetric soil moisture, aggregated by month and treatment type. Horizontal lines represent collection months included in study.

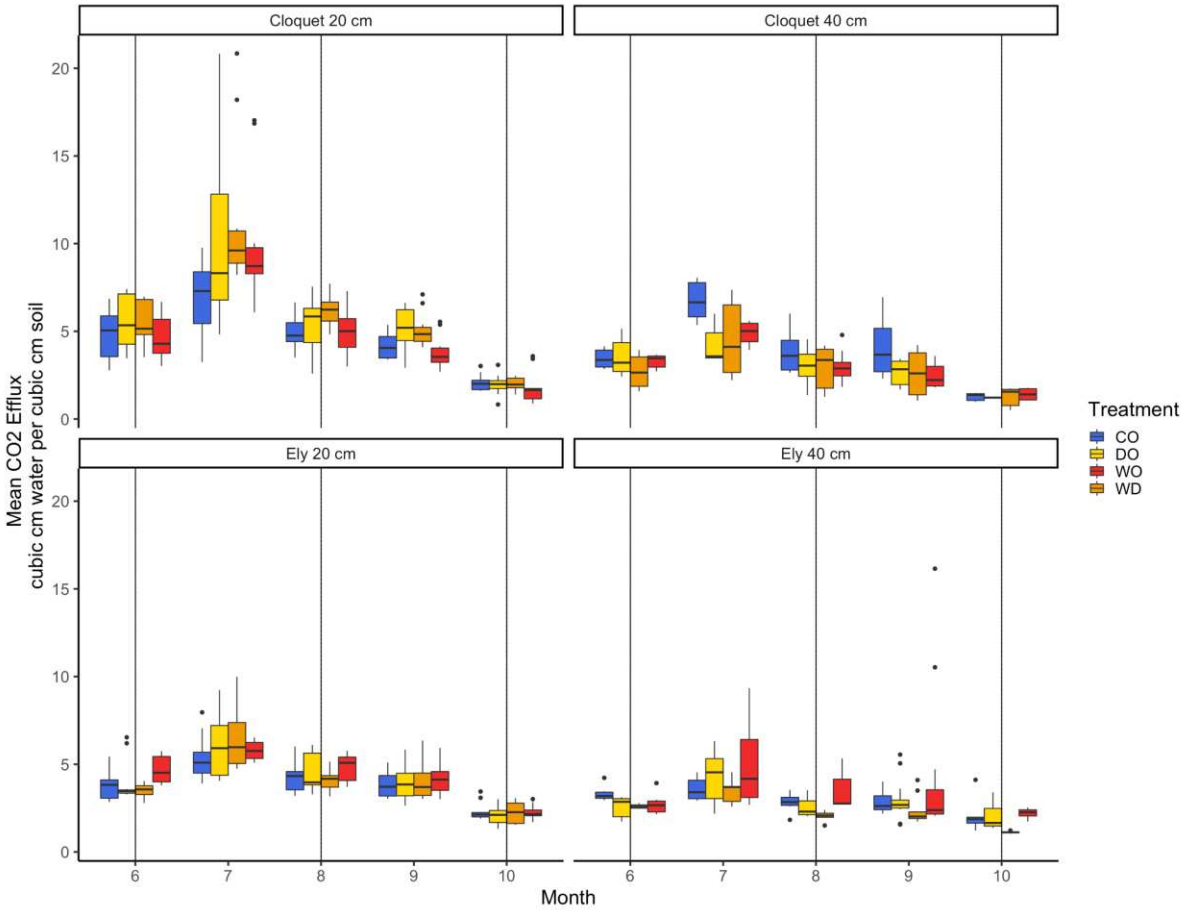

Figure S3: 2017 Monthly averages of growing season measurements of CO2 efflux by site, collar depth, and treatment. Horizontal lines represent collection months included in study.

Supplemental Tables

Table S1: B4WarmED Site Conditions (2010-2018 mean values)
Site elevations, along with annual means of Temperature and mm precipitation, over a 2008-2018 measuring period.

Location	*Elevation (m a.s.c.)*	*MAT (°C)*	*Annual ppt (mm)*
Cloquet Forestry Center	382	4.5	807
Hubacheck Wilderness Research Center	415	3	722

Table S2: GLM-based Response Group Enrichment (Significant Cases Only).
Table shows instances of significant ($p < 0.05$) Enrichment or Depletion of phyla in Linear model correlation-based response groups. "*Group*" represents response classifications as based on generalized linear model correlation to warming and drying effects; the "Enrichment" and "Depletion" values given are hypergeometric probability scores for over- or underrepresentation in the response group, compared to the overall distribution of taxa in each site.
Table S2.1: Bacterial Results

site	type	group	phylum	enrichment	depletion
Cloquet	16S	- - -	p__Proteobacteria	0.020797274	0.982274522
Cloquet	16S	- - -	p__Firmicutes	0.983800408	0.041696062
Cloquet	16S	- - -	p__Chlamydiae	0.998113834	0.012221269
Cloquet	16S	- - -	p__OD1	0.998682741	0.007038962
Cloquet	16S	- - W:D	p__Elusimicrobia	0.008064286	0.996897619
Cloquet	16S	- D -	p__Acidobacteria	0.006365893	0.995430227
Cloquet	16S	- D -	p__Actinobacteria	0.982267112	0.02579258
Cloquet	16S	- D W:D	p__Bacteroidetes	0.023252659	0.989683164
Cloquet	16S	W - -	p__OD1	3.83E-04	0.999949636
Cloquet	16S	W - -	p__Acidobacteria	0.999339199	0.001038235
Cloquet	16S	W - W:D	p__BHI80-139	0.041771952	0.99955911
Cloquet	16S	W - W:D	p__Planctomycetes	0.99380227	0.022039598
Cloquet	16S	W D -	p__Planctomycetes	0.02822504	0.980649267
Cloquet	16S	W D -	p__Actinobacteria	0.029587361	0.979162974
Cloquet	16S	W D -	p__Bacteroidetes	0.999571869	7.84E-04
Ely	16S	- - -	p__Verrucomicrobia	2.37E-04	0.999835825
Ely	16S	- - -	p__Armatimonadetes	0.007326516	0.997719244
Ely	16S	- - -	p__Planctomycetes	0.984863574	0.019679392
Ely	16S	- - -	p__OD1	0.992146521	0.028862651
Ely	16S	- - -	NA	0.996990221	0.01236856
Ely	16S	- - -	p__Actinobacteria	0.998641339	0.001877484
Ely	16S	- - W:D	p__Actinobacteria	0.003692654	0.997597565
Ely	16S	- - W:D	p__Firmicutes	0.007268534	0.99821332
Ely	16S	- D -	NA	0.015880551	0.996382736
Ely	16S	- D -	p__AD3	0.016574656	0.997718025
Ely	16S	- D -	p__Gemmatimonadetes	0.986215568	0.039386571
Ely	16S	- D W:D	p__FCPU426	0.02874291	0.997828762
Ely	16S	- D W:D	p__BHI80-139	0.035598752	0.999681498
Ely	16S	W - -	p__Gemmatimonadetes	0.019084396	0.990660293
Ely	16S	W - -	p__WS3	0.037086718	0.988382386
Ely	16S	W - -	p__WPS-2	0.037193139	0.995829464
Ely	16S	W - -	p__Actinobacteria	0.971896878	0.039425479

Ely	16S	W - W:D	p__Bacteroidetes	1.13E-06	0.999999713
Ely	16S	W D -	p__Actinobacteria	0.001191028	0.999260972
Ely	16S	W D -	p__Planctomycetes	0.004539655	0.997105936
Ely	16S	W D -	p__Verrucomicrobia	0.992638809	0.011371336
Ely	16S	W D W:D	p__Bacteroidetes	2.20E-06	0.99999955

Table S2.2: Fungal Results

site	type	group	phylum	enrichment_ response_group	depletion_ response_group
Cloquet	ITS	W D W:D	p__Basidiomycota	0.007549645	0.998087539
Ely	ITS	- - -	p__Basidiomycota	0.986623772	0.023939254

Table S3: Enrichment by Large Network Subnetwork—Taxonomic. Table shows instances of significant ($p < 0.05$) Enrichment or Depletion by phylum in Bacterial Spearman network subnetworks. "*Cluster Index*" represents contiguous clusters of taxa in each site's co-occurrence network, organized by size (e.g. C.1, n = 70, is the largest bacterial subnetwork in Cloquet). "Enrichment" and "Depletion" values given are hypergeometric probability scores for over- or underrepresentation in the response group, compared to the overall distribution of taxa in each site.

Site	cluster_index	count	phylum	enrichment_ cluster	depletion_ cluster
Cloquet	1	70	p__Bacteroidetes	4.70E-14	1
Cloquet	1	70	p__Verrucomicrobia	0.997828096	0.010378963
Cloquet	1	70	p__Acidobacteria	0.993152029	0.021150129
Cloquet	2	32	p__Proteobacteria	0.01277717	0.995607538
Cloquet	2	32	p__Bacteroidetes	0.999106281	0.005747499
Cloquet	2	32	p__Actinobacteria	0.004381415	0.99928372
Cloquet	3	29	p__Proteobacteria	0.999958875	3.80E-04
Cloquet	3	29	p__Bacteroidetes	0.999822947	0.002129623
Cloquet	3	29	p__Verrucomicrobia	8.59E-05	0.999988849
Cloquet	3	29	p__Acidobacteria	0.002353139	0.999483373
Cloquet	4	8	p__Actinobacteria	1.93E-06	0.999999971
Cloquet	5	8	p__Verrucomicrobia	0.008483973	0.999226196
Ely	1	39	p__Proteobacteria	0.999988357	1.17E-04
Ely	1	39	p__Gemmatimonadetes	0.036771342	0.996661992
Ely	1	39	p__Bacteroidetes	0.999983528	2.54E-04
Ely	1	39	p__Acidobacteria	0.002259392	0.999326932
Ely	1	39	p__Verrucomicrobia	4.40E-05	0.999993399
Ely	2	37	p__Proteobacteria	0.023902201	0.990761855
Ely	2	37	p__Bacteroidetes	3.57E-06	0.999999454
Ely	2	37	p__Acidobacteria	0.999998734	2.49E-05
Ely	3	30	p__Proteobacteria	0.99765595	0.010956196
Ely	3	30	p__Bacteroidetes	1.64E-06	0.999999797
Ely	5	22	p__Bacteroidetes	0.997305004	0.022203292
Ely	5	22	p__Acidobacteria	0.019134326	0.994332035

Table S4: Instances of significant ($p < 0.05$) Enrichment or Depletion by class in Bacterial Spearman network subnetworks. "*Cluster Index*" represents contiguous clusters of taxa in each site's co-occurrence network, organized by size (e.g. C.1, n = 70, is the largest bacterial subnetwork in Cloquet). "Enrichment" and "Depletion" values given are hypergeometric probability scores for over- or underrepresentation in the response group, compared to the overall distribution of taxa in each site.

Site	cluster_index	count	class	enrichment_ cluster	depletion_ cluster
Cloquet	1	70	c__Flavobacteriia	0.012164833	0.999200491
Cloquet	1	70	c__Sphingobacteriia	3.39E-14	1
Cloquet	1	70	c__Betaproteobacteria	0.02707495	0.990640604
Cloquet	1	70	c__Acidobacteriia	0.995660586	0.034499877
Cloquet	2	32	c__Thermoleophilia	0.006565014	0.999246964
Cloquet	2	32	c__Alphaproteobacteria	2.80E-06	0.999999731
Cloquet	3	29	c__Acidobacteriia	3.88E-06	0.9999998
Cloquet	3	29	c__[Spartobacteria]	1.89E-07	0.999999991
Cloquet	3	29	c__Planctomycetia	0.04481275	0.998022967
Cloquet	4	8	c__Thermoleophilia	0.003222544	0.999868252
Cloquet	4	8	c__Actinobacteria	5.76E-04	0.999990015
Cloquet	5	8	c__[Pedosphaerae]	0.006486119	0.999880859
Cloquet	5	8	c__Cytophagia	9.90E-04	0.999977042
Cloquet	5	8	c__Opitutae	0.010615008	0.999707214
Ely	1	39	c__Gemmatimonadetes	0.036771342	0.996661992
Ely	1	39	c__Acidobacteriia	1.36E-04	0.999979756
Ely	1	39	c__[Spartobacteria]	4.77E-06	0.999999472
Ely	2	37	c__Sphingobacteriia	8.30E-10	1
Ely	2	37	c__Betaproteobacteria	1.78E-05	0.999997822
Ely	3	30	c__[Saprospirae]	1.31E-07	0.999999992
Ely	3	30	c__[Chloracidobacteria]	0.019659742	0.997481638
Ely	4	25	c__Alphaproteobacteria	5.53E-04	0.999930722
Ely	5	22	c__DA052	0.002543894	0.9996833
Ely	5	22	c__Solibacteres	0.021549383	0.99805515

Supplemental Material and Methods:

Indicator Value Color Scale Assignment

Indicator Value assignments and associated p values were translated to a (-1,1) scale, with -1 corresponding to the minimum p value (approaching 0.0) for the Control condition, +1 to the minimum Treatment value, and 0 to a p value of 1.0 for either condition. These derived metrics were used to create a two-axis color gradient, with assigned colors being a mix of Warming (spanning Cyan (RGB 0,1,1) to Red (RGB 1,0,0)), and Drying (spanning Blue (RGB 0,0,1) to Yellow (RGB 1,1,0)). A value of 0 (i.e. p value of 1.0) on either treatment gradient was assigned a neutral grey (RGB 0.5,0.5,0.5). These color values were selected to give a neutral grey at the midpoint, as well as intuitive color mixes between gradients (e.g. a 1:1 mix of Yellow and Cyan yields Green, while Yellow and Blue give grey). To prevent highly significant (e.g. $p < 0.0001$) edge cases from overextending the range of available colors and desaturating larger, but still significant, *p* values (e.g. 0.05), raw p values were transformed with $-\log_{10}(p)$ when translating to RGB coordinates on a 0-1 scale, and had an artificial upper limit of $p = 0.05$ imposed.

Supplemental Results:

Indicator Value Scores Suggest Broad OTU-Level Resistance to Warming and Drying Effects

Classifying taxa using the Dufrene-Legendre indicator value *D*, with a probability threshold $p \leq 0.05$ for reactive taxa, revealed that in both cases the majority of taxa were resistant to warming and drying perturbations, at considerably higher proportions than those seen using negative binomial linear model correlations as the metric, likely due to accounting for taxon relative abundance and distribution across samples. Cloquet's bacterial community had 295 significant warming indicator taxa (97 opportunistic, 198 sensitive), and 296 drying indictors (132 opportunistic, 164 sensitive), or just ~6.8% in total for both cases. Despite the similarity in ratios, these indicator groups were largely distinct, with only 19 indicators OTUs sharing a

positive or negative association with both treatments. Ely's bacterial community similarly had 6/8% of OTUs indicating for warming (121 opportunistic, 164 sensitive), and only 4.9% significant drying indicators (83 opportunistic, 122 sensitive).

Taxonomic enrichment within the groups also showed a fair degree of accord across sites. Warming-resistant taxa were enriched for Actinobacteria and Planctomycetes at both Cloquet ($p = 0.001$, $p = 0.003$) and Ely ($p = 0.003$, $p = 0.02$); drying-resistant taxa showed more site-dependence for enrichment, with Cloquet being enriched for Actinobacteria ($p = 0.001$), while Ely followed the warming-resistant taxa in a Planctomycetes enrichment ($p = 0.006$). Warming-opportunistic indicators were enriched for Acidobacteria (Cloquet $p = 0.009$, Ely $p < 0.001$), while Ely was additionally enriched for Gemmatimonadetes; drying-opportunistic taxa were enriched in Acidobacteria ($p < 0.001$ in both sites), with Cloquet's community additionally being enriched for Verrucomicrobia ($p < 0.001$) and Nitrospirae ($p = 0.041$). Warming-sensitive indicators were enriched for Bacteroidetes in both sites (Cloquet $p = 0.026$, Ely $p < 0.001$), while there was no significant enrichment of taxa for water-sensitive bacteria, indicating a fairly proportional cross-section of the community (with the exception of depletion for Actinobacteria in Ely—$p = 0.04$).

The fungal community saw a comparable distribution; 6.8% of Cloquet OTUs were indicators for warming (32 opportunistic, 25 sensitive), and 5.8% for drying (9 opportunistic, 20 sensitive). Ely was slightly more reactive by this metric, with 8.8% of OTUs being a significant indicator for warming (24 opportunistic, 20 sensitive) , and 6.2% being indicators for drying (8 opportunistic, 23 sensitive). As with Ely, very few (1-2) OTUs shared a significant opportunistic or sensitive response to both treatment effects. Shared instances enrichment, and enrichment in general, of phyla in fungal indicator groups across sites was rarer than bacteria; the only commonality was a depletion of Ascomycota (Cloquet $p = 0.036$, Ely $p = 0.047$) among warming-sensitive taxa, and enriched Mucoromycota (Cloquet $p = 0.017$, Ely $p = 0.016$) among drying-opportunistic indicators. Cloquet had more cases of significant enrichment in indicator

groups, including Mucoromycota among warming opportunistic taxa ($p < 0.001$), and of Mortierellomycota in warming sensitive taxa ($p = 0.012$); this phylum was enriched among Cloquet's water-opportunistic taxa as well ($p = 0.017$).

Overall, major implications of this distribution include more bacterial taxa being negatively than positively correlated with warming or drying pressure. This would make sense with diminished alpha diversity and shifting community structure at the community level, as it suggests a dynamic along the ones observed in Sheik et al (2012) or De Angelis etal (2015), or a small subset of heat-tolerant taxa proliferating as a larger subset diminish. While most bacterial taxa were not significantly over- or underrepresents, major exceptions like Acidobacteria, Actinobacteria, and Bacteroidetes point to an important relationship between the taxonomic makeup of a community and its response to climate-related perturbations. In the fungal community, there appeared to be a preferential enrichment for warming-opportunistic, and drying-sensitive response profiles, but these were generally not linked to taxa.