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Soil bacterial and fungal response to wildfires in the Canadian boreal forest across a burn severity gradient

Thea Whitman[a,*], Ellen Whitman[b,c], Jamie Woolet[a], Mike D. Flannigan[b], Dan K. Thompson[c], Marc-André Parisien[c]

[a] Department of Soil Science, University of Wisconsin-Madison, 1525 Observatory Dr., Madison, WI, 53703, USA
[b] Department of Renewable Resources, University of Alberta, 751 General Services Building, Edmonton, AB, T6G 2H1, Canada
[c] Northern Forestry Centre, Canadian Forest Service, Natural Resources Canada, 5320 122 Street, Edmonton, AB, T6H 3S5, Canada

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ABSTRACT

Global fire regimes are changing, with increases in wildfire frequency and severity expected for many North American forests over the next 100 years. Fires can result in dramatic changes to carbon (C) stocks and can restructure plant and microbial communities, with long-lasting effects on ecosystem functions. We investigated wildfire effects on soil microbial communities (bacteria and fungi) in an extreme fire season in the northwestern Canadian boreal forest, using field surveys, remote sensing, and high-throughput amplicon sequencing in upland and wetland sites. We hypothesized that vegetation community and soil pH would be the most important determinants of microbial community composition, while the effect of fire might not be significant, and found that fire occurrence, along with vegetation community, moisture regime, pH, total carbon, and soil texture are all significant predictors of soil microbial community composition. Burned communities become increasingly dissimilar to unburned communities with increasingly severe burns, and the burn severity index (an index of the fractional area of consumed organic soils and exposed mineral soils) best predicted total bacterial community composition, while whether a site was burned or not was the best predictor for fungi. Globally abundant taxa were identified as significant positive fire responders in this system, including the bacteria *Massilia* sp. (64 × more abundant with fire) and *Arthrobacter* sp. (35 ×), and the fungi *Penicillium* sp. (22 ×) and *Fusicladium* sp. (12 ×). Bacterial and fungal co-occurrence network modules were characterized by fire responsiveness as well as pH and moisture regime. Building on the efforts of previous studies, our results consider a particularly wide range of soils, vegetation, and burn severities, and we identify specific fire-responsive microbial taxa and suggest that accounting for burn severity improves our understanding of microbial response to fires.

1. Introduction

The boreal forests of Canada hold roughly 10% (between 168 and 200 Pg) of total global terrestrial carbon (C) stocks (Bradshaw and Warkentin, 2015) and cover about 55% of the country's landmass (Brandt et al., 2013). Much of this soil C is stored in peatlands (peat-forming wetlands), which cover as much as 50% of the land surface in some boreal forest landscapes (Tarnocai et al., 2011). Wildfire is a natural part of these ecosystems, playing a key role in structuring vegetation communities and affecting above- and belowground C stocks through combustion and persistent effects on the subsequent forest recovery (Bond-Lamberty et al., 2007; Burton et al., 2008; de Groot et al., 2013; Pellegrini et al., 2017). Because soil microbes play an important role in these ecosystems, both governing soil C cycling and also interacting directly with plants, it is important to understand how soil microbes are affected by wildfire.

Microbial response to fire has been studied for over a century (Seaver, 1909; Boudier, 1877). Across studies, wildfires usually decrease soil microbial biomass (Dooley and Treseder, 2011; Holden and Treseder, 2013; Pressler et al., 2018) and microbial communities can take decades to recover to pre-fire states (Dooley and Treseder, 2011; Ferrenberg et al., 2013; Dove and Hart, 2017). There are numerous mechanisms through which fires can affect soil microorganisms. Briefly, we can organize these mechanisms into three categories: (1) fire directly killing microbes and destroying microbial habitat; (2) altered post-fire chemico-physical belowground environment (*e.g.*, increased

* Corresponding author. Observatory Dr., Madison, WI, 53703, USA.
E-mail address: twhitman@wisc.edu (T. Whitman).

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pH, changes to water permeability, changed nutrient inputs or competition for nutrients from plants) (3) altered post-fire biological environment (*e.g.*, competitors removed or loss of symbiont plants) (Ryan, 1991; Hart et al., 2005).; For the purposes of this paper, we are interested in all of these mechanisms, the results of which we consider as "effects" of fire. Investigating specific fire-responsive taxa and beginning to decipher their ecological strategies may help us predict the long-term ecological and biogeochemical effects of fire. For example, if specific plant symbionts are consistently affected by fires, this may help explain plant recolonization post-fire. If changes in methanotrophic bacteria are associated with fires, this could have important biogeochemical implications.

Although numerous field studies have investigated how microbes are affected by fires, few consider multiple ecosystem types, more than one fire, or how these effects change with increasing burn severity. Wildfires can range from lightly burned surface fires with no tree mortality, to fires that kill all trees and remove all or most of the soil litter layer (O horizon). As fire regimes around the world are affected by climate change (Flannigan et al., 2009; de Groot et al., 2013), fire frequency, size, and intensity (and thus, indirectly, burn severity) are expected to increase in many areas of the boreal forest (Flannigan et al., 2009; Wotton et al., 2017). Burn severity is the degree of fire-induced change to vegetation and soils (Keeley, 2012; Parks et al., 2014). When past studies have considered burn severity, some have observed that increasing burn severity is associated with reduced fungal abundance (Bergner et al., 2004) or changes to community composition (Holden et al., 2016). For bacteria, while some studies have found only negligible effects of burn severity on community composition (Kennedy et al., 2014) others have noted distinct changes to bacterial community composition with different levels of burn severity (Weber et al., 2014). Furthermore, burn severity may interact with plant communities to affect microbial communities post-fire. For example, Knelman et al. (2015) reported interactive effects between burn severity (low *vs*. high) and the effect of plant colonization (*Corydalis aurea* presence *vs*. absence) on bacterial communities and potential activity in a Colorado, USA *Pinus ponderosa* forest.

There are numerous metrics of burn severity used by fire scientists, each of which represents different aspects of the effects of fire. Field-based ground metrics include single measurements such as percent exposed mineral soil or mean duff depth (O horizon); the burn severity index (BSI) integrates the burn severity of the forest floor and soil surface (Loboda et al., 2013). Canopy fire severity index (CFSI) estimates the intensity of the combustion of large trees (Kasischke et al., 2000). Composite burn index (CBI) is a generalized measure of burn severity, mortality, and combustion across all forest strata from soils to large trees (Key and Benson, 2006; Kasischke et al., 2008) The remotely-sensed relativised burn ratio (RBR (Parks et al., 2014)) combines satellite imagery from before and after burns, capturing changes in reflectance due to vegetation combustion and mortality, and combustion of soil organic matter and changes in soil moisture. Few or no studies have investigated the relative utility of these different burn severity metrics for predicting microbial community response to fire. Determining the effects of fire on soil microbial communities across a wide range of sites and assessing the utility of different burn severity metrics could underpin efforts to predict and characterize the effects of wildfires and changing wildfire regimes on soil microbes in boreal upland and wetland soils.

The first objective of our study was to determine the relative importance of soil, vegetation, and wildfire severity metrics in predicting soil microbial community composition one year post-fire across five vegetation types in the boreal forests of northwestern Canada. Our studied regions have high pedodiversity, spanning wide ranges of pH, texture, and organic horizon thicknesses. We hypothesized that vegetation community and soil pH would be the most important determinants of microbial community composition, while the effect of fire might not be significant after controlling for vegetation community and soil properties across such a wide range of sites. If fire were to be found to be a significant predictor, we hypothesized that burn metrics associated with the ground surface would reflect microbial community composition better than remotely sensed metrics or canopy burn metrics, because they are constrained to the scale of the microbial habitat in question. The second objective of our study was to identify specific fire-responsive taxa. To achieve these objectives, we sampled six large wildfires one year after they burned in the Northwest Territories and northern Alberta, Canada, characterizing soil, vegetation, and fire properties, and sequencing microbial (bacterial/archaeal and fungal) communities using the ribosomal RNA gene. The one year sampling timepoint offers one observation of post-fire community dynamics, with the high number and diversity of sites and fire conditions enhancing the utility and novelty of this study.

2. Methods

2.1. Study region

We selected sites in the Northwest Territories and northern Alberta (Wood Buffalo National Park), Canada, and sampled them one year post-fire, in 2015 (Fig. 1; Supplemental Table 1; Supplemental Note 1). The fires and the drivers of burn severity are described in detail in Whitman et al. (2018a), while their effects on understory vegetation are described in detail in Whitman et al. (2018b). The study region has long, cold winters and short, hot summers, with mean annual temperatures between −4.3 °C and −1.8 °C and annual precipitation ranging from 300 to 360 mm (ESWG, 1995; Wang et al., 2012) The fire regime of the study region includes infrequent stand-replacing fires every 40–350 years on average (Boulanger et al., 2012). The majority (~97%) of the burned area is contributed by ~3% of fires (Stocks et al., 2002). 2014 was an extreme year for wildfires in this region, with particularly numerous (57% more than 20-year mean) and large (6 × the 20-year mean area) wildfires (NTENR, 2015). The six large wildfires in this study ranged in size from 14 000 to 700 000 ha. The soils in these regions are mostly classified as Typic Mesisols (32 sites), Orthic Gleysols (16 sites), or Orthic Grey Luvisols (8 sites) (Soil Landscapes of Canada map v.3.2). The sampled sites span a wide range of soil properties, with pH values ranging from 3.2 (wetlands) to 8.1 (uplands with calcareous plant material), total C ranging from 0.5% (mineral horizon) to 52% (organic horizon), and a wide range of textures (Supplemental Table 2). The vegetation community and topoedaphic context (upland or wetland class) of a plot were described according to the Field Guide to Ecosites of Northern Alberta (Beckingham and Archibald, 1996). We generalized ecosites for each site as being treed wetlands, open wetlands, upland jack pine-dominated (*Pinus banskiana* Lamb.), upland black spruce-dominated (*Picea mariana* (Mill.)), or composed of an upland mix of coniferous and broadleaf trees ("mixedwood").

2.2. Site assessment methodologies

Sites were selected and characterized as described in detail by Whitman et al. (2018a; 2018b). Briefly, field sites were selected to represent the local range of burn severity and vegetation communities, resulting in a total of 50 burned field sites. We selected an additional 12 control sites (not burned within the last 38 years before sampling, mean time since last fire 95 years; "unburned"), chosen to reflect the range of vegetation communities sampled in the burned plots, for a total of 62 sites (properties described in Supplemental Table 1). At each site we established a 30 × 30 m square plot with 10 × 10 m subplots at the four corners. We measured post-fire organic horizon depth (up to 10 cm) at the inner corners of the 10 × 10 m subplots. Understory vegetation percent cover was assessed in five 1 × 1 m plots at the same four points as organic soil depth, and at the plot centre (Whitman et al., 2018b). We assessed burn severity in the four subplots (described in

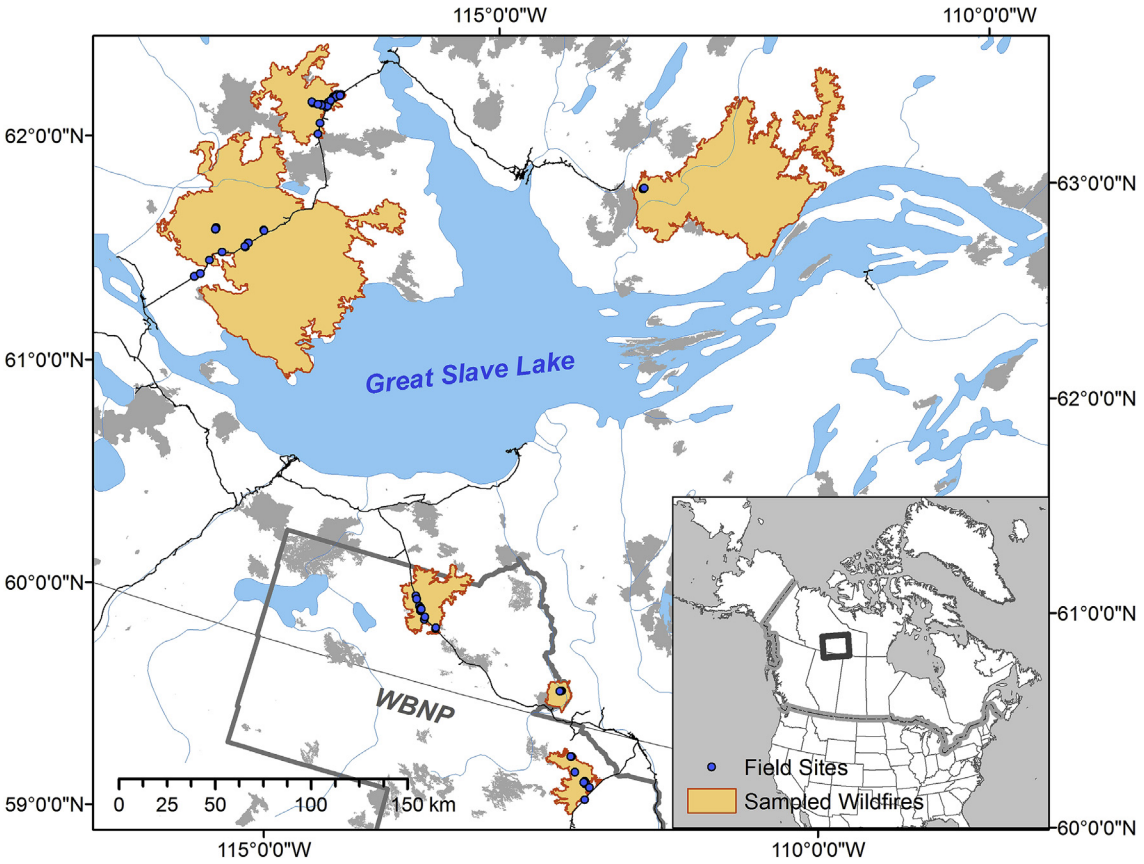

Fig. 1. Study region of northern Alberta and the Northwest Territories, Canada, including Wood Buffalo National Park (WBNP – grey outline). Blue points indicate sampled sites, orange shapes indicate sampled fires, and grey shapes indicate other 2014 fires in the region. Inset indicates relative location within North America. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

detail in Whitman et al. (2018a); Supplemental Table 1), using severity metrics of canopy fire severity index (CFSI (Kasischke et al., 2000), burn severity index (BSI (Loboda et al., 2013)), and percent exposed mineral soil. We also assessed the composite burn index (CBI; understory, overstory, and mean Key and Benson, 2006; Kasischke et al., 2008)) in the entire 30 × 30 m plot area. We used the relativised burn ratio (RBR) to represent remotely sensed burned severity at each site (Parks et al., 2014). RBR was produced using multispectral Landsat 8 Operational Land Imager and Landsat 5 Thematic Mapper images (Landsat Level-1 imagery, courtesy of the USGS).

At each plot, we took soil cores (5.5 cm diameter, 13.5 cm depth) at three locations (centre, SW and NE subplots). Soil cores were gently extruded and separated into organic (O) horizons (where present) and mineral (M) horizons (where present in the top 13.5 cm of soil profile). The three samples were combined by horizon at each site and mixed gently by hand in a bag. From these site-level samples, sub-samples were collected for microbial community analysis and stored in LifeGuard Soil Preservation solution (QIAGEN, Germantown, MD) in a 5 mL tube (Eppendorf, Hamburg, Germany). Tubes were kept as cold as possible while in the field (usually for less than 8 h, but up to 2 days for remote sites) and then stored frozen. The remaining soil samples were air-dried and analyzed for a range of properties, including, pH and total C (Supplemental Table 2; Supplemental Note 1).

2.3. DNA extraction, amplification, and sequencing

Duplicate DNA extractions were performed for each sample, with two blank extractions for every 24 samples (identical methods but using empty tubes, half of which were sequenced), using a DNEasy PowerLyzer PowerSoil DNA extraction kit (QIAGEN, Germantown, MD) following manufacturer's instructions. Extracted DNA was amplified in triplicate PCR, targeting the 16S rRNA gene v4 region (henceforth, "16S") with 515f and 806r primers (Walters et al., 2015), and targeting the ITS2 gene region with 5.8S-Fun and ITS4-Fun primers (Taylor et al., 2016) with barcodes and Illumina sequencing adapters added as per (Kozich et al., 2013) (all primers in Supplemental Tables 3–5). The PCR amplicon triplicates were pooled, purified and normalized using a SequalPrep Normalization Plate (96) Kit (ThermoFisher Scientific, Waltham, MA). Samples, including blanks, were pooled and library cleanup was performed using a Wizard SV Gel and PCR Clean-Up System A9282 (Promega, Madison, WI). The pooled library was submitted to the UW Madison Biotechnology Center (UW-Madison, WI) for 2x250 paired end (PE) Illumina MiSeq sequencing for the 16S amplicons and 2x300 PE for the ITS2 amplicons. (See Supplemental Note 1 for full details.)

2.4. Sequence data processing and taxonomic assignments

For 16S reads, we quality-filtered and trimmed, dereplicated, learned errors, determined operational taxonomic units (OTUs), and removed chimeras using dada2 (Callahan et al., 2016) as implemented in R. For ITS2 reads, we first merged reads using PEAR (Zhang et al., 2014), and then performed the same steps as for 16S. These sequence processing steps were performed on the UW-Madison Centre for High Throughput Computing cluster (Madison, WI). After confirming that the paired DNA extraction replicates were very similar to each other, we combined the community composition data from paired extractions additively and proceeded with a single sequencing dataset for each soil sample. Taxonomy was assigned to the 16S reads using a mothur (Schloss et al., 2009) classify.seqs knn method, with the aligned 515f-806r region of the 99% ID OTUs from the Silva nr 128 database (Quast

et al., 2013; Yilmaz et al., 2014). We used BLAST to determine the closest > 97% ID match, if present, in the database of globally abundant bacterial phylotypes (Delgado-Baquerizo et al., 2018). For the ITS2 reads, we first ran them through ITSx (Bengtsson-Palme et al., 2013) to identify fungi and to remove plant sequences, and then assigned taxonomy using the UNITE species hypothesis 99% threshold database version 7.2 (UNITE Community, 2017), using the parallel_assign_taxonomy_uclust.py script in QIIME1 (Caporaso et al., 2010) with default settings to the genus level. We also classified ITS2 taxonomic assignments using the FunGuild database (Nguyen et al., 2016). (All sequences are deposited in the NCBI SRA under bioproject number PRJNA564811 (accessions SRR10097729-SRR10097958 (16S) and SRR10097961-SRR10098185 (ITS2)).See Supplemental Note 1 for full details of bioinformatics, including sequences retained at each step.)

2.5. Quantitative PCR

To estimate the relative abundance of bacteria *vs.* fungi in a given sample, extracted DNA was amplified via quantitative PCR (qPCR) in triplicate, targeting the 16S rRNA gene v4 region with 515f and 806r primers (Carini et al., 2016) and targeting the 18S gene region with FR1 and FF390 primers (Prévost-Bouré et al., 2011) (Supplemental Note 1; qPCR primers in Supplemental Table 3; raw Cq values and calibration curves in Supplemental File 1).

2.6. Bioinformatics and statistics

We worked primarily in Jupyter notebooks, with phyloseq (McMurdie and Holmes, 2013), ggplot (Wickham, 2016), and dplyr (Wickham et al., 2019) being instrumental in working with the data in R (R Core Team, 2017) (See Supplemental Note 1 for full bioinformatics details).

We compared community composition across samples using Bray-Curtis dissimilarities on Hellinger-transformed relative abundances (Legendre and Gallagher, 2001), which we represented using NMDS ordinations. We tested for significant effects of vegetation community, moisture regime (as a continuous variable), pH, total C, texture (% sand), and burned/unburned using a permutational multivariate ANOVA (PERMANOVA; the adonis function in vegan (Oksanen et al., 2018). Because the order of the terms in the PERMANOVA model affects the partial R^2 of a given term, to compare the relative explanatory power of each component, we also compared the R^2 of single-component models for each factor. We predicted 16S rRNA gene copy numbers using the ribosomal RNA operon database (rrnDB) (Stoddard et al., 2015). We calculated the abundance-weighted mean predicted copy number for each sample using the approach of Nemergut et al. (2016). We tested for the relationship between weighted mean predicted copy number for each sample and burn severity (understory composite burn index) with a linear model. In their study of burn severity effects on fungi, Holden et al. (2016) used a method of estimating “fire severity tolerance” for different fungal phyla. To compare our findings with those of Holden et al. (2016), we calculated the mean understory CBI value for all sites at which each OTU within that phylum was present, and determined whether there were significant differences in these values between different fungal phyla, using an ANOVA and Tukey's HSD for multiple comparison correction. We tested whether vegetation community dissimilarity was significantly correlated with bacterial or fungal community dissimilarity for all pairs of sites in mineral and in organic soil horizons using Mantel tests, with 999 permutations. (Briefly, this addresses the question, for a given pair of samples, when their plant communities are similar (or dissimilar), are their microbial communities also similar (or dissimilar)?)

To examine the relative explanatory power of different burn severity metrics for predicting microbial community composition, we used a simple linear model, which controlled for parameters we expected to influence community composition – vegetation community, moisture regime (as a continuous variable), pH, texture (% sand), and total C – and then tested the inclusion of each severity metric, comparing the partial R^2 values for the severity metric. The severity metrics we tested included: Burned/Unburned, RBR, CFSI, CBI, Understory CBI, Overstory CBI, BSI, % exposed mineral soil, and mean duff depth. To determine whether communities become increasingly dissimilar with increasing burn severity, we fit a linear model to Bray-Curtis dissimilarity (to unburned samples from sites with the same vegetation community and soil horizon) *vs*. BSI. We calculated the relationship between BSI and log(16S abundance: 18S abundance) using a linear model in R. We also did the same with pH and log(16S abundance: 18S abundance).

We estimated richness and its associated standard error in each sample using the *breakaway* function in R (Willis et al., 2016). We estimated Pielou's evenness as Shannon index/log(observed OTUs) as implemented in *vegan* (Oksanen et al., 2018). We determined which OTUs were significantly enriched in burned plots (*vs.* unburned plots) using metagenomeSeq (Paulson et al., 2013), after controlling for (including as variables) vegetation community (categorical variable), pH (continuous variable), and %C (continuous variable), resulting in an estimate of the $\log_2$-fold change in the abundance of each OTU in burned vs. unburned plots, across samples. For a small subset of OTUs, we investigated the relationship between their log(relative abundance) and BSI using a linear model.

To determine which fungal and bacterial OTUs and understory vegetation co-occurred across samples, we used a network analysis approach, following Connor et al. (2017) to avoid false positives and establish conservative network cutoff parameters. After simulating a null model network to choose an appropriate rho value, we determined a consensus network by adding random tie-breaking noise to the matrix 2000 times, selecting only the co-occurrences that occurred in 95% of the 2000 replications. We determined standard network characterization metrics (Guimera and Amaral, 2005; Olesen et al., 2007; Zhou et al., 2010; Deng et al., 2012; Shi et al., 2016). To determine modules (groups of nodes, or taxa, that significantly co-occur), we used a greedy agglomerative algorithm (Clauset et al., 2004). For the purposes of this paper, we interpret co-occurrences or modules as generally representing shared niches rather than necessarily representing direct interactions between organisms (Faust and Raes, 2012; Montoya et al., 2015). We plotted the network using igraph R package (Csardi and Nepusz, 2006).

3. Results

3.1. Community-level characteristics and predictors

Bacterial and fungal communities were dominated by typical soil organisms (Bahram et al., 2018; Delgado-Baquerizo et al., 2018) (Supplemental Figs. 1–4). All tested factors (vegetation community, moisture regime, pH, total C, texture, and burned/unburned) were significant predictors of community composition for both bacteria (Fig. 2) and fungi (Fig. 2) in the combined model (PERMANOVA, $p < 0.01$ for all factors, Supplemental Tables 6 and 7). Vegetation community provided the most explanatory power for bacteria and for fungi (R^2 values between 0.12 and 0.14 for the individual models). After these factors, pH provided the most explanatory power ($R^2_{pH,16S} = 0.07$; $R^2_{pH,ITS2} = 0.04$ Fig. 2A and B).

Different fungal phyla were present at different levels of burn severity. OTUs within *Chytridiomycota* and *Mucoromycota* occurred at sites with significantly higher mean BSI than *Ascomycota, Mortierellomycota*, or *Rozellomycota* in upland sites (Supplemental Fig. 5A). In wetland sites, OTUs within *Basidiomycota* occurred at sites with significantly lower mean BSI than for all other phyla except *Rozellomycota*. This trend largely mirrored that of the same approach with moisture regime instead of BSI (Supplemental Fig. 5B), but even after controlling for moisture regime, similar trends persisted (Supplemental

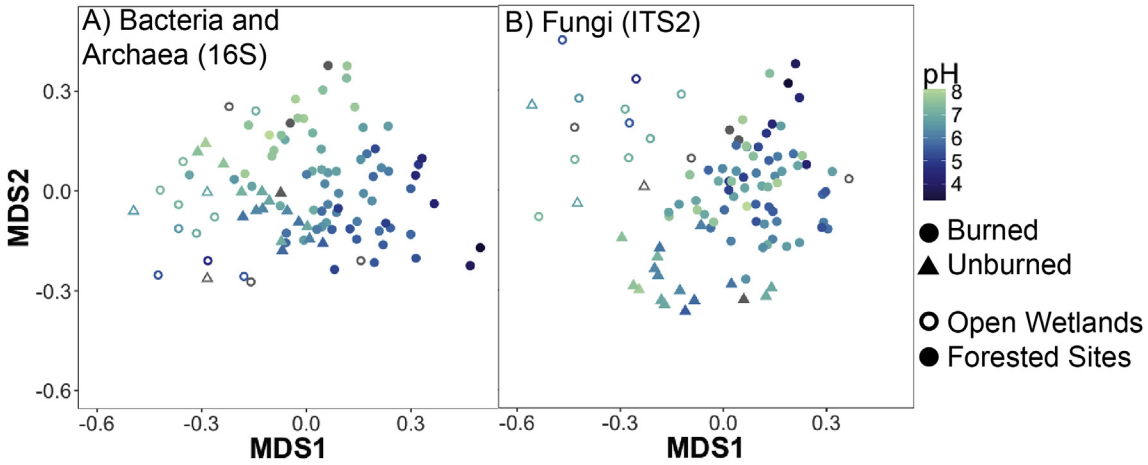

Fig. 2. (A) NMDS ordination of Bray-Curtis distances between bacterial/archaeal (16S rRNA gene v4 region) communities for all samples (k = 2, stress = 0.16). (B) NMDS ordination of Bray-Curtis distances between fungal (ITS2) communities for all samples (k = 3, stress = 0.14). (For both panels) Circles indicate burned plots, while triangles indicate unburned plots, and open points indicate open wetland sites. Points are shaded by pH, with darker colours indicating lower pH values. Grey points indicate samples for which pH values were not attainable, due to insufficient sample mass. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

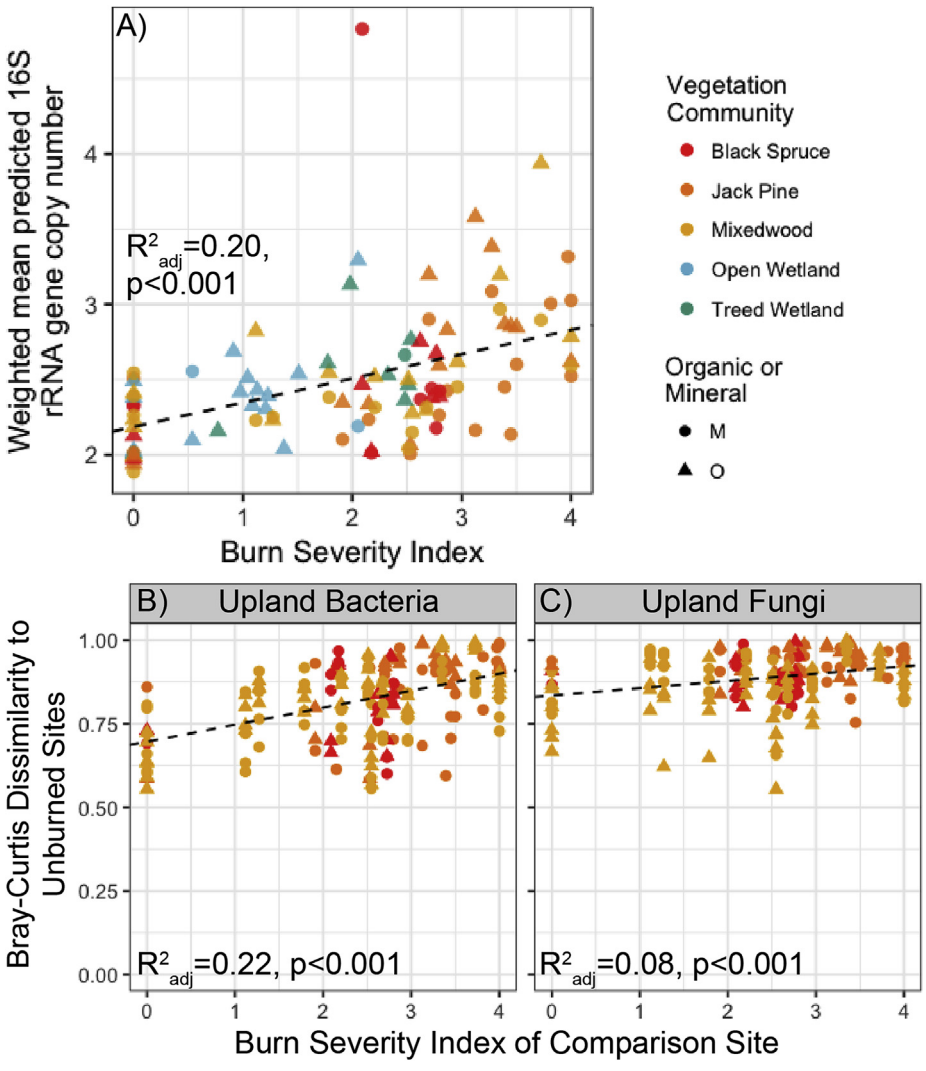

Fig. 3. (A) Relationship between weighted mean predicted 16S rRNA gene copy number and burn severity index (BSI). Dashed line indicates linear fit ($y = 2.19 + 0.16x$, $R^2_{adj} = 0.20$, $p < 0.001$). (B) Bray-Curtis dissimilarity to unburned sites (within the same vegetation community and the same soil horizon type) for bacteria in uplands vs. burn severity index of comparison sites. Dashed lines indicate linear fit ($y = 0.0.05\ x + 0.70$, $p < 0.001$, $R^2_{adj} = 0.22$). (C) Bray-Curtis dissimilarity to unburned sites (within the same vegetation community and the same soil horizon type) for fungi in uplands vs. burn severity index of comparison sites. Dashed lines indicate linear regressions ($y = 0.02\ x + 0.83$, $p < 0.001$, $R^2_{adj} = 0.08$). For all figures, points are coloured by vegetation community; circles represent mineral horizon samples, triangles represent organic horizon samples.

Table 1
Predictive value of burn severity metrics in models of Hellinger-transformed microbial community Bray-Curtis dissimilarities, after controlling for vegetation community, moisture regime, pH, total C, and texture (% sand). The best models for each group are highlighted in bold text. $N_{bacteria} = 94$, $N_{fungi} = 92$, except for Overstory CBI, where $N_{bacteria} = 90$, $N_{fungi} = 88$.

Severity metric	$p_{severity}$	Partial $R^2_{severity}$	R^2_{full}
Bacteria (16S rRNA gene v4 region)			
Burned/Unburned	**0.001**	**0.037**	**0.29**
Relativised Burn Ratio	0.001	0.028	0.29
Canopy Fire Severity Index	0.001	0.020	0.28
Composite Burn Index (CBI)	0.001	0.032	0.29
Understory CBI	0.001	0.033	0.29
Overstory CBI	0.001	0.031	0.29
Burn Severity Index	**0.001**	**0.039**	**0.30**
% exposed mineral soil	0.001	0.026	0.28
Mean duff depth	0.034	0.013	0.27
Fungi (ITS2)			
Burned/Unburned	**0.001**	**0.044**	**0.28**
Relativised Burn Ratio	0.001	0.036	0.27
Canopy Fire Severity Index	0.001	0.030	0.27
Composite Burn Index (CBI)	0.001	0.040	0.28
Understory CBI	0.001	0.041	0.28
Overstory CBI	0.001	0.039	0.27
Burn Severity Index	**0.001**	**0.043**	**0.28**
% exposed mineral soil	0.001	0.026	0.26
Mean duff depth	0.001	0.021	0.26

Fig. 6).

There was a significant positive relationship between burn severity and weighted mean predicted 16S copy number (Fig. 3A). The OTUs identified as positive fire-responders had significantly higher mean predicted 16S copy number than those identified as negative fire-responders (3.6 *vs.* 2.6, $p = 0.01$).

Bacterial and fungal communities become increasingly dissimilar from unburned sites with increasing burn severity in upland sites ($p < 0.001$, Fig. 3B and C). We did not detect such a relationship for wetland sites ($p > 0.05$). Across all sample pairs, there was a significant positive relationship between understory vegetation community dissimilarity and organic horizon microbial community dissimilarity in wetlands ($R_{16S} = 0.62$, $p_{16S} < 0.001$; $R_{ITS2} = 0.44$, $p_{ITS2} < 0.001$) but not in uplands ($p_{16S} = 0.1$; $p_{ITS2} = 0.6$) – *i.e.*, wetland sites with similar understory plant communities have similar microbial communities.

All burn severity metrics added significant (but relatively little) additional predictive power to the model explaining microbial community composition. For bacteria, burn severity index was the best predictor of microbial community composition (partial $R^2_{BSI} = 0.039$, $p = 0.001$), marginally better than burned/unburned (Table 1). For fungi, a simple burned/unburned metric was the best predictor of microbial community composition (partial $R^2_{B/U} = 0.044$, $p = 0.001$), marginally better than burn severity index (Table 1).

There was not a significant relationship ($p > 0.05$) between BSI (or other measures of burn severity) and 16S:18S gene copy numbers as determined by qPCR ($p_{uplands} = 0.19$, $p_{wetlands} = 0.74$). However, there was a significant but weak positive relationship between pH and 16S:18S gene copy numbers as determined by qPCR in uplands ($R^2_{adj} = 0.13$, $p = 0.001$), suggesting an increasing relative abundance of fungi in more acidic upland soils.

There were no significant detectable changes in estimated richness across different vegetation communities or with increasing burn severity for bacteria or fungi (Supplemental Figs. 7 and 8). Evenness was significantly negatively correlated with burn severity index, controlling for vegetation community (ANOVA, $p_{TS2} = 0.01$, $p_{16S} = 0.002$; Supplemental Figs. 7 and 8).

3.2. Specific fire-responsive microbes

There were wide ranges of responses to wildfire within individual phyla. Numerous bacterial OTUs were identified as being significantly enriched (160 OTUs) or depleted (133 OTUs) in burned *vs.* unburned sites, after controlling for vegetation community, total C, and pH (Fig. 4A; Supplemental Table 8). About half of the responsive OTUs were at least 97% ID similar to the globally dominant phylotypes identified by Delgado-Baquerizo et al. (2018). The most abundant bacterial OTU across samples (average 4% in burned samples *vs.* average 0.09% in unburned samples) was identified as a positive fire responder and was classified as an *Arthrobacter* sp. The third most abundant OTU (average 2% of the community in burned samples and not detected in unburned samples) was also identified as a positive fire responder and was classified as a *Massilia* sp. Of the bacterial taxa identified as being fire-responsive, different OTUs also showed different trends with burn severity (Fig. 4B): the relative abundance of the *Arthrobacter* sp. (OTU sq1 and sq7 (we use the arbitrarily numbered "sq#" to distinguish specific OTUs)) increased with increasing burn severity ($p < 0.05$). *Aeromicrobium* (OTU sq8), *Blastococcus* (OTU sq20), and *Massilia* (OTU sq3) also increased in relative abundance with increasing burn severity ($p < 0.05$), with the *Massilia* and *Blastococcus* OTUs not even being detectable at any unburned sites.

Numerous fungal OTUs were identified as being significantly enriched (79 OTUs) or depleted (60 OTUs) in burned *vs.* unburned sites, after controlling for vegetation community, total C, and pH (Fig. 5A; Supplemental Table 9). There were wide ranges of responses within classes, with the exceptions of *Dothideomycetes* and *Cystobasidiomycetes* OTUs (which tended to be enriched in burned sites) and *Mortierellomycotina* subdivision (which tended to be depleted within burned sites). Certain fungal OTUs also stood out as fire responders. For example, fire-responsive OTUs included *Neurospora* and *Geopyxis* – genera that include well-known fire-responsive fungi (Jacobson et al., 2004; Vrålstad et al., 1998; Supplemental Note 2). The third most abundant OTU was identified as a fire responder and was classified as *Penicillium* sp. Different fire-responsive OTUs showed different trends with burn severity (Fig. 5B): the relative abundance of *Penicillium* (OTUs sq4 and sq65) and one *Fusicladium* (OTU sq24) increased significantly with increasing burn severity ($p < 0.05$), whereas another *Fusicladium* (OTU sq31), *Calyptrozyma* (OTU sq9), and *Sordariomycetes* (OTU sq6) had a significant fire response, but did not continue to increase in relative abundance with increasing fire severity ($p = 0.11$, 0.15, and 0.23, respectively). There were not consistent response patterns within putative mycorrhizal fungi (Supplemental Table 9).

3.3. Co-occurrence network

The bacterial and fungal consensus network included 3454 edges or connections, 351 bacterial OTU nodes (~2% of all bacterial OTUs), and 250 fungal OTU nodes (~4% of all fungal OTUs) (Fig. 6A, Supplemental Figs. 21–24, Supplemental Tables 10 and 11). However, the OTUs that were retained represented an average of 40% (maximum of 74%) of the community for bacteria and 30% for fungi (maximum of 70%). Of the bacteria in the network, 48% were at least a 97% ID match for one of the globally abundant phylotypes designated by Delgado-Baquerizo et al. (2018). The network had a modularity of 0.58, which is above the threshold of 0.4 suggested to define modular structure (Newman, 2006). We report the properties of the 8 largest modules in Supplemental Table 11. Two of the larger modules (1 and 11) contain numerous OTUs that were identified as fire-responders using the $\log_2$-fold-change approach (55% and 82% of OTUs in the module, respectively), while one of the other larger modules (4) is characterized by negative fire-responders (63%). Module 4 also contains OTUs that were prevalent in wetter sites (Fig. 6C). Module 11 contains OTUs more prevalent at high pH sites, while Module 1 is characterized by OTUs that are more prevalent at low pH sites (Fig. 6B).

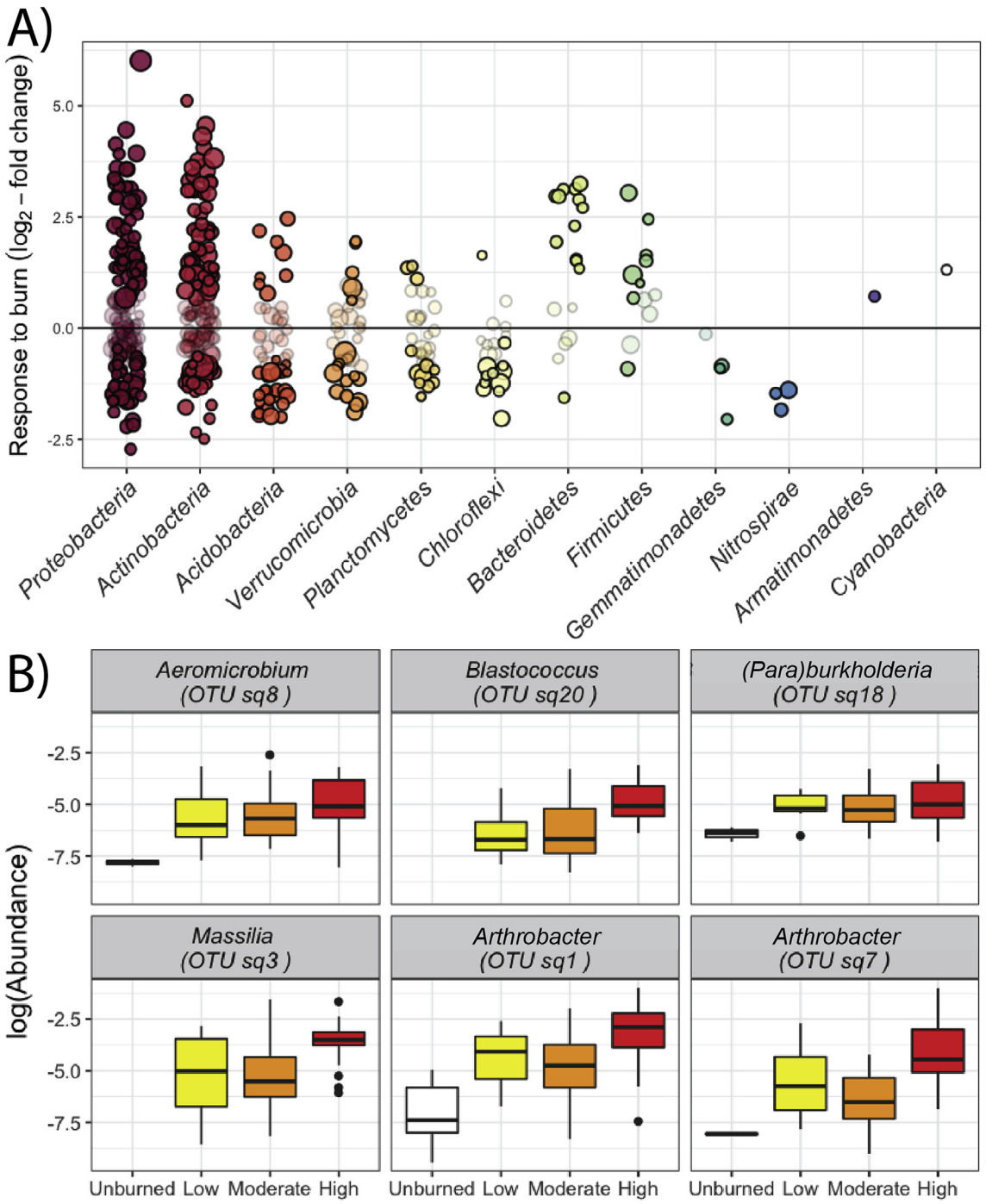

Fig. 4. Bacterial response to fire. (A) Log $_2$-fold change in burned *vs*. unburned plots, controlling for vegetation community, total C, and pH. Each point represents a single 16S rRNA gene v4 region OTU, and the size of each point represents the mean relative abundance of that OTU across all samples. Faint points represent OTUs that were not significantly different in abundance in burned *vs*. unburned plots. (B) Relative abundance (note log scale) of selected fire-responsive bacterial OTUs across BSI ranges (unburned = 0, 0–2 low, 2–3 moderate, 3–4 high).

4. Discussion

4.1. Burn severity metrics are significant predictors of microbial community composition

Given the wide range of soil properties and vegetation communities spanned by our study, we were impressed that the effects of burning on soil microbial communities stood out so clearly, which was counter to our hypothesis that consistent fire effects might not be detectable across this range of sites (Fig. 2). In addition to the effects of fire, our observation that soil communities were most strongly structured by vegetation community, then by pH (although less strongly so for fungi), was consistent with our hypothesis and with previous findings (Fierer and Jackson, 2006; Bahram et al., 2018). High-throughput sequencing data from this region are rare, but Masse et al. (2017) and Turney et al. (2018) report broadly similar microbial communities in northern Alberta to those observed in this study (Supplemental Figs. 1–4). However, the novelty of this study lies not only in characterizing the soil microbial communities of - and the effects of fire in - the northern boreal forest in Canada across a very wide range of conditions. The

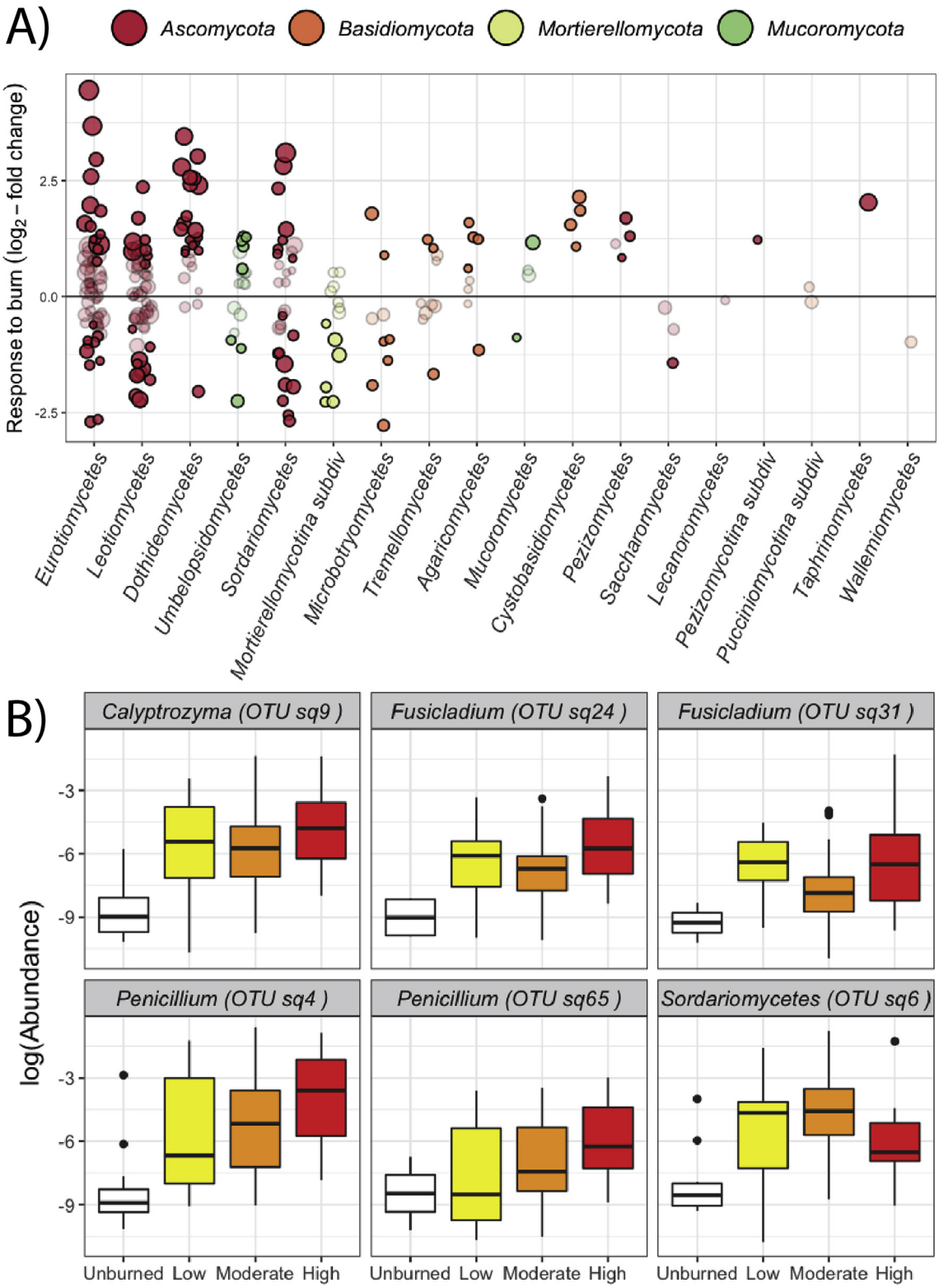

Fig. 5. Fungal response to fire. (A) Log_2-fold change in burned *vs*. unburned plots, controlling for vegetation community, total C, and pH, arranged by class and coloured by phylum. Each point represents a single ITS2 OTU, and the size of each point represents the mean relative abundance of that OTU across all samples. Faint points represent OTUs that were not significantly different in abundance in burned *vs*. unburned plots. (B) Relative abundance (note log scale) of selected fire-responsive fungal OTUs across BSI ranges (unburned = 0, 0–2 low, 2–3 moderate, 3–4 high).

inclusion of burn severity is an important but often "missing piece" in assessing the ecological effects of wildfires, which can range from barely-detectable light surface burns to total tree mortality and complete O horizon losses.

Our data suggest that the predicted increases in burn severity for the boreal forest (Flannigan et al., 2009; Wotton et al., 2017) may be accompanied by increasingly disturbed microbial communities (Fig. 3A and B), although there is little differentiation between different burn

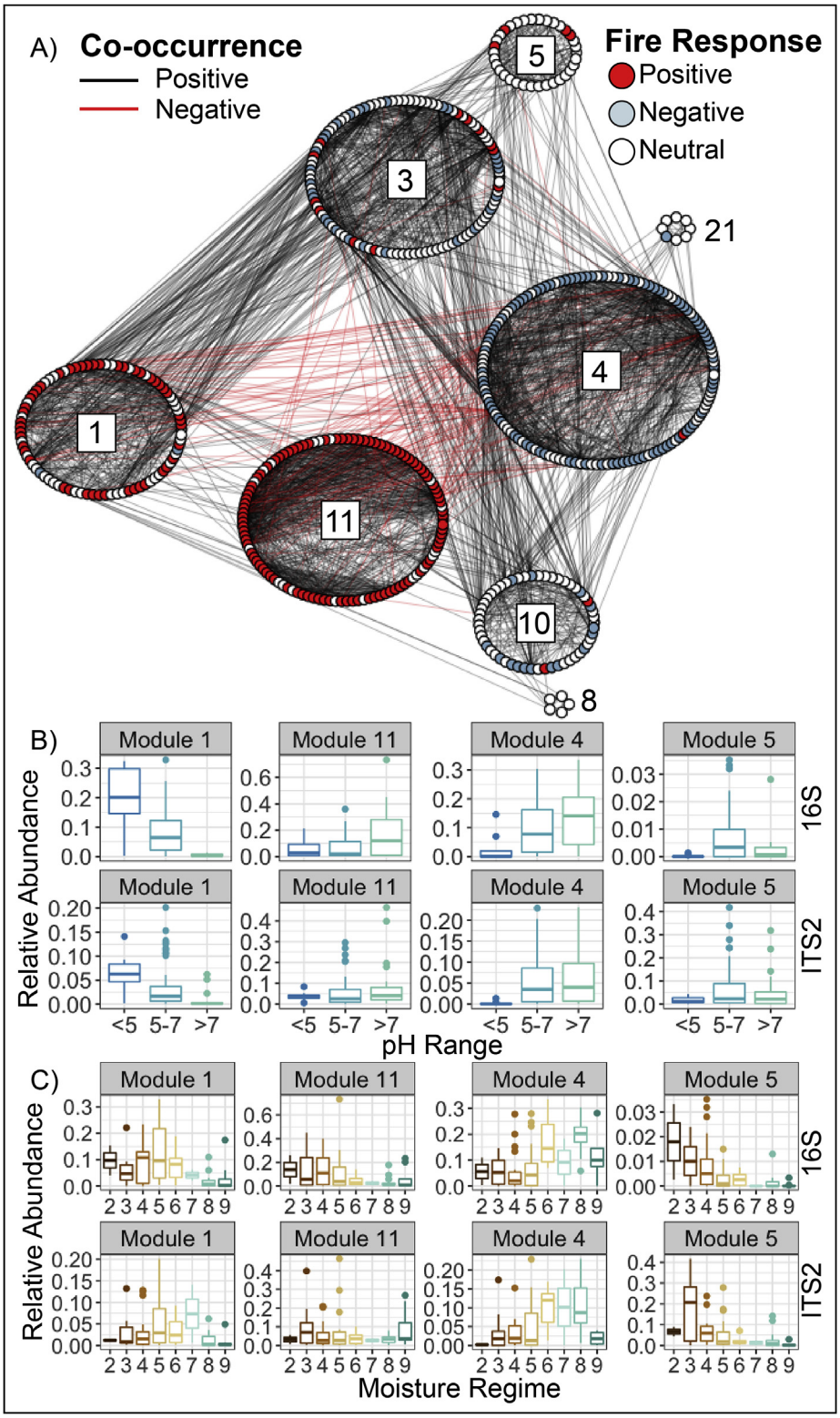

Fig. 6. A) Co-occurrence network [16S and ITS2 - Organic and Mineral Horizons], arranged into greedy clustering-defined modules. Each point represents an OTU. Points are coloured by whether they were identified as being significantly more abundant in burned samples (red) and those significantly less abundant in burned samples (light blue) or no significant response (white). Lines between points indicate co-occurrences (black) or co-exclusion (red). Module IDs are indicated with numbers for reference. B) Module representation across pH values: Fraction of total community represented by all bacterial (top, 16S) and fungal (bottom, ITS2) OTUs within selected modules grouped by pH range. C) Module representation across moisture regimes: Fraction of total community represented by all bacterial (top, 16S) and fungal (bottom, ITS2) OTUs within selected modules, grouped by moisture regime, 2 being very dry, and 9 being very wet. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

severity metrics for predicting microbial community composition. We observed different response trends with severity for different taxa (Figs. 4B and 5B), suggesting that fires evoke non-linear responses with increasing severity, and that the shapes of these responses differ for different fire-responsive taxa. Future studies could be designed explicitly to investigate these nonlinear relationships between community composition and burn severity, possibly developing response-based severity categories. Additionally, the study's sampling timeline (one year post-fire) will affect our detection of different community responses to different severity levels (Ludwig et al., 2017). For example, if the strongest effects of fires on soil microbes are driven by changes to the vegetation community (Hart et al., 2005), these effects may continue to emerge many years post-fire, while the strongest short-term effects of direct killing of microbes from the fire's heat may no longer be detectable one-year post-fire. Furthermore, burn severity metrics – originally developed for plant communities – integrate effects that are

not as relevant to microbes as to plants, diluting their efficacy as predictors of microbial community composition. Focusing on soil-based severity metrics (Vega et al., 2013; Kolka et al., 2014), calculated at sample-level scales, might help strengthen their predictive value. Future investigations could decompose the sub-components of burn severity (*e.g.*, degree of understory vegetation survival *vs*. magnitude of combustion of the organic horizon), to determine which are most influential on microbial community composition, and perhaps develop microbially-specific burn severity metrics.

4.2. Specific bacterial and fungal taxa can be identified as fire-responders

Globally abundant organisms that we identified as positive fire responders are likely relevant across diverse ecosystems. For example, Fernández-González et al. (2017) also observed that both *Arthrobacter* sp. and *Blastococcus* sp. were significantly enriched in post-fire soils, but in a very different ecosystem – holm-oak forests in the Sierra Nevada of Spain. Of their 55 sequenced strains of *Arthrobacter*, 41 isolates were a 100% ID match for our first *Arthrobacter* (OTU sq1) and 11 were a 99% ID match for the second (OTU sq7) (Fernández-González et al., 2017). They speculate that *Arthrobacter* may be able to survive fires due to its ability to resist starvation, desiccation and oxidative stress (Mongodin et al., 2006; SantaCruz-Calvo et al., 2013; Weber et al., 2014; Manzanera et al., 2015). Then, it may thrive on the fire-affected aromatic C sources (Westerberg et al., 2000) and may also play a role in post-fire nitrogen cycling (Cobo-Díaz et al., 2015) and phosphorus solubilization. These activities could have important effects on plant growth: Fernández-González et al. (2017) demonstrated 40% or greater increases in plant biomass in alfalfa plants inoculated with a subset of their *Arthrobacter* strains. Although alfalfa is not a relevant plant to the boreal forest, it will be interesting to see whether these post-fire *Arthrobacter* might affect boreal plants as well. Our results support the suggestion of Fernández-González et al. (2017) that *Arthrobacter* may play an important role in the post-fire microbial ecosystem and expand their findings to a very different ecosystem – the boreal forest.

Our most abundant fungal fire-responder likely also has broad ecological relevance. Ten *Penicillium* sp. OTUs were identified as positive fire-responders (Supplemental Table 9), including two that were particularly abundant (OTUs sq4 and sq65; Fig. 5B). *Penicillium* is a common saprotrophic forest microfungus (Lumley et al., 2011), and may be taking advantage of the post-fire nutrient and C availability. Mikita-Barbato et al. (2015) also noted a *Penicillium* sp. that was found at severely burned pine-oak forests in New Jersey, USA, but was not detected at the unburned sites.

In addition to the two taxa discussed above, many of the fire-responsive genera we identified have previously been identified as being enriched post-fire in other studies of fungi (*e.g.*, *Neurospora* sp. (Jacobson et al., 2004) or *Geopyxis* sp. (Greene et al., 2017); Supplemental Note 2) or bacteria (Weber et al., 2014; Cobo-Díaz et al., 2015; Mikita-Barbato et al., 2015; Sun et al., 2016; Guo et al., 2017) (Supplemental Note 3). Just like well-established fire-response strategies for plants, there are likely a series of fire-response strategies for microbes (conceptual model illustrated in Fig. 7). The first possible trait – fast growth post-fire (as suggested by significantly higher mean predicted 16S gene copy numbers for communities from more severely burned sites, and within positive fire-responders) – may allow a microbe to take advantage of a habitat newly depleted of competitors. Some of the strongest fire-responders (Fig. 4) have particularly high predicted copy numbers – *e.g.*, *Massilia* sp. (OTU sq2) has a predicted copy number of 7, while the *Arthrobacter* sp. (OTU sq1) has one of 5.71. This trait has been previously associated with the bacteria that make up early-successional communities, including another post-fire system (Nemergut et al., 2016). It has been suggested that this trait may allow bacteria to grow more quickly (Klappenbach et al., 2000; Vieira-Silva and Rocha, 2010), allowing them to rapidly take advantage of post-fire resources. Because some of the fire-responders were not even detected in the unburned sites, the question must be asked how they came to dominate the burned sites. Possible explanations include: (1) They were present in the unburned sites, but at numbers so low as to remain undetected with our current sequencing depth; (2) They were transported to the site post-fire, either through standard mechanisms of microbial transport, or even possibly mediated by fire-transported particles and smoke (Kobziar et al., 2018).

A second trait that could allow microbes to thrive post-fire would be the ability to exploit resources created by the fire – for example, changes in nutrient availability (Allison et al., 2010) (Supplemental Note 4) or fire-affected organic matter, which is characterized by an increased abundance of aromatic C structures (Reisser et al., 2016). Many of our most abundant fire-responsive bacterial taxa (*Aeromicrobium*, *Massilia*, and *Burkholderia-Paraburkholderia*) are genetically identical in the sequenced region to organisms that have been identified as aromatic C-degraders (Liu et al., 2014; Guo et al., 2017; Thijs et al., 2018) (Supplemental Note 5). Similarly, numerous fungi with lignolytic capabilities are also able to degrade other aromatic C structures, and include species within the genera *Penicillium* and *Mucor*, for which we identified positive fire-responsive OTUs (Wang et al., 2012; Aydin et al., 2017) (Fig. 5; Supplemental Table 9).

A third potential fire responder trait is survival at elevated temperatures (Peay et al., 2009; Glassman et al., 2016; McGee et al., 2006) (although increased temperatures from fire rapidly attenuate with soil depth (Merino et al., 2018)). Because we sampled sites one-year post-fire, the longer-term effects of fire (changes to the soil environment or vegetation) may be playing a larger role than the immediate post-fire effects of the direct killing of organisms during the fire, which might be most important in the weeks or months right after the fire.

In addition to heat survival, fast growth, and the ability to take advantage of post-fire resources, interactions between fire-responders and other members of the ecosystem, including plants and animals, will structure post-fire communities. For example, one fungal OTU identified as a 100% match with *Fimetariella rabenhorstii* was significantly enriched with fire, and has commonly been found in the dung of boreal herbivores (Krug, 1995). This is consistent with animal studies that suggest that herbivory may increase post-fire as plants regenerate (Strong and Gates, 2009; Eby et al., 2014; Leverkus et al., 2018) and the observation that our study region is inhabited by wood bison that likely benefit from fire clearing grazing areas. With respect to plants, two of the fungal taxa we identified as significant fire-responders were classified as *Fusicladium* sp., and two as *Phoma* sp. These genera are rated as "probable" and "highly probable" plant pathogens within the FUNGuild classification system (Tedersoo et al., 2014; Nguyen et al., 2016), and one interpretation could be that they are exploiting damaged trees post-fire. However, *Fusicladium* has also been isolated from pine litter (Koukol, 2009), and may just as likely be living as a saprotroph on fire-killed litter (Crous et al., 2007) Similarly, *Phoma* is also known to exhibit saprotrophic strategies (Weber et al., 2004). Other putative plant-associated fungi (pathogenic or non-pathogenic endophytes) were enriched in burned sites (three *Venturia* or *Fusicladium* OTUs). However, we are not able to point to clear broad trends across plant-associated fungal guilds, including ectomycorrhizae, in this dataset (Supplemental Table 9). Additionally, we stress that even 100% ID matches may not have the same functional potential or activity as the organisms identified in reference databases; further study would be required to demonstrate these suggestions. Still, such inter-kingdom interactions merit further study, as they could have implications for the post-fire community assembly in plants and fungi, and the effects of post-fire microbial communities on the broader ecosystem.

4.3. Co-occurrence network clusters by fire effects, pH, and moisture regime

The most interesting observation for the network is that the taxa cluster in modules that are associated with fire effects (Fig. 6), and include numerous taxa that are close matches for globally abundant

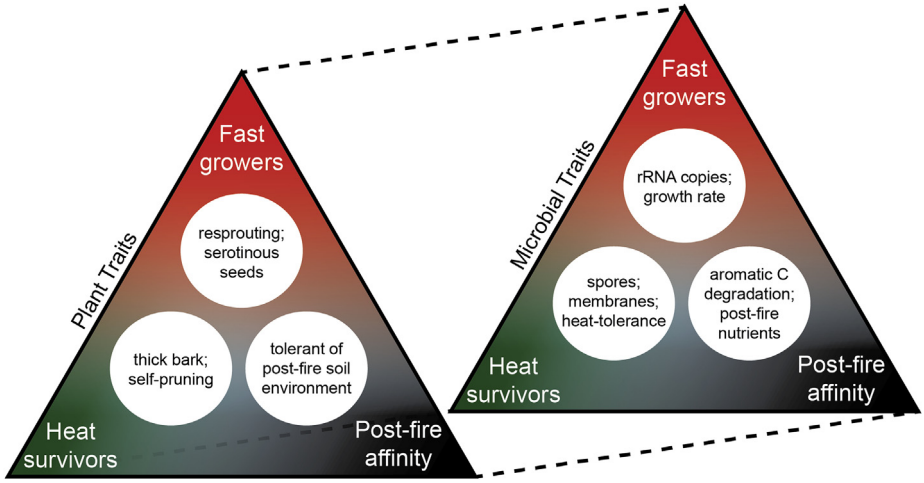

Fig. 7. Conceptual figure of hypothesized parallels between fire response strategies for plants and microbes. Layered on top of these traits would be ecological interactions between organisms in the post-fire community.

taxa. This could prompt future research asking whether the remaining taxa in these modules also respond positively to fire. We also noticed that the two fire-responsive modules (1 and 11) clustered separately – *i.e.*, despite both containing a large proportion of fire-responsive taxa, there are few co-occurrences between the two modules. Our most likely explanation for this is pH: bacterial OTUs from module 1 tend to be more abundant in lower pH soils from across a wide moisture gradient, while bacterial OTUs from module 11 tend to be more abundant in higher pH soils specific to drier ecosystems (Fig. 6C). Thus, we might interpret bacterial OTUs in module 11 as broadly representing the high pH fire-responders, and the bacterial OTUs in module 1 as broadly representing the low pH fire-responders.

Many negative fire-responders are captured by Module 4 (Fig. 6A), which also includes OTUs associated with neutral-high pH (Fig. 6B). This module has a higher abundance in wetlands than other modules do (Fig. 6C; Supplemental Table 11), but this largely reflects that wetlands tended to be less severely burned, not that negative fire-responders are generally adapted to wetlands, *per se*. This raises an interesting question of whether wetlands, which tend to burn less severely, play any notable role in seeding the post-fire recovery and reestablishment of microbial communities within the larger, patchwork, landscape.

5. Conclusions

Despite high cross-site variability, we identified an effect of fire severity on microbial community composition. Building on the efforts of previous studies, our results identify specific fire-responsive microbial taxa and provide support for possible successful post-fire ecological strategies, including fast growth, post-fire resource use, and fire survival. Our findings suggest that accounting for burn severity could improve our understanding of their response to fires, with potentially important implications for ecosystem functions. Overall, the network analysis identifies several clusters of fire- and pH-responsive taxa, which could inform future investigations of whether similar patterns are found in different ecosystems that are also affected by fire and to further disentangle the effects of fire on microbes as mediated by changes to vegetation communities and soil properties. Future studies might investigate the most microbially-relevant sub-components of burn severity metrics, continue to classify and test for specific ecological strategies of fire-responsive microbes, establish the timescale over which these effects persist, and determine how prevalent these specific microbial responses to fire are across different ecosystems. For example, anecdotally, we have observed patches of jack pine in this region that grow back as unusually dense stands of slow-growing trees after very severe fires. It would be fascinating to determine whether this "stalled growth" could possibly be related to shifts in the microbial community, such as the loss of necessary symbionts.

Conflicts of interest

The authors declare no competing interests.

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Appendix A. Supplementary data

All sequences are deposited in the NCBI SRA under bioproject number PRJNA564811 (accessions SRR10097729-SRR10097958 (16S) and SRR10097961-SRR10098185 (ITS2)). Code for the analyses conducted in this paper are available at GitHub.com/TheaWhitman/WoodBuffalo/Paper_Analyses_Figures.

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