SOIL BACTERIAL RESPONSE TO FIRE UNDER HIGH AND LOW MOISTURE CONDITIONS IN THE CANADIAN BOREAL FOREST

by

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CHAPTER 1

INTRODUCTION

Boreal zones cover around 14% of the earth's terrestrial surface and store large amounts of carbon (C) (between 370 and 1700 Pg of C), most of which is held in the soil (Bradshaw and Warkentin 2015). C storage within the boreal forest ecosystem is a balance of C inputs and outputs. Soil bacteria impact ecosystem C cycling by decomposing soil organic matter (OM) and releasing C back to the atmosphere as CO_2. Wildfires play an integral part in C cycling in boreal forest ecosystems. Wildfires vary in intensity, duration, and size, from surface fires that burn litter and duff to crown fires that consume entire trees, reach very high temperatures, and cover tens of thousands of hectares (Whitman et al. 2018; Johnson et al. 2001; Kafka et al. 2001). Wildfires impact soil through multiple processes, including loss of organic matter via combustion, increase in pH, change in nutrient availability, and shifts in vegetation types and microbial community composition (Certini 2005; DeBano 1991; Janzen and Tobin-Janzen 2008).

My research focuses on the impact of fire on soil bacterial communities under high and low moisture conditions in the Canadian boreal forest of Wood Buffalo National Park (WBNP). WBNP is located at the border of Alberta and the Northwest Territories, Canada and spans 44,700 km^2 of boreal plains, boreal shield, and taiga shield ecozones (ESWG 1995). The park is a mosaic of sandy, acidic soils dominated by jack pine forests and organic-rich peat soils (DeLuca and Boisvenue 2012; ESWG 1995). Fires play an integral role in vegetative reproduction and nutrient cycling in this ecosystem. Although fire passes through areas of the

park at seemingly long 50-150 year intervals (Johnstone and Chapin 2006), each fire resets the clock on vegetation succession and alters temporarily nutrient cycles, soil structure, and biological activity (Rowe and Scotter 1973; Zackrisson 1977).

Fire intensity, severity, and temperature

Wildfires are classified by fire intensity, fire severity, and/or burn severity. Fire intensity relates to the rate of energy release and depends on rates of aboveground fuel consumption (Keeley 2009). A fire that burns quickly has a higher intensity than a slower burn. It is rarely possible, let alone practical, to measure the energy release of wildfire. Instead, flame height at a fire's leading front is used to estimate fire intensity. Fire severity is a qualitative measure of the impact of fire on vegetation and soil (Keeley 2009). Metrics such as canopy loss, tree mortality, OM combustion, and watershed stability are used to qualitatively assess the effect of fire (Table 1.1) on the ecosystem (Keeley 2009; Neary et al. 1999). Burn severity is a measure of OM loss from the soil surface (Keeley 2009). Both fire severity and burn severity can be assessed post-fire.

The effect of wildfires on soil surface temperatures can range from no increase in temperature at the soil surface to temperatures greater than 675 °C (Neary et al. 1999; DeBano 1991). The combustion of aboveground fuels – vegetation, coarse woody debris, and litter on the soil surface – produces heat, which is transferred downward into the soil (DeBano et al. 1998). Soil temperatures decimeters below the surface rarely exceed pre-fire levels due to the insulative properties of soil (Neary et al. 1999; Neary et al. 2005). The depth of heat transfer into soil

depends on fire intensity, fire residence time, the abundance and types of OM present, and soil moisture. Higher intensity fires and longer residence times can lead to higher soil temperatures (Keeley 2009). In a laboratory simulation by Hatten and Zabowski (2010), soils subjected to low intensity burns experienced a surface temperature of 272 ºC, which dropped to 23 ºC just 2 cm below the surface. Under a high severity burn, the same soil reached 406 ºC at the surface and 141 ºC at a depth of 2 cm.

Table 1.1. The impact of light, moderate, and high fire severity on temperature, organic matter (OM), plant roots, microorganisms, and nutrients in soil (Adapted from Neary et al. (1999)).

		Fire severity		
Parameters	**Depth in soil**	**Low**	**Moderate**	**High**
Temperature	Surface	250°C	400°C	675°C
	25 mm	100°C	175°C	190°C
	50 mm	< 50°C	50°C	75°C
OM	Surface	partially scorched	mostly consumed	totally consumed
	25 mm	OM distillation started	partially scorched	consumed/scorched
	50 mm	not affected	OM distillation started	OM distillation started
Microbes	Surface	dead	dead	dead
	25 mm	live	selective die-off	dead
	50 mm	live	selective die-off	selective die-off
Roots	Surface	dead	dead	dead
	25 mm	dead	dead	dead
	50 mm	live	live	dead
Nutrient volatilization	Surface	N	N, org P	N, P
	25 mm	none	none	none
	50 mm	none	none	none

Generally, temperatures below the soil surface decrease with increasing soil moisture (Busse et al. 2010). Vaporization of water in soil surface layers and subsequent condensation deeper in the soil is a primary mechanism of downward heat transfer in soil (Neary et al. 2005; DeBano et al. 1998). The likelihood of soil surface OM ignition decreases with increasing mineral content and moisture (Neary et al. 2005). If OM does not combust, it acts as an insulative layer for deeper mineral horizons; however, OM combustion acts as a heat source for the underlying soil (Neary et al. 2005). In summary, the relationships between fire intensity, soil properties, and soil heating are complex.

The impact of fire on soil physical and chemical properties

Wildfires can affect a range of soil properties, including nutrient availability, the presence of soil surface OM, water infiltration, and soil pH. Physical temperature thresholds for alteration of clay, silt, and sand particles are high enough that soil texture is very rarely changed by fire. The lattice structure of clay begins to collapse at temperatures greater than 400 ºC, which are rarely reached below the soil surface (Neary et al. 2005). Common cations in the soil, such as Ca, Mg, Na, P, and K, have high temperature thresholds and are not lost during fire (Neary et al. 1999). Some soil nutrients, such as N, are more easily lost from soils during fires due to low temperature thresholds and OM combustion (Dannenmann et al. 2018; Bormann et al. 2008). OM is vulnerable to fire not only because it is combustible at lower temperatures (Neary et al. 1999), but also because it is usually concentrated in the surface horizon of soil, which experiences the greatest temperatures during a fire (Hatten and Zabowski 2010; Amiro et al.

2011). Peat soils, which are common in WBNP, are usually saturated, which promotes the accumulation of organic material over time and renders these areas less susceptible to fire; however, during periods of long drought, these soils can dry out and become vulnerable to fire (Neary et al. 2005). Dry OM will combust during even low intensity fires (Neary et al. 2005). Once started, fires in peaty soils are extensive and of long duration (McCarty et al. 2020).

Heating and combustion of soil organic matter (SOM) during wildfires impacts nutrient availability, carbon sequestration, water availability, and infiltration (Neary et al. 2005). SOM can be partially or fully combusted during a wildfire. During the combustion of plants and SOM, large quantities of nitrogen are volatilized and lost as N_2 gas. A portion of this nitrogen is translocated downwards in the soil as vaporized OM where it condenses in the underlying soils. N loss is proportional to OM combustion at high temperatures, although at lower temperatures, this relationship does not hold because OM will decompose and N will not volatilize (DeBano 1991). OM begins to break down at temperatures approaching 100 ºC (Neary et al. 2005). The incomplete combustion of organic materials during wildfires produces charcoal, or pyrogenic organic matter (PyOM). PyOM is characterized by polycyclic aromatic structures that lend it biological and chemical stability over centuries to millennia (Ohlson et al. 2009). Carboxylic groups on the aromatic structure increase the nutrient and water holding capacity of PyOM (Zackrisson et al. 1996).

OM combustion and the production of ash during wildfires also impact soil water regimes. Loss of vegetative cover and soil surface litter can lead to an increase in soil erosion (Neary et al. 1999). Heating of organic compounds to 200 to 250 ℃ can also increase hydrophobicity of mineral soil and decrease water infiltration rates (Certini 2005; Neary et al. 1999). Ash

deposition on the soil surface can also decrease water infiltration by clogging soil pores (Bodí et al. 2014).

Soil pH is important in controlling microbial diversity, composition, and abundance as well as nutrient availability (Lauber et al. 2009; Delgado-Baquerizo et al. 2018; Braus and Whitman 2020). Bacterial diversity has been observed to positively correlate with a pH increase from 4 to 8 (Rousk et al. 2010) and peak near a pH of 7 (Lauber et al. 2009). During wildfires, the combination of heat-driven denaturation of organic acids and retention of base cations causes an increase in soil pH (Certini 2005; USDA Forest Service 2005). pH has been shown to influence microbial community composition in boreal forests 1 year post-fire (Whitman et al. 2019).

Soil Microorganisms

In the soil, microorganisms cycle nutrients, decompose OM, form symbioses with plant roots, and influence soil structure. There is evidence that fire can cause a decrease in fungal abundance as well as a decline in microbial richness and diversity (Day et al. 2019; Dooley and Treseder 2012). Wildfires in boreal forests influence microbial communities by causing microbial death, changing soil properties, and producing long-term changes in vegetation (Pingree and Kobziar 2019; Sáenz de Miera et al. 2020; Dooley and Treseder 2012; Hart et al. 2005).

Soil bacterial mortality is influenced by soil temperature, duration of heating, soil moisture,

and the physiological state of bacteria. Mortality generally increases with increasing temperature (Pingree and Kobziar 2019; Köster et al. 2021; Holden et al. 2016; Dunn et al. 1985). Soil bacterial mortality can occur at high temperatures of short duration as well as lower temperatures over longer durations (Pingree and Kobziar 2019). Moisture in the soil can act as an insulator, because large amounts of heat energy are consumed to vaporize water. However, moist heat increases microorganism mortality compared to dry heat (Beadle 1940). In soil heating trials, bacteria that are physiologically active and residing in moist soil are more susceptible to mortality with increasing temperatures than dormant bacteria in dry soil (Dunn et al. 1985; Sella et al. 2014). In general, bacterial endospores are comparatively resistant to damage by heat or desiccation. Spores can survive dry heat of >100 ℃ for 24 hours but are more susceptible to moist heat, which can reduce survival time at a given temperature 1000-fold (Nicholson et al. 2000).

Fire can also alter microbial communities via the production of PyOM (Woolet and Whitman 2020). There is evidence that PyOM may increase microbial activity and decomposition of non-pyrogenic OM (Whitman et al. 2016; Zackrisson et al. 1996), but microbial community response is not consistent across varying soil types and with PyOM derived from various sources and heat treatments (Woolet and Whitman 2020). The effects of burning on soil properties such as pH and carbon availability can persist for years post-fire (Bodí et al. 2014; DeLuca and Aplet 2008; Dove et al. 2020; Úbeda et al. 2005), and there is evidence that microbial community composition may take years to recover to its pre-burn state (Dooley and Treseder 2012; Pressler et al. 2019; Adkins et al. 2020).

Conclusion

In the Canadian boreal forests, fire plays an integral role in the success and maintenance of the ecosystem by influencing chemical, physical, and biological properties of soil. Climate change is driving changes in fire severity and area burned (Flannigan et al. 2000; Gillett 2004). Given the susceptibility of soil microorganisms to high temperatures, increasing fire severity stands to impact bacteria community composition and, by extension, C cycling in boreal forests.

Previous work in WBNP and the surrounding area has found an effect of fires and burn severity on soil microbial communities and identified specific fire-responsive bacterial taxa 1 year post-fire (Whitman et al. 2019). We are interested in specific microbial traits linked to bacterial taxa enriched in the post-fire environment. Microbial traits such as spore-formation, high thermotolerance, fast-growth, and the ability to degrade PyOM may confer an advantage to specific bacterial taxa to survive fires and/or colonize the post-fire environment. This thesis will explore key mechanisms that may be controlling bacterial community composition in the days to months post-fire.

CHAPTER 2

THE EFFECT OF HIGH AND LOW SEVERITY FIRES ON SOIL PROPERTIES AND MICROBIAL RESPIRATION

Introduction

During a wildfire, the combustion of aboveground fuels – vegetation, coarse woody debris, and litter on the soil surface – produces heat that is transferred downward into the soil. Transfer of heat downwards is dependent not only on surface level temperatures during a burn, but also on soil moisture and soil type (DeBano et al.1998; Beadle 1940). Soil surface temperatures during a wildfire can range from less than 250 °C to greater than 675 °C (Neary et al. 1999; DeBano 1991). Temperatures just centimeters below the soil surface can remain low during fire due to the insulative properties of soil (Beadle 1940). Bacterial mortality generally occurs within a temperature range of 50-121 ºC (Neary et al. 1999). Temperatures meeting or exceeding this range result in higher bacterial morality and lower respiration.

This chapter contains a detailed overview of the entire study design and the effects of simulated burns in a cone calorimeter on soil properties including pH, total carbon (C), total nitrogen (N), and microbial respiration. We hypothesized that (a) higher burn temperatures would have a larger effect on soil properties than lower burn temperatures, and (b) increasing burn temperatures would have a negative effect on microbial respiration. We predicted that the

microbial respiration rates in the months following the burns would be lowest in soil that experienced the highest burn temperatures.

Methods

Overview

We used simulated burns in a cone calorimeter, soil incubations, and nucleic acid sequencing to assess microbial traits necessary to thrive in a post-fire environment (Figure 2.1). First, we compared microbial community nucleic acid sequences from soil cores one day after being subjected to laboratory burns under varying moisture conditions and paired control (unburned) cores to identify fire-surviving bacteria (Ch. 3). Second, we used a 5-week incubation post-burn to test for fast-growing bacteria immediately post-fire (Ch. 4). And third, to identify bacteria with an affinity for the post-burn environment with and without the ability to survive

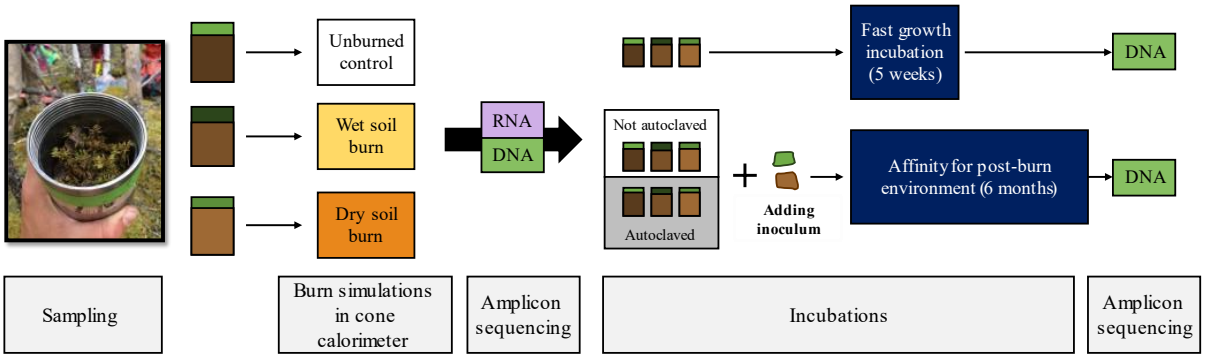

Figure 2.1. Soil samples were collected from Wood Buffalo National Park, and two cores from each sampling site were subjected to simulated burns in a cone calorimeter under wet and dry soil conditions. A third core was used as an unburned control. Soil samples for RNA-based and DNA-based 16S rRNA gene sequencing were collected one day post-burn. Each core was then divided into thirds for a fast growth incubation (5 weeks) and a paired affinity for post-fire environment incubation (6 months). Soil samples for DNA-based 16S rRNA gene sequencing were collected at the end of both incubations.

fire, we autoclaved soil cores one day after the burn treatment to deplete the surviving microbial community, added an unburned soil inoculum, and incubated cores for 6 months (Ch. 5). We used nucleic acid sequencing pre- and post-incubation to identify changes in microbial community composition over these three timepoints.

Site description and sample collection

Soil cores were collected from nineteen sites across Wood Buffalo National Park (WBNP) (Figure 2.2A), Canada over a 6-day period in June 2019. WBNP spans 44,700 km^2 of boreal plains, boreal shield, and taiga shield ecozone (ESWG 1995) sitting at the border of Alberta and the Northwest Territories, Canada. The park has both sandy, acidic soils (Eutric Gleysols and Eutric Cambisols, FAO) and organic-rich peatlands (Dystric Histosols, FAO) underlain by discontinuous permafrost (Box 2.1.; ESWG 1995; DeLuca and Boisvenue 2012; Soil Survey Staff 2014). The dominant vegetation of the sampled regions of the park is jack pine (*Pinus banksiana* Lamb.), trembling aspen (*Populus tremuloides* Michx.), black spruce (*Picea mariana* (Mill.)), and white spruce (*Picea glauca* (Moench) Voss). Mean temperatures range from 13 °C in the summer to -17.5 °C in the winter, and annual precipitation is 300-400 mm (ESWG 1995). Most precipitation falls in the summer (June-September)

Box 2.1. Soil type by system of classification

FAO: Eutric Gleysols (n=7), Eutric Cambisol (n=6), Dystric Histosol (n=6)
Soil Landscapes of Canada map v.3.2: Dystric Brunisol (n=11), Gleysol (n=4), Mesisol (n=2), Organic Cryosol (n=1), Gray Luvisol (n=1)
U.S. soil taxonomy: Inceptisol (Cambisol/ Brunisol), Aqu-suborders (Gleysol), Histosol (Histosol/Mesisol), Cryochrepts/ Eutrochrepts (Eutric Cambisol/Brunisol), Aquents/Fluvents/Aqualfs (Eutric Gleysol)

months. Precipitation during the summer averages 50 mm per month but can range from as little as no precipitation during droughts to >140 mm per month (Environment and Climate Change Canada 2021). For example, in Hay River, located approximately 35 km north of WBNP, summer precipitation in 2014 dropped to 27 mm per month from a summer monthly average of 41 mm and in September 2014, no precipitation was recorded. In Fort Smith, located on the eastern edge of WBNP, monthly precipitation < 3 mm was recorded in June 2007, August 2009, July 2010, and September 2011 (Environment and Climate Change Canada 2021).

Sites were selected using stratified random sampling using dominant vegetation from the Canadian National Forest Inventory and soil type from FAO soil survey data (Table 2.1; Supplementary Information Table S2.1). We used the Canadian National Fire Database (2019)

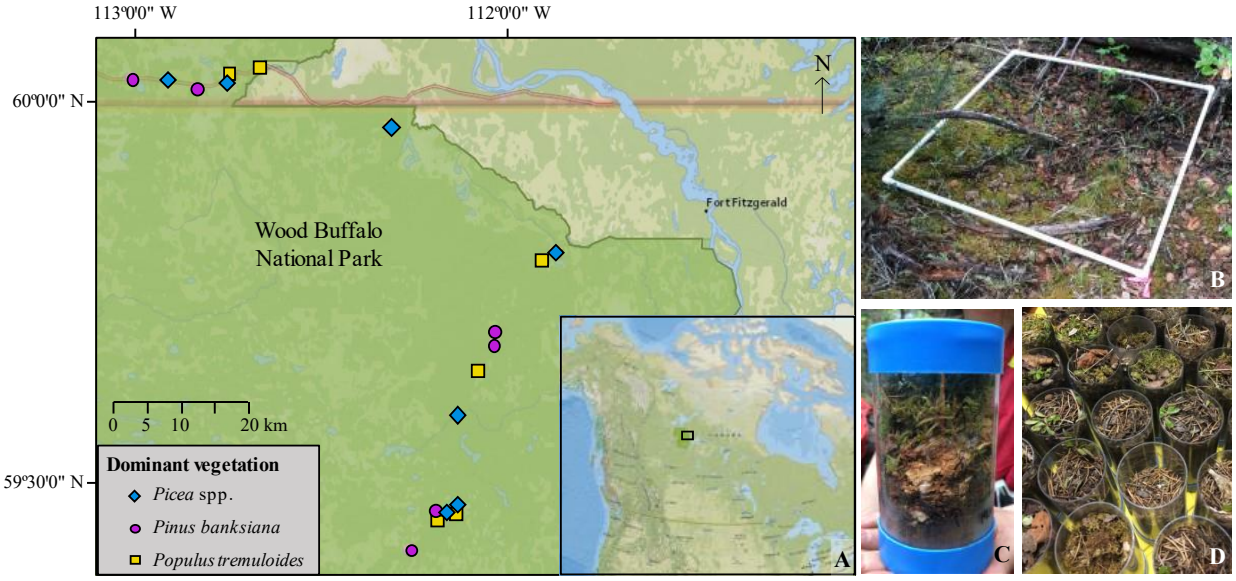

Figure 2.2. (A) Sampling locations within Wood Buffalo National Park, Canada. Blue diamonds, purple circles, and yellow squares indicate *Picea* spp., *Pinus banksiana*, and *Populus tremuloides*-dominated sites, respectively. Inset indicates the relative location of the sampling region within North America. (B) 1 x 1 m grid used during sampling. (C) Example of soil cores collected during field campaign. (D) Soil cores air drying in Madison, WI.

to choose sites that had not burned in at least the previous 30 years. Sites were located between 0.1 and 1 km from roads and > 0.5 km from other sampling sites. We used a Garmin GPSMAP 64 GPS finder to reach each designated location. Upon arriving, we confirmed the dominant tree species and recorded slope and aspect. Samples were collected across a 2 x 2-meter grid. A collapsible PVC pipe square was used to map out the sampling grid (Figure 2.2B). After sampling most field sites, we produced a second selection of random points in a more limited region with field-validated tree species dominance. This second random sample was designed to address identified gaps in species dominance in the initial sample that were the result of errors and limitations in the map products used, bringing us to a total of 19 sites, with 6-7 sites under each dominant vegetation type (Table 2.1).

At each site, ten soil cores (15.24 cm x 7.62 cm dia.) were collected using a soil core sampler with clear plastic core liners and plastic end caps (Product IDs 405.09 and 418.09; AMS, American Falls, ID, USA) every 1 m within (and two at the center of) a 2 m x 2 m grid (Figure 2.2B, C, and D). Cores were labeled and capped, with the top core cap perforated to prevent anoxic conditions from developing. Cores were stored at room temperature or cooler and

Table 2.1. The mean and standard deviation sand, silt, and clay percentages across the three classes of dominant vegetation.

Dominant vegetation	**Sand (%)**	**Silt (%)**	**Clay (%)**
Picea spp. (n=7)	55.3 ± (19.8)	29.7 ± (9.1)	15.0 ± (13.2)
Populus tremuloides (n=6)	63.8 ± (7.1)	26.5 ± (6.0)	9.7 ± (2.3)
Pinus banksiana (n=6)	53.4 ± (22.5)	30.8 ± (14.5)	15.8 ± (10.2)

transported by vehicle to Madison, WI within 14 days of collection. Cores were then placed in a dark room and allowed to dry with top cap removed for 1.5 months, to simulate extreme dry conditions with no precipitation.

Fire simulations

Three cores from each site were chosen for burn simulation based on similarity in soil horizon thickness. Using a cone calorimeter, two of the three cores selected from each site were subjected to a burn treatment under wet and dry soil moisture conditions, respectively, to capture a range of burn temperatures and severities. The first core, referred to as the "wet soil", was saturated with MilliQ water and allowed to free-drain to field capacity 24 hours before the burn treatment, and the second core, the "dry soil", was not wet up (Supplementary Information Figure S2.1). The third core was not wet up and not burned ("control").

In preparation for the burn, the upper 10 cm of each core was extruded (saving the lower soil for moisture measurements), wrapped in perforated ceramic paper (ArtGlassSupplies, NH, USA) to hold the core together while not constraining airflow and combusting itself, and temporarily held together with a string (Figure 2.3, A and B). Thermocouples were inserted 1 cm above the base of the core, to track the least-affected portions of the core, and at the transition between the O horizon and top mineral horizon. For cores that were completely organic, the second thermocouple was placed 5 cm above the core base. The soil core and thermocouples were then placed into a rigid metal mesh cylinder to maintain stability during the burn treatment, the string was removed, and the core was topped with a 0.5 cm diameter

metal mesh to reduce loss of material during burn (Figure 2.3C). Each core was placed under a Mass Loss Calorimeter (Fire Testing Technology Limited, West Sussex, UK) cone calorimeter and exposed to a heat flux of 60 kW m^{-2} for 2 minutes, and a spark igniter located between the top of the core and the cone heater was used to trigger combustion (Figure 2.3E). This heat flux was chosen to represent typical fire front propagation (Silvani and Morandini 2009) and simulate a mid- to high-range crown fire (Thompson et al. 2015; Frankman et al. 2012; Butler et al. 2004). All the dry soil cores experienced some degree of combustion. Some but not all the wet soil cores ignited during the burn treatment.

At the end of the combustion period, each core was removed from the cone calorimeter and left in a fume hood overnight as it continued to burn or smolder and cool (Figure 2.3D). We recorded the temperature from the thermocouples for at least 6 hours, or until the temperature

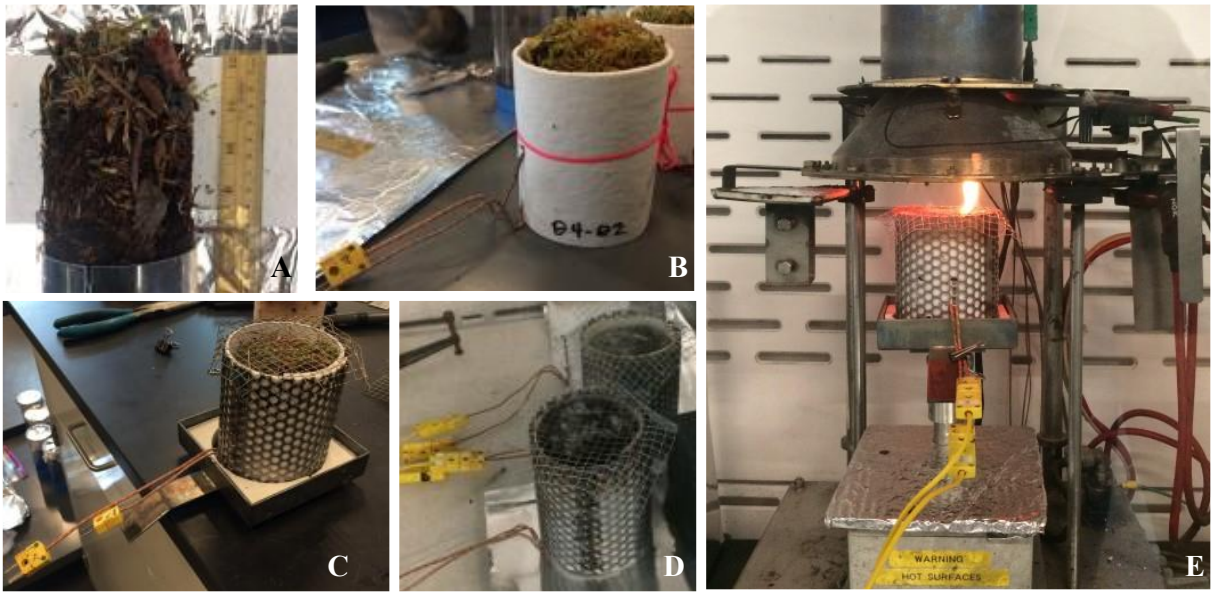

Figure 2.3. The top 10 cm of each soil core was (A) extruded and measured, (B) wrapped in ceramic paper and fitted with two thermocouples, and (C) placed in a metal mesh cylinder to maintain structure and topped with metal mesh to prevent loss of material during burning. (E) Cores were placed in a cone calorimeter and exposed to a heat flux for 2 minutes and (D) then allowed to smolder for up to 24 hours.

returned to within 2 °C of room temperature. We calculated degree hours (DH) as the cumulative degrees above room temperature (21 °C) for each soil horizon as the sum of the difference between each temperature data point (*T)* and 21 °C multiplied by the change in time (Δt) (Equation 2.1; modified from Busse et al. 2005).

$$\sum_{t=t_0}^{t_f} (T(t) - 21) \bullet \Delta t \tag{2.1}$$

Soil Subsampling

Twenty-four hours after the burn, each cooled core was reweighed and the decrease in core height due to the burn was recorded. The O horizon material was separated from the underlying mineral soil and each portion was weighed and homogenized. Subsamples of the O horizon and mineral soil were immediately collected for later nucleic acid extraction and sequencing (testing for survival; Ch. 3) and placed in a -80 °C freezer in a Whirl-pak bag (Nasco®, Fort Atkinson, WI, USA). Additionally, 1-2 g of the O horizon and mineral soil were weighed, placed in a drying oven at 105 °C for 24 hours, and reweighed to calculate post-burn moisture content. The remaining soil was placed in a 4 °C refrigerator for 24 hours prior to initiation of the incubations.

Because we could not destructively sample the cores to calculate their exact moisture content before the burns, we estimated it two ways. First, the unburned soil remaining below the 10 cm burned core was bagged and mixed. 1-2 g of this soil was portioned out, weighed, placed in a drying oven at a temperature of 105 °C for at least 24 hours, and reweighed to calculate pre-burn moisture content at the bottom of the core. For the second method, we measured the

thickness of the organic and mineral horizons in the burned cores before the burn treatment. We then used the soil moisture and bulk density of the organic and mineral horizons of the control cores as an estimate for the pre-burn soil moisture of the burned cores normalized by soil horizon thickness.

Incubations

Parallel incubation experiments of two durations were set up to test for fast growth (1 jar; 5 weeks; Ch. 4) and affinity for the post-fire environment (paired jars; 6 months; Ch. 5). Soil from the horizons of each core was divided equally between the three jars to recreate original soil profiles proportionally. From this point, the methods differed based on the specific incubation and treatment as follows:

I. **Fast growth incubation (5 weeks):** Mineral soil from each sample (where present) was placed into a sterilized and acid-washed 60 mL glass jar and then topped with soil from the sample's respective O horizon.

II. **Post-fire environment (6 months):** Mineral soil and the O horizon from each core were separated and divided equally into two sterilized and acid-washed 60 mL glass jars. The first jar was capped with tinfoil and autoclaved at 121 °C and 20 psi for 20 minutes. Samples were removed from the autoclave and allowed to cool to room temperature. The second jar was not autoclaved. All samples were then inoculated with soil from an unburned core from the same site. Of the remaining cores from each site,

we selected the one that best resembled the three experimental cores to use as the unburned inoculum core and deconstructed it into homogenized O horizon and mineral soil. Each long-term incubation sample received an inoculum representing 10% on a dry sample mass basis by horizon (Lankau et al. 2011; Lankau 2009)**.** Sample and inoculum were thoroughly mixed, and then the mineral and O horizons were recombined into a single jar, resulting in two paired jars – one autoclaved and one not – for all cores.

For all incubations, we ensured that soil was packed to match the wet bulk density of the original core as recorded during the destructive sampling process post-burn treatment. Bulk densities ranged from 0.005-0.29 g cm^{-3} for the O horizon and 0.25-0.61 g cm^{-3} for the mineral soil. Finally, deionized water was added to each sample to bring all samples to equivalent soil moisture across all incubations from a given site. Target soil moistures were based on a water holding capacity of 175% g H_2O per g dry soil for O horizons and 55% water filled pore space for mineral soil. Seven of the 19 sites contained O horizons that we classified as Fibric Histosols, in which case we used a target moisture of 285% g H_2O per g dry soil, which is consistent with peatland moistures (Rydin and Jeglum 2013).

Gas flux tracing

Each sample jar was placed in a pint-sized Mason jar with 15 mL of 0.5 M KOH solution in a scintillation vial and 5 mL of CO_2-depleted (via boiling) water in the bottom of the jar to maintain humidity. Mason jars were sealed and stored in the dark. We measured CO_2 absorbed

by the KOH base trap after three days and replaced the trap with a fresh vial of 0.5 M KOH (Anderson 1983). We continued these measurements every 7-14 days. The fast growth incubations were stopped after 5 weeks. The post-fire environment incubations were continued for 6 months.

Base trap absorption of grams of CO_2 was calculated as the proportion (*P*) of the total base trap capacity for CO_2 absorption (*CCA*) that is used, multiplied by the trap capacity (modified from Strotmann et al. 2004). *P* is calculated as (Equation 2.2):

$$P = \frac{EC_{init} - EC_{sample}}{EC_{init} - EC_{sat}} \quad (2.2)$$

Where EC_{init}, EC_{sample}, EC_{sat} are the electrical conductivity of the absorbing OH ion solution initially (0.5 M KOH), at the time of measurement, and at full saturation (0.25 M KOH), respectively. The base trap capacity for absorption of g CO_2 is calculated as (Equation 2.3):

$$CCA = V \times C \times R \times M \quad (2.3)$$

Where *V* is the volume of the absorbing OH ion solution (0.015 L), *C* is the initial concentration of KOH, *R* is the mole to mole ratio of KOH present to CO_2 absorbed (0.5), and *M* is the molecular mass of CO_2 (Strotmann et al. 2004).

Soil property measurements

Soil pH measurements were taken following the protocol laid out in Braus and Whitman (2020). Briefly, we mixed mineral and organic soil with a 0.1 M $CaCl_2$ solution in 1:1 and 1:5 soil:solution ratios, respectively. Samples were incubated at room temperature and vortexed every 20 minutes for 1 hour. Samples were centrifuged at 10,000 x g for 1 minute, and pH of the supernatant was measured using a pH electrode (Orion Star A215, Thermo Fisher Scientific, MA, USA).

Two additional unburned cores from each site were destructively sampled for a texture analysis. Mineral horizon soil was oven dried at 105 °C for 24 hours. The sample was hand-ground to break up large aggregates and sieved to ≤ 2 mm diameter. Soil clay, silt, and sand content was determined at the UW-Madison Soil and Forage Lab using a physical analysis hydrometer with readings taken at 40 seconds and 7 hours. Soil texture of mineral horizons was classified as sand, sandy loam, loam, or clay loam based on percentage clay, silt, and sand. For cores lacking mineral horizons, we designated the soil texture as 'organic' (Table 2.2).

To measure C and N, soil was first dried at 105 °C for 24 hours. A subsample of 7-50 mg soil was finely ground for 2 minutes using a SpexMill 8000D ball mill (Spex, Metuchen, NJ, USA) and analyzed for total C and total N via combustion at 900 °C using a Flash EA 1112 CN Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy). Results were standardized against medium and low organic content soil and clay (Product IDs B2178, B2152, and B2184; EA Consumables, Pennsauken, NJ, USA).

Soil C pools

A 2-pool decay model was used to calculate respiration rate constants and fractional size of active and slow C pools (Equation 2.4).

$$M_t = (M_1 \times e^{-k_1 t}) + (M_2 \times e^{-k_2 t}) \quad (2.4)$$

Where M_t is the total C pool, M_1 and M_2 are the active (or fast) and slow C pools, respectively, *k1* and *k2* are the respiration rate constants for the fast and slow C pools, respectively, and *t* is time. This model was fit using the *nls.lm* function in the *minpack.lm* package in R.

Statistical analyses

We used two-way ANOVAs with Tukey's post-hoc tests to test the relationship between burn treatment, dominant vegetation, and fractional dry mass loss. pH change was calculated as the difference in pH of burned cores and the corresponding control core for each horizon and site. This calculation was repeated for total C, total N, and C:N. Simple linear regressions were used to test for significant correlations between DH and fractional dry mass loss,

Table 2.2. Dominant vegetation and soil texture at each site.

Site ID	Dominant Vegetation	Soil texture
1	*P. banksiana*	clay loam
2	*P. tremuloides*	sandy loam
3	*P. banksiana*	loam
4	*Picea* spp.	sandy loam
5	*P. tremuloides*	sandy loam
6	*Picea* spp.	sandy loam
7	*Picea* spp.	organic
8	*P. tremuloides*	sandy loam
9	*P. banksiana*	sandy loam
10	*P. banksiana*	loam
11	*P. tremuloides*	sandy loam
12	*Picea* spp.	organic
13	*Picea* spp.	organic
14	*P. tremuloides*	sandy loam
15	*P. tremuloides*	sandy loam
16	*P. banksiana*	loam
17	*Picea* spp.	organic
18	*Picea* spp.	clay loam
19	*P. banksiana*	sand

soil pH, total C, total N, and C:N. We used QQ-plots and Levene's test to test for normal distribution of data and variance homogeneity prior to ANOVAs and simple linear regressions. We tested for normality using QQ plots and used Kruskal-Wallis to test for differences in pH change and C:N change between the dry and wet soil burn treatment controlling for dominant vegetation.

Results

Description of burns

Most of the dry and wet soil burn cores experienced some degree of flaming combustion. Dry soil burns ignited within seconds of the beginning of the simulation and continued to smolder after being removed from the heat source. Most combustion was observed to be constrained to the O horizon. Wet soil combustion was less extensive than that of the dry soil burns. We observed no ignition in two wet soil burns, both of which were organic-rich cores from *Picea* spp.-dominated sites.

Maximum temperatures during the dry soil burns ranged from 30-587 °C at the O horizon-mineral soil interface (Supplementary Information Figure S2.3). Four of the 7 dry soil burns from *Picea* spp.-dominated sites exceeded 500 °C, and 2 of the 6 dry soil burns from *P. banksiana*-dominated sites exceeded 500 °C. The average cumulative time above 500 ºC was less than 1 hour (Supplementary Information Table S2.2). At the core base, maximum temperatures ranged from 26-529 °C across all dry soil burns. Dry soil burn DH ranged from

25-1199 and 27-1128 °C·hrs for the O horizons and mineral soil, respectively (Supplementary Information Table S2.3). Soil temperatures in the dry soil burns returned to room temperature (21 °C) within 7.5 hours of the burn treatment.

Temperatures during the wet soil burns were lower (Figure 2.4). Maximum temperatures during the wet soil burn treatments ranged from 23-42 °C at the organic-mineral interface (Supplementary Information Figure S2.2). At the core base, maximum temperatures ranged from 24-35 °C across all wet soil burns. Wet soil burn DH ranged from 4-26 and 5-39 °C·hrs for O horizons and mineral soil, respectively (Supplementary Information Table S2.3). Soil temperatures in the wet soil burns returned to room temperature within 5 hours of the burn treatment.

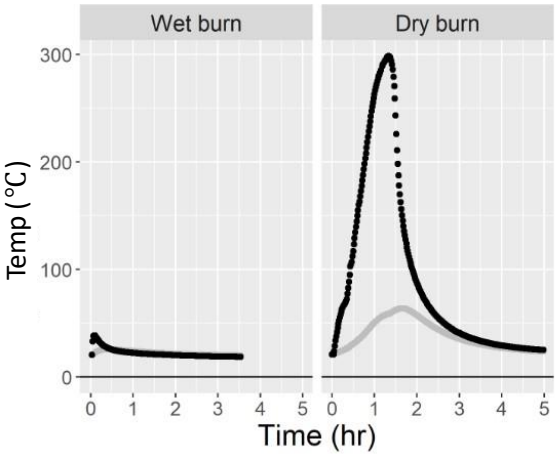

Figure 2.4. Soil temperatures in a wet soil core (left) and dry soil core (right) during and up to 5 hours after the burn treatment. The soil surface was exposed to a 60 kW m^{-2} heat flux for 2 min., after which the cores were left to cool. Temperature was recorded 5 cm (black) and 9 cm (grey) below the surface.

Soil Mass Loss

Mean soil mass of the unburned O horizons ranged from 25.0 g (SD = 16.8 g) in the *P. banksiana*-dominated sites to 63.2 g (SD = 46.1 g) in the *Picea* spp.-dominated sites. Mean soil mass of the unburned mineral horizons ranged from 83.4 g (SD = 94.1 g) in the *Picea* spp.-dominated sites to 247 g (SD = 69.3 g) in the *P. banksiana*-dominated sites. Soil fractional dry mass loss was significantly higher in the dry soil burns (mean = 0.19, SD = 0.182) than in the wet soil burns (mean = 0.10, SD = 0.06) (Figure 2.5; Supplementary Information Table S2.4).

Soil fractional dry mass loss of combined O horizon and mineral soil (where present) increased significantly with DH (y = 0.0002x + 0.078; simple linear regression, $p < 0.001$). Within the wet soil burns, fractional dry mass loss was significantly higher in samples from *Picea* spp.-dominated sites than in samples from *P. banksiana*-dominated sites (ANOVA, $p = 0.001$). There was no significant difference in dry mass loss between vegetation types during the dry soil burns.

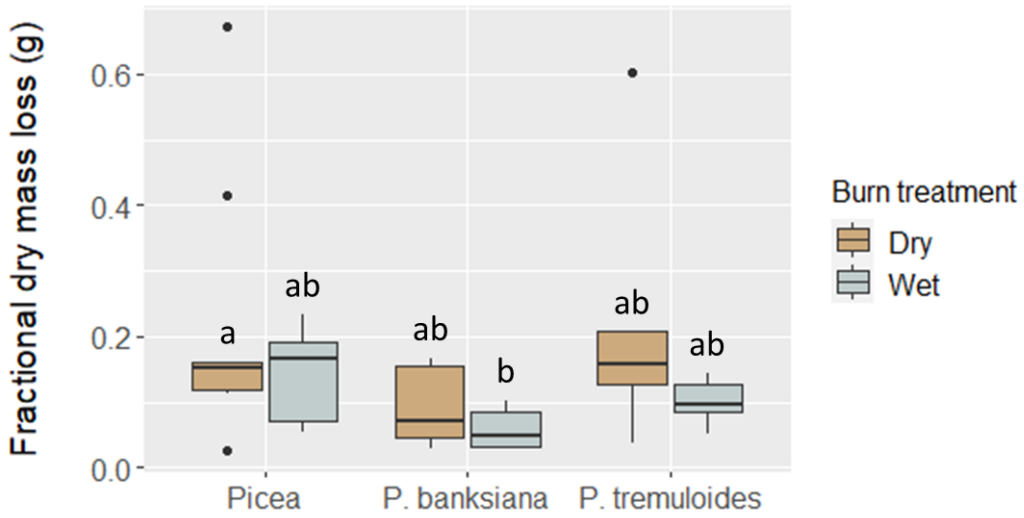

Figure 2.5. Boxplot and results of Tukey HSD post-hoc test between fractional dry mass loss, dominant vegetation, and burn treatment. Different letters indicate statistically significant differences ($p < 0.05$).

Soil pH

Mean soil pH of unburned organic horizons was 4.2 units (SD = 0.5) in the *P. banksiana*-dominated sites and 4.8 units (SD = 1.2) in the *P. tremuloides*- dominated sites (Table 2.3). Mean soil pH of unburned mineral horizons was 3.9 units (SD = 0.1) in the *Picea* spp.-dominated sites and 4.8 units (SD = 1.2) in the *P. tremuloides*-dominated sites. Soil pH was significantly affected by burn treatment in both O horizons and mineral soil compared to

Table 2.3. Unburned soil pH, total C and N, and soil horizon thickness. Standard deviation in parentheses.

Dominant vegetation	**Soil horizon**	**pH (SD)**	**total C, % (SD)**	**total N, % (SD)**	**Horizon thickness (cm)**
Picea spp.	O (n=7)	4.5 ± (1.0)	37.9 ± (9.3)	1.7 ± (0.5)	8.9 ± (1.9)
	Mineral (n=2)	3.9 ± (0.1)	1.6 ± (0.0)	0.1 ± (0.0)	3.8 ± (1.1)
P. tremuloides	O (n=6)	4.8 ± (0.3)	19.6 ± (9.7)	1.4 ± (0.8)	7.3 ± (3.0)
	Mineral (n=3)	4.8 ± (1.2)	1.3 ± (0.4)	0.1 ± (0.0)	5.3 ± (1.2)
P. banksiana	O (n=6)	4.2 ± (0.5)	23.2 ± (9.6)	1 ± (0.5)	5.4 ± (0.5)
	Mineral (n=6)	4.2 ± (0.8)	0.9 ± (0.2)	0.1 ± (0.0)	4.6 ± (0.5)

unburned soil pH. pH in O horizons subjected to the dry soil burn was higher than in the control soils by a mean of 2.4 units (SD = 1.4; ANOVA, $p < 0.0001$), and pH in O horizons subjected to the wet soil burn was higher than in the control soils by a mean of 0.34 units (SD = 0.38; ANOVA, $p < 0.0001$) (Figure 2.6; Supplementary Information Table S2.5). pH in mineral soil subjected to the dry soil burns was higher than in control soils by a mean of 0.36 units (SD = 0.73; ANOVA, $p < 0.0001$), and pH in mineral soil subjected to the wet soil burns did not differ significantly from pH in control soils.

Soil pH increased significantly with DH in both O horizons and mineral soil of wet soil and dry soil burns compared to control soil pH. pH increases in O horizons with DH ranged from -0.2 units / °C•hr to 4.2 units / °C•hr (simple linear regression, $p < 0.001$) (Figure 2.6). Soil pH increases in mineral horizons with DH ranged from -0.8 units / °C•hr to 2.0 units / °C•hr

(simple linear regression, $p < 0.001$). The pH increase did not differ significantly across vegetation types for either organic (ANOVA, $p = 0.76$) or mineral horizons (ANOVA, $p = 0.49$).

Soil carbon and nitrogen

Mean soil C content of unburned O horizons ranged from 19.6 % (SD = 9.7) in the *P. tremuloides*- dominated sites to 37.9 % (SD = 9.3) in the *Picea* spp.-dominated sites. Mean soil C content of unburned mineral soil ranged from 0.9 % (SD = 0.2) in the *P. banksiana*-dominated sites to 1.6 % (SD = 0.0) in the *Picea* spp.-dominated sites. Mean C content was significantly affected by burn treatment in the O horizons. O horizons subjected to the dry soil burn had a mean decrease in C of 11.7 % (SD = 14.8; ANOVA, $p = 0.011$). Mean C content of O horizons subjected to the wet soil burn ranged from 7.92 to 47.3 % and did not differ significantly from the unburned soil. The mean C content of mineral soil did not differ significantly by burn treatment. Mineral soil subjected to dry soil burns and wet soil burns had a mean C content of 2.0 % (SD = 1.7) and 1.7 % (SD = 0.8), respectively. Mean C content of O horizons significantly decreased with increasing DH (simple linear regression, $p = 0.002$), but this correlation also ranged widely from -33.4 % / °C•hr to +32.0 % / °C•hr. Mean C content of mineral soil ranged from -0.76 % / °C•hr to +1.19 % / °C•hr and did not differ significantly with DH ($p = 0.14$).

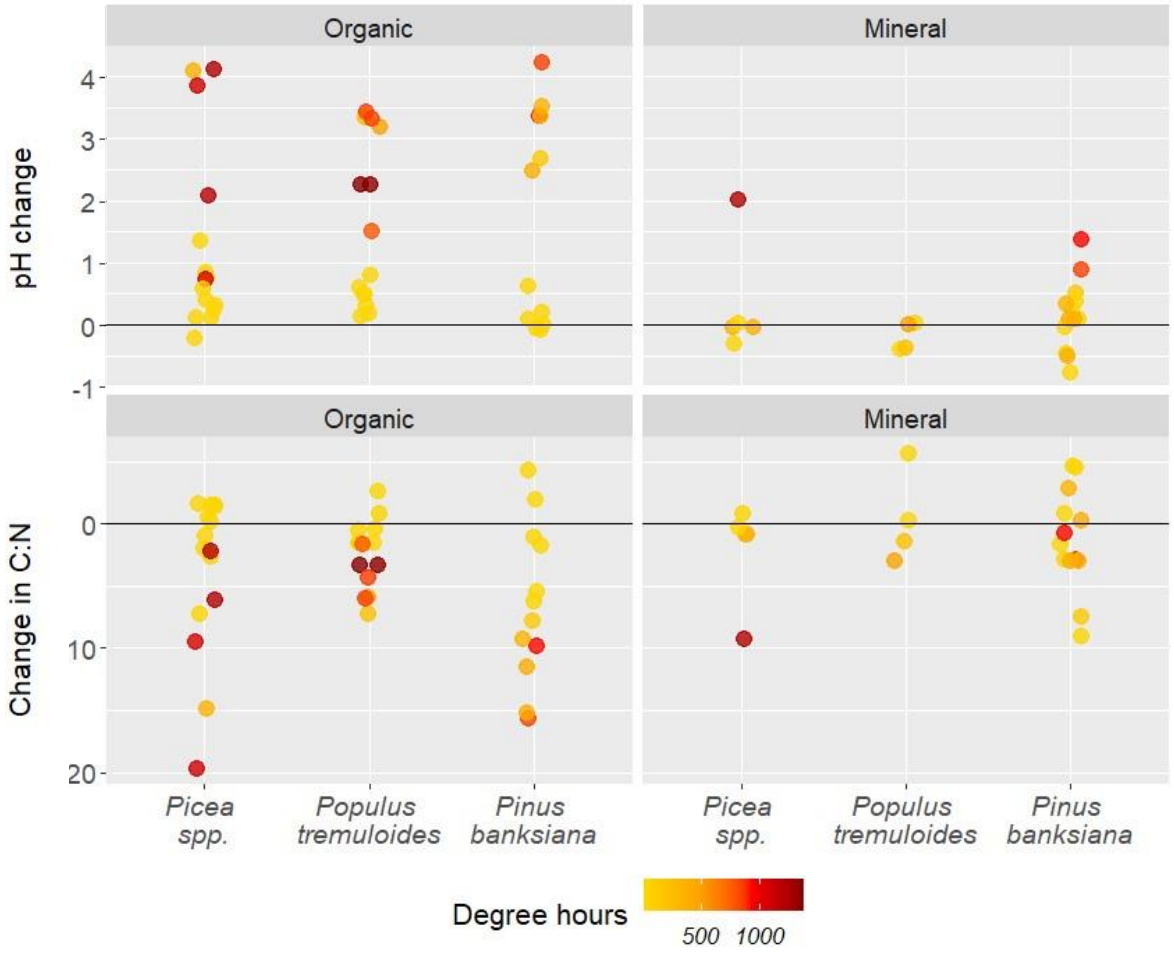

Figure 2.6. Change in pH and C:N in the O horizon and mineral soil of dry and wet soil burns across the three dominant vegetation types. Color represents DH with darker colors indicating higher DH.

Mean soil N content of unburned O horizons ranged from 1.0 % (SD = 0.5) in the *P. banksiana*-dominated sites to 1.7 % (SD = 0.5) in the *Picea* spp.-dominated sites. Mean soil N content of unburned mineral soil was 0.1 % (SD = 0.0) across all dominant vegetation classes. Mean N content was not significantly affected by burn treatment or DH in either the O horizon or mineral soil. O horizons subjected to dry soil burns and wet soil burns had a mean N content of 1.3 % (SD = 0.9) and 1.5 % (SD = 0.7), respectively. Mineral soil subjected to dry soil burns

and wet soil burns had a mean N content of 0.2 % (SD = 0.4) and 0.1 % (SD = 0.1), respectively.

Mean soil C:N of unburned O horizons ranged from 14.8 (SD = 2.1) in the *P. tremuloides*-dominated sites to 24.1 (SD = 4.0) in the *P. banksiana*-dominated sites. Soil C:N was significantly lower in the O horizons of the dry soil burns compared to control cores. C:N in O horizons subjected to the dry soil burn was lower than in the control cores by a mean of 8.0 (SD = 5.5; ANOVA, $p < 0.0001$). Mean difference in soil C:N in the O horizons subjected to the wet soil burn compared to the control cores ranged from -7.3 to +4.4 but did not differ significantly from zero. Mean soil C:N of unburned mineral soil ranged from 12.5 (SD = 2.5) in the *P. tremuloides*-dominated sites to 16.3 (SD = 4.7) in the *P. banksiana*-dominated sites. Soil C:N did not differ significantly in the mineral soil with burning. Mean difference in soil C:N in the mineral soil compared to the control cores subjected to the dry soil burns and wet soil burns ranged from -9.3 to +2.9 units and -9.0 to +5.7 units, respectively. Soil C:N decreased significantly with DH in both O horizons (simple linear regression, $p = 0.0005$) and mineral soil ($p = 0.03$). The decrease in C:N did not differ significantly across vegetation types for either O horizons or mineral soil.

Soil Microbial Respiration

Two-pool exponential decay models resulted in good fits across all samples (R^2 of 0.98 to 1) (Supplementary Information Figure S2.4 and S2.5), with about 1% of the total C in the fast

pools. Respiration rates were highest at the beginning of the incubation and decreased with time (Supplementary Information Figure S2.7 and S2.8).

In the fast-growth incubation, the fractional size of the active (*i.e.*, fast) C pool (M_1) of the unburned cores ranged from 0.006 to 0.008 of the total C pools and was significantly affected by burn treatment (Figure 2.7A; Supplementary Information Table S2.6). The fast C pool of the wet soil burn cores (mean 0.014, SD = 0.008) was larger than in the dry soil burn cores (mean = 0.005, SD = 0.004; Wilcoxon signed-rank test, $p = 0.012$) or in the unburned controls (mean = 0.007, SD = 0.007; Wilcoxon signed-rank test, $p = 0.003$). The size of the fast C pool increased with DH in the dry soil burn cores from the fast-growth incubation (simple linear regression, $p = 0.001$; Supplementary Information Figure S2.9). The fast C pool decay rate coefficient (k_1) was lower for the dry soil burn cores (mean = 0.074, SD = 0.067) than for the wet soil burn cores (mean = 0.057, SD = 0.118; Wilcoxon signed-rank test, $p < 0.001$) or the unburned cores (mean = 0.128, SD = 0.23; $p < 0.001$) (Figure 2.7C). There was no significant difference between the fast C pool size or decay rate coefficient across vegetation types in the fast-growth incubation.

In the fast-growth incubation, the slow C pool decay rate coefficient (k_2) was slightly higher for the unburned soil (mean = 4.6×10^{-5}, SD = 6.1×10^{-5}) than for the wet soil burn cores (mean = 2.8×10^{-5}, SD = 7.7×10^{-5}; Wilcoxon signed-rank test, $p = 0.03$) (Supplementary Information Table S2.7). There was no significant difference between k_2 of the dry soil burn cores and the wet soil burn cores (Wilcoxon signed-rank test, $p = 0.11$) or the unburned soil (Wilcoxon signed-rank test, $p = 0.10$).

In the post-fire environment incubation, the fractional size of the fast C pool of the unburned cores ranged from 0.005 to 0.011 in the autoclaved cores and 0.011 to 0.017 in the unautoclaved cores. The fast C pool was significantly affected by autoclaving, burn treatment, and O horizon thickness. The fast C pool was significantly smaller in autoclaved cores (mean = 0.008, SD = 0.006) than in unautoclaved cores (mean = 0.014, SD = 0.21; Wilcoxon signed-rank test, $p < 0.001$). The fast C pool of the dry soil burn cores (mean = 0.005, SD = 0.005) was significantly smaller than the wet soil burn cores (mean = 0.017, SD = 0.024; Wilcoxon

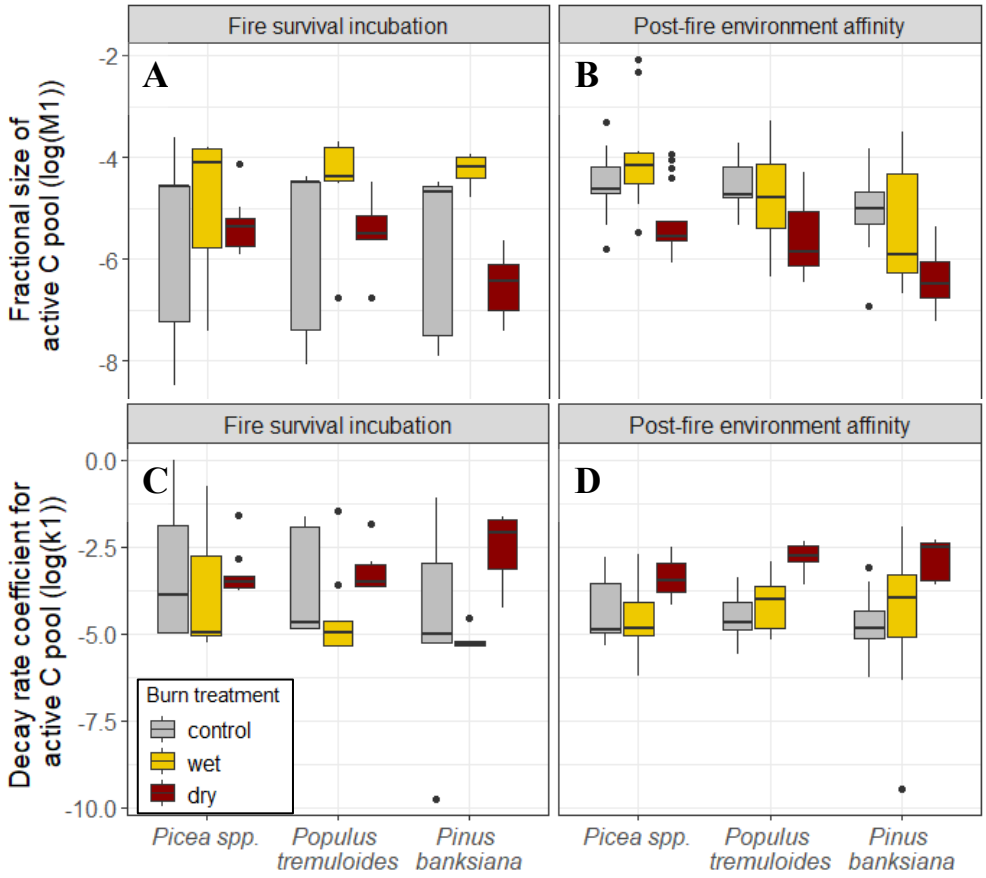

Figure 2.7. Fractional size of C in the fast pool (note log scale) grouped by dominant vegetation in the (A) fire-survival incubation and (B) post-fire environment affinity incubation. Decay rate coefficient for the fast C pool (note log scale) grouped by dominant vegetation in the (C) fire-survival incubation and (D) post-fire environment affinity incubation. Colors indicate burn treatment with grey, yellow, and red representing unburned soil, wet soil burn cores, and dry soil burn cores, respectively.

signed-rank test, $p < 0.001$) and the unburned cores (mean = 0.011, SD = 0.008; Wilcoxon signed-rank test, $p = 0.004$). There was no significant difference between the size of the fast C pool of wet soil burn cores and unburned cores (Figure 2.7B). The fast C pool of soil cores containing only O horizon and no mineral soil (mean = 0.019, SD = 0.029) was significantly larger than soil cores with both O and mineral horizons (mean = 0.032, SD = 0.032; Wilcoxon signed-rank test, $p = 0.0001$). There was no significant difference between the size of the fast C pool across vegetation types. The size of the fast C pool increased with increasing DH in both the autoclaved and not autoclaved dry soil burn samples from the post-fire environment incubation (simple linear regression, $p < 0.0001$; Supplementary Information Figure S2.9).

In the post-fire environment incubation, k_1 of the control cores ranged from 0.006 to 0.062 in the autoclaved cores and 0.002 to 0.044 in the unautoclaved cores. k_1 was significantly affected by autoclaving, burn treatment, dominant vegetation, and soil texture. Autoclaved cores had a higher k_1 (mean = 0.04, SD = 0.031) than unautoclaved cores (mean = 0.027, SD = 0.028; Wilcoxon signed-rank test, $p = 0.0002$). In the dry soil burn cores, k_1 (mean = 0.059, SD = 0.027) was significantly higher than for the wet soil burn cores (mean = 0.025, SD = 0.029; Wilcoxon signed-rank test, $p < 0.0001$) or the control cores (mean = 0.017, SD = 0.015; Wilcoxon signed-rank test, $p < 0.0001$) (Figure 2.7D). There was no significant difference between k_1 of soil cores containing only O horizon and those with mineral soil. There was a small significant difference in k_1 between *P. banksiana*-dominated sites (mean = 0.039, SD = 0.038) and *Picea* spp.-dominated sites (mean = 0.029, SD = 0.023; Wilcoxon signed-rank test, $p = 0.04$).

In the post-fire environment incubation, k_2 was significantly higher in the dry soil burn cores (mean = 2.6 x 10^{-5}, SD = 3.4 x 10^{-5}) than in the unburned soil (mean = 2.3 x 10^{-5}, SD = 4.0 x 10^{-5}; Wilcoxon signed-rank test, p = 0.001). There was no significant difference between k_2 of the wet soil burn cores and the dry soil burn cores (Wilcoxon signed-rank test, p = 0.45) or the unburned soil (Wilcoxon signed-rank test, p = 0.23). k_2 increased with DH in both the autoclaved and not autoclaved dry soil burn samples from the post-fire environment incubation (simple linear regression, $p < 0.05$; Supplementary Information Figure S2.10).

Discussion

Higher severity fires under dry conditions caused an increase in soil pH and decreased rates of soil respiration in 18 of the 19 cores compared to wet soil burns, which could be driven by microbial mortality at high temperatures and a reduction in easily-mineralizable C by combustion. We consider the effects of heating on soil properties and on microbial respiration below.

Soil temperature and resulting change in soil properties

Burning under low and high moisture conditions successfully produced a wide range of maximum temperatures and DH, spanning those typically reported in studies of wildfires (Neary et al. 1999; DeBano 1991). Consistent with our hypothesis, we saw an increase in fractional dry mass loss with increasing DH. As more of the soil reaches temperatures at or

above the combustion threshold of OM, more mass is lost. Under higher moisture conditions, more energy is required to reach this temperature threshold (Neary et al. 2005), which is reflected in the lower fractional dry mass loss observed following the wet soil burns. Wildfires in the Canadian boreal forests are predicted to increase in intensity and in rates of forest floor fuel consumption (De Groot et al. 2013). Increasing mass loss at higher temperatures, as we have observed here, has implications for long-term OM stocks in the boreal forest.

Soil pH also increased with DH and maximum temperatures, as predicted. At high temperatures, the complete combustion of organic C and formation of mineral ash has been observed to increase soil pH by 3 units, and ash produced at temperatures from 350 to 500 ºC can increase pH by 2-3 units (Bodí et al. 2014). Under the dry soil burn conditions, temperatures exceeded 500 ºC, causing OM combustion, ash production, and an increase in pH. Shifting pH can impact soil microbial communities and nutrient availability. For example, bacterial diversity has been observed to positively correlate with a pH increase from 4 to 8 (Rousk et al. 2010) and peak near a pH of 7 (Lauber et al. 2009). Increasing pH from acidic to neutral or slightly alkaline also decreases Fe and Al availability and increases N, P, and Mg availability (Brady and Weil 1996). In boreal forest soils, net nitrification has been shown to increase with increasing pH (Ste-Marie and Paré 1999). Thus, it is likely that increasing pH via burning impacts multiple soil properties and processes in the post-burn environment.

At the high temperatures, C and N will both volatilize. However, the temperature threshold for C is lower than that of N. Combustion of OM and associated C losses begin at around 100 ºC with near complete combustion occurring at temperatures of 450 ºC for a duration of 2 hours or 500 ºC for a duration of 30 mins (Neary et al. 2005). N volatilization begins at around 200

ºC and near complete loss is reached at around 500 ºC (Neary et al. 2005). Although temperatures at the organic-mineral interface (or core midpoint in the absence of a mineral horizon) in 15 of the 19 dry soil burns exceeded 200 ºC, large losses of N were not recorded, so the decrease in C:N with DH is driven by a decrease in C, rather than an increase in N. There could be several explanations for this. First, the duration of high temperatures may have been sufficient to cause C loss without N volatilization. Second, the homogenization of the O horizon during the post-burn subsampling may have diluted the effect of burning on surface N concentrations, especially for the subset of all-organic cores.

Microbial respiration

The proportion of C in the fast pool was smaller in the dry soil burn cores compared to the wet soil burn cores, suggesting that the higher severity burns that resulted from the higher temperatures achieved in the dry soil burn treatment reduced the proportion of readily available C and/or altered the microbial community. We will consider the evidence for each of these possibilities.

A reduction in the proportion of readily available C is supported by the higher fractional dry soil mass loss and larger decrease in O horizon thickness (Supplementary Information Table S2.5) observed in the dry soil burns than in the wet soil burns. In the unburned soil, respiration rates were higher in the *Picea* spp. and *P. tremuloides*-dominated sites, which overall had thicker O horizons than the *P. banksiana*-dominated sites. Microbial respiration rates have been shown to decline with soil depth and decreasing total organic carbon (Fang and Moncrieff

2005). Combustion of OM in the O horizon removed readily available C from the system and may have also produced pyrogenic organic matter (PyOM).

PyOM is produced via the partial combustion of organic materials (Knicker 2011). PyOM has a polycyclic aromatic structure that lends it biological and chemical stability over centuries (Ohlson et al. 2009). PyOM additions to soil have been shown to impact soil microbial community composition (Lanza et al. 2016) and mineralization rates (Maestrini et al. 2015; Whitman et al. 2015), and the conversion of OM to PyOM during burning changes its bioavailability for bacteria (Whitman et al. 2013). At most sites, we saw a decrease in total respiration rate as well as an increase in fractional C loss upon burning in dry soil burn cores compared to control cores, which is consistent with loss of readily available C and increase in PyOM.

Unlike trends seen across the study, the proportional size of the fast C pool of cores collected at site 12 was highest in the dry soil burn core. Site 12 is characterized as *Picea* spp.-dominated, and our sampling depth did not reach mineral soil. In this case, it is difficult to compare microbial respiration rates on a mass basis across the control, wet, and dry soil burn cores due to the high level of combustion seen in the dry soil burn (Figure 2.8). The dry soil burn core lost 4.5 cm of its O horizon to combustion compared to the wet soil burn, which lost 0.5 cm. Bacterial mortality caused by high temperatures in the dry soil burn core may have led to a temporary increase in nutrient availability that fueled respiration for surviving bacteria. Alternatively, soil bacteria at the base of the dry soil burn, wet soil burn, and control cores may have been relatively unaffected by the burn; equal proportions of the fast C pool in the lower,

unburned portion of the three cores may have resulted in a disproportionately high respiration rate in the dry soil burn core due to its lesser post-burn mass.

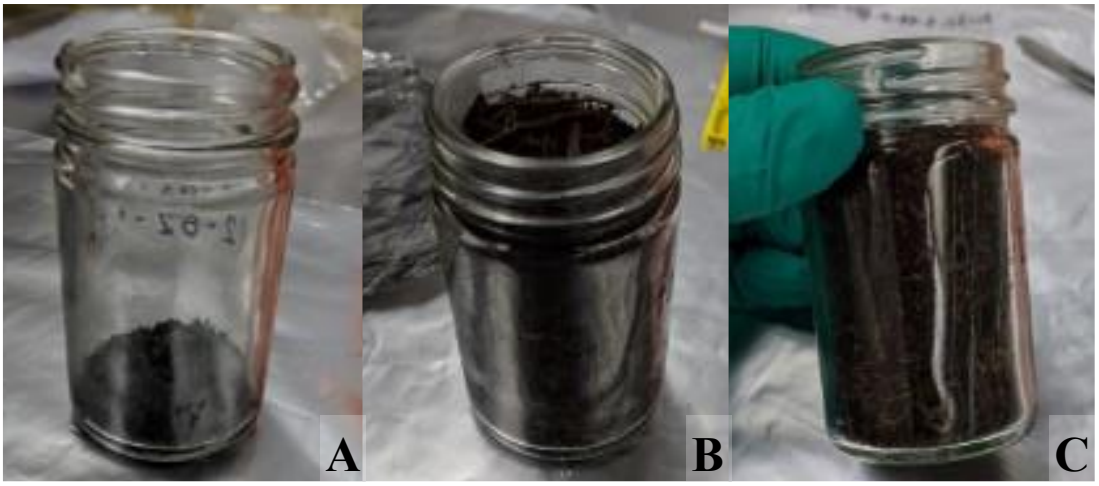

Figure 2.8. Post-incubation photos of (A) dry soil burn, (B) wet soil burn, and (C) unburned control soil demonstrating the high variability of soil mass used in the post-burn incubations.

Changes in microbial community composition could also be affecting the proportion of C in the fast pool in the wet and dry soil burn cores. Differences in microbial community composition between burned and unburned soil have been observed 1 year post-fire (Whitman et al. 2019; Xiang et al. 2014) and have been found to persist 15 years post-fire (Dooley and Treseder 2012). Overall, temperatures reached in the dry soil burns were high enough to cause bacterial mortality. Microbial survival during heating has been shown to decrease with increasing temperatures (Dunn et al. 1985; Pingree and Kobziar 2019). Increasing burn severity has been seen to correlate with decreasing microbial biomass (Holden et al. 2016). Thus, it is likely that a larger portion of the microbial community was killed during the dry soil burns than during the wet soil burns. (Chapter 3 will explore the effect of burning under high and low moisture conditions on soil bacterial community composition and identify fire-surviving bacterial taxa.) While the proportion of C in the fast pool was overall smaller for the

dry soil burn cores than the wet soil burn cores, the proportion of C in the fast pool (Supplementary Information Figure S2.9) and the slow C pool decay rate (Supplementary Information Figure S2.10) increased with DH within the dry soil burn cores. This could be explained by a decrease in PyOM due to more complete combustion at higher temperatures and an increase in relative abundance of bacterial taxa with the ability to degrade PyOM. (Chapter 5 will discuss the potential for fire-surviving taxa to degrade PyOM and PyOM degradation as an advantageous microbial trait in colonizing the post-fire environment.)

There is evidence that the effect of PyOM additions to soil on bacterial community composition vary with pyrolysis temperature (Dai et al. 2021). Higher pyrolysis temperatures have been shown to correlate with longer PyOM persistence in soil (Mimmo et al. 2014; Whitman et al. 2013). Thus, it is likely that the decrease in the proportion of C in the fast pool in the dry soil burn cores compared to the wet soil burn cores is caused by a combination of the decrease in readily available C, increased conversion of OM to PyOM, and changes in microbial community composition.

Conclusion

Decreasing soil moisture leads to an increase in the impact of burning on soil properties such as pH, O horizon thickness, and nutrient availability. We found that increasing burn severity causes a decrease in post-burn respiration rates and changes the proportional size of the fast and slow C pools in the soil. Our findings support previous work showing larger impacts of high severity fires on soil properties such as pH and C availability than low severity fires

(Verma and Jayakumar 2012; Knicker 2011).

This chapter focused on the effect of burning on soil properties and post-burn respiration rates, leading us to hypothesize that changes in microbial community composition and in C composition explain the smaller proportion of the fast C pool in the dry soil burns. Burning causes changes in soil pH and carbon availability, both of which have been shown to shape post-burn soil microbial communities (Dai et al. 2021; Certini 2005). The next chapter will explore the impact of burning under high and low moisture conditions and bacterial mortality on soil microbial community composition 24 hours post-burn and identify fire-surviving bacterial taxa.

CHAPTER 3

BACTERIAL FIRE SURVIVAL UNDER HIGH AND LOW MOISTURE CONDITIONS

Introduction

Survival is one important component determining post-fire soil microbial community composition. Soil bacterial mortality is affected by temperature and duration of temperature exposure, generally increasing with both (Pingree and Kobziar 2019; Köster et al. 2021; Holden et al. 2016; Dunn et al. 1985). Thus, soil bacterial mortality can occur at high temperatures of short duration as well as lower temperatures over longer durations: in a review of 23 studies, Pingree and Kobziar (2019) found that soil bacterial mortality can occur across a wide range of temperatures (80-400 ℃) and duration (2-30 mins).

In addition to the degree and length of heat exposure, the physiological state of bacteria is another factor in susceptibility to high temperature. For example, in soil heating trials, bacteria that were physiologically active and residing in moist soil were more susceptible to mortality with increasing temperatures than dormant bacteria in dry soil (Dunn et al. 1985; Sella et al. 2014). Spore-formation is found in a range of bacteria including aerobic heterotrophs (*Bacillus* spp. and *Paenibacillus* spp.) and anaerobes (*Clostridium* spp. and *Anaerobacter* spp.) (Nicholson et al. 2000; Logan and Halket 2011), and spore-forming taxa *Clostridium* and *Paenibacillus* have been shown to increase in abundance in burned soils versus unburned controls (Yeager et al. 2005). Bacterial endospores are generally more resistant to damage by

heat or desiccation than vegetative cells. Dry conditions (such as those imposed in the 1.5 months leading up to the burn, as described in Chapter 2) trigger vegetative cells to sporulate. Moisture also moderates the effects of heating on bacteria: some spores can survive dry heat of >100 ℃ for 24 hours but are more susceptible to moist heat, which can reduce survival time at a given temperature 1000-fold (Nicholson et al. 2000).

A soil bacterial community is composed of bacteria in active and dormant physiological states. Under conditions of low organic carbon availability, bacterial growth and activity will be low and many soil organisms will exist in a dormant phase (van Elsas and van Overbeek 1993). More than 50% of the cells within a soil sample can be inactive at a time (Jones and Lennon 2010). The persistence of genomic DNA in metabolically inactive soil bacteria means that DNA-based community composition assays include both active, dormant, and dead taxa (unless measures are taken to remove DNA from damaged cells or extracellular DNA). RNA offers a snapshot of living (active or dormant) bacteria. RNA has been used to identify active bacteria within the soil, and rRNA:DNA gene ratios in soil have been used as a measure of microbial activity, with higher ratios indicting higher activity and associated with an increase in soil organic matter decomposition (Loeppmann et al. 2018). A 16S rRNA:16S rRNA gene ratio ≤ 1 has been used to define dormant taxa (Kearns et al. 2016). However, the relationship between RNA and bacterial activity is complex and variable (Blazewicz et al. 2013; Sukenik et al. 2012). Generally, rRNA:DNA ratios can be used to identify active microbial populations, but the ratios are less robust tools when used to identify dormant populations (Steven et al. 2017). Although rRNA makes up a large fraction (82-90%) of a cell's total RNA (Neidhardt and Magasanik 1960; Tissieres and Watson 1958), and the concentration of rRNA is generally

proportional to the number of ribosomes in a cell (Kerkhof and Ward 1993), rRNA is not a clear predictor of microbial growth rate or activity (Blazewicz et al. 2013). The concentration of rRNA correlates well with growth rate of bacteria in pure culture in an unchanging environment (Ramos et al. 2000; Bremer and Dennis 2008) and a decrease in rRNA has been shown to correlate with a decrease in growth rate for certain taxa in nutrient-limited conditions (Tolker-Nielsen et al. 1997). However, RNA concentrations are not a robust proxy for growth rate or activity for all bacterial taxa in all conditions. Still, the use of RNA in determining microbial community composition has an advantage over DNA because extracellular RNA in the soil degrades very quickly compared to DNA (Kunadiya et al. 2021; Levy-Booth et al. 2007). RNA-based community composition will include both active and dormant taxa but few dead organisms. In this chapter, both RNA and DNA-based community composition will be used to identify fire surviving bacterial taxa. DNA collected from soil 24 hours post-burn contains the signatures of active and dormant taxa as well microbes that perished during the burn, whereas RNA should include active and dormant taxa only. Therefore, analyzing RNA-based communities allows us to screen out dead taxa.

This chapter explores the effect of burning soil under high and low moisture conditions on bacterial survival and the importance of burn survival on microbial community composition. We hypothesized that (a) burning soil under low moisture conditions would have a greater effect on soil microbial community composition than burning soil under high moisture conditions, (b) burning would have minimal impact on mineral horizon microbial communities, and (c) the relative abundance of *Bacillus* and *Paenibacillus* would increase more following the dry soil burn than the wet soil burn. DNA- and RNA-based 16S rRNA gene

sequencing was used to construct soil microbial communities and screen for differential abundance of individual taxa between burn treatments 24 hours after burning (Ch. 2).

Methods

In brief, soil cores under high and low moisture conditions were subjected to burn simulations in a cone calorimeter. After the burn, soil horizon subsamples for each core were collected for nucleic acid sequencing to identify positive and negative fire-responding bacteria.

Sampling

After the burn, cores were removed from the cone calorimeter and left to cool to room temperature for 24 hours. The O horizon material was separated from the underlying mineral soil and each portion was homogenized. Subsamples of the O horizon and mineral soil were immediately collected for later nucleic acid extraction and sequencing and stored at -80 °C.

RNA and DNA extractions

RNA and genomic DNA (gDNA) co-extractions were performed for each soil horizon of each sample with a blank extraction (identical methods but with empty tubes) for every 8 samples using RNeasy PowerSoil Total RNA Kits and RNeasy PowerSoil DNA Elution Kits (QIAGEN, Germantown, MD, USA), respectively. In brief, 0.5 – 1 g of O horizon or 2 g of

mineral soil was added to a PowerBead tube. A phenol/chloroform nucleic acid extraction was performed following manufacturer's instructions. Homogenization and lysis were done using a FastPrep-24™ 5G bead beating grinder and lysis system (MP Biomedicals, Irvine, CA, USA) at a frequency of 6.5 m s^{-1} for 45 s at room temperature.

We were unable to extract amplifiable gDNA from 8 of the 57 O horizon samples. We did a second gDNA extraction for these 8 samples (plus 7 additional samples to compare sequencing results from the two extraction kits) and two blanks using a DNeasy PowerLyzer PowerSoil DNA extraction Kit (QIAGEN, Germantown, MD, USA). Sample volume was reduced to 0.1 g following manufacturer's instructions and samples underwent homogenization and lysis under the same conditions reported above.

RNA clean-up and reverse transcription

Residual gDNA contamination was removed from the RNA extracts using DNase Max Kits (QIAGEN, Germantown, MD, USA) following manufacturer's instructions. In brief, 40 µL of sample was combined with a DNase enzyme, buffer, and nuclease-free water and incubated at 37 ℃ for 20 min. The sample was then combined with 5 µL of DNase removal resin, incubated for 10 mins on a vortex adapter set to low, and centrifuged at 13,000 x g for 1 min. Supernatant was collected in a clean tube for further analysis.

RNA reverse transcription was carried out using Invitrogen SuperScript IV VILO Master Mix (ThermoFisher Scientific, Waltham, MA, USA) following manufacturer's instructions. A

control reaction using no reverse transcriptase enzyme was carried out on one of every three RNA extracts to ensure minimum contamination by gDNA in the RNA sample. A Quant-iT RiboGreen RNA Assay Kit (ThermoFisher Scientific, Waltham, MA, USA) was used to assess copy DNA (cDNA) concentration and gDNA contamination levels.

RNA and gDNA amplification and sequencing

The resulting cDNA and extracted gDNA were amplified via triplicate PCR targeting the 16S RNA gene v4 region with 515f and 806r primers (Walters et al. 2016) with barcodes and Illumina sequencing adapters added as per Kozich et al. (2013). In brief, PCR mixes contained 1.25 µL 515f forward primer (10 µM), 1.25 µL 806r reverse primer (10 µM), 1 µL cDNA or gDNA template, 12.5 µL Q5 Hot Start High-Fidelity 2X Master mix (New England BioLabs INC., Ipswich, MA, USA), 1.25 µL Bovine Serum Albumin (BSA) (20 mg mL^{-1}) (VWR, Radnor, PA, USA), and 7.75 µL nuclease-free water, in a 96-well plate. The plate was sealed and placed on an Eppendorf Mastercycler nexus gradient thermal cycler (Hamburg, Germany). Reactions were run at 98 ℃ for 2 minutes + (98 ℃ for 30 seconds + 58 ℃ for 15 seconds + 72 ℃ for 10 seconds) x 30 cycles + 72 ℃ for 2 minutes and 4 ℃ hold. The quality of PCR amplicon triplicates was assessed using gel electrophoresis, and triplicates were then pooled, purified, and normalized using SequalPrep Normalization Plate Kits (96-well) (ThermoFisher Scientific, Waltham, MA, USA). Samples were then pooled, and library cleanup was performed using a Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). The pooled library, including blanks, was submitted to the UW-Madison Biotechnology Center

(Madison, WI, USA) for 2x250 paired end Illumina MiSeq sequencing. The library was sequenced twice using identical protocols to improve sequencing depth. Reads from the two sequencing runs were pooled by sample after sequence processing and before analysis.

Sequence processing and taxonomic assignments

We quality filtered, trimmed (left trim 13 for both reads; truncation length 182 for forward reads, 161 for reverse reads), and dereplicated, learned errors (1M reads, randomized), picked operational taxonomic units (OTUs), and removed chimeras (consensus method) using dada2 (Callahan et al. 2016) as implemented in Quantitative Insights Into Microbial Ecology (QIIME2) (Bolyen et al. 2019). A total of 18,830,558 reads were obtained from amplicon sequencing. 6,360,490 reads were removed during the filtering processing, and taxonomy was assigned to the remaining 12,470,068 reads (24,940 mean 16S reads per sample) using the naïve Bayes classifier (Bokulich et al. 2018) in QIIME2 with the aligned 515f-806r region of the 99% OTUs from the SILVA database (SIVLA 138 SSU) (Quast et al. 2013; Glöckner et al. 2017; Yilmaz et al. 2014). We excluded 1 cDNA and 2 gDNA samples from further analysis due to low 16S reads per sample (<1000).

Bioinformatics and statistics

We worked primarily in R (Team 2020) relying extensively on R packages *phyloseq* (McMurdie and Holmes 2013), *dplyr* (Wickham et al. 2021), *ggplot2* (Wickham 2016), and

vegan (Oksanen et al. 2020). We compared community composition across samples using weighted UniFrac dissimilarities on RNA- and DNA-based relative abundances and tested for significant effects of horizon (O horizon versus mineral soil), dominant vegetation, pre-burn horizon thickness, pH, total C and N, soil texture, and degree hours (DH) using a permutational multivariate ANOVA (PERMANOVA using the *adonis* function in *vegan* (Oksanen et al. 2020)) and used the *betadisp()* function to test if variables differed in their dispersion. We used single-component models to compare the R^2 for each factor.

We calculated the 16S rRNA:16S rRNA gene ratio (RNA:DNA) by dividing the relative abundance of RNA-based OTUs by the relative abundance of DNA-based OTUs. To prevent division by 0, we identified phantom taxa as OTUs with non-zero RNA reads that were absent in the corresponding sample DNA-based community and set the RNA:DNA ratio for phantom taxa to 100 (Bowsher et al. 2019). We used two-way ANOVAs with Tukey's post-hoc tests to test the relationship between burn treatment and mean sample RNA:DNA and used simple linear models to test the relationship between DH and mean sample RNA:DNA in the dry soil burns.

For both RNA- and DNA-based community profiles, we identified differentially abundant individual bacterial taxa across the three treatments (unburned control vs. wet soil burn or vs. dry soil burn) in each soil horizon using the *corncob* package (Martin et al. 2020) in R and controlling for dominant vegetation and texture. We used a mean relative abundance cutoff of 0.000001 to filter out rare taxa and used a controlled false discovery rate cutoff of 0.05 to adjust p values of differentially abundant taxa to account for potential false positives. We identified enrichment of bacterial taxa in the dry soil burns with increasing DH in the same way.

Results

Predictors of bacterial community composition

All tested factors (dominant vegetation, pre-burn horizon thickness, pH, soil texture, burn treatment, and DH) except total C and N were significant predictors of RNA-based community composition for bacteria (Figure 3.1A and B) in the combined model (PERMANOVA, $p \leq 0.01$ for all factors except total C ($p = 0.07$) and N ($p = 0.06$)) (Supplementary Information Table S3.8). All tested factors were significant predictors for DNA-based community composition (Figure 3.1C and D) (PERMANOVA, $p < 0.05$ for total N; $p \leq 0.001$ for all other factors) (Supplementary Information Table S3.9). Soil texture provided the most explanatory power for RNA- and DNA-based community composition ($R_{text, RNA}^2 = 0.10$, $R_{text, DNA}^2 = 0.11$) followed by pH ($R_{pH, RNA}^2 = 0.05$, $R_{pH, DNA}^2 = 0.05$). The PERMANOVA results for burn soil moisture and horizon could be affected by non-homogenous dispersion of data. The dispersion of RNA-based communities across treatments differed significantly depending on the burn treatment (*betadisper*, $F = 6.31$, $p = 0.001$) and horizon (*betadisper*, $F = 3.96$, $p = 0.04$). The dispersion of DNA-based communities differed significantly depending on burn treatment (*betadisper*, $F = 5.67$, $p = 0.002$).

RNA-based community composition

In the dry soil burn samples, multiple bacterial OTUs were identified as being significantly enriched (24 OTUs, O horizon; 45 OTUs, mineral horizon) or depleted (89 OTUs, O horizon;

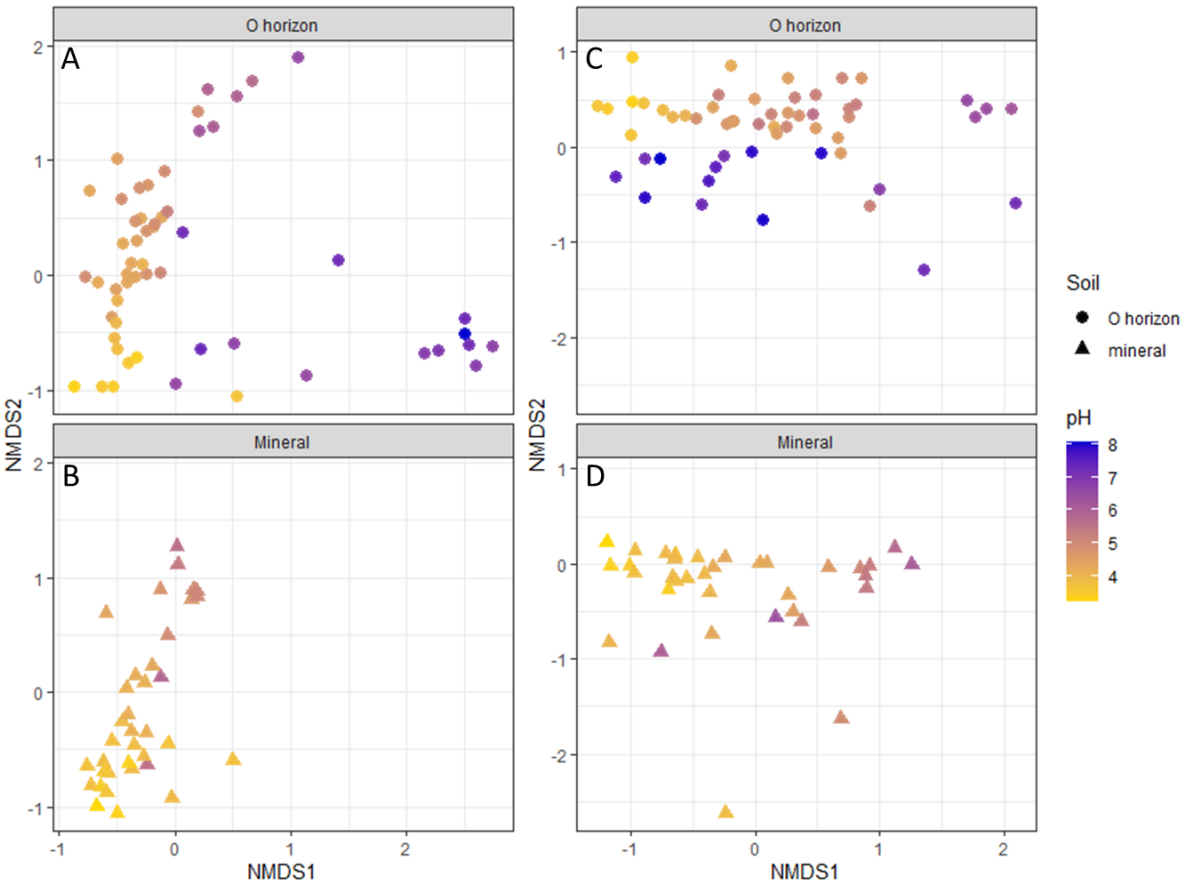

Figure 3.1. First two axes of NMDS ordination on weighted UniFrac dissimilarities between RNA-based bacterial communities for the (A) O horizon and (B) mineral soil (k = 3, stress = 0.095). NMDS ordination on weighted UniFrac dissimilarities between DNA-based bacterial communities for the (C) O horizon and (D) mineral soil (k = 3, stress = 0.097). Circles and triangles indicate O and mineral horizons, respectively. Points are shaded by pH with darker colors indicating higher pH.

52 OTUs, mineral horizon) in the RNA-based communities compared to the unburned soil after controlling for dominant vegetation and soil texture (Figure 3.2). In the wet soil burn samples, multiple bacteria OTUs were identified as being significantly enriched (62 OTUs, O horizon; 52 OTUs, mineral horizon) or depleted (51 OTUs, O horizon; 45 OTUs, mineral horizon) compared to the unburned soil after controlling for dominant vegetation and soil texture (Supplementary Information Figure S3.11).

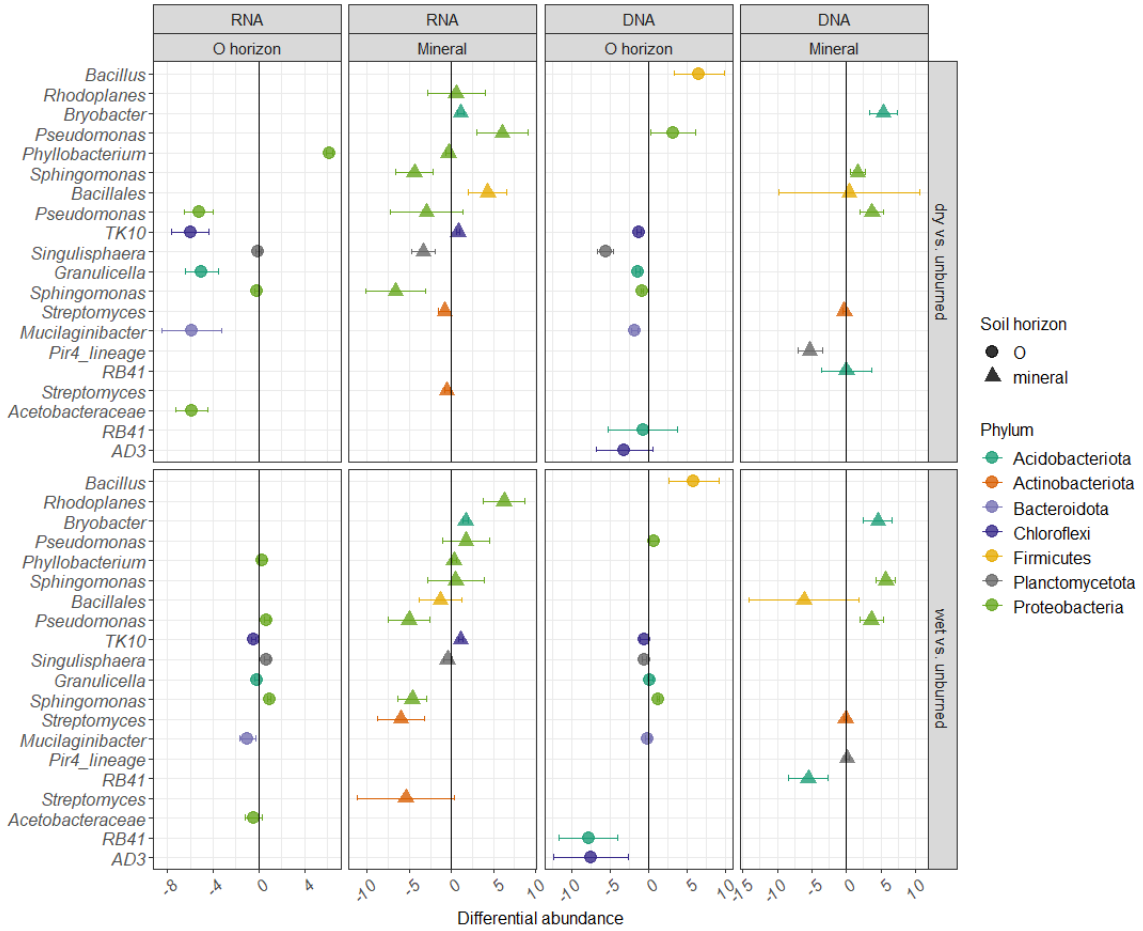

Figure 3.2. Differential abundance of bacterial OTUs (with a mean relative abundance > 0.1 %) identified as positive or negative fire responders in either soil horizon of the dry soil burns (top) and the wet soil burns (bottom) in the RNA- and DNA-based community analyses after controlling for soil texture and dominant vegetation. Each point represents an individual bacterial OTU. Colors represent phyla-level classification. Error bars represent 95% prediction intervals for the differential abundance factor by sample. Y-axis is labelled with finest available taxonomy. Circles and triangles indicate O horizon and mineral soil, respectively.

In the RNA-based communities, the most abundant taxa in the dry soil burns were a *Pseudomonas* sp., *Phyllobacterium* sp., and *Bacillus* sp. (mean abundances of 8.7%, 5.9% and 3.0%, respectively). The second most abundant OTU was identified as a positive fire-responder and classified as a *Phyllobacterium* sp. (mean abundances of 5.9% dry soil burn samples; 0.1% unburned soil in the O horizon) (Figure 3.3).

DNA-based community composition

More bacterial OTUs were identified as positive or negative fire responders in the DNA-based communities than in the RNA-based communities. In the dry soil burn samples, multiple bacterial OTUs were identified as being significantly enriched (58 OTUs, O horizon; 73 OTUs, mineral horizon) or depleted (120 OTUs, O horizon; 70 OTUs, mineral horizon) compared to the unburned soil after controlling for dominant vegetation and soil texture (Supplementary Information Figure S3.11).

In the wet soil burn samples, multiple bacterial OTUs were identified as being significantly enriched (92 OTUs, O horizon; 63 OTUs, mineral horizon) or depleted (86 OTUs, O horizon; 80 OTUs, mineral horizon) compared to the unburned soil after controlling for dominant vegetation and soil texture. In the DNA profile, the most abundant taxa in the dry soil burns were classified as *Xanthobacteraceae*, *Bacillus* sp., and *Firmicutes* (mean abundances of 2.1%, 2.1% and 1.9%, respectively).

RNA and DNA community comparisons

Thirteen OTUs were significantly enriched in the O horizons of burned soil compared to the unburned samples in both the RNA- and DNA-based communities after controlling for soil texture and dominant vegetation. The most abundant taxon in both RNA- and DNA-based communities was classified as a *Xanthobacteraceae* (mean abundance 3.5% in RNA samples and 3.3% in DNA samples). This taxon was identified as a negative fire responder in both O

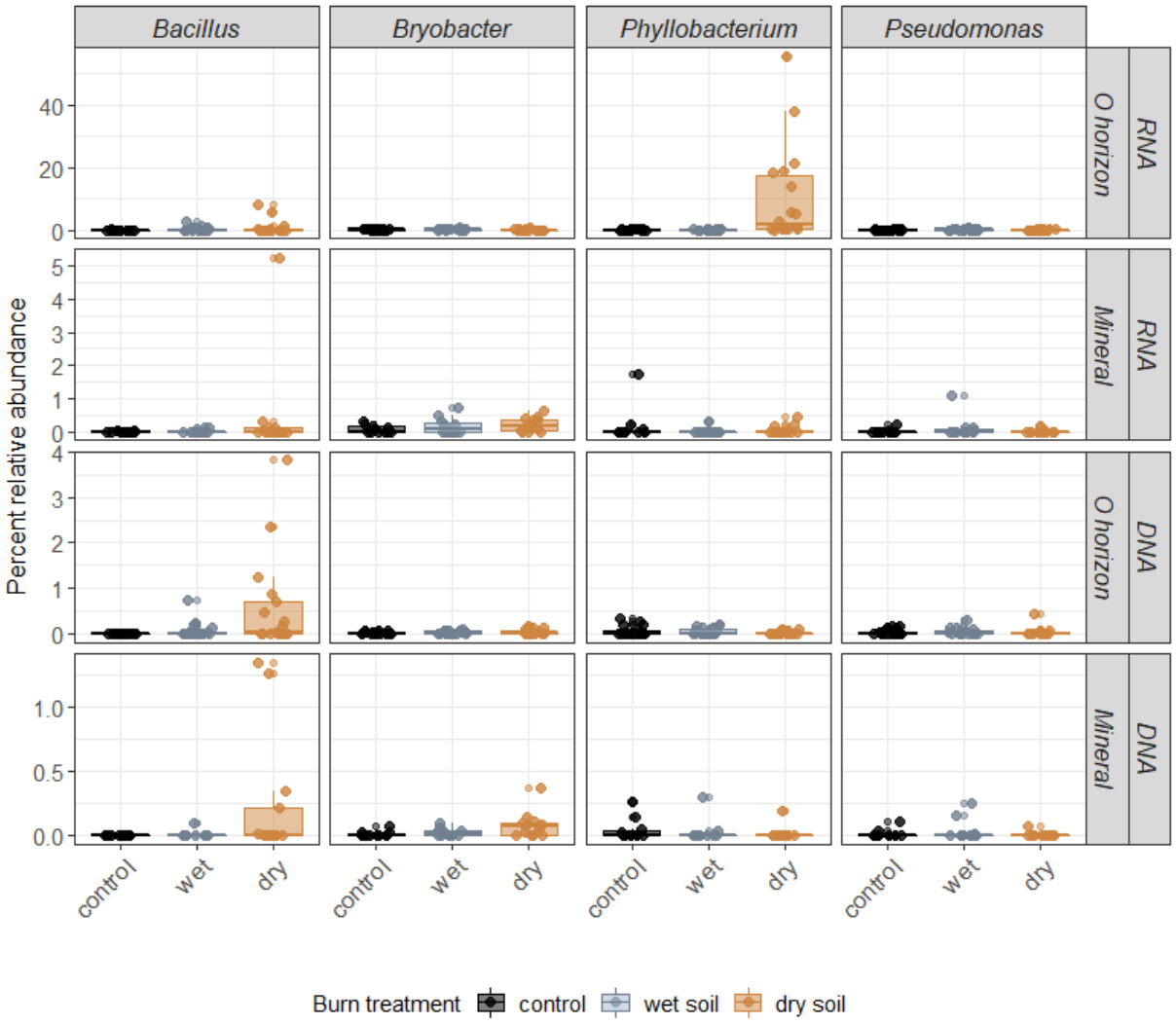

Figure 3.3. Percent relative abundance in RNA- and DNA-based communities of the positive fire-responding OTUs with the highest relative abundance across all samples. Colors represent burn treatment.

horizons (mean abundance 1.7% in dry soil burn samples and 2.2% in unburned samples) and mineral soil (mean abundance 2.5% in dry soil burn samples and 3.1% in unburned samples) in the RNA-based communities and in both O horizons (mean abundance 2.1% in dry soil burn samples and 3.5% in unburned samples) and mineral soil (mean abundance 2.2% in dry soil burn samples and 4.2% in unburned samples) in the DNA-based communities.

Out of a total 20,106 OTUs present in the burned RNA- and/or DNA-based communities, 3990 were phantom taxa (present in the RNA- and not the DNA-based communities), and 7500 were present in the DNA- and not the RNA-based communitites. In the O horizon, 16S rRNA:DNA gene ratios varied widely from 0, indicating no RNA present for that taxon, to 284, indicating a putative highly active taxon (mean = 0.74). The range of RNA:DNA ratios was smaller in the mineral horizon (0 to 77.7, mean = 0.91). RNA:DNA ratios were significantly lower in the O horizon than in the mineral soil (ANOVA, $p < 0.0001$). RNA:DNA ratios were significantly lower in the dry soil burn samples (mean 0.68, SD = 3.39) than in the unburned soil (mean = 0.89, SD = 1.87, (ANOVA, p. adj. < 0.0001)) or the wet soil burn samples (mean = 0.86, SD = 1.73; ANOVA, p. adj. < 0.0001). In both the O horizon and mineral soil, RNA:DNA ratios decreased with increasing DH in the dry soil burn samples (simple linear regression, O horizon, $p < 0.0001$; mineral soil, $p < 0.0001$).

Change in Bacillus *and* Paenibacillus

Bacilli (which contains both *Bacillus* and *Paenibacillus*) was identified as one of the top positive fire responding classes in the O horizon of dry soil and wet soil burn samples in the both the RNA- and DNA-based communities (Supplementary Information Figure S3.12). In the RNA-based communities, 109 OTUs were classified as either *Bacillus* or *Paenibacillus*. Only one of these OTUs was identified as a positive fire responder (classified as *Bacillales*). The relative abundance of this *Bacillales* OTU was higher in the dry soil burn samples (mean = 0.7%, O horizon; 4.0% mineral soil) than in the wet soil burn samples (mean = 0.002%, O

horizon; 0.03%, mineral soil; ANOVA, p = 0.003) and the unburned soil (mean = 0.004%, O horizon; 0.04% mineral soil; ANOVA, p = 0.003). There was not a significant difference in relative abundance of this *Bacillales* OTU between the wet soil burn samples and the unburned soil (p = 1).

In the DNA-based communities, 148 OTUs were classified as either *Bacillus* or *Paenibacillus*, and at the OTU-level, 3 were classified as positive fire responders. The most abundant positive fire responder across all samples was classified as *Bacillus* sp. (Figure 3.3). The relative abundance of this *Bacillus* sp. was higher in the dry soil burn samples (mean = 0.6%, O horizon; 0.2% mineral soil) than in the wet soil burn samples (mean = 0.07%, O horizon; 0.008%, mineral soil) and the unburned soil (mean = 0.0004%, O horizon; undetected in the mineral soil).

Overall, of all 109 OTUs classified as either *Bacillus* or *Paenibacillus*, 33 OTUs were identified in the RNA- but not the DNA-based communities, and 72 OTUs were identified in the DNA- but not the RNA-based communities.

Discussion

The effects of burning and soil moisture on bacterial community composition.

Soil bacterial communities were structured most strongly by soil texture and then by pH, which is consistent with previous findings (Xia et al. 2020; Bach et al. 2010; Lauber et al. 2009). Burning and soil moisture also had a measurable effect on both RNA- and DNA-based soil

bacterial communities (Figure 3.1 and 3.2). This supports my hypothesis that the microbial communities of burned and unburned soil would differ and that burning would have a different effect on microbial communities of low and high moisture soil. This can be explained by the temperature differences in the burns. The dry soil burns reached higher temperatures and stayed hot for longer than the wet soil burns (as described in Ch. 2). For example, in over half (11 of 19) of the dry soil burns, temperatures of the O horizon exceeded 400 °C (Ch. 2), at which temperature widespread bacterial mortality is expected (Pingree and Kobziar 2019). In contrast, none of the wet soil burns reached temperatures expected to be lethal to bacteria at the O horizon-mineral interface. Thus, it is likely that heat-induced bacterial mortality impacted soil bacterial community composition in this study.

Impact of burning on O horizon versus mineral soil

The microbial community composition of the O horizon and mineral soil differed before burning, which is expected given the differences in substrate. After burning, we see more OTUs enriched and fewer depleted in the mineral soil than the O horizon in both the RNA- and DNA-based communities, suggesting that bacteria in the mineral soil were more likely to survive the burns than bacteria in the O horizon. This is supported by the significantly lower RNA:DNA gene ratios in the O horizon compared to the mineral soil. Decreased RNA:DNA gene ratios have been shown to correlate with decreased bacterial activity (Bowsher et al. 2019), and a decrease in ratios could indicate an increase in dead taxa included in the DNA-

based community and absent in the RNA-based community due to differences in the persistence of DNA and RNA in the soil (Kunadiya et al. 2021; Levy-Booth et al. 2007).

In total, 11 of the 51 cores included in this analysis did not contain mineral soil. The absence of mineral soil in 20% of the comparisons may have limited our ability to detect significant differences in microbial composition across soil horizons. Additionally, the three samples removed from the analyses early on due to very low 16S reads per sample were O horizon samples. These three samples were organic-rich soils with no mineral horizons that were subjected to the dry soil burn treatment. We measured a large decrease in O horizon thickness with burning in each of these samples, which indicates that a large portion of these cores was combusted. Based on burn temperatures and O horizon loss, these were relatively high severity burns. It is possible that including these samples would have increased our ability to detect changes in microbial community composition with burning.

The effect of burning on spore-forming bacteria

At the class-level, there was a clear increase in the relative abundance of *Bacilli* in both dry soil and wet soil burns compared to the unburned soil. This increase was not as clear at the individual OTU level. Only three taxa in the *Bacilli* class were identified as positive fire-responders (Figure 3.3). We see an increase in the relative abundance of *Bacillus* sp. (Figure 3.2) in the O horizon and mineral soil of both the dry and wet soil burns in the DNA-based communities. This is not mirrored in the RNA-based communities, suggesting that if this OTU was in a dormant phase, burning did not induce germination.

The increase in relative abundance of *Bacilli* could be driven by small increases in relative abundance across a multitude of taxa. Alternatively, deeper sequencing depth in dry soil burns caused by an overall decrease in absolute abundance of microbes could be enabling detection of more *Bacilli*. This is supported by the lack of *Bacillus* spp. and *Paenibacillus* spp. detected in either the O horizon or mineral soil of 5 of the 19 unburned soil cores – *i.e.*, it is a relatively rare taxon to begin with.

Spore-forming bacteria, such as *Paenibacillus* spp. and *Bacillus* spp., and thermophilic bacteria are common in fire-impacted soils (Yeager et al. 2005; Norris et al. 2002; Janzen and Tobin-Janzen 2008). *Bacillus* spp. are able to form endospores, which are able to withstand high temperatures (Nicholson et al. 2000), and are frequently reported in burned soils (Lucas-Borja et al. 2019; Smith et al. 2008; Vázquez et al. 1993; Prendergast-Miller et al. 2017). All soil cores were exposed to drought conditions in the month preceding the burn, which is known to trigger the formation of endospores (Nicholson et al. 2000). Bacteria present in a dormant phase during the burn may have been more resistant to heat-induced death. In preparation for the wet soil burns, cores were wet up the day before the burns. Moisture can trigger the germination of an endospore to its vegetative phase, and this phase change can occur rapidly under optimal conditions (< 3 hours) (Logan and Halket 2011; Keynan et al. 1964; Sinai et al. 2015). The similar increase in relative abundance of *Bacilli* in both the dry and wet soil burns could be driven by different mechanisms. The increase in relative abundance of *Bacilli* following the dry soil burns compared to the unburned soil could be driven by fire survival, whereas increased germination rates triggered by increased soil moisture could explain the increased relative abundance of *Bacilli* following the wet soil burns.

Conclusion

We can see an effect of burning on soil bacterial community composition immediately (24 hours) post-burn. This effect is evident despite variable pH and texture across samples. Microbial community composition also varies with soil horizon, which is in line with higher temperatures and larger changes in soil pH and C:N in the O horizon versus the mineral soil. Changes in microbial community composition between the O horizon and mineral soil, paired with decreased RNA:DNA gene ratios in the O horizon and with greater DH in the dry soil burns, suggests that heat-induced microbial mortality impacts post-burn community composition. Our results identify fire-surviving taxa, supporting previous work showing that bacterial spore-formation is a potential fire-survival trait structuring microbial community composition 24 hours post-burn. Fire-survival via spore-formation and heat-induced bacterial mortality may shape microbial community composition in the days following burning, but other microbial traits may become more important with time. The next chapter will explore the effects of burning on soil community composition and the persistence of fire-surviving taxa 1 month post-burn.

CHAPTER 4

FAST GROWING BACTERIA IN THE POST-FIRE ENVIRONMENT

Introduction

Burning changes soil properties such as pH, C:N, and horizon thickness (Ch. 2) and causes an immediate change in bacterial community composition via heat-induced bacterial mortality (Ch. 3). In Ch. 3, we showed that burn degree hours (DH) is a significant driver of bacterial community composition and identified fire-surviving bacterial taxa. Fire survival (or post-fire colonization) is a prerequisite for fast growth in the post-fire environment. Burning causes some degree of heat-induced bacterial mortality, which may release nutrients for use by surviving bacteria. Bacteria that survive the burn, either due to a high thermotolerance or because they are located deeper in the soil and thus avoid lethal temperatures may or may not be well-suited to rapidly colonize the post-fire environment. Fast-growing bacteria will recolonize the burned environment most quickly. Under lower burn severities, there will be less selective pressure for fast growing bacteria, due to (1) lower expected mortality during the fire in the first place resulting in fewer open niches and (2) less release of nutrients of which fast-growing bacteria could take advantage.

In soil bacterial isolates, a higher number of 16S ribosomal RNA (rRNA) gene copies has been found to correlate with a faster response to nutrient availability (Klappenbach et al. 2000). The number of rRNA gene copies in a bacterial genome is also positively correlated with growth

rate and efficiency (Roller et al. 2016). Copiotrophic bacteria, which are classified by fast growth, rapid reproduction, and the ability to thrive in resource-abundant ecosystems, tend to have multiple rRNA gene copies. Oligotrophic bacteria dominate under conditions of resource scarcity, are slower growing, and tend to have fewer rRNA gene copies than copiotrophs (Roller et al. 2016).

In this chapter, we use DNA-derived microbial community composition data to test whether changes in bacterial community composition post-burn persist after a 5-week incubation. We identify fast-growing bacteria in the post-fire environment and use mean rRNA operon number as a proxy for bacterial growth rate.

We hypothesized that (a) 5 weeks post-burn, the effect of burning soil on microbial community composition would be greater for soil burned at low moisture than at high moisture, (b) bacterial taxa identified as fire-survivors in both the dry and wet soil burns (Ch. 3) would continue to be enriched in the burned soil compared to the unburned soil 5 weeks after the burn, (c) bacterial community mean 16S rRNA gene copy number would be higher in the dry soil burn samples than in the unburned soil or the wet soil burn samples, and (d) mean 16S rRNA gene copy number within bacterial communities would increase with DH.

We used weighted predicted mean 16S rRNA gene copy numbers and a taxon regression model to identify fast-growing bacteria and test for differential abundance across burn treatments and DH. We used a taxon regression model to test for differential abundance of individual fire-surviving bacterial taxa (Ch. 3) across burn treatments and DH at the end of the 5-week incubation.

Methods

In brief, soil cores under high and low moisture conditions were subjected to burn simulations in a cone calorimeter. Horizon-weighted subsamples of each core were placed into a jar and incubated for 5 weeks to identify fast-growing bacteria in the post-fire environment (see Ch. 2, Methods for further details). After 5 weeks, the incubations were dismantled, and samples were separated into O horizon and mineral soil (where present). Samples for nucleic acid sequencing were immediately stored at -80 ℃ until processing.

DNA extraction, amplification, and sequencing

DNA extractions were performed for each soil horizon of each sample, along with a blank extraction (identical methods but with empty tubes) for every 15 samples using DNeasy PowerLyzer PowerSoil DNA extraction kits (QIAGEN, Germantown, MD, USA). In brief, 0.1 or 0.25 g of O horizon or mineral soil, respectively, were added to a PowerBead tube. Sample underwent homogenization and lysis on a FastPrep-24™ 5G bead beating grinder and lysis system (MP Biomedicals, Irvine, CA, USA) at a frequency of 6.5 m s^{-1} for 45 sec at room temperature. DNA was cleaned of non-DNA organic and inorganic material and concentrated following the manufacturer's instructions. Extracted DNA was amplified via triplicate PCR targeting the 16S RNA gene v4 region with 515f and 806r primers (Walters et al. 2016). Barcodes and Illumina sequencing adapters were added as per Kozich et al. (2013). In brief, PCR contained 1.25 µL 515f forward primer (10 µM), 1.25 µL 806r reverse primer (10 µM), 1 µL DNA template, 12.5 µL Q5 Hot Start High-Fidelity 2X Master mix (New England

BioLabs INC., Ipswich, MA, USA), 1.25 µL Bovine Serum Albumin (BSA) (20 mg mL^{-1}) (VWR, Radnor, PA, USA), and 7.75 µL nuclease-free water, in a 96-well plate. The plate was sealed and placed on an Eppendorf Mastercycler nexus gradient thermal cycler (Hamburg, Germany). Reactions were run at 98 ℃ for 2 minutes + (98 ℃ for 30 seconds + 58 ℃ for 15 seconds + 72 ℃ for 10 seconds) x 30 cycles + 72 ℃ for 2 minutes and 4 ℃ hold. The quality of PCR amplicon triplicates was assessed using gel electrophoresis, and triplicates were then pooled, purified, and normalized using SequalPrep Normalization Plate Kits (96-well) (ThermoFisher Scientific, Waltham, MA, USA). Samples were then pooled, and library cleanup was performed using a Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). The pooled library including blanks was submitted to the UW-Madison Biotechnology Center (Madison, WI, USA) for 2x250 paired end Illumina MiSeq sequencing. The library was sequenced twice using identical protocols to improve sequencing depth. Reads from the two sequencing runs were pooled by sample after sequence processing and before analysis.

Sequence processing and taxonomic assignments

We quality filtered, trimmed (left trim 13 for both reads; truncation length 193 for forward reads, 160 for reverse reads), and dereplicated, learned errors (1M reads, randomized), picked operational taxonomic units (OTUs), and removed chimeras (consensus method) using dada2 (Callahan et al. 2016) as implemented in Quantitative Insights Into Microbial Ecology (QIIME2) (Bolyen et al. 2019). A total of 23,167,332 reads were obtained from amplicon

sequencing. 6,862,949 reads were removed during the filtering processing, and taxonomy was assigned to the remaining 16,304,383 reads (26,904 mean 16S reads per sample) using the naïve Bayes classifier (Bokulich et al. 2018) in QIIME2 with the aligned 515f-806r region of the 99% OTUs from the SILVA database (SILVA 138 SSU) (Quast et al. 2013; Glöckner et al. 2017; Yilmaz et al. 2014).

Bioinformatics and statistics

We worked primarily in R (Team 2020) relying extensively on R packages *phyloseq* (McMurdie and Holmes 2013), *dplyr* (Wickham et al. 2021), *ggplot2* (Wickham 2016), and *vegan* (Oksanen et al. 2020). We compared community composition across samples using weighted UniFrac dissimilarities on relative abundances and tested for significant effects of horizon (O horizon versus mineral soil) dominant vegetation, pre-burn horizon thickness, pH, total C and N, soil texture, and DH using a permutational multivariate ANOVA (PERMANOVA using the *adonis* function in *vegan* (Oksanen et al. 2020)). We used single-component models to compare the R^2 for each factor.

We analyzed the effects of burning and increasing DH on differential abundance of bacterial taxa within each soil horizon using the *corncob* package (Martin et al. 2020) in R and controlling for dominant vegetation and soil texture class (Ch. 2). We used a mean abundance cutoff of 0.000001 to filter out rare taxa and used a controlled false discovery rate cutoff of 0.05 to adjust p values of differentially abundant taxa to account for potential false positives.

We used the rrnDB RDP Classifier tool (version 2.12) to obtain a mean 16S rRNA gene copy number for each genus in the fire-survival dataset (Stoddard et al. 2015). Briefly, we used a 0.8 confidence cutoff to assign taxonomy. For taxa with a genus-level assignment, we used the genus mean copy number in the RDP database as the predicted copy number. In the absence of a genus-level assignment, we used the mean copy number for all other taxa in this study as the predicted copy number. OTU counts were normalized by dividing by the predicted copy number and calculating the community weighted mean rRNA gene copy number by summing the product of the predicted copy number and the relative abundance of each OTU for all OTUs in each sample. Finally, we calculated the relationship between weighted mean predicted copy number and DH with a simple linear model for each soil horizon.

Results

Predictors of bacterial community composition

All tested factors (dominant vegetation, pre-burn horizon thickness, pH, total C and N, soil texture, burn treatment, and DH) were significant predictors of community composition for bacteria (Figure 4.1) in the combined model (PERMANOVA, $p < 0.05$ for total N; $p \leq 0.01$ for all other factors; Supplementary Information Table S10). Soil pH provided the most explanatory power for bacterial community composition ($R^2 = 0.10$) followed by texture ($R^2 = 0.09$), burn treatment ($R^2 = 0.08$), and DH ($R^2 = 0.08$) (Supplementary Information Figure S4.13). The PERMANOVA results for burn soil moisture could be affected by non-homogenous dispersion of data. The dispersion of bacterial communities across treatments

differed significantly depending on the burn treatment (*betadisper*, F = 8.01, p = 0.001) and dominant vegetation (*betadisper*, F = 3.81, p = 0.03).

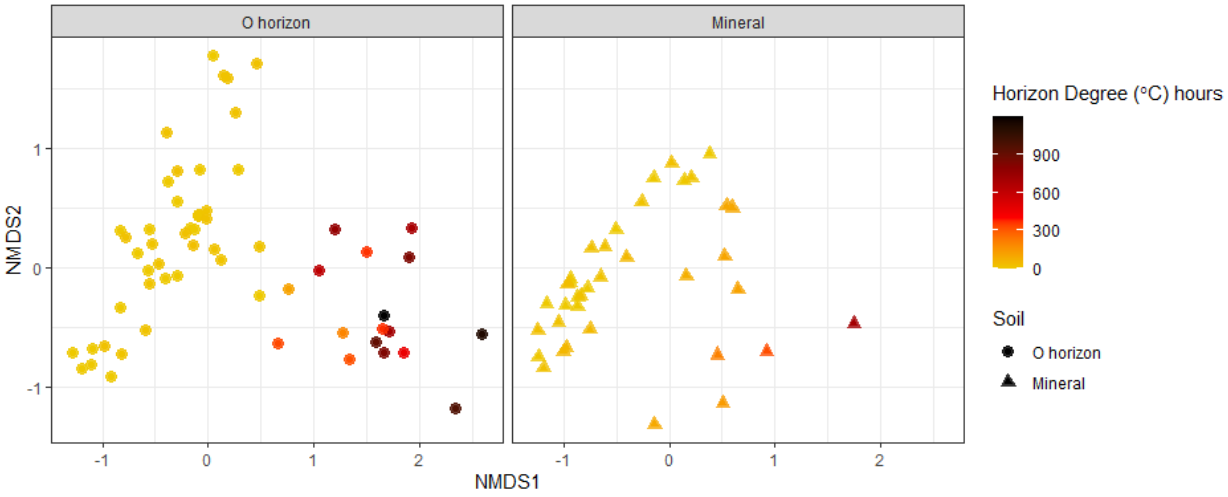

Figure 4.1. First two axes of NMDS ordination on weighted UniFrac dissimilarities between bacterial communities for the O horizon and mineral soil (k = 3, stress = 0.09). Circles and triangles indicate O and mineral horizons, respectively. Points are shaded by degree hours (DH) with darker colors indicated higher DH.

Differential abundance of bacterial taxa with burning

In the dry soil burn samples, multiple bacterial OTUs were identified as being significantly enriched (46 OTUs, O horizon; 59 OTUs, mineral horizon) or depleted (113 OTUs, O horizon; 74 OTUs, mineral horizon) compared to the unburned soil after controlling for dominant vegetation and soil texture (Supplementary Information Figure S4.14). In the wet soil burn samples, more bacterial OTUs in the O horizon were identified as being significantly enriched (81 OTUs, O horizon; 56 OTUs, mineral horizon) and fewer OTUs were identified as being significantly depleted (78 OTUs, O horizon; 77 OTUs, mineral horizon) than in the dry soil burn samples (Figure 4.2).

The most abundant taxa in the dry soil burns are *Symbiobacterium* sp. (mean rel. abundance 6.4% in O horizons; 1.6% in mineral soil), *Domibacillus* sp. (mean rel. abundance 3.8% in O horizons; 0.1% in mineral soil), and *Pseudomonas* sp. (mean rel. abundance 2.3% in O horizons; 2.7% in mineral soil). The OTU identified as having the largest positive response in the dry soil burn compared to the unburned soil was classified as *Massilia* sp. (mean rel. abundance 1.8% in dry soil burn O horizon; 0.005% in unburned O horizon). *Massilia* sp. was not significantly enriched in the mineral soil of the dry soil burn (mean rel. abundance 1.3%) compared to the unburned mineral soil (mean rel. abundance 0.09%) (Figure 4.2 and 4.4). The OTU with the second largest response was classified as *Cohnella* sp. (mean rel. abundance 1.3% in dry soil burn O horizon; 0.04% in unburned O horizon). *Cohnella* sp. was also enriched in the dry soil burn mineral soil (mean rel. abundance 0.74%) compared to the unburned mineral soil (mean rel. abundance 0.08%) (Figure 4.4). *Cohnella* sp. was identified as a positive and negative fire responder in the O horizon and mineral soil, respectively, of the wet soil burns (Figure 4.2); however, *Cohnella* sp. was not detected in most of the unburned soil horizon (undetected in 23 of 30 horizons) or the wet soil burn horizons (undetected in 24 of 30 horizons). In comparison, *Cohnella* sp. was detected in 31 of 34 dry soil burn horizons.

The OTU identified as having the largest negative response in the dry soil burn compared to the unburned soil was classified as WD260 (mean rel. abundance 0.0004% in dry soil burn O horizon; 0.2% in unburned O horizon) (Figure 4.2). WD260 was slightly depleted in the dry soil burn mineral soil (mean rel. abundance 0.04%) compared to the unburned mineral soil (mean rel. abundance 0.1%).

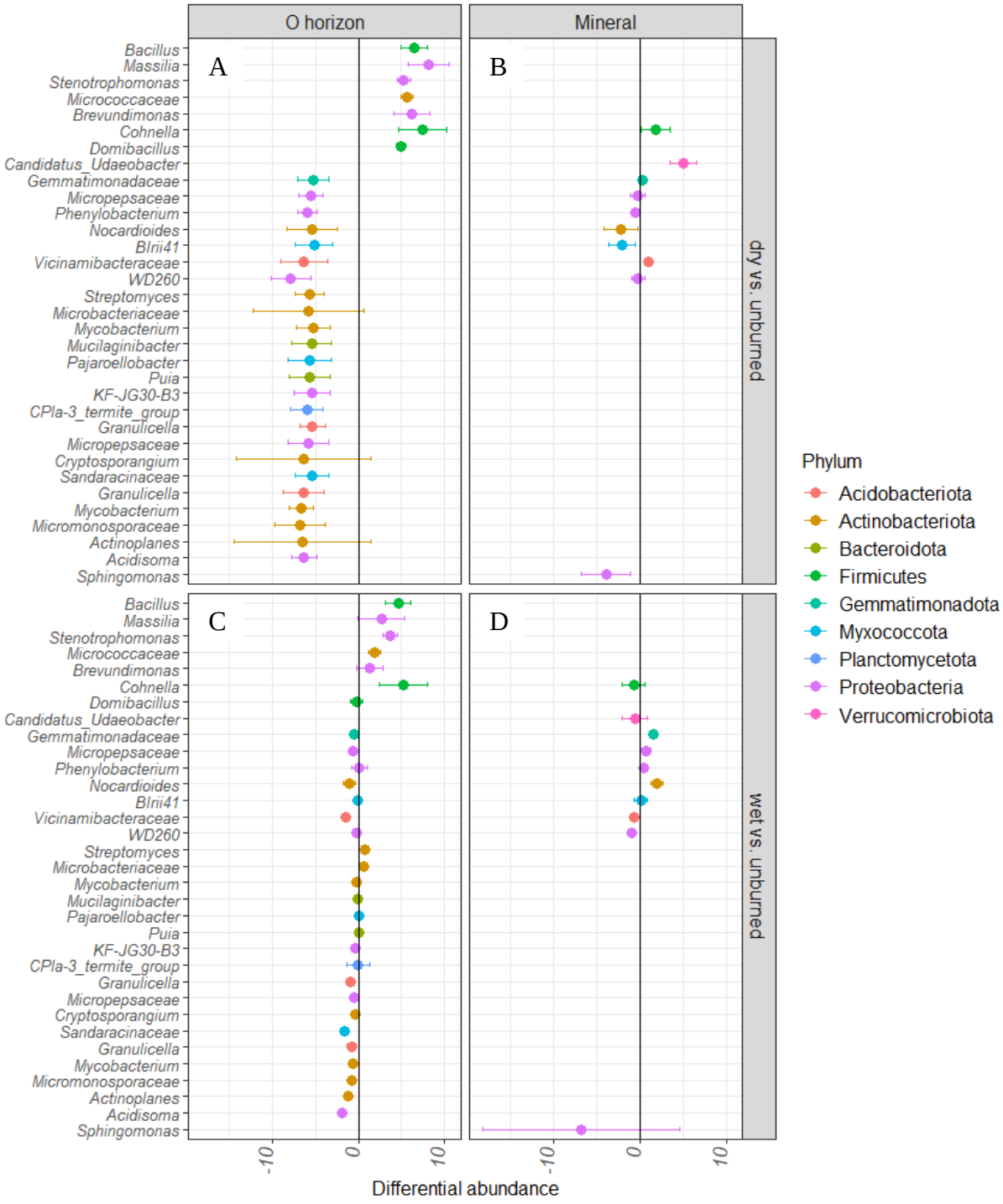

Figure 4.2. Differential abundance of bacterial OTUs (with a mean relative abundance > 0.1%) between the dry soil burn samples and unburned soil in the (A) O horizon and (B) mineral soil and between the wet soil burn samples and unburned soil in the (C) O horizon and (D) mineral soil, controlling for dominant vegetation and soil texture. Each point represents an individual bacterial OTU. Colors represent phyla-level classification. Error bars represent 95% prediction intervals for the differential abundance factor by sample. Y-axis is labelled with finest available taxonomy.

Differentially abundant bacterial taxa and fire survival

In the dry soil burn samples, multiple bacterial OTUs were also identified as being significantly enriched or depleted compared to the unburned soil 24 hours post-burn (Ch. 3) as well as after this fast-growth incubation – *i.e.*, were also putative strong or weak fire survivors, respectively (enriched: 11 OTUs, O horizon (24%); 31 OTUs, mineral soil (53%); depleted: 60 OTUs, O horizon (53%); 38 OTUs, mineral soil (51%)). *Cohnella* sp. and *Massilia* sp. were both significantly enriched at both timepoints in the O horizon and mineral soil (Supplementary Information Figure S4.15). *Bacillus* sp. was identified as a fire-survivor post-burn (Ch. 3, Figure 3.3) and was also significantly enriched at the end of the fast-growth incubation.

In the wet soil burn samples, multiple bacterial OTUs were also identified as being significantly enriched (17 OTUs, O horizon (21%); 24 OTUs, mineral soil (43%)) or depleted (46 OTUs, O horizon (59%); 34 OTUs, mineral soil (44%)) 24 hours post-burn (Ch. 3) as well as after the fast-growth incubation, compared to the unburned soil.

The effect of burning on mean 16S rRNA gene copy number

Weighted mean rRNA gene copy number was a significant predictor of bacterial community composition after controlling for dominant vegetation, pre-burn horizon thickness, pH, total C and N, soil texture, burn treatment, and DH (PERMANOVA, $p = 0.001$, $R^2 = 0.10$). In both the O horizon and the mineral soil, weighted mean rRNA gene copy number was significantly higher in the dry soil burn samples (mean = 3.4, O horizon; mean = 2.6, mineral soil) than in

the unburned soil (mean = 2.0, O horizon (ANOVA, p. adj. < 0.0001); mean = 1.6, mineral soil (ANOVA, p. adj. = 0.0002) and the wet soil burn samples (mean = 2.2, O horizon (ANOVA, p. adj. < 0.0001)); mean = 1.6, mineral soil (ANOVA, p. adj. = 0.0001); Supplementary Information Table S4.11). Mean RNA gene copy number was not significantly different between the wet soil burn samples and the unburned soil for either the O horizon (ANOVA, p = 0.70) or mineral soil (ANOVA, p = 1.0).

There was a significant positive relationship between DH and change in weighted mean predicted rRNA gene copy numbers for the O and mineral horizons of dry soil burn samples (Figure 4.3, A and B). There was a weak negative relationship between DH and change in predicted 16S rRNA gene copy numbers for the O horizon of wet soil burn samples ($y = -0.04x + 0.99$, $R^2_{adj} = 0.20$, $p = 0.03$) (Figure 4.3C). DH was not a significant predicter of weighted mean rRNA gene copy number in the mineral horizon of wet soil burn samples (Figure 4.3D).

Discussion

Burn effect at high and low moisture conditions

Burning under high and low soil moisture conditions had significant effects on bacterial community composition 24 hours post-burn (Ch. 3). At the end of the 5-week fast growth incubation, there is still a measurable effect of burning on bacterial community composition (Figure 4.1). There are several key differences between burned and unburned soils, including increased pH (Ch. 2), direct killing of taxa (Ch. 3), and potentially other changes that were not measured in this study, such as the production of pyrogenic organic matter (PyOM).

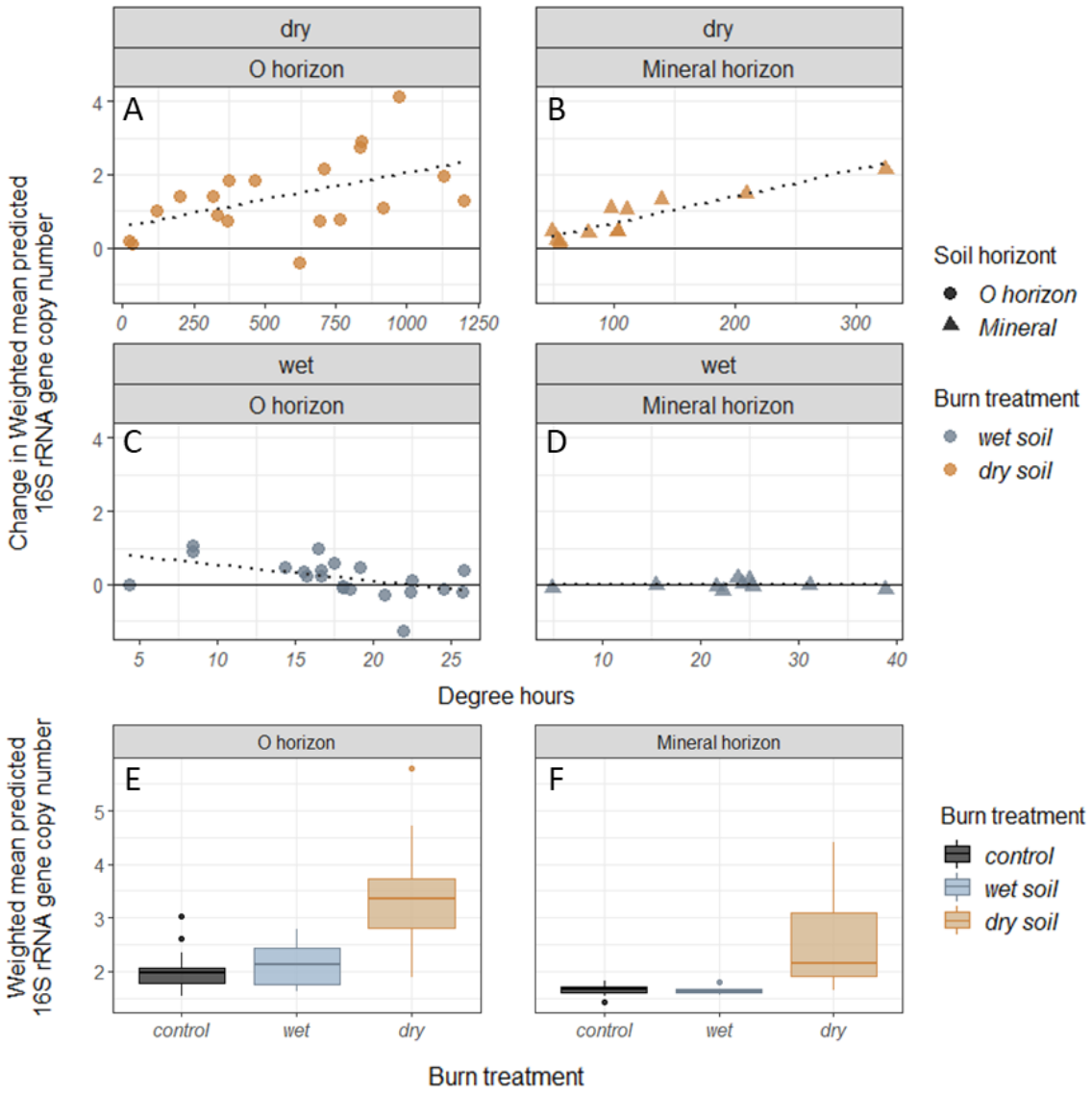

Figure 4.3. (A) Relationship between DH for the dry soil burns and difference in weighted mean 16S rRNA gene copy number from corresponding control core for the O horizon. Dotted line shows the linear fit ($y = 0.002x + 0.24$, $R^2_{adj} = 0.45$, $p < 0.001$). (B) Relationship between DH for the dry soil burns and difference in weighted mean 16S rRNA gene copy number from corresponding control core for the mineral horizon. Dotted line shows the linear fit ($y = 0.007x - 0.06$, $R^2_{adj} = 0.83$, $p < 0.001$). (C) Weak negative relationship between DH for the wet soil burns and difference in weighted mean 16S rRNA gene copy numbers from corresponding unburned control core for the O horizon. Dotted line shows the linear fit ($y = -0.04x+0.99$, $R^2_{adj} = 0.20$, $p = 0.03$). (D) DH versus difference in weighted mean 16S rRNA gene copy number. DH is not a significant predicter of weighted mean rRNA gene copy number in the mineral horizon of wet soil burn samples. (E) and (F) Weighted mean predicted 16S rRNA gene copy number for unburned controls, wet soil, and dry soil burns in the O and mineral horizons, respectively. For figures A-D, circles and triangles indicate O and mineral horizons, respectively. For all figures, points are colored by burn treatment.

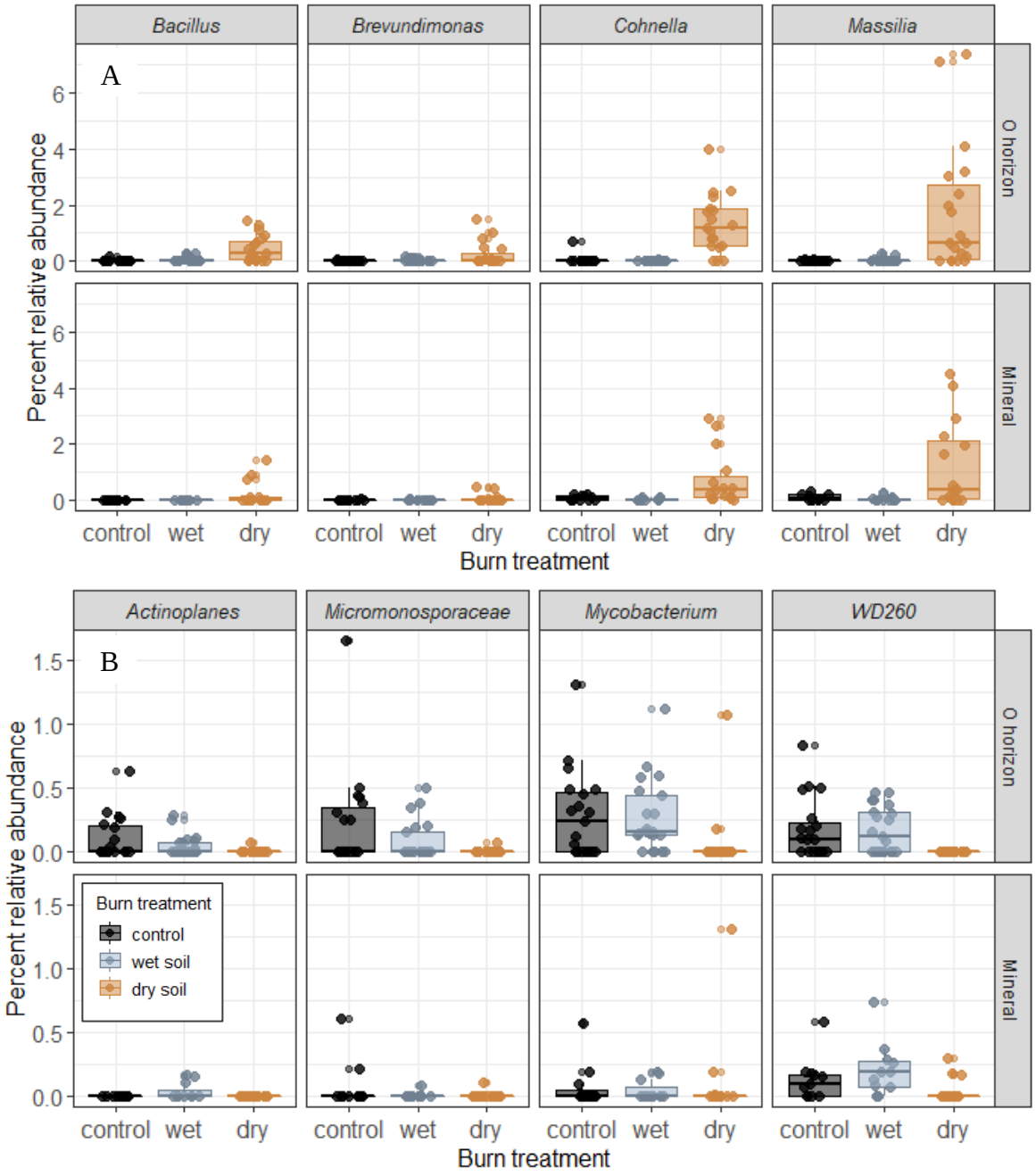

Figure 4.4. Relative abundance (%) of 4 selected bacterial OTUs (with mean relative abundance > 0.1%) identified as (A) positive fire-responders and (B) negative fire-responders in either the O horizon (top) and/or mineral soil (bottom) in the dry soil burn samples compared to the unburned soil. Each point represents an individual OTU within a single sample. Color represents burn treatment.

Burning caused an increase in soil pH in both the dry and wet soil burns, and soil pH (measured 24 hours post-burn; Ch. 2) was the strongest predictor of bacterial community composition at the end of the fast-growth incubation. This suggests that either increased pH at the time of the burn caused a shift in community composition that persisted for the duration of the fast-growth incubation, and/or soil pH remained higher in the burned soil than in the unburned soil. An increase in soil pH in burned soils has been shown to persist for months to years after burning (Úbeda et al. 2005; Certini 2005), so we would expect the same to be the case in this study, suggesting selection for a higher pH-adapted community in the burned samples. This strong effect of pH (here, driven by burning) is consistent with numerous studies of soil pH and bacterial community composition. For example, increasing pH from 4 to 8 correlates with increased levels of bacterial richness and total abundances (Rousk et al. 2010; Bahram et al. 2018).

There is evidence that recovery of bacterial richness and microbial biomass to pre-burn levels may take years to decades (Pressler et al. 2019). Microbial dispersal is one mechanism that has been shown to increase soil bacterial community resilience to disturbance (Sorensen and Shade 2020). After controlling for pH, burning and DH had a significant effect on bacterial community composition. The greatest number of bacterial OTUs were identified as depleted in the dry soil burn samples 24-hours post-burn. Some of these OTUs recovered by the end of the incubation and were no longer depleted, but a subset did not. The taxa that did not recover may have low temperature tolerances, as observed in Chapter 2, in addition to low pH thresholds (or other burn-affected soil conditions) that render them unsuitable to the post-burn environment, as seen in this chapter. Dispersal from the mineral soil to the O horizon was a

possible mechanism for replenishment of bacterial taxa with low thermotolerance that did not survive the burn in this study. (We will explore the potential effect of dispersal from outside the soil cores further in Chapter 5.)

Another distinction between the burned and unburned soil is the presence of PyOM, which is produced via incomplete combustion of OM. Generally, increase in PyOM has been shown to favor microbes with the ability to decompose aromatic substrates (Czimczik and Masiello 2007; Woolet and Whitman 2020). While we did not characterize PyOM in this study, some of the enriched taxa do include putative PyOM-degraders, such as *Massilia* sp. (Liu et al. 2014). (We will further discuss the ability to degrade PyOM as a potentially important trait in shaping microbial community composition in the weeks to years post-fire in the next chapter.)

Persistence of fire-surviving taxa

Fewer OTUs were enriched or depleted in both the dry and wet soil burn samples 5 weeks after burning compared to 24 hours post-burn, suggesting that fire-survival does not necessarily also indicate the ability to persist in the post-fire environment. Most of the taxa identified as fire-survivors (Ch. 3) were no longer enriched in the dry soil burns compared to the unburned soil. High temperature tolerance and spore-formation are possible microbial fire-survival traits, while rapid growth may interact with these traits to allow certain taxa to dominate post-fire communities over timescales of weeks. We identified 11 OTUs in the O horizon of the dry soil burns that were enriched at both timepoints, indicating that these taxa are both fire-survivors and also able to persist in the post-fire environment. For example, *Bacillus* sp., *Cohnella* sp.,

and *Massilia* sp. were enriched in the dry soil burn samples compared to the unburned soil at both timepoints (Supplementary Information Figure S4.15). As noted in Ch. 3, endospores are able to survive higher temperatures than vegetative cells (Nicholson et al. 2000), and spore-forming bacteria are common in fire-impacted soils. *Bacillus* and *Cohnella* are both aerobic, spore-forming bacteria in the phylum *Firmicutes* (Kämpfer et al. 2006; García-Fraile et al. 2008; Khianngam et al. 2010) and have previously been identified in burned soils (Lucas-Borja et al. 2019; Smith et al. 2008; Vázquez et al. 1993; Prendergast-Miller et al. 2017; Villadas et al. 2019). The ability to form spores would likely confer a fire-survival advantage to *Bacillus* and *Cohnella*. The similar level of relative abundance and degree of enrichment of *Bacillus* sp. 24 hours and 5 weeks post-burn suggests that *Bacillus* sp. are persisting as an endospore throughout the fast-growth incubation. The degree of enrichment of *Cohnella* sp. was higher 5 weeks post-burn, suggesting that the addition of water during the incubation could have induced germination and an increase in overall abundance.

Copiotrophic growth strategies is another potential microbial trait enabling bacterial taxa to persist in and colonize the post-fire environment. For example, *Massilia* are characterized as aerobic, non-spore forming, root-colonizing bacteria and have been characterized as copiotrophs (Ofek et al. 2012). Increase in relative abundance of *Massilia* with burning has been found in previous studies (Whitman et al. 2019; Sáenz de Miera et al. 2020; Weber et al. 2014). Heat-induced death of bacteria and fungi paired with incomplete combustion of dead cells may be creating a soil environment favorable to copiotrophic growth strategies. Thus, it is likely that a combination of microbial traits enables bacterial taxa to persist in and colonize the post-fire environment.

Relationship between burning and fast-growing bacteria

At the end of the incubation, we found a clear increase in mean predicted 16S rRNA gene copy number in the dry soil burn samples compared to the unburned soil. The increase in gene copy number was positively correlated with DH in the O horizon and mineral soil of the dry soil burn samples. Higher copy numbers of rRNA operons have been found to correlate with faster sporulation and growth rates (Yano et al. 2013; Roller, Stoddard, and Schmidt 2016) and have been proposed as a mechanism allowing bacteria to quickly colonize burned environments (Pérez-Valera et al. 2019). This is supported by the relatively high respiration rates at the beginning of the incubation and the decrease in respiration rates with time since burn (Ch. 2; Supplementary Information Figure S2.7 and S2.8). Burning under low moisture conditions depleted total biomass in the original bacterial communities (Ch. 3). Any bacterial cells that were killed during the burn but not fully combusted (*i.e.*, "necromass") become available nutrients for surviving bacteria. Additionally, combustion of OM and production of ash increases the bioavailability of nutrients at the soil surface (Neary et al. 2005). Bacteria with higher rRNA gene copy numbers may have a selective advantage in responding to increased nutrient availability (Nemergut et al. 2015). For example, the taxon with the greatest increase in relative abundance in the dry soil burns was identified as *Massilia* sp. Mean rRNA gene copy number for species within this genus is 6.4 (SD = 1.9), which is higher than the community-weighted mean for all samples.

However, community-weighted mean 16S rRNA copy numbers would not be expected to remain high in the dry soil burns over long periods of time. Previous work has found a decrease in copy number in the months and years post-burn (Nemergut et al. 2015; Pérez-Valera et al.

2019), suggesting that copiotrophic bacteria are responding to a temporary pulse in nutrient availability. Thus, the trait of potential fast growth may be most important at intermediate timescales, with adaptation to soil conditions being more important over longer timescales.

Conclusion

An effect of burning under high and low moisture conditions on soil bacterial community composition persisted for 5 weeks after the burn. Our results identify fire-surviving taxa with the ability to colonize the post-fire environment and show an increase in community-mean 16S rRNA gene copy number with burning. Our findings support previous work showing that bacterial spore formation and fast growth are potential fire-survival traits structuring post-burn community composition. Our findings also demonstrate that fire survival alone is not sufficient to allow a taxon to dominate the soil community post-fire. This chapter focused on the fast-growing bacteria in the 5 weeks post-burn. In our fast-growth incubation, dispersal was limited to dispersal between the O and mineral horizons of an individual sample. In boreal forests, spatial heterogeneity in fire severity leads to complete combustion of soil O horizon in some areas while other areas are unburned (Lavoie and Mack 2010; Carlson et al. 2011). Microbial dispersal from unburned to burned soils is one mechanism that has been shown to increase soil bacterial community resilience to disturbance (Sorensen and Shade 2020). In the next chapter, we will explore the ability of fire-susceptible bacteria to recolonize and thrive in the post-fire environment.

CHAPTER 5

BACTERIAL AFFINITY FOR THE POST-FIRE ENVIRONMENT

Introduction

In boreal forests, spatial heterogeneity in fire severity results in complete combustion of soil O horizons in some areas while other areas remain unburned (Lavoie and Mack 2010; Carlson et al. 2011). Microbial dispersal from unburned to burned soils is one mechanism that has been shown to increase soil bacterial community resilience to disturbance (Sorensen and Shade 2020; Moore et al. 2021). Microbial traits such as high thermotolerance or the ability to form spores that confer a fire-survival advantage may become less important if microbial dispersal reintroduces fire-susceptible taxa and restructures the post-fire soil environment.

The effects of burning on soil properties such as pH and carbon availability can be large (Ch. 2) and can persist for years post-fire (Bodí et al. 2014; DeLuca and Aplet 2008; Dove et al. 2020; Úbeda et al. 2005). During fires, pyrogenic organic matter (PyOM) is produced via the incomplete combustion of soil organic carbon (Goldberg 1985). Generally, PyOM contains a high proportion of aromatic compounds (Knicker 2011), whose chemical recalcitrance increase the persistence of PyOM in soil (Skjemstad et al. 1996). The addition of PyOM to soil has been shown to affect microbial community composition (Whitman et al. 2016; Lanza et al. 2016), but microbial community response is not consistent across varying soil types and with PyOM derived from various sources and heat treatments (Woolet and Whitman 2020). Soil bacteria with the ability to degrade PyOM may show an affinity for the post-fire environment.

In this chapter, we use DNA-derived microbial community composition data to test whether changes in bacterial community composition post-burn persist after a 6-month incubation, and whether there are taxa that do not survive burning but do well under post-fire soil conditions. We used unburned soil as an inoculum to reintroduce the original soil bacterial community to the post-fire environment to explore the ability of fire-susceptible bacteria to recolonize and thrive in the post-fire environment. We also looked for the presence of fire-surviving (Ch. 3) and fast-growing (Ch. 4) bacterial taxa 6 months post-fire.

We hypothesize that (a) burning would have an effect on microbial community composition and the effect would be greater for burning under low soil moisture conditions than under high soil moisture conditions 6 months post-burn, (b) bacterial taxa identified as fire-survivors (Ch. 3) and fast-growing in the post-fire environment (Ch. 4) would be enriched in the dry soil burn samples 6 months post-fire, and (c) bacterial taxa that were depleted 24 hours post-burn would not thrive in the burned environment.

To identify bacteria with an affinity for the post-burn environment with and without the ability to survive fire, we autoclaved soil cores one day after the burn treatment to deplete the surviving microbial community and added an unburned soil inoculum. We used nucleic acid sequencing pre- and post-incubation to identify changes in microbial community over these three timepoints. We used a taxon regression model to test for differential abundance of individual bacterial taxa across burn treatments and degree-hours (DH; Ch. 2) at the end of the 6-month incubation.

Methods

In brief, soil cores under varying moisture conditions were subjected to burn simulations in a cone calorimeter. Horizon-weighted subsamples of each core were used for a paired 6-month long incubation with the goal of identifying bacteria with an affinity for the post-fire environment (see Ch. 2, Methods for further details). Mineral soil and the O horizon from each core were separated and divided equally into two sterilized and acid-washed 60 mL glass jars. The first jar was capped with tinfoil and autoclaved at 121 °C and 20 psi for 20 minutes. Samples were removed from the autoclave and allowed to cool to room temperature. The second jar was not autoclaved. All samples were then inoculated with soil from an unburned core from the same site. Of the remaining cores from each site, we selected the one that best resembled the three experimental cores (dry soil burn, wet soil burn, control) to use as the unburned inoculum core and deconstructed it into homogenized O horizon and mineral soil. Each long-term incubation sample received an inoculum representing 10% on a dry sample mass basis by horizon (Lankau et al. 2011; Lankau 2009). Sample and inoculum were thoroughly mixed, and then the mineral and O horizons were recombined into a single jar resulting in two paired jars – one autoclaved and one not – for all cores (burn treatment x site combinations) and incubated for 6 months. After 6 months, the incubations were dismantled, and samples were separated into O horizon and mineral soil (where present). Samples for nucleic acid sequencing were immediately stored at -80 °C until processing.

DNA extraction, amplification, and sequencing

DNA extractions were performed for each soil horizon of each sample, along with a blank extraction (identical methods but with empty tubes) for every 15 samples using DNeasy PowerLyzer PowerSoil DNA extraction kits (QIAGEN, Germantown, MD, USA). In brief, 0.1 or 0.25 g of O horizon or mineral soil, respectively, were added to a PowerBead tube. Sample underwent homogenization and lysis on a FastPrep-24™ 5G bead beating grinder and lysis system (MP Biomedicals, Irvine, CA, USA) at a frequency of 6.5 m s^{-1} for 45 sec at room temperature. DNA was cleaned of non-DNA organic and inorganic material and concentrated following the manufacturer's instructions. Extracted DNA was amplified via triplicate PCR targeting the 16S RNA gene v4 region with 515f and 806r primers (Walters et al. 2016). Barcodes and Illumina sequencing adapters were added as per Kozich et al. (2013). In brief, PCR contained 1.25 µL 515f forward primer (10 µM), 1.25 µL 806r reverse primer (10 µM), 1 µL DNA template, 12.5 µL Q5 Hot Start High-Fidelity 2X Master mix (New England BioLabs INC., Ipswich, MA, USA), 1.25 µL Bovine Serum Albumin (BSA) (20 mg mL^{-1}) (VWR, Radnor, PA), and 7.75 µL nuclease-free water, in a 96-well plate. The plate was sealed and placed on an Eppendorf Mastercycler nexus gradient thermal cycler (Hamburg, Germany). Reactions were run at 98 ℃ for 2 minutes + (98 ℃ for 30 seconds + 58 ℃ for 15 seconds + 72 ℃ for 10 seconds) x 30 cycles + 72 ℃ for 2 minutes and 4 ℃ hold. The quality of PCR amplicon triplicates was assessed using gel electrophoresis, and triplicates were then pooled, purified, and normalized using SequalPrep Normalization Plate Kits (96-well) (ThermoFisher Scientific, Waltham, MA, USA). Samples were then pooled, and library cleanup was performed using a Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA).

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Bioinformatics and statistics

We worked primarily in R (Team 2020) relying extensively on R packages *phyloseq* (McMurdie and Holmes 2013), *dplyr* (Wickham et al. 2021), *ggplot2* (Wickham 2016), and

vegan (Oksanen et al. 2020). We compared community composition across samples using weighted UniFrac dissimilarities on relative abundances and tested for significant effects of horizon (O horizon versus mineral soil) dominant vegetation, pre-burn horizon thickness, pH, total C and N, soil texture, DH, and autoclaving using a permutational multivariate ANOVA (PERMANOVA using the *adonis* function in *vegan* (Oksanen et al. 2020)). We used single-component models to compare the R^2 for each factor. We analyzed the effects of burning and DH on differential abundance of bacterial taxa within each soil horizon using the *corncob* package (Martin et al. 2020) in R and controlling for dominant vegetation, soil texture class, and autoclave treatment. We used a mean abundance cutoff of 0.000001 to filter out rare taxa and used a controlled false discovery rate cutoff of 0.05 to adjust p values of differentially abundant taxa to account for potential false positives.

Results

Predictors of bacterial community composition

All tested factors (dominant vegetation, soil horizon and texture, pH, total C and N, burn treatment, DH, and autoclave treatment) were significant predictors of community composition for bacteria (Supplementary Information Table S5.12) in the combined model (PERMANOVA, $p = 0.001$ all factors). Soil pH and texture provided the most explanatory power for bacterial community composition ($R^2 = 0.08$ and 0.07, respectively; Supplementary Information Figure S5.17) followed by DH ($R^2 = 0.06$, Figure 5.1). Autoclaving provided the least explanatory power for bacterial community composition ($R^2 = 0.02$; Supplementary

Information Figure S5.16). The PERMANOVA results for burn treatment and soil texture could be affected by non-homogenous dispersion of data. The dispersion of bacterial communities across treatments differed significantly depending on the burn treatment (*betadisper*, F = 9.30, p = 0.002) and soil texture (*betadisper*, F = 3.03, p = 0.015).

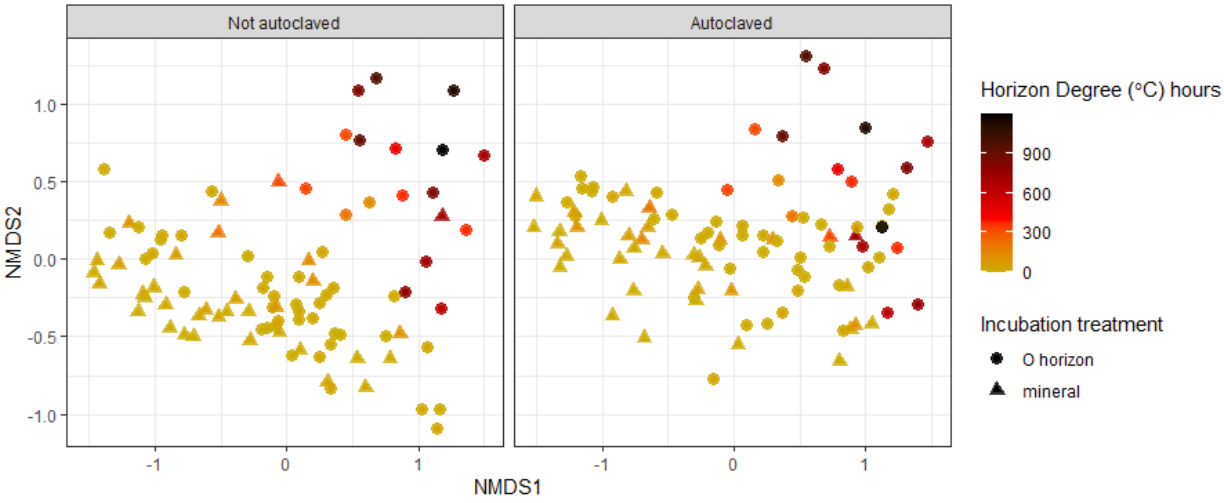

Figure 5.1. First two axes of NMDS on weighted UniFrac dissimilarity (k =3, stress = 0.11). Color represents degree hours with darker colors indicating higher degree hours. Circles and triangles represent the O horizon and mineral soil, respectively.

Differential abundance of individual bacterial taxa in burned soil

In the dry soil burn samples, multiple bacterial OTUs were identified as being significantly enriched (51 OTUs, O horizon; 34 OTUs, mineral horizon) or depleted (75 OTUs, O horizon; 43 OTUs, mineral horizon) compared to the unburned soil after controlling for dominant vegetation, soil texture, and autoclaving (Supplementary Information Figure S5.18). In the wet soil burn samples, multiple bacterial OTUs were identified as being significantly enriched (68 OTUs, O horizon; 35 OTUs, mineral horizon) or depleted (58 OTUs, O horizon; 42 OTUs,

mineral horizon) compared to the unburned soil after controlling for dominant vegetation, soil texture, and autoclaving (Figure 5.2).

The most abundant taxa in O horizons of the dry soil burns were classified as *Streptosporangium* (mean abundance 3.6 %, O horizon; 0.2% mineral soil) and *Bacillus* sp. (mean abundance 1.4%, O horizon; 0.8 % mineral soil) (Figure 5.3). Relatively few of the taxa that were enriched in relative abundance 24 hours or 5 weeks after the burns remained enriched 6 months post-fire and after inoculation with unburned soil. Of the 51 OTUs identified as significantly enriched in the O horizon 6 months post-burn, 47 OTUs were not identified as enriched 24 hours (Ch. 3) and 40 were not identified as enriched 5 weeks (Ch. 4) post-burn. Of the 119 OTUs identified as enriched 24 hours post-burn (Ch. 3) in the O horizon and/or mineral soil of the dry soil burns, only 4 were enriched (Supplementary Information Figure S5.19) and 10 were depleted in the O horizon 6 months after the dry soil burn compared to the unburned soil. Of the 95 OTUs identified as enriched 5 weeks post-burn (Ch. 4) in the O horizon or mineral soil of the dry soil burns, only 11 were enriched and 10 were depleted in the O horizon 6 months after the dry soil burn compared to the unburned soil.

Relatively few of the taxa that were depleted in relative abundance 24 hours after the burns were enriched 6 months post-fire and after inoculation with unburned soil. Of the 169 OTUs identified as depleted 24 hours post-burn (Ch. 3) in the O horizon and/or mineral soil of the dry soil burns, only 3 were enriched and 24 were depleted in the O horizon 6 months after the dry soil burn compared to the unburned soil (Figure 5.4).

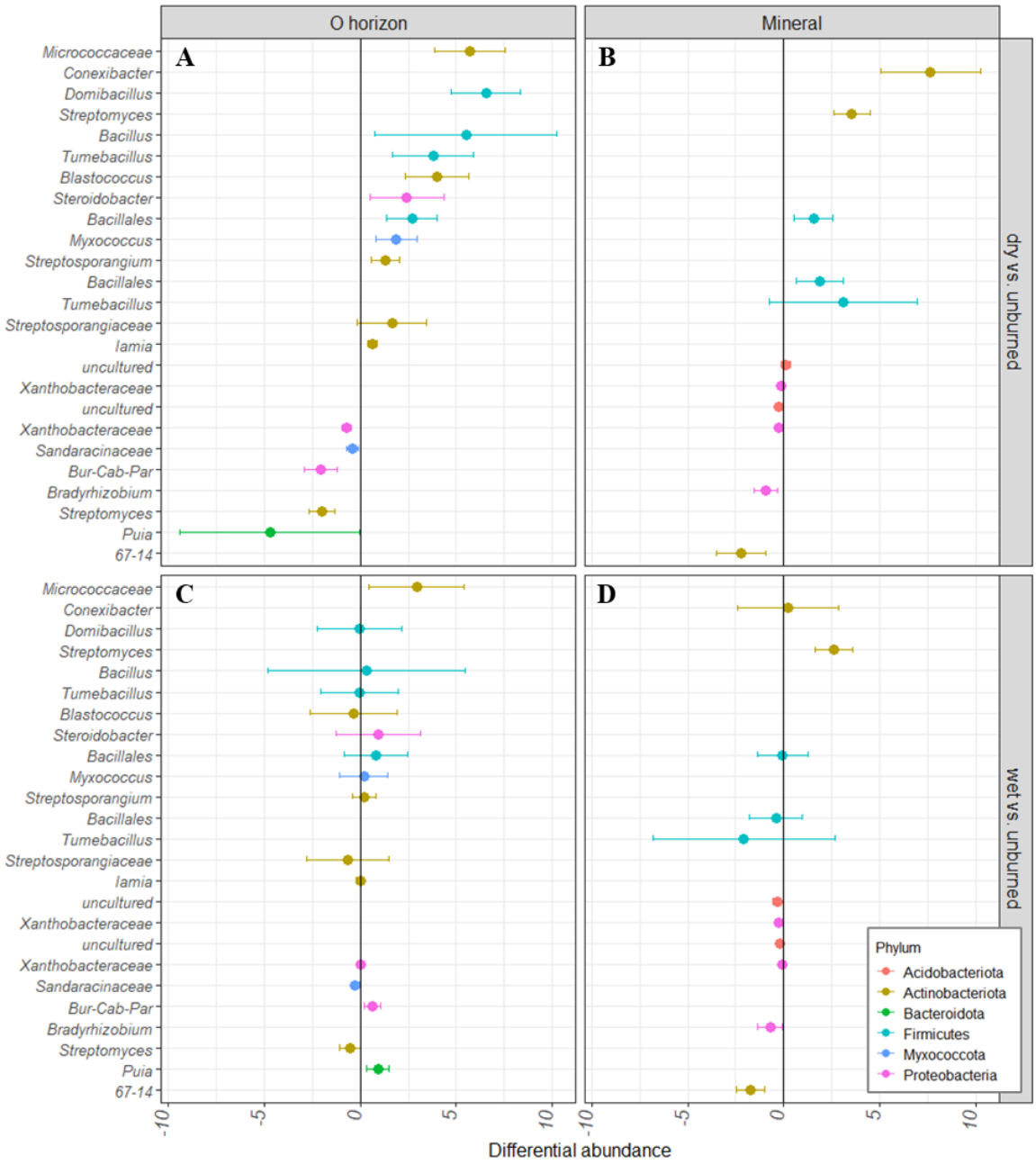

Figure 5.2. Differential abundance of bacterial OTUs (with mean relative abundance > 0.5%) between the dry soil burn samples and unburned soil in the (A) O horizon and (B) mineral soil and between the wet soil burn samples and unburned soil in the (C) O horizon and (D) mineral soil controlling for dominant vegetation and soil texture. Each point represents an individual bacterial OTU. Colors represent phyla-level classification. Error bars represent 95% prediction intervals for the differential abundance factor by sample. Y-axis is labelled with finest available taxonomy. The genus *Burkholderia-Caballeronia-Paraburkholderia*" has been abbreviated to "*Bur-Cab-Par*".

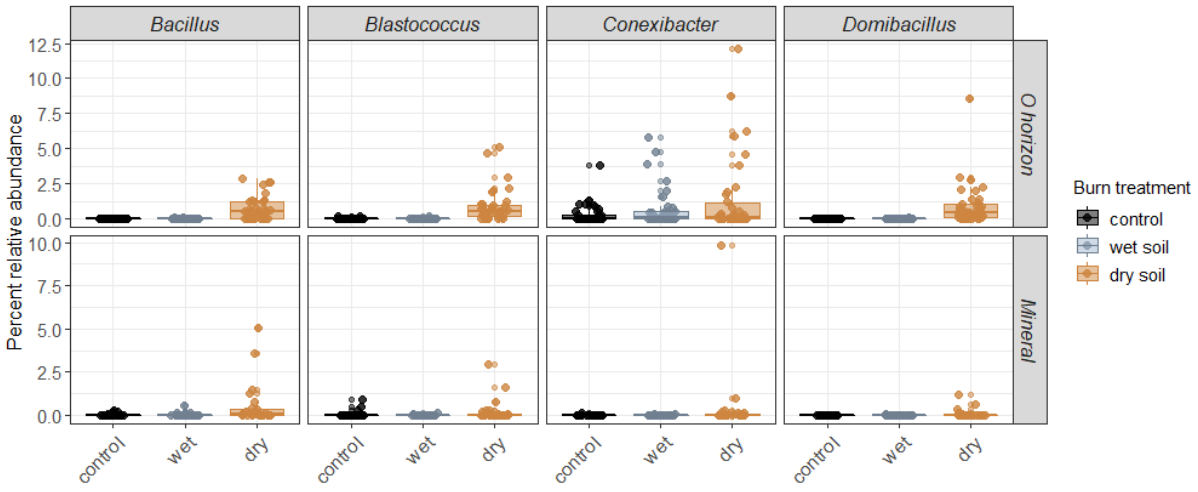

Figure 5.3. Percent relative abundance of selected bacterial taxa (with mean relative abundance > 0.1%) in the O horizon (top) and mineral soil (bottom) identified as significantly enriched in the 6 months post-burn of the dry soil burn samples compared to the unburned soil. Each point represents an individual OTU within a single sample. Color represents burn treatment.

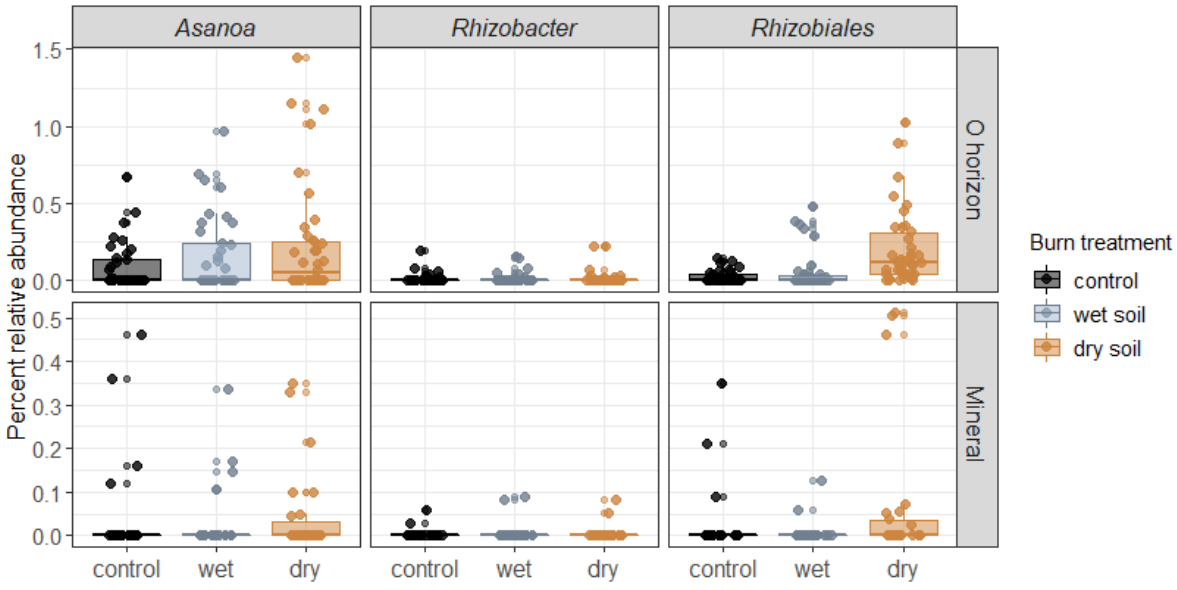

Figure 5.4. Percent relative abundance of fire-susceptible bacterial taxa (identified as depleted 24 hours post-burn; Ch. 3) identified as significantly enriched in the O horizon (top) and/or mineral soil (bottom) of the dry soil burn samples compared to the unburned soil 6 months post-burn and after inoculation with unburned soil. Each point represents an individual OTU within a single sample. Color represents burn treatment.

Discussion

Persistence of the effect of burning at high and low soil moisture

Soil bacterial communities were structured most strongly by soil pH and then by soil texture, which is consistent with our findings both 24 hours (Ch. 3) and 5 weeks (Ch. 4) post-burn. Burning and soil moisture had a measurable effect on soil bacterial communities 24 hours (Ch. 3) and 5 weeks (Ch. 4) post-burn. The effect of burning on soil bacterial communities is still evident 6 months post-burn and with inoculation of unburned communities (Figure 5.1 and 5.2). This indicates that there is likely meaningful selection for microbes adapted to post-fire conditions due to post-fire soil properties and beyond the simple ability to survive the fire. It also supports our hypothesis that the microbial communities of burned and unburned soil would still differ 6 months post-burn and with inoculation with unburned communities, and that burning would have a different effect on microbial communities of low and high moisture soil.

Burning could be affecting community composition via long-term changes in soil pH and/or the production of PyOM. Increasing the thermal degradation temperature of OM from 200 to > 400 ℃ leads to increasing aromaticity and decreasing microbial degradation rates of PyOM (Wiedemeier et al. 2015; McBeath et al. 2011; Baldock and Smernik 2002). The temperatures during the dry soil burns were high enough to produce PyOM (Keiluweit et al. 2010), and all dry soil burns resulted in some degree of O horizon combustion (Ch. 2). The wet soil burns also experienced combustion, but the loss in O horizons across all samples was smaller for the wet soil burns than for the dry soil burns. Thus, it is likely that the dry soil burn samples

contained more carbon in the form of PyOM than the unburned soil (although it is also possible that smoldering conditions during wet soil burns could help support the low-O_2 conditions required for incomplete combustion). This is supported by the increase in the size of the slow C pool of the dry soil burns compared to the wet soil burns and unburned soil (Ch. 2). Thus, traits such as the ability to degrade PyOM may become increasingly important with time since burning, as the easily-mineralizable C pools are depleted over time. Bacteria with the ability to degrade complex, aromatic compounds may be better suited to the post-fire environment than copiotrophic bacteria, particularly over long timescales.

Persistence/depletion of fire-surviving and fast-growing bacterial taxa

By inoculating the cores with unburned soil, we reintroduced the original, unburned bacterial community to the burned environment, in order to identify bacteria that were unable to survive burning but able to thrive in the post-fire environment. The majority of taxa identified as fire-survivors (Ch. 3) and fast-growers in the post-burn environment (Ch. 4) were not enriched 6 months post-burn. This does not support our hypothesis that fire-surviving and fast-growing bacterial taxa would be enriched in the burned soil 6 months post-fire.

The ability to form spores and a high thermotolerance may be important traits for fire-survival, as evidenced by the increase in relative abundance of spore-forming taxa in burned versus unburned soils (Yeager et al. 2005) and relatively low temperature thresholds of some soil bacterial taxa (Pingree and Kobziar 2019). We found an increase in relative abundance of spore-forming taxa 24 hours post-burn (Ch. 3), but most of these taxa were no longer enriched

6 months post-burn. Two of the 4 taxa enriched 24 hours and also 6 months post-burn were spore-forming bacteria. This suggests that spore-formation may allow some bacteria to persist in the soil but does not necessarily convey a long-term advantage in the post-fire environment.

In the short-term (5 weeks), we saw that fast-growth may be an advantage to bacteria in colonizing the post-burn environment (Ch. 4), as evidenced by an increase in community-mean 16S rRNA gene copy numbers in the dry soil burns with increasing DH. Most of the OTUs identified as being enriched 5 weeks after the burn were no longer enriched 6 months after the burn and with inoculation from unburned soils, suggesting that fast growth does not confer an increased affinity for the post-fire environment. As available nutrients in the soil decrease and the soil environment becomes nutrient-poor, bacteria with oligotrophic growth strategies will have an advantage (Fierer et al. 2007). Oligotrophic bacteria are slow growing and can outcompete copiotrophic bacteria in low nutrient environments due to higher substrate affinities. For example, some bacterial genera have been shown to increase in relative abundance in the presence of PyOM (Woolet and Whitman 2020). In this study, one taxon classified as *Micrococcaceae* was significantly enriched in the O horizons of the dry and wet soil burns (Figure 5.2). There is evidence that some members of the family *Micrococcaceae* are able to degrade aromatic hydrocarbons (Storey et al. 2018; Aryal and Liakopoulou-Kyriakides 2013) and *Micrococcaceae* have been found in increased relative abundance in burned soils (Whitman et al. 2019; Sáenz de Miera et al. 2020). It is likely that bacteria with the ability to degrade PyOM will have an increased affinity for the post-burn environment.

Ability of fire-susceptible bacterial taxa to colonize post-fire environment

The composition of enriched taxa changes with time since burn. However, most of the taxa that were identified as fire-susceptible (*i.e.,* depleted 24 hours post-burn, Ch. 3) were also not enriched at the end of this incubation. Thus, the addition of unburned taxa to the burned soil does not appear to be driving changes in community composition in the months following burning. This supports our hypothesis that even if fire-susceptible bacteria are reintroduced to the burned environment, these taxa will not necessarily be able to colonize the post-fire environment. This could be explained by changes in soil chemical and physical properties with burning. Burning increases soil pH, which changes nutrient availability – increasing pH from 5 to 8 decreases the bioavailability of iron, magnesium, and phosphorus (Neary et al. 2005).

Some taxa from the phylum *Acidobacteria* thrive at low pH and have been found to decrease in relative abundance with an increase in soil pH (Rousk et al. 2010; Bang-Andreasen et al. 2017; Brais et al. 2015). We found multiple taxa of the phylum *Acidobacteria* to be significantly depleted in the O horizon of the dry soil burns compared to the unburned soil (Supplementary Information Figure S5.18). If increased pH rendered low-pH *Acidobacteria* unviable or dormant, then these taxa would not be expected to flourish in the post-burn environment for several reasons. First, we added the unburned inoculum at the same timepoint that we measured soil pH, so the unburned bacteria were added to relatively high pH environments in the case of the dry soil burns, and so would not be expected to flourish. Second, increased soil pH in the burned soils likely persisted for the duration of the incubations. Elevated pH following burning has been measured 4-9 years post-fire (Dove et al. 2020; Úbeda et al. 2005; Humphreys and Lambert 1965). Post-burn soil pH is one of the

strongest drivers of soil bacterial community composition 24 hours post-burn and after 6 months and with inoculation. Thus, it is likely that the same conditions that made the soil inhospitable to certain taxa 24 hours post-burn prevented reintroduced unburned taxa from thriving in the post-burn environment 6 months later.

Following wildfires, bacterial dispersal is not limited to a single timepoint. Fire-susceptible taxa may be more likely to thrive in the post-fire environment if these taxa are introduced weeks to month versus hours post-burn. For example, as soil pH decreases to pre-burn levels, there may be an optimal time period for bacterial dispersal to occur to maximize proliferation of acidophilic bacterial taxa.

Conclusion

An effect of burning under high and low moisture conditions on soil bacterial community composition persisted for 6 months after the burn and with inoculation of unburned bacteria. Our results identify taxa with an affinity for the post-fire environment, regardless of their ability to survive burning. We found that neither fire-susceptible nor fire-surviving taxa reintroduced to the burned environment showed consistently strong affinities for the post-burn environment. Thus, our findings demonstrate that the presence of fire-surviving taxa in the burned environment is not sufficient to allow a taxon to dominate the soil community 6 months post-fire. Similarly, some taxa that are fire-susceptible may do well if reintroduced to burned soils, highlighting the potential importance of dispersal for these taxa. Fire-survival and fast-growth likely become less important microbial traits with increasing time since burning. Other

traits such as a tolerance for high pH and the ability to degrade PyOM may be more important for bacterial taxa to persist in the post-fire environment over time. The effects of burning on soil properties and bacterial communities are complex and variable. Understanding how bacterial community composition will shift following burning is an important step towards predicting changes in bacterial community function under changing wildfire regimes.

CHAPTER 6

CONCLUSION

Our findings support previous work showing that bacterial spore formation and fast growth are potential fire-survival traits structuring post-burn soil bacterial community composition, but fire survival alone is not sufficient to allow a taxon to dominate the soil community post-fire. We found that neither fast-growing nor fire-surviving taxa reintroduced to the burned environment showed consistently strong affinities for the post-burn environment. Fire-survival and fast-growth likely become less important microbial traits with increasing time since burning. Other traits such as a tolerance for high pH and the ability to degrade pyrogenic organic matter may be more important for bacterial taxa to persist in the post-fire environment over time. We suggest future work investigate the importance of various microbial traits, such spore-formation, copiotrophic growth strategies, and pyrogenic organic matter degradation, in shaping soil bacterial community composition in the months to years following fire.

We identified a small subset of fire-susceptible taxa that may do well if reintroduced to burned soils, highlighting the potential importance of dispersal for these taxa. Following wildfires, bacterial dispersal is not limited to a single timepoint. Fire-susceptible taxa may be more likely to thrive in the post-fire environment if these taxa are introduced weeks to month versus hours post-burn. We suggest future work investigate the impact of bacterial dispersal at multiple timepoints post-burn on soil bacterial community composition.

The effects of burning on soil properties and bacterial communities are complex and variable. Understanding how bacterial community composition shifts following burning is an important step towards predicting changes in bacterial community function under changing wildfire regimes.

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SUPPLEMENTARY INFORMATION

CHAPTER 2.

Table S2.1. Site characteristics, location, and soil properties grouped by dominant vegetation type.

Site	Slope position	Aspect	Overstory density (%)	Latitude	Longitude	Sand (%)	Silt (%)	Clay (%)
				***Picea* spp.**				
4	Middle	ESE	98	59° 27' 44.62" N	112° 17' 51.10" W	62.5	32.5	5
6	Lower	NW	52	59° 28' 19.14" N	112° 16' 23.58" W	70.5	19.5	10
7	Level	NNW	96	59° 35' 29.05" N	112° 16' 13.72" W		NA	
12	Toe		91	59° 48' 28.97" N	112° 00' 18.41" W		NA	
13	Level		79	59° 58' 24.99" N	112° 26' 57.74" W		NA	
17	Level		92	60° 01' 48.23" N	112° 53' 21.29" W		NA	
18	Level		92	60° 00' 54.13" N	112° 52' 42.88" W	33	37	30
				Populus tremuloides				
2	Level		94	59° 27' 07.18" N	112° 19' 35.98" W	54.5	32	13.5
5	Middle	NW	97	59° 27' 41.18" N	112° 17' 00.31" W	59.5	31	9.5
8	Level	S	99	59° 38' 59.28" N	112° 12' 45.89" W	68	25	7
11	Level		99	59° 47' 45.65" N	112° 02' 27.45" W	59	32	9
14	Level		89	60° 02' 54.21" N	112° 47' 50.94" W	72	20	8
15	Lower	NW	93	60° 02' 21.03" N	112° 52' 45.09" W	70	19	11
				Pinus banksiana				
1	Upper	W	96	59° 24' 38.19" N	112° 23' 50.15" W	23	43	34
3	Level		100	59° 27' 25.16" N	112° 19' 27.27" W	51.5	36	12.5
9	Level		97	59° 41' 04.03" N	112° 10' 18.51" W	54	29.5	16.5
10	Level		100	59° 42' 04.70" N	112° 10' 13.50" W	49	41	10
16	Level		85	60° 01' 17.15" N	112° 58' 14.23" W	50	32.5	17.5
19	Middle	SSE	91	60° 02' 05.49" N	113° 08' 40.44" W	93	3	4

Table S2.2. Cumulative hours above 500 ºC for the nine dry soil burns that reached temperatures >500 ºC at the upper thermocouple.

	Time over 500 ºC (hrs)	
Core ID	5 cm above core base	1 cm above core base
19UW-WB-03-02	0.8	0.0
19UW-WB-08-10	1.1	0.0
19UW-WB-09-08	0.4	0.0
19UW-WB-11-10	0.6	0.3
19UW-WB-12-02	2.5	0.0
19UW-WB-13-03	0.3	0.0
19UW-WB-14-07	0.6	0.0
19UW-WB-17-01	1.1	0.0
19UW-WB-18-05	0.5	0.0

Table S2.3. Mean soil temperature and degree hours above 21 ℃ at the O horizon-mineral soil interface and 1 cm above core base. Standard deviation in parentheses.

Burn treatment	Soil horizon	Maximum temp. at O/Mineral interface (℃)	Maximum temp. 1 cm above base of core (℃)	Degree hours at O/Mineral interface	Degree hours 1 cm above base of core
			***Picea* spp.**		
Dry soil	O (n=7)	357.3 (234.1)	152 (179)	699.3 (545.3)	242.2 (297.5)
	Mineral (n=3)	292.3 (249.1)	75.9 (68.2)	521 (606.4)	135.2 (163.8)
Wet soil	O (n=7)	29.9 (6.3)	28.6 (3.3)	19.3 (7.2)	26.3 (7.6)
	Mineral (n=2)	28.1 (2.7)	29 (1.7)	25.8 (0)	34.9 (5.4)
			Pinus banksiana		
Dry soil	O (n=6)	359.6 (157.6)	71.5 (35.1)	487.5 (314.4)	108.4 (53.3)
	Mineral (n=6)	359.6 (157.6)	71.5 (35.1)	487.5 (314.4)	108.4 (53.3)
Wet soil	O (n=6)	30.5 (5.3)	26.8 (1.9)	15 (5.5)	19.2 (7.8)
	Mineral (n=6)	30.5 (5.3)	26.8 (1.9)	15 (5.5)	19.2 (7.8)
			Populus tremuloides		
Dry soil	O (n=6)	439.5 (159)	199.6 (210.4)	745.9 (396.5)	323.5 (334.8)
	Mineral (n=4)	413.8 (195.9)	176.1 (230.5)	584.1 (309.3)	224.1 (246.5)
Wet soil	O (n=6)	28.1 (3.4)	25.9 (0.4)	16.7 (4.8)	20.2 (7)
	Mineral (n=4)	28 (4.1)	25.8 (0.5)	14.3 (3.9)	18.5 (7.5)

Table S2.4. Mean fractional dry mass loss during burning grouped by dominant vegetation type and burn treatment. Standard deviation in parentheses.

Dominant Vegetation	**Burn treatment**	**Fractional dry mass loss**
Picea sp.	dry	0.2 ± (0.19)
Picea sp.	wet	0.14 ± (0.07)
Pinus banksiana	dry	0.09 ± (0.05)
Pinus banksiana	wet	0.06 ± (0.03)
Populus tremuloides	dry	0.23 ± (0.21)
Populus tremuloides	wet	0.1 ± (0.03)

Table S2.5. Mean soil pH, total C, total N, and horizon thickness for dry and wet soil burn samples and unburned soil grouped by dominant vegetation. Standard deviation in parentheses.

Burn treatment	Soil horizon	C:N	Total C (%)	Total N (%)	pH	Pre-burn horizon thickness (cm)	Post-burn horizon thickness (cm)
			***Picea* spp.**				
Control	O (n=7)	22.6 (6.1)	37.8 (9.3)	1.7 (0.5)	4.5 (1)	8.9 (1.8)	8.9 (1.8)
	Mineral (n=2)	14.8 (5.4)	1.5 (0)	0.1 (0)	3.9 (0)	3.7 (1)	3.7 (1)
Dry soil	O (n=7)	14.9 (5.1)	20.5 (13.5)	1.3 (0.9)	6.6 (1.4)	8 (2.6)	5.5 (2.2)
	Mineral (n=3)	13.4 (6.3)	1.8 (1.1)	0.1 (0)	5.1 (1.2)	4.5 (1.8)	4.5 (1.8)
Wet soil	O (n=7)	21.8 (4.8)	35.2 (11.5)	1.6 (0.5)	5.1 (1)	9.2 (1.6)	8.1 (1.7)
	Mineral (n=2)	15.1 (4.6)	1.6 (0.1)	0.1 (0)	3.7 (0.3)	2.7 (2.4)	2.7 (2.4)
			Pinus banksiana				
Control	O (n=6)	24 (4)	23.1 (9.6)	0.9 (0.5)	4.1 (0.4)	5.4 (0.5)	5.4 (0.5)
	Mineral (n=6)	16.3 (4.7)	0.8 (0.2)	0	4.1 (0.7)	4.6 (0.5)	4.6 (0.5)
Dry soil	O (n=6)	12.5 (3.9)	12.2 (9.8)	0.9 (0.6)	7.4 (0.3)	5.5 (0.8)	3.5 (0.8)
	Mineral (n=6)	14.4 (2.6)	1.2 (0.3)	0	4.6 (0.5)	4.5 (0.8)	4.5 (0.8)
Wet soil	O (n=6)	22.7 (0.5)	29.8 (7.7)	1.3 (0.3)	4.3 (0.6)	4.7 (0.8)	3.5 (0.9)
	Mineral (n=6)	15.7 (3.7)	1.2 (0.3)	0	4.1 (0.7)	5.2 (0.8)	5.2 (0.8)
			Populus tremuloides				
Control	O (n=6)	14.8 (2.1)	19.6 (9.7)	1.3 (0.8)	4.7 (0.3)	7.3 (3)	7.3 (3)
	Mineral (n=4)	12.5 (2.4)	1.3 (0.3)	0.1 (0)	4.7 (1.1)	5.3 (1.1)	5.3 (1.1)
Dry soil	O (n=6)	10 (1.9)	13.7 (9.3)	1.4 (1)	7.5 (0.5)	7.5 (2.5)	5.3 (2)
	Mineral (n=4)	9 (3.7)	3.1 (2.5)	0.5 (0.6)	4.7 (1.1)	3.5 (2.6)	3.5 (2.6)
Wet soil	O (n=6)	14.7 (2.4)	23.6 (15.2)	1.6 (1)	5.1 (0.2)	6.9 (1.7)	5.9 (1.9)
	Mineral (n=4)	15.2 (2.4)	2.4 (1)	0.1 (0)	4.2 (0.3)	3.8 (1.1)	3.8 (1.1)

Table S2.6. Mean (SD) 2-pool fast C pool decay coefficients for modelling microbial respiration post-burn. M_1 is the proportional size of the active (or fast) C pools, and *k1* is the respiration rate constants for the active C pools.

	Fast growth incubation		Affinity for post-fire environment incubation - Autoclaved		Affinity for post-fire environment incubation – Not autoclaved	
	k1	M1	k1	M1	k1	M1
			Picea spp.			
Unburned soil	0.173 (0.321)	0.009 (0.008)	0.023 (0.02)	0.011 (0.005)	0.015 (0.014)	0.016 (0.012)
Wet soil	0.086 (0.155)	0.014 (0.01)	0.026 (0.025)	0.014 (0.005)	0.008 (0.005)	0.036 (0.043)
Dry soil	0.049 (0.055)	0.006 (0.004)	0.044 (0.021)	0.006 (0.005)	0.032 (0.018)	0.006 (0.005)
			Populus tremuloides			
Unburned soil	0.08 (0.087)	0.007 (0.006)	0.018 (0.01)	0.008 (0.002)	0.008 (0.004)	0.015 (0.006)
Wet soil	0.031 (0.071)	0.016 (0.008)	0.018 (0.016)	0.012 (0.005)	0.022 (0.01)	0.01 (0.011)
Dry soil	0.057 (0.055)	0.005 (0.003)	0.065 (0.026)	0.004 (0.004)	0.063 (0.022)	0.005 (0.004)
			Pinus banksiana			
Unburned soil	0.066 (0.123)	0.007 (0.005)	0.018 (0.016)	0.005 (0.003)	0.007 (0.004)	0.011 (0.006)
Wet soil	0.006 (0.002)	0.015 (0.004)	0.059 (0.046)	0.003 (0.002)	0.008 (0.008)	0.016 (0.012)
Dry soil	0.114 (0.07)	0.002 (0.001)	0.066 (0.029)	0.002 (0.001)	0.073 (0.031)	0.002 (0.001)

Table S2.7. Mean (SD) 2-pool slow C pool decay coefficients for modelling microbial respiration post-burn. M_2 is the proportional size of the slow C pools, and *k2* is the respiration rate constants for the slow C pools.

	Fast growth incubation		Affinity for post-fire environment incubation - Autoclaved		Affinity for post-fire environment incubation – Not autoclaved	
	k2	M2	k2	M2	k2	M2
			Picea spp.			
Unburned soil	0.00005 (0.00007)	0.99 (0.009)	0.00004 (0.00005)	0.988 (0.005)	0.00005 (0.00008)	0.983 (0.012)
Wet soil	0.00007 (0.00011)	0.985 (0.009)	0.00003 (0.00005)	0.984 (0.004)	0.00001 (0.00003)	0.963 (0.043)
Dry soil	0.00002 (0.00005)	0.994 (0.004)	0.00004 (0.00005)	0.992 (0.007)	0.00004 (0.00006)	0.993 (0.007)
			Populus tremuloides			
Unburned soil	0.00005 (0.00007)	0.993 (0.006)	0.00002 (0.00002)	0.991 (0.003)	0 (0.00001)	0.984 (0.006)
Wet soil	0.00002 (0.00007)	0.984 (0.008)	0.00002 (0.00003)	0.987 (0.006)	0.00004 (0.00003)	0.989 (0.011)
Dry soil	0.00001 (0.00001)	0.994 (0.003)	0.00002 (0.00001)	0.995 (0.004)	0.00002 (0.00001)	0.994 (0.005)
			Pinus banksiana			
Unburned soil	0.00004 (0.00005)	0.993 (0.005)	0.00002 (0.00002)	0.995 (0.003)	0.00002 (0.00003)	0.988 (0.006)
Wet soil	0 (0)	0.985 (0.004)	0.00003 (0.00001)	0.997 (0.002)	0.00002 (0.00003)	0.984 (0.012)
Dry soil	0.00001 (0.00001)	0.997 (0.001)	0.00001 (0)	0.998 (0.001)	0.00001 (0)	0.998 (0.002)

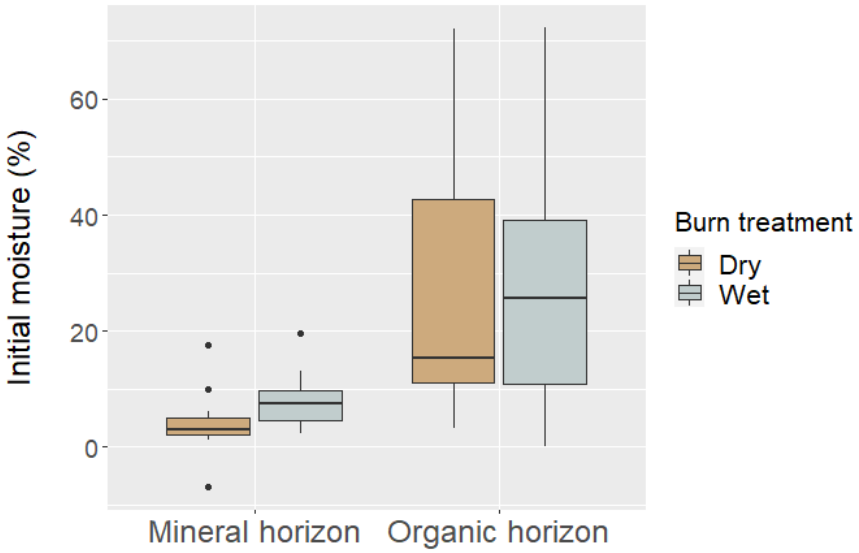

Figure S2.1. Mean soil moisture (percent water mass per mass dry soil) for the O horizon and mineral soil at the start of the burn.

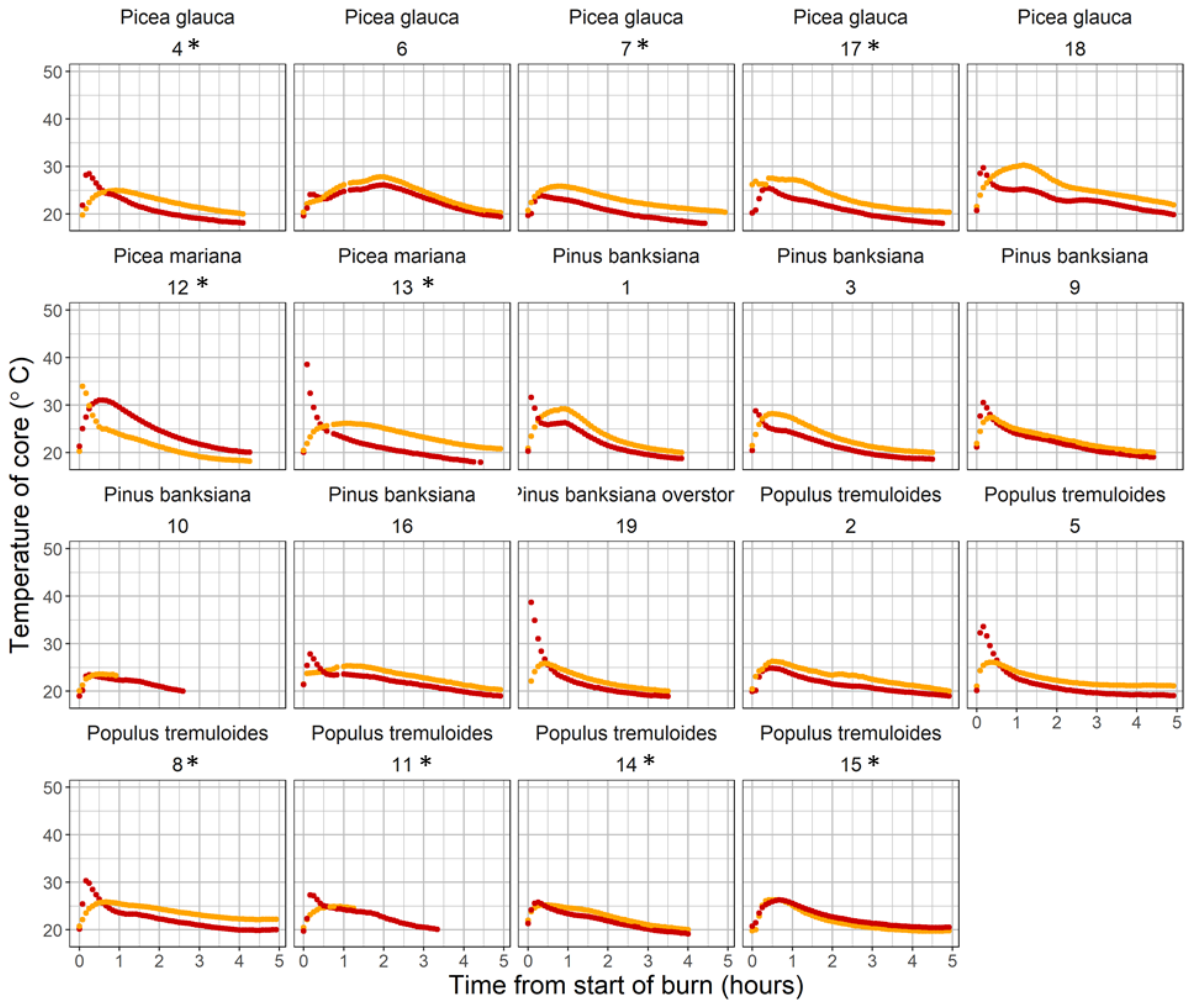

Figure S2.2. Soil temperature during the burn and following 5 hours for wet soil. Plots are labelled with dominant vegetation and site ID (Table 1). Red and yellow points indicate the organic-mineral horizon interface (where present) and 1 cm above core base, respectively. Asterisks (*) indicate cores with no mineral soil, in which case thermocouples were placed 1 cm and 5 cm from the core base.

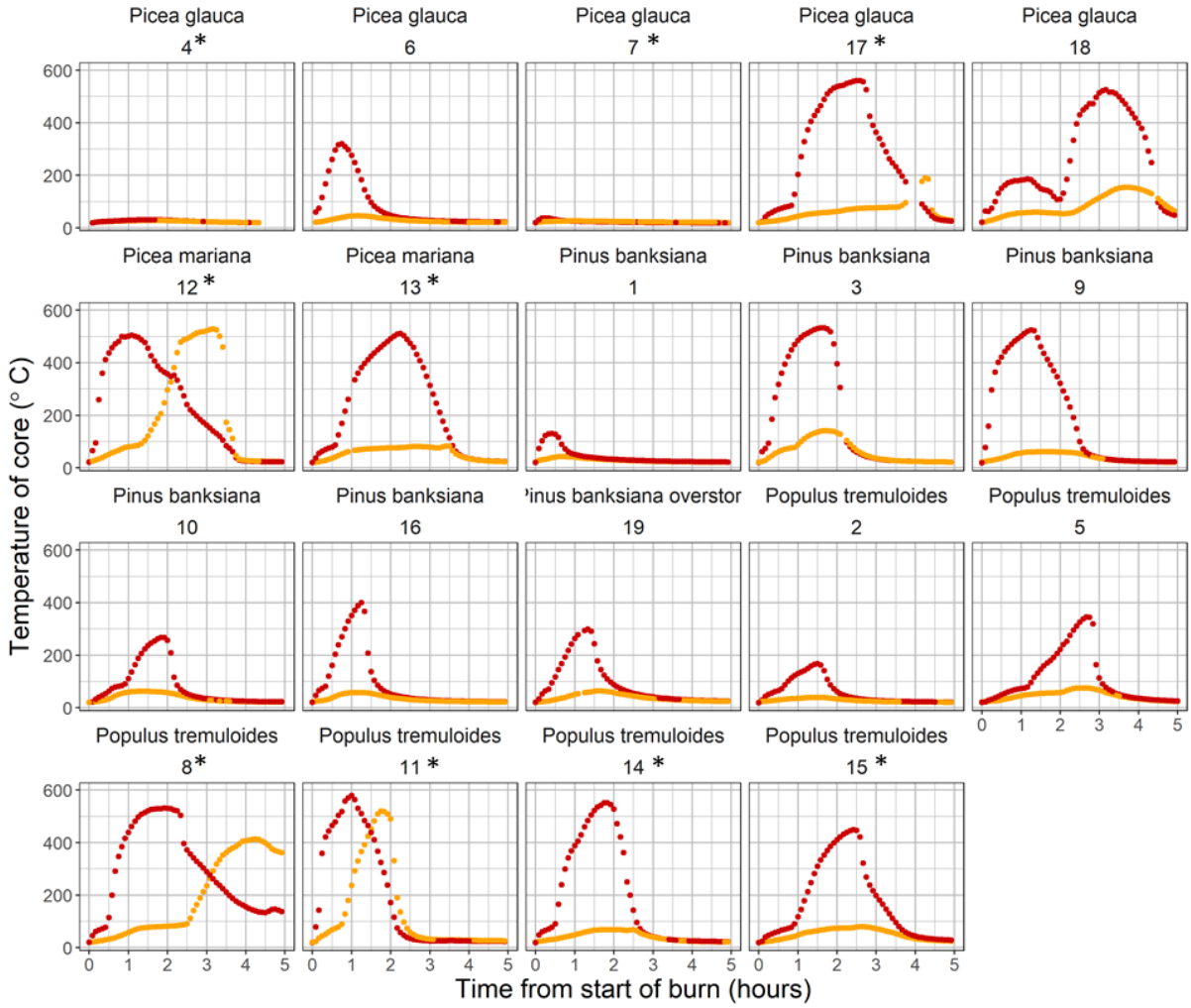

Figure S2.3. Soil temperature during the burn and following 5 hours for dry soil. Plots are labelled with dominant vegetation and site ID (Table S2.1). Red and yellow points indicate the organic-mineral horizon interface (where present) and 1 cm above core base, respectively. Asterisks (*) indicate cores with no mineral soil, in which case thermocouples were placed 1 cm and 5 cm from the core base.

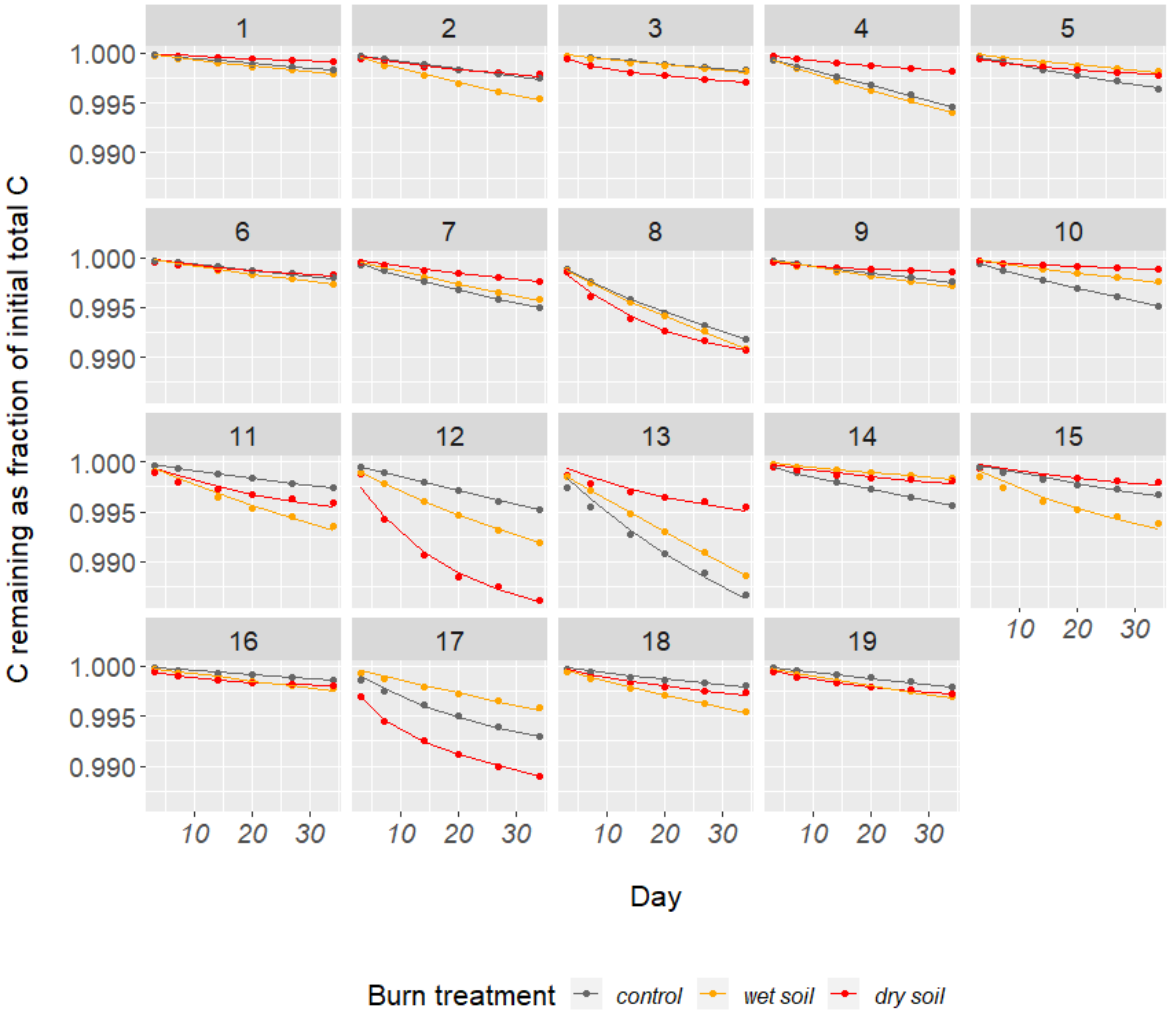

Figure S2.4. Fractional C remaining during the 5-week fast-growth incubation for each site (Site IDs in Table S2.1). Points indicate measured C remaining. Lines indicate the 2-pool decay model fits.

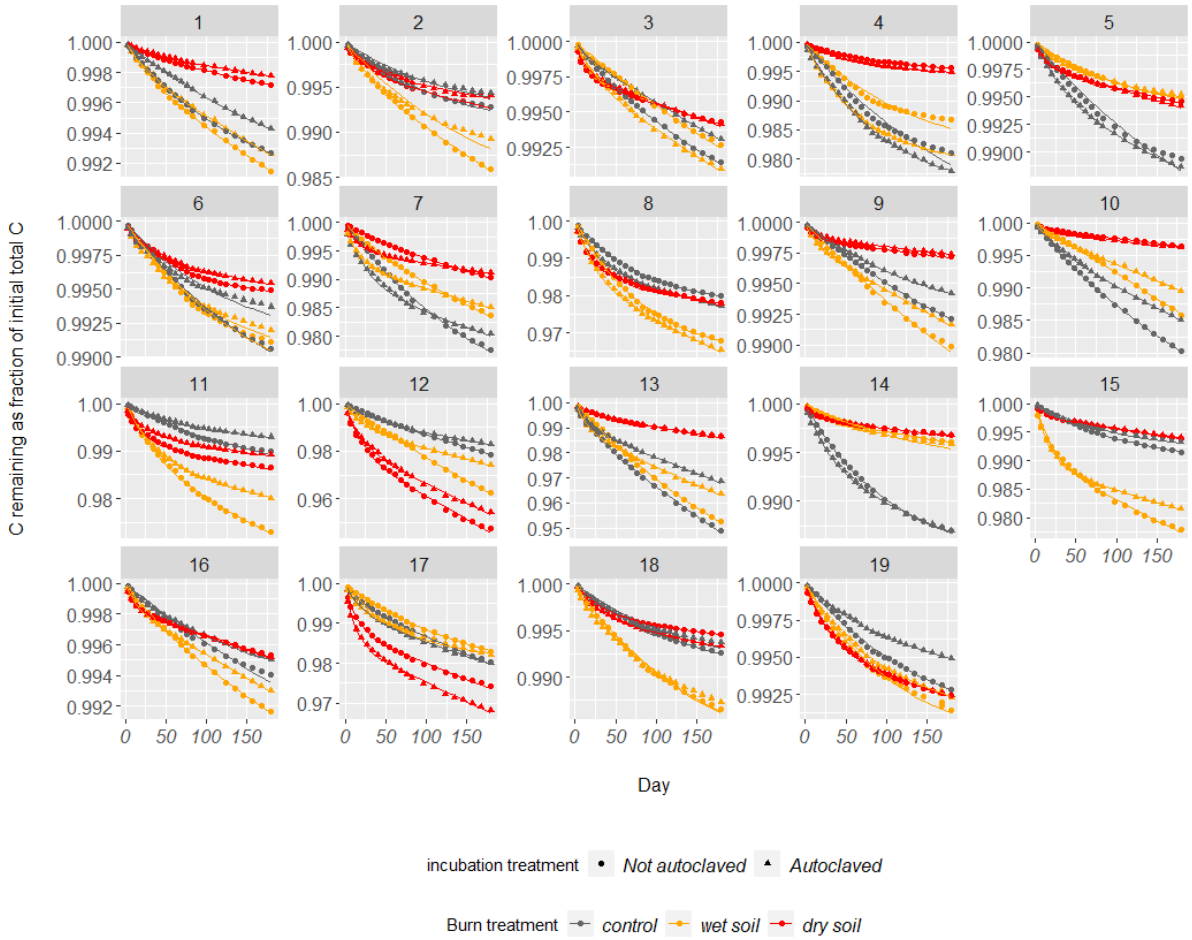

Figure S2.5. Fractional C remaining during 6-month incubation for each site (Site IDs in Table S2.1). Points indicate measured C remaining. Lines indicate the 2-pool decay model fits.

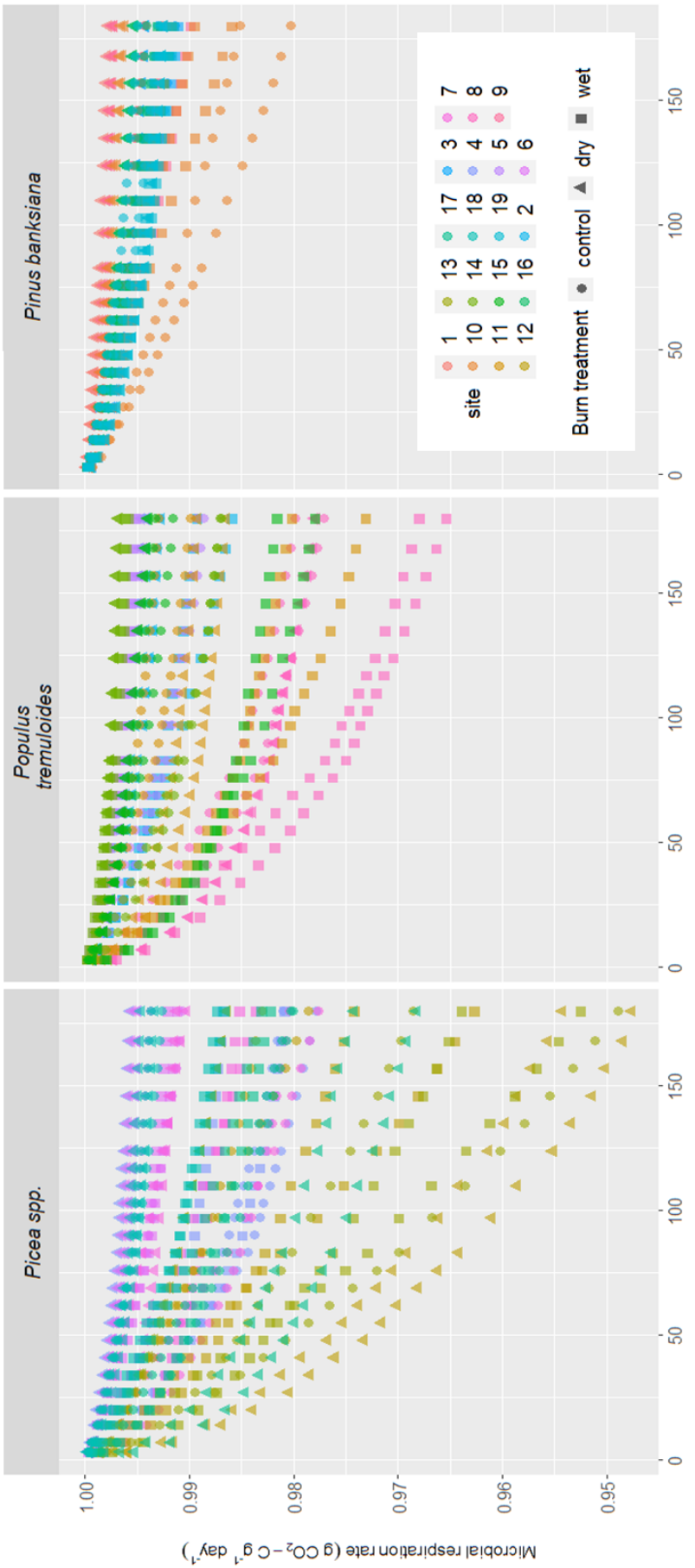

Figure S2.6. Fractional C remaining during 6-month incubation for each dominant vegetation type. Points indicate measured C remaining. Colors indicate sites (site IDs in Table S2.1).

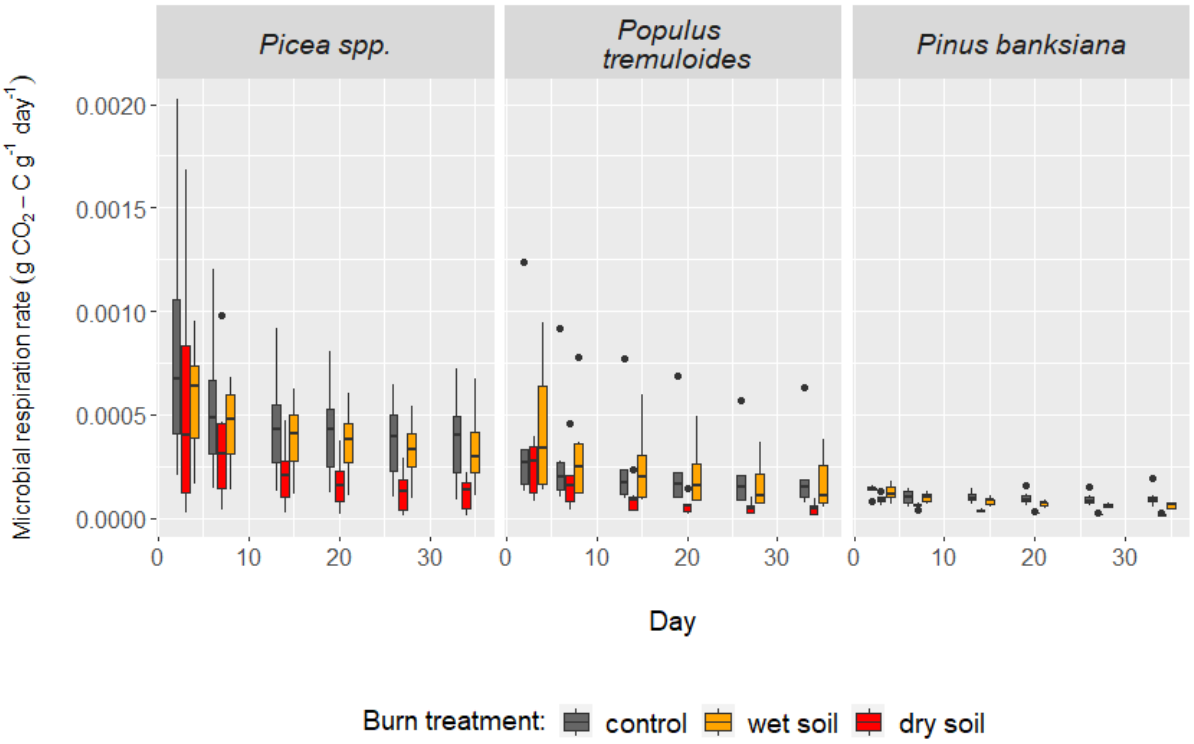

Figure S2.7. Mean respiration rate over 5-week fast-growth incubation grouped by dominant vegetation. Color indicates burn treatment.

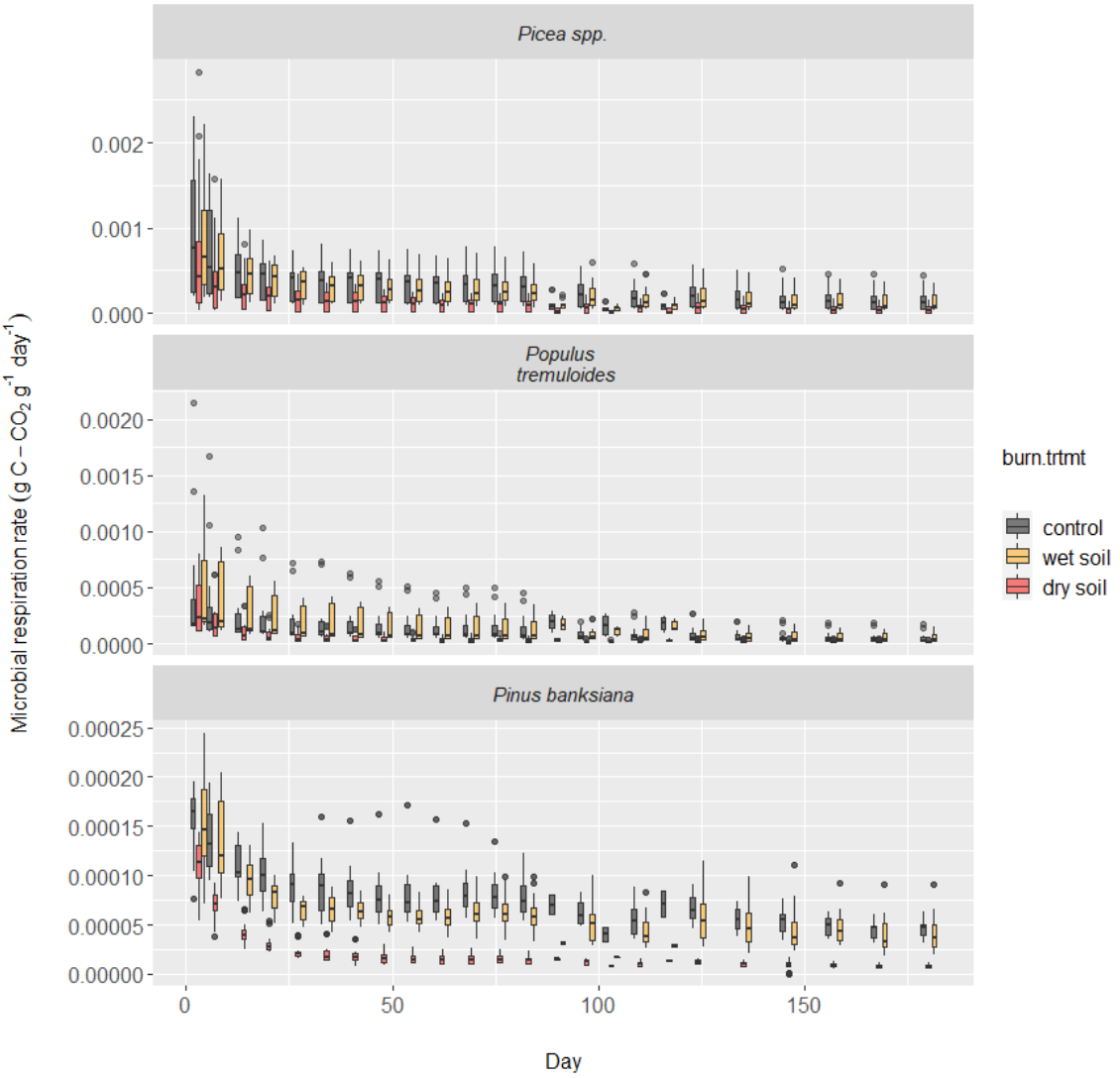

Figure S2.8. Mean respiration rate over 6-month fast-growth incubation grouped by dominant vegetation. Color indicates burn treatment.

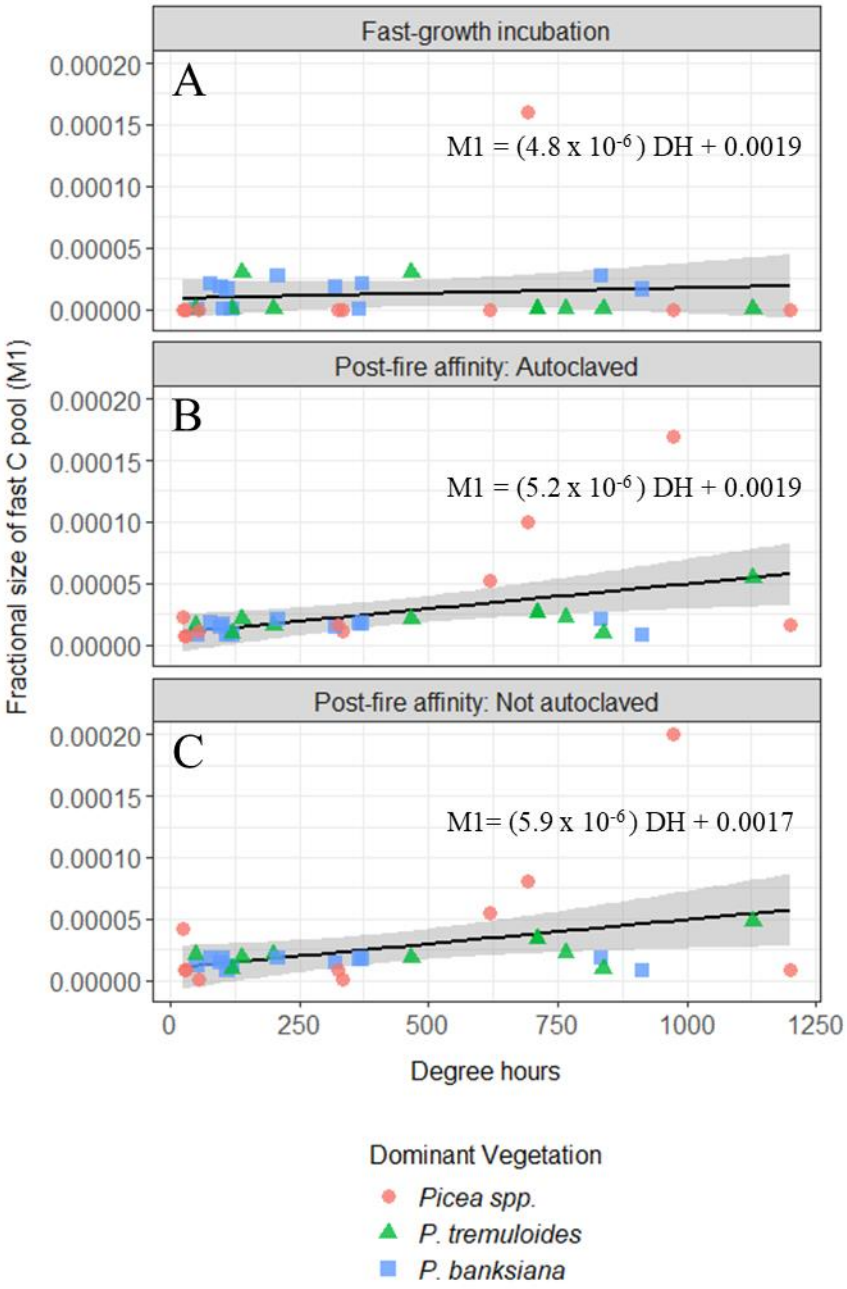

Figure S2.9. Fractional size of the fast C pool (M1) with increasing DH in the (A) fast-growth incubation ($p = 0.001$, R^2 adjusted = 0.28), (B) the autoclaved cores in the post-fire affinity incubation ($p = 0.005$, R^2 adjusted = 0.21), and (C) the not autoclaved cores in the post-fire affinity incubation ($p = 0.002$, R^2 adjusted = 0.25). Colors indicate dominant vegetation.

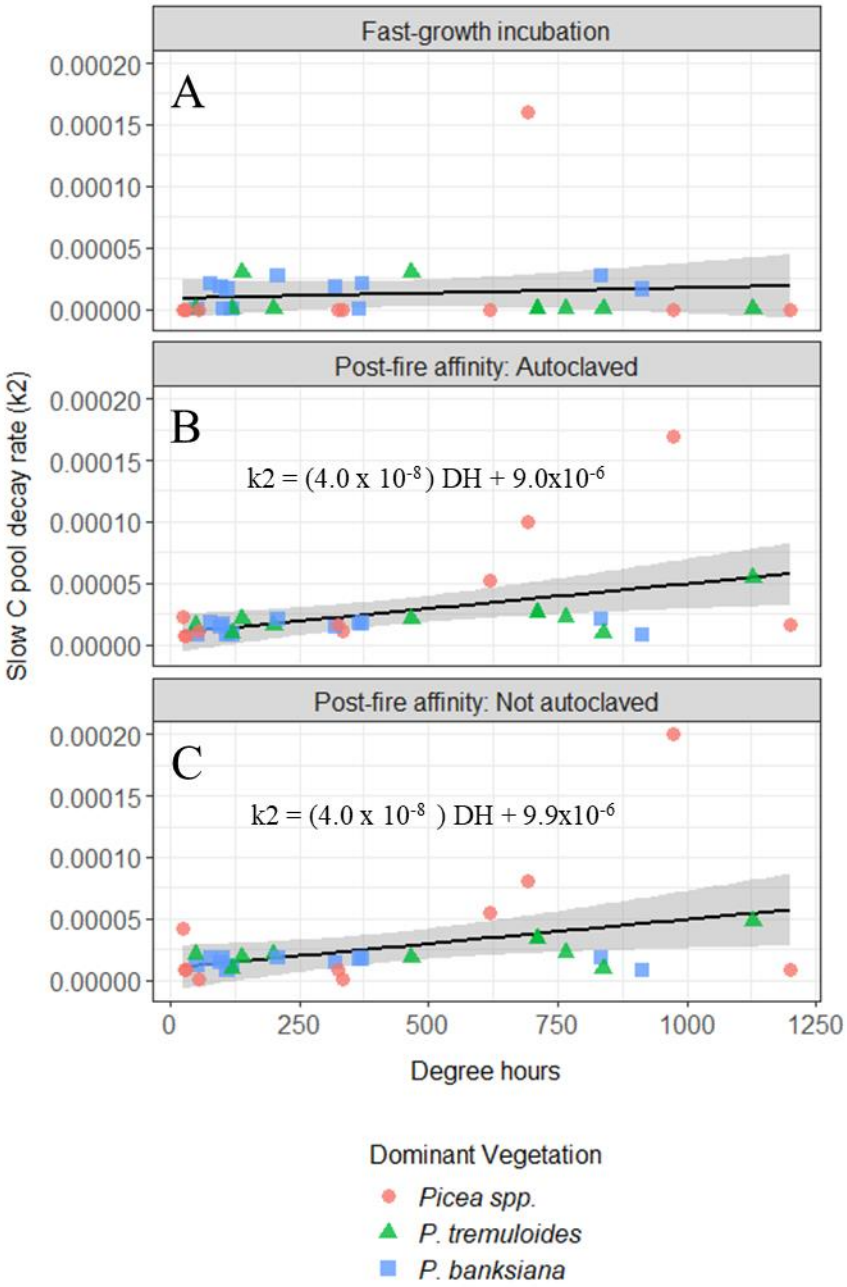

Figure S2.10. Slow C pool decay coefficient (k2) with increasing DH in the (A) fast-growth incubation ($p = 0.56$), (B) the autoclaved cores in the post-fire affinity incubation ($p = 0.008$, R^2 adjusted = 0.18), and (C) the not autoclaved cores in the post-fire affinity incubation ($p = 0.02$, R^2 adjusted = 0.13). Colors indicate dominant vegetation.

CHAPTER 3.

Table S3.8. PERMANOVA results for full model for 16S for RNA-based community composition 24 hours post-burn

	Df	Sums of sqs	Mean sqs	F.Model	R^2	Pr(>F)	R^2 single-component model
Soil horizon	1	0.001052	0.001052	5.8677	0.05259	0.001	0.05402
Dominate Vegetation	2	0.000803	0.000402	2.2398	0.04015	0.001	0.04586
Pre-burn horizon thickness (cm)	1	0.000391	0.000391	2.181	0.01955	0.01	0.03994
pH	1	0.000936	0.000936	5.2193	0.04678	0.001	0.0567
Total C (%)	1	0.00032	0.00032	1.7852	0.016	0.025	0.04481
Total N (%)	1	0.000311	0.000311	1.7344	0.01554	0.042	0.04683
Soil texture	4	0.001236	0.000309	1.7235	0.06178	0.004	0.11038
Horizon DH	1	0.000792	0.000792	4.4191	0.0396	0.001	0.04978
Residuals	79	0.014165	0.000179		0.70801		
Total	91	0.020006			1		

Table S3.9. PERMANOVA results for full model for 16S DNA-based community composition 24 hours post-burn

	Df	Sums of sqs	Mean sqs	F.Model	R^2	Pr(>F)	R^2 single-component model
Soil horizon	1	0.001112	0.001112	4.9527	0.04405	0.001	0.04405
Dominant Vegetation	2	0.001135	0.000567	2.5282	0.04498	0.001	0.04723
Pre-burn horizon thickness (cm)	1	0.00067	0.000669	2.9829	0.02653	0.001	0.04071
pH	1	0.001134	0.001134	5.0522	0.04494	0.001	0.05007
Total C (%)	1	0.000496	0.000496	2.212	0.01968	0.005	0.04394
Total N (%)	1	0.000389	0.000389	1.7343	0.01543	0.021	0.04395
Soil texture	4	0.001857	0.000464	2.069	0.07362	0.001	0.11478
Horizon DH	1	0.000933	0.000933	4.1558	0.03697	0.001	0.03946
Residuals	78	0.017506	0.000224		0.69381		
Total	90	0.025232			1		

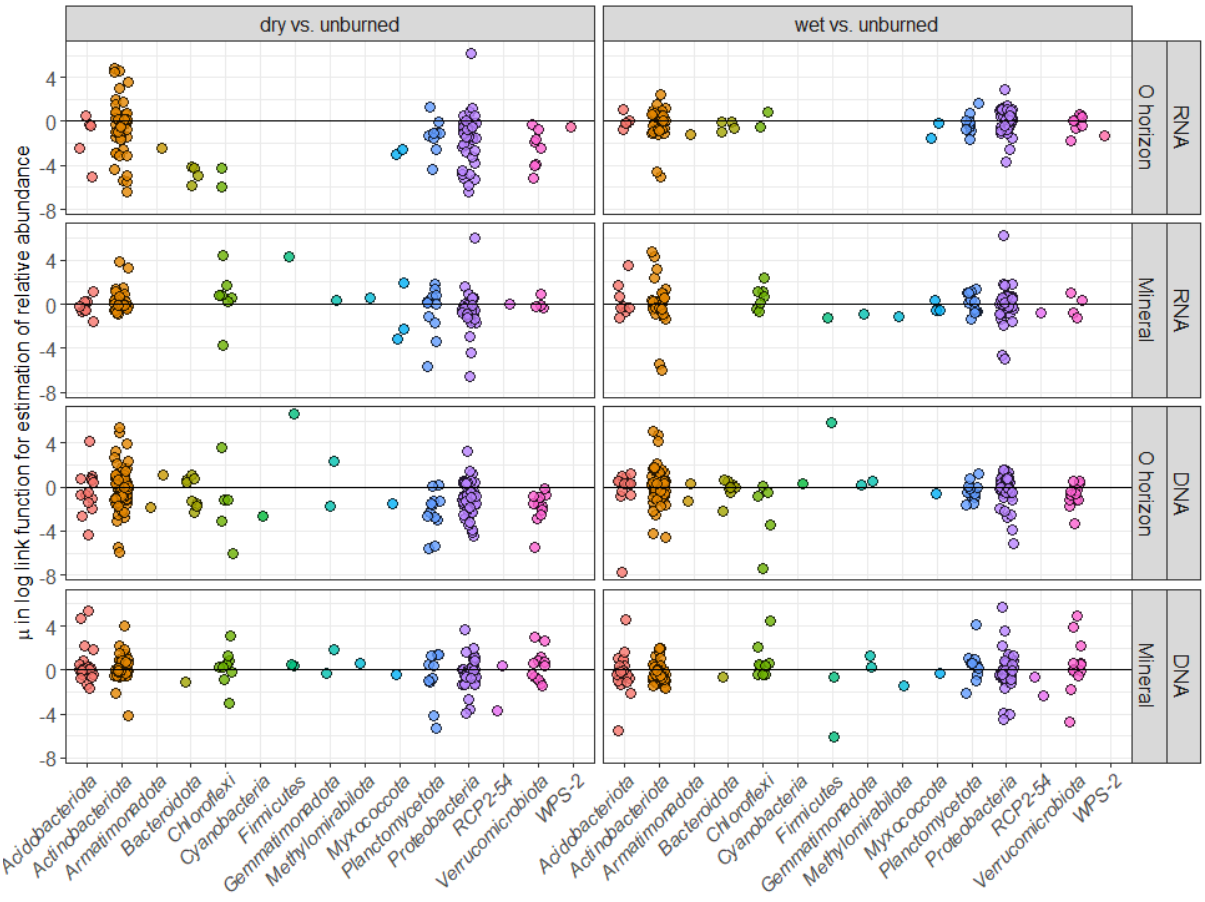

Figure S3.11. Differential abundance between dry soil burn samples and unburned control and between wet soil burn samples and unburned control of bacterial taxa in RNA- and DNA-based community analysis in the O horizon and mineral soil. Each point represents a bacterial taxon. Colors represent phyla-level classification of each OTU.

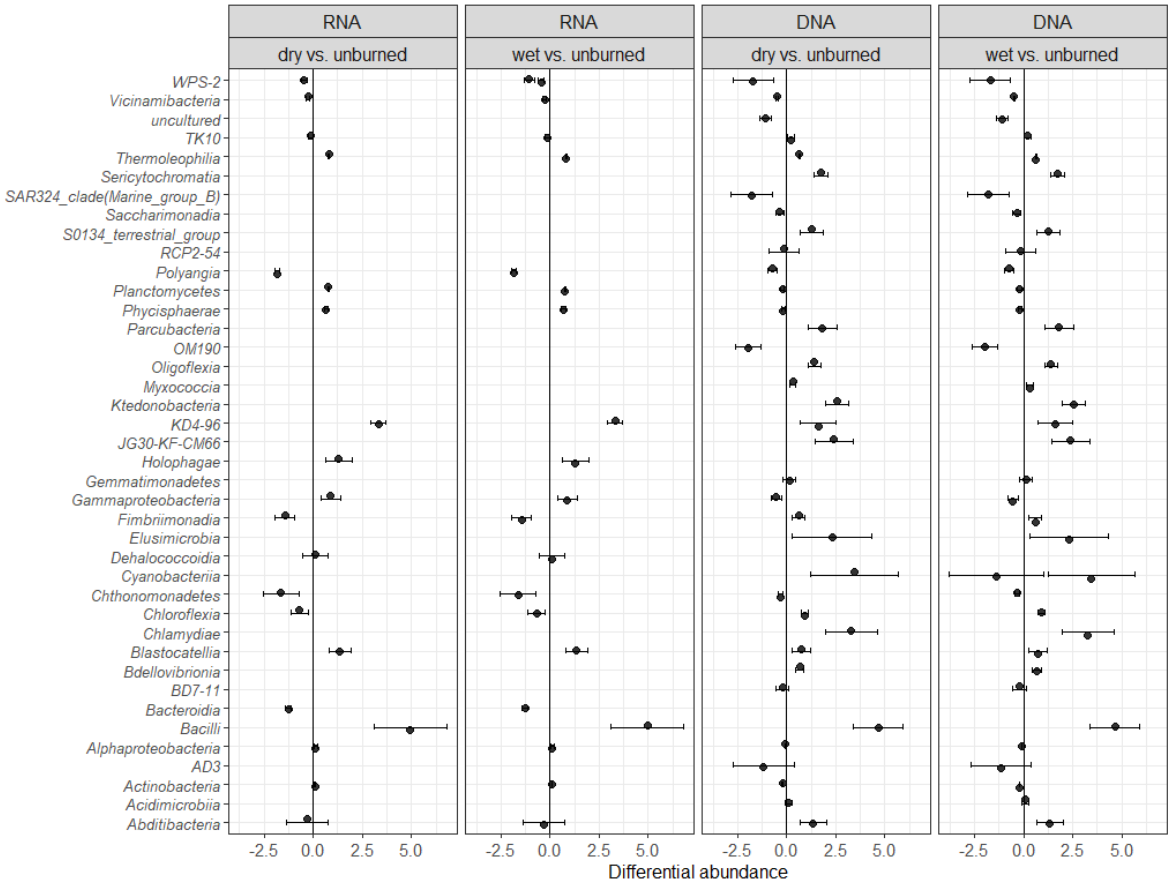

Figure S3.12. Differential abundance of bacterial Classes in the O horizon of the RNA- (left two panels) and DNA-based (right two panels) communities between dry soil burn samples and unburned controls and between wet soil burn samples and unburned controls. A mean relative abundance of > 0 at the Class level was used. Each point represents an individual bacterial OTU. Error bars represent 95% prediction intervals for the differential abundance factor by sample.

Table S4.10. PERMANOVA results for full model for microbial community composition at end of fire-survival incubation.

	Df	Sums of sqs	Mean sqs	F.Model	R^2	Pr(>F)	R^2 single-component model
Burn treatment	2	0.004268	0.002134	5.2766	0.08166	0.001	0.08166
Soil horizon	1	0.003312	0.0033116	8.1885	0.06337	0.001	0.06194
Dominant vegetation	2	0.001744	0.0008721	2.1565	0.03337	0.001	0.03416
Pre-burn horizon thickness (cm)	1	0.000916	0.0009165	2.2662	0.01754	0.003	0.03093
pH	1	0.002784	0.0027842	6.8844	0.05327	0.001	0.10266
Total C (%)	1	0.001003	0.0010034	2.4812	0.0192	0.005	0.05286
Total N (%)	1	0.000685	0.0006854	1.6947	0.01311	0.025	0.05141
Soil texture	4	0.003051	0.0007628	1.8861	0.05838	0.001	0.09125
Horizon DH	1	0.00174	0.0017398	4.3019	0.03329	0.001	0.0779
Residuals	81	0.032758	0.0004044		0.6268		
Total	95	0.052262			1		

Table S4.11. Weighted predicted mean 16S ribosomal RNA (rRNA) gene copy number across burn treatments and soil horizons.

	Weighted Mean Copy Num	
Burn Treatment	**O horizon**	**Mineral horizon**
Control	2.0 ± (0.36)	1.7 ± (0.11)
Wet soil	2.1 ± (0.39)	1.6 ± (0.06)
Dry Soil	3.4 ± (0.94)	2.6 ± (0.83)

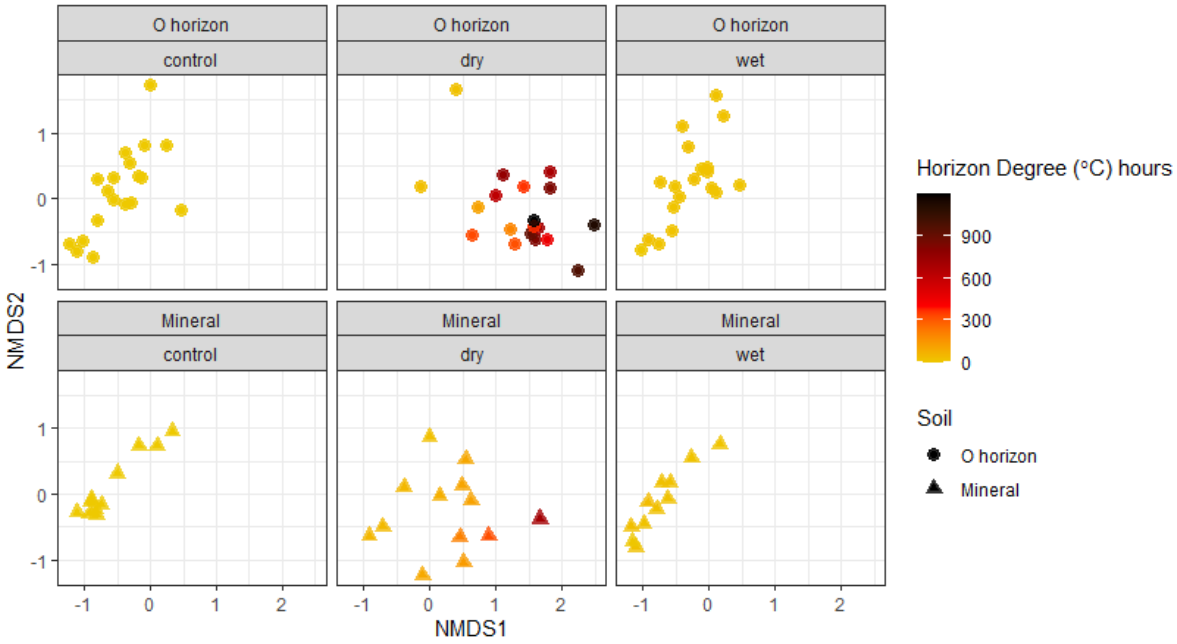

Figure S4.13. First two axes of NMDS ordination on weighted UniFrac dissimilarities between bacterial communities 5 weeks post-burn (k = 3, stress = 0.09). Circles and triangles indicate O horizon and mineral soil, respectively. Colors indicate DH with darker colors representing higher DH.

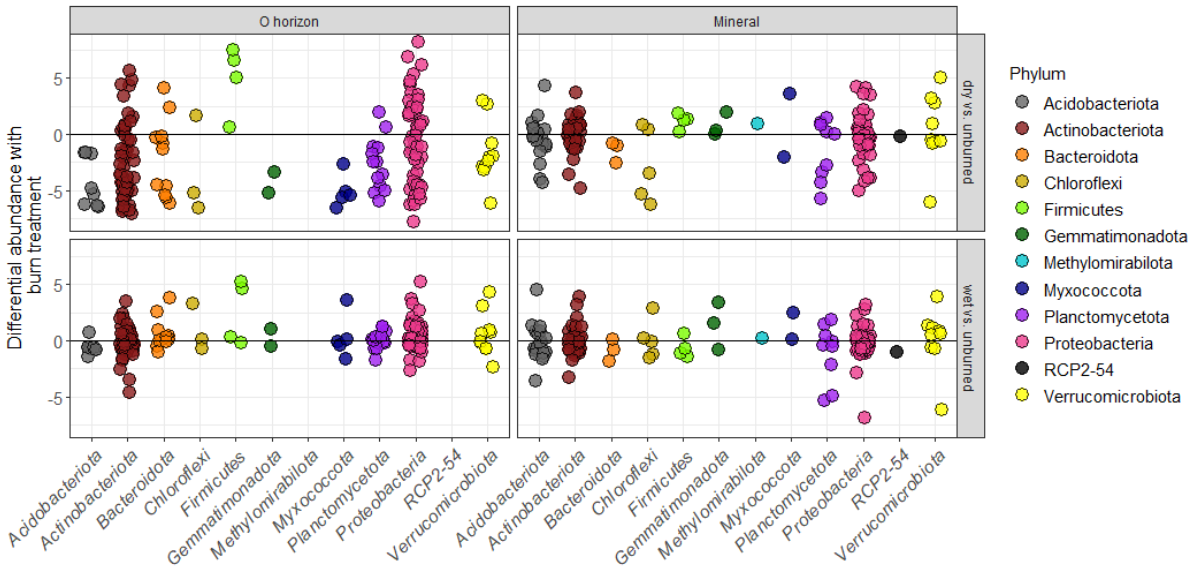

Figure S4.14. Differential abundance in the O horizon and mineral soil between dry soil burn samples and unburned control and between wet soil burn samples and unburned control of bacterial taxa in DNA-based communities at the end of the 5-week fast-growth incubation. Each point represents an individual bacterial taxon. Colors represent phyla-level classification of each OTU.

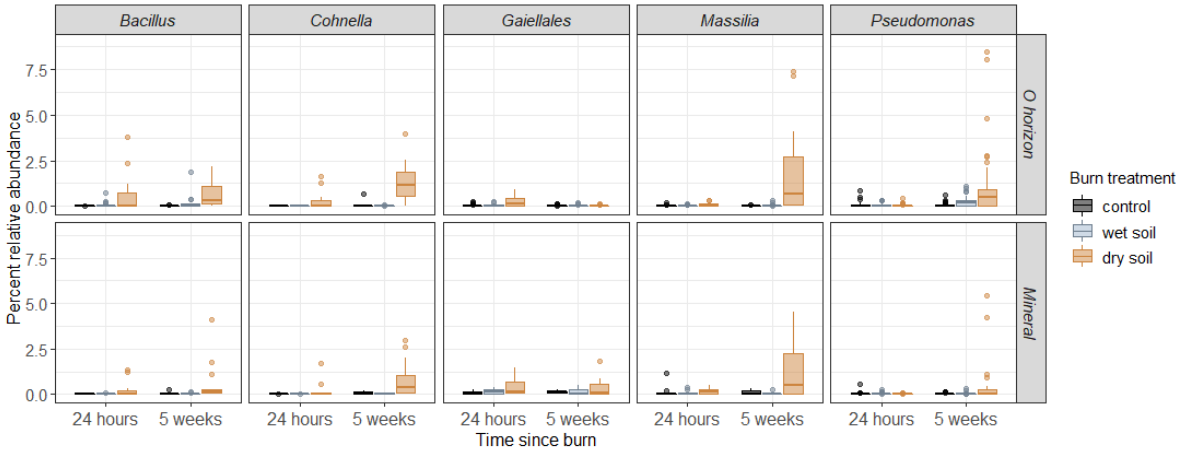

Figure S4.15. Relative abundance (%) of bacterial OTUs (with mean relative abundance > 0.1%) in the O horizon (top) and mineral soil (bottom) that were enriched (μ in log link function > 3) 24 hours and 5 weeks post-burn in the dry soil burn samples compared to the unburned soil. Each point represents an individual OTU within a single sample. Color represents burn treatment. The relative abundance of *Pseudomonas* sp. at 5 weeks is not shown for three samples (relative abundance > 10%).

CHAPTER 5

Table S5.12. PERMANOVA results for full model for microbial community composition at end of post-affinity incubation.

	Df	Sums of sqs	Mean sqs	F.Model	R^2	Pr(>F)	R^2 single-component model
Dominant vegetation type	2	0.001957	0.0009786	3.9446	0.03325	0.001	0.03325
Soil pH	1	0.004687	0.0046866	18.891	0.07962	0.001	0.08353
Total C (%)	1	0.00197	0.00197	7.9408	0.03347	0.001	0.03623
Total N (%)	1	0.000785	0.0007852	3.1649	0.01334	0.001	0.03741
Soil texture	4	0.00339	0.0008476	3.4167	0.0576	0.001	0.07233
Burn soil moisture	2	0.001034	0.0005168	2.0831	0.01756	0.001	0.04938
Soil horizon	1	0.000733	0.0007333	2.9559	0.01246	0.001	0.05185
Horizon DH	1	0.001005	0.0010046	4.0493	0.01707	0.001	0.05783
Autoclaving	1	0.001127	0.0011274	4.5445	0.01915	0.001	0.01904
Residuals	170	0.042174	0.0002481		0.71649		

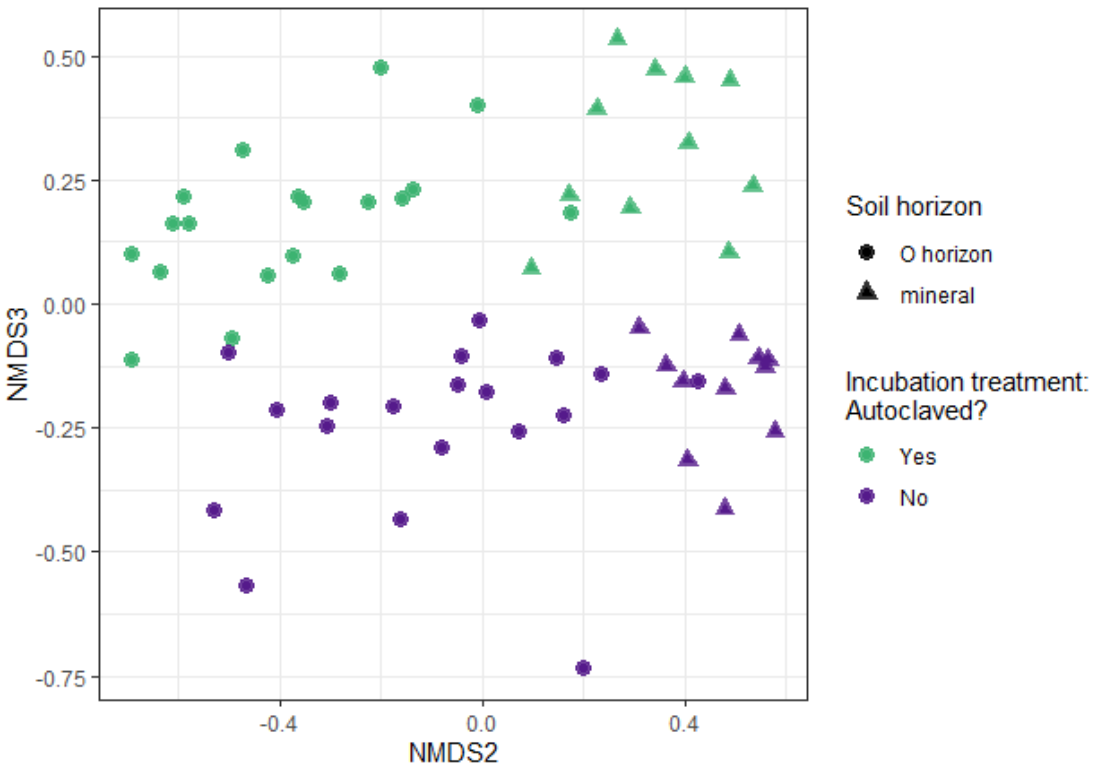

Figure S5.16. Second and third axes of NMDS ordination on weighted UniFrac dissimilarities between bacterial communities for the control (unburned) cores after the post-fire affinity incubation (k =3, stress = 0.09). Circles and triangles indicate O and mineral horizons, respectively. Teal and purple coloring indicated autoclaved and unautoclaved samples.

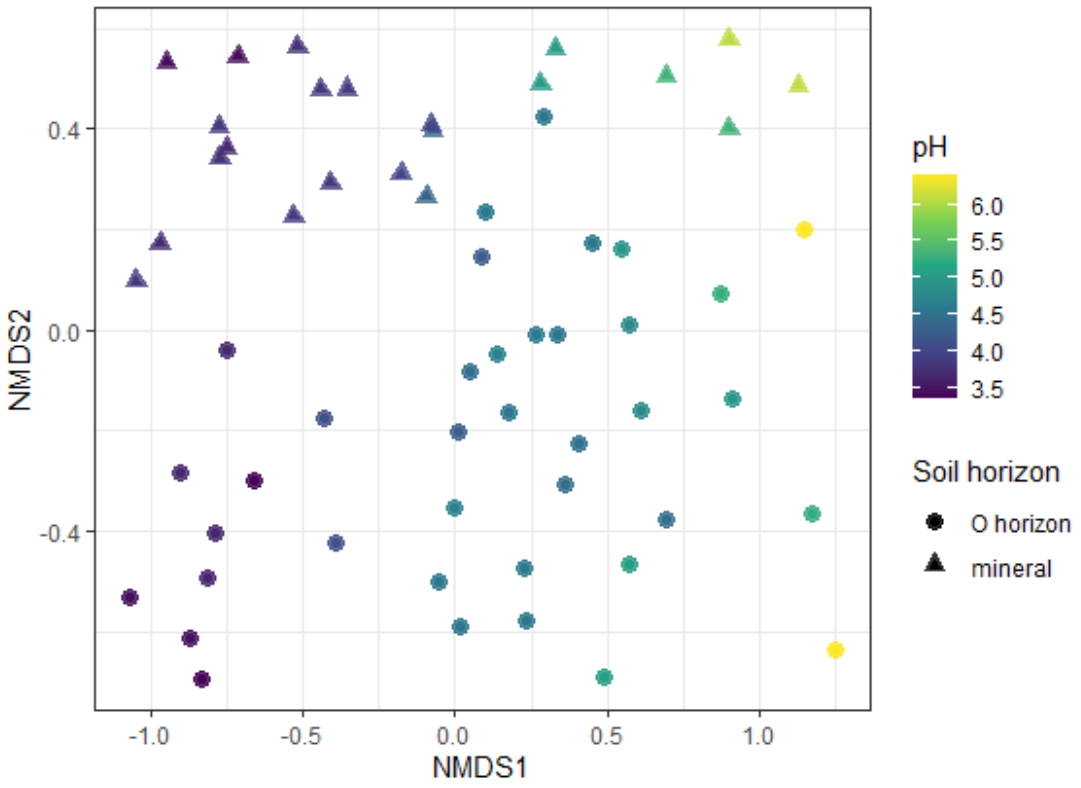

Figure S5.17. First two axes of NMDS ordination on weighted UniFrac dissimilarities between bacterial communities for the control (unburned) cores after the post-fire affinity incubation for the O horizon and mineral soil (k = 3, stress = 0.09). Circles and triangles indicate O and mineral horizons, respectively. Points are shaded by pH with lighter colors indicated higher pH.

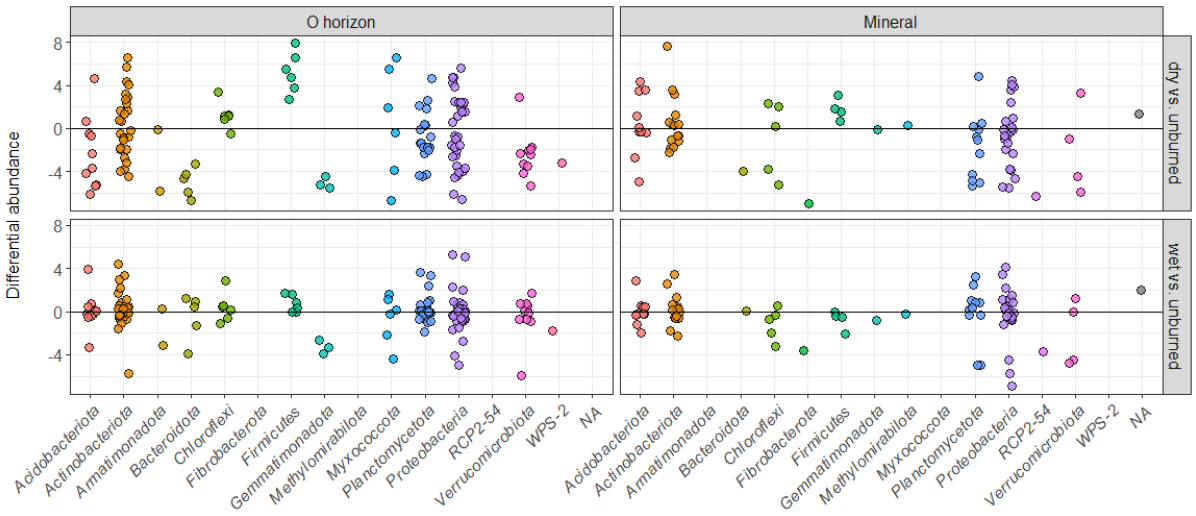

Figure S5.18. Differential abundance between dry soil burn samples and unburned control and between wet soil burn samples and unburned control of bacterial taxa in both autoclaved and not autoclaved cores. Each point represents a bacterial taxon. Colors represent phyla-level classification of each OTU.

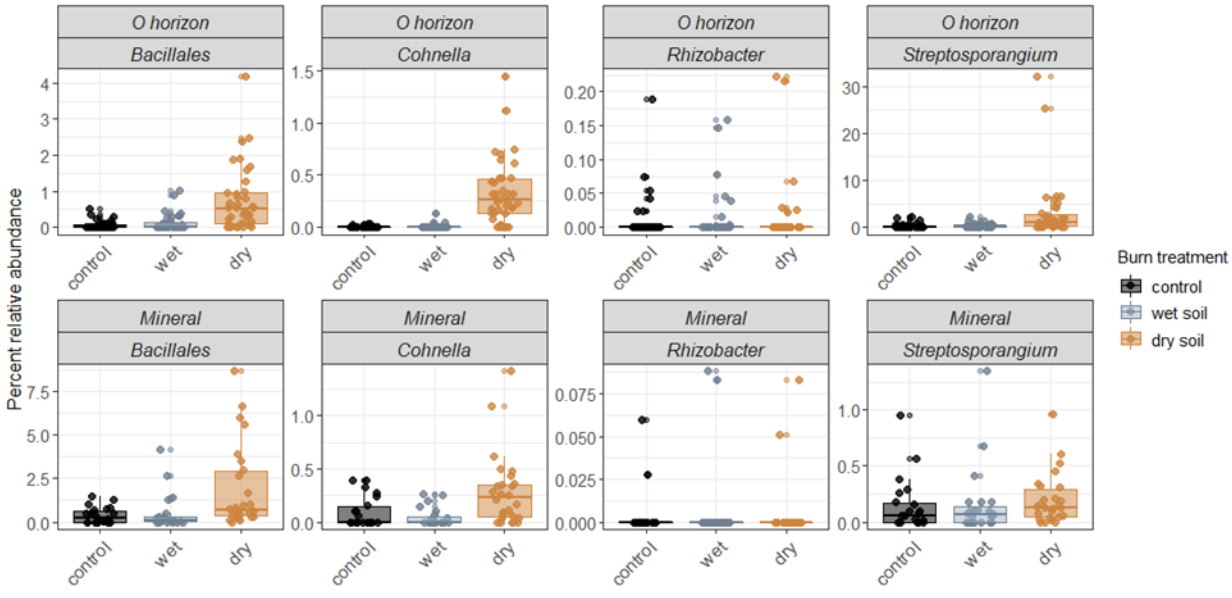

Figure S5.19. Relative abundance of bacterial OTUs identified as both fire-survivors (enriched 24 hours post-burn; Ch. 3) and enriched 6 months post-burn in the O horizon of the dry soil burns. Colors indicate burn treatment.