WILDFIRE IMPACTS ON SOIL MICROBIAL COMMUNITIES: POTENTIAL FOR DISRUPTIONS TO NUTRIENT CYCLING AND ANTIBIOTIC RESISTANCE GENE PROPAGATION IN BACTERIA

By

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Abstract

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Wildfires naturally regulate nutrient cycling in ecosystems, but anthropogenic influences have caused wildfires to threaten the United States with increasing regularity and wildfires regularly impact soil bacteria. Wildfire-affected soils were studied with two primary objectives: 1) Determine the community composition in soils affected by wildfire intensities (i.e., low, moderate, high) to examine the impact on nutrient-cycling bacteria and 2) examine the effect of wildfires on the development of antibiotic resistance genes (ARGs) in soils. Objective 1 was achieved by characterizing soil bacterial communities in control (i.e., unburned) and burned soils using Illumina MiSeq *16S* sequencing. Intensity (i.e., heat yield) was used rather than severity (i.e., burn impact) to examine the impact of fire temperatures on bacteria abundance. Six families and 17 genera were significantly (Spearman $r_s > |0.4|$; $p < 0.05$) negatively associated with wildfire intensity and three families and six genera were significantly positively associated with wildfire intensity. Many of these taxa contain species that are known to be critical contributors to maintaining global nutrient cycles (i.e., nitrogen, sulfur, and phosphorus). Objective 2 was achieved by performing polymerase chain reaction and gel electrophoresis on isolated DNA. We targeted ARGs conferring resistance to common antibiotics (i.e., *sul1*, *sul2*, *tetM*, *tetB*, *tetO*, *tetW*, and *ermF*) as well as last resort

antibiotics (i.e., *mcr-1, optrA, fosA2*, *cfr*). ARGs could increase after wildfires due to bacteria exposure to antibiotic fungal secondary metabolites, co-selection of ARGs on plasmids, increased rates of horizontal gene transfer, and exposure to antibiotics in runoff. No ARGs were detected in control soils. However, several ARGs were detected in burned soils. S*ul1* was prominent in low and moderate intensity soils, while *sul2* dominated in low intensity soils. *TetW* was prominent in moderate fire intensity soils. These results suggest that wildfires significantly alter microbial community structures and functions. A decrease in average relative abundance of nutrient cyclers after high intensity wildfires could slow ecosystem recovery, while the prominence of ARGs in wildfire affected soils suggests that wildfires increase ARG abundance. Increases in ARGs in the environment pose health risks to humans as the spread of antibiotic resistant infections grows.

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CHAPTER ONE: INTRODUCTION

Wildfires are a necessary and natural phenomenon that help to regulate nutrient cycling in ecosystems (Pausas & Bond, 2020; Pereira et al., 2021), but climate change and anthropogenic influences have exacerbated the negative effects of wildfires in recent years. Widespread increases in the number of fire events (Moritz et al., 2012), fire season length (Westerling et al., 2006), and total burn area (Dennison et al., 2014) have created unprecedented challenges for protecting human health and the environment (Arnold et al., 2014). An increase in drought occurrence (Kountouris, 2020) and overall global temperature due to climate change, coupled with an accumulation of wildfire fuel (i.e., dried grasses and dead trees), has resulted in more intense and frequent fires throughout the western United States (Keane & Karau, 2010). Since 1986, the average area of land burned during uncontrolled fire events across the western United States has increased by 355 km^2 annually (Dennison et al., 2014) and recent studies have shown that anthropogenic climate change will continue to drive these trends upwards (Abatzoglou & Williams, 2016) (Figure 1). However, this escalation in wildfires is not limited to this region of the United States. Thus, additional research that evaluates the holistic impacts of wildfire on environmental and human health are urgently needed.

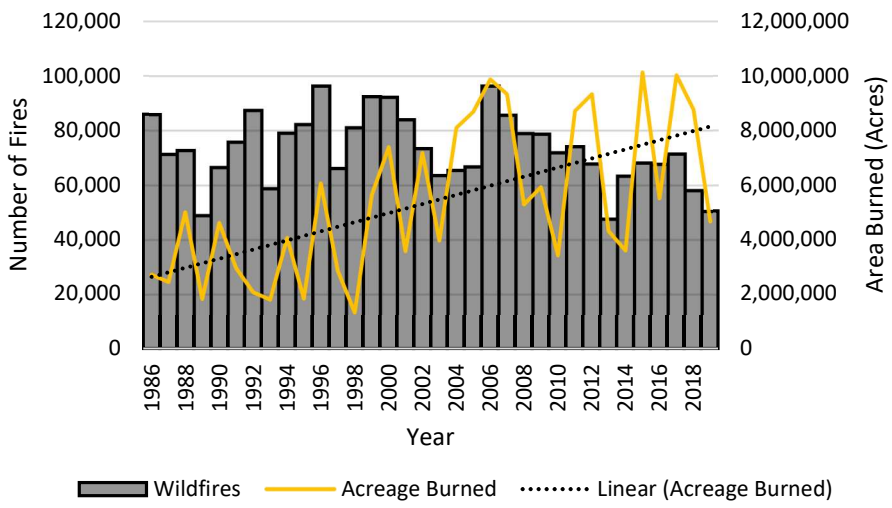

Figure 1. Wildfires and acreage burned in the US from 1986 – 2019, without data from North Carolina in 2004 (National Interagency Fire Center*, 2021*).

Figure 1 shows that the acreage burned in the U.S. during wildfire events has increased steadily since 1986. During a preliminary analysis of wildfire trends, we assessed the relationships among years, fires, and acreage burned using raw data from the National Interagency Fire Center using IBM SPSS Statistics 26 software (2021). Our analysis showed a correlation of 0.600 between acreage burned and years elapsed with a strong association among these variables ($p < 0.001$). These increases have also resulted in a corresponding increase in emissions of carbon dioxide, carbon monoxide, and other greenhouse gases that contribute to further climate change (Guo et al., 2017).

The environmental impacts of wildfires are not limited only to increases in global burn area. For example, many species make their habitats in wildfire fuel and increased acreage destroyed by wildfires results in the destruction of those habitats (Rochelmeyer et al., 2019). Additionally, the annual economic costs and losses (e.g., agriculture loss, injuries and deaths, evacuation costs, etc.) of wildfires in the U.S. are estimated to be between $71.7 and $346 billion (Thomas, 2017). Wildfires affect drinking water supplies as reservoirs are polluted with sediments and ash and many drinking water treatment plants are not equipped to deal with an increase in pollutants (EPA, 2019). Most past wildfire research has focused

on loss of animal life and habitat, destruction of property, and economic cost of recovery. This research has largely ignored the potential impacts of wildfires on environmental microbes which provide critical services for maintaining sustainable ecosystems. These microbes are known as the soil microbiome, a collection of microorganisms and their associated genes that support ecosystem health through nutrient cycling (Jansson & Hofmockel, 2020). Soil microorganisms are affected by wildfires in varying ways, often experiencing reduced or eliminated populations after wildfire events (Kara & Bolat, 2009). Several studies have already noted that as high intensity fires are more frequent, damage to soil ecosystems will become more common and more severe (Bárcenas-Moreno & Bååth, 2009; Santín & Doerr, 2016). Wildfires can cause soils to reach extremely high temperatures, potentially releasing toxic compounds stored in the soil (Terzano et al., 2021) and changing many soil physiochemical characteristics (Whitman et al., 2019). Post-fire effects on soil quality can persist for more than a decade, compounding these threats and highlighting the need to better understand how these changes can significantly alter the health of soil microbial communities (Bladon et al., 2014; Bormann et al., 2008; Ice et al., 2004).

1.1 Wildfire Impacts on Microbial Community Structures and Nutrient Cycling Activity

Soil bacteria can be affected by wildfire-induced alterations to soil chemistry and structure in multiple ways. First, fire heat can directly impact bacteria by killing soil microbes outright (Nelson et al., 2021), decreasing microbial biomass in the soil, and altering community structure (Köster et al., 2021). This alters microbial dynamics across both small (e.g., cell metabolism and communication) and large (e.g., community dynamics and microbial evolution) scales. The most marked shift in microbial communities following wildfires has been seen in the cycling of soil nutrients such as nitrogen, sulfur, phosphorus, and carbon (H. Xu et al., 2021; Yadav et al., 2021). In the nitrogen cycle, bacteria transform organic nitrogen from decomposed plant and animals into an inorganic form that plants can use. As plants decompose, assimilated nitrogen returns to the soil in an organic form and the cycle continues (Killpack & Buchholz,

1993). Bacteria can also fix atmospheric nitrogen converting it to more reactive compounds (e.g., ammonia) (Natwora & Sheik, 2021). Plant growth is benefitted by this process (Yadav et al., 2021).

During the sulfur cycle, sulfur is deposited into the soil from the atmosphere through mineral weathering and organic material decomposition (Edwards, 1998). Bacteria are primarily responsible for sulfur mineralization, the transformation of organic sulfur often bound to hydrocarbons, to inorganic sulfur (Gu et al., 2017). When the sulfur is transformed to an inorganic form, plants can utilize it and release it back into the soil in an organic form upon death (Edwards, 1998). Genera that can decompose organic sulfur include species of *Methylophaga* (Schäfer, 2007) and *Arthrobacter* (Yang et al., 2007). Sulfide-oxidizing bacteria and sulfate-reducing bacteria are heavily involved in the sulfur cycle (Aranda et al., 2015; Tang et al., 2009). Examples of sulfide-oxidizing bacteria include *Candidatus Isobeggiatoa, Candidatus Parabeggiatoa*, and *Arcobacter* (Aranda et al., 2015). Complete oxidizers include *Desulfobacter* and *Desulfococcus* and incomplete oxidizers include *Desulfovibrio* and *Desulfofustis* (Tang et al., 2009).

The phosphorus cycle is similar to the sulfur cycle, as weathering breaks down minerals and causes phosphate ions to be released (Flicoteaux & Lucas, 1984). Plants can use phosphate ions, but the phosphorus is released in a carbon-bonded organic form upon death. Bacteria can utilize carbon-bound phosphate, transforming the organic phosphorus back to inorganic phosphorus, and the cycle continues (Zeng et al., 2022). Genera that can degrade organic phosphorus include species of *Flavobacterium*, *Enterobacter*, and *Arthrobacter*, and *Pseudomonas* (Singh & Walker, 2006).

Finally, in the carbon cycle, plants fix atmospheric carbon during photosynthesis and store it in the soil (Johnston et al., 2004). Some lithotrophic bacteria can also fix carbon dioxide and use it for metabolism (Rubin-Blum et al., 2019). Heterotrophic bacteria use organic sources of carbon (S. Wang et

al., 2021) and these bacteria can convert soil carbon back to atmospheric carbon dioxide, helping the carbon cycle continue (Ecoss, 2015). Some species involved in the conversion of soil to atmospheric carbon are *Bacteroides succinogenes, Clostridium butyricum,* and *Syntrophomonas spp.* (Abatenh et al., 2018). Additionally, when plants and animals die, soil microorganisms recycle the nutrients from the dead matter back into the environment so they can be reused by other species in the form of DNA, RNA, and amino acid construction (Yadav et al., 2021).

Microorganisms manage nutrient cycling in the soil (Kara & Bolat, 2009) and a reduction in healthy soil microbial populations leads to a loss of soil nutrients and a decrease in ecosystem diversity. Runoff and soil erosion is also extremely common after wildfires, carrying away vital nutrients from recovering areas (Shakesby, 2011). Fires can indirectly impact microbes by changing soil chemistry to become inhospitable for bacteria (Nelson et al., 2021). These changes in soil chemistry depend on the soil temperature caused by wildfires and include alterations to pH and soil organic matter content and decreases in total nitrogen (Nelson et al., 2021; Xu et al., 2012) Wildfire heating of soil can also cause changes in soil organic matter structure, increasing the ratio of carbon to combustible organic material (Neff et al., 2005). Long term effects of fire may also impact microbial communities by destroying native plants and encouraging the growth of invasive species (Hart et al., 2005). Bacteria play an active role in plant development, with plant growth being promoted by bacteria that fix nitrogen in the rhizosphere, the immediate area surrounding plants roots (Moreira et al., 2020). High intensity fires can decrease nitrogen-fixing gene abundance in soil bacteria (Nelson et al., 2021), indicating that nitrogen cycling activity is likely significantly altered in soils exposed to wildfires. As a result, this decrease in nitrogen fixation, combined with the erosion of surface soil nutrients after wildfires, could prove detrimental for already struggling plant life post-fire as plants are unable to obtain the required nutrients from their environment.

Previous studies have shown that bacteria taxa have varying responses to fire intensity (Bárcenas-Moreno & Bååth, 2009; Certini et al., 2021; Whitman et al., 2019). Phyla that have been observed to increase post-fire include Actinobacteria and Proteobacteria groups (Nelson et al., 2021). Most Actinobacteria and Proteobacteria are highly involved breaking down a variety of organic materials (Ul-Hassan, 2009), a process that would be beneficial for a recovering ecosystem post-fire. However, Proteobacteria includes pathogenic species like *E. coli* (K. G. Patel & Pyrsopoulos, 2019), indicating that an increase in Proteobacteria post-fire could have downstream risks for human health and environmental quality. *Solirubrobacteraceae* and *Frankiaceae* families belonging to the phylum Actinobacteria are often more abundant in fire exposed soils compared to control soils (Nelson et al., 2021). *Solirubrobacter,* part of the *Solirubrobacteraceae* family, supports a role in the nitrogen cycle (Maquia et al., 2021). *Frankiaceae* are nitrogen fixing bacteria that live in root nodules of plants (Normand, 2006) and would likely encourage ecosystem recovery post-fire. The genus *Massilia*, part of the *Oxalobacteraceae* family, also increases post-fire (Whitman et al., 2019), and species of *Oxalobacteraceae* promote nitrogen acquisition in plants (Yu et al., 2021). The Actinobacteria genera *Arthrobacter* has also shown to increase following wildfires (Nelson et al., 2021). Some *Arthrobacter* spp. capable of nitrogen metabolism assist in ecosystem recovery after wildfires (Fernández-González et al., 2017). Many bacteria decrease following wildfire exposure. The Acidobacteria phylum has been shown to decrease with wildfire intensity (Adkins et al., 2020). Acidobacteria are oligotrophs, which can survive on low levels of nutrients, and their decrease post-fire could be due to low reproduction rates in response to environmental stress from wildfires (D. A. Gray et al., 2019). However, interphylum taxa have been demonstrated to be disparately affected by wildfire, suggesting high-level phylogenetic relationships cannot predict microbial responses to wildfires. For example, Chloroflexia and Ktedonobacteria both belong to the Chloroflexi phylum, but Chloroflexia spp. have decreased abundance after fires while Ktedonobacteria spp. have increased abundance (Zhou et al.,

2020).

Wildfires that burn surface soils for long durations can cause surface soils to reach extremely high temperatures (Terzano et al., 2021), indicating that impacts to soil microbiomes will differ across soil depths. Fire disproportionately affects surface soils (Fairbanks et al., 2020) and high temperatures can effect soil and chemical properties 15 cm below the surface and up to 30 cm below during extremely severe (i.e., destructive) wildfires (Terzano et al., 2021). While wildfires can cause soil temperatures at the surface to reach 850°C, and even 1,000°C for high intensity fires, dry soil is usually fairly insulating and soil temperatures below a 5 cm depth are unlikely to exceed 150°C (N. R. Smith et al., 2008; Terzano et al., 2021). A study by Qin & Liu (2021) examined wildfire effects on varying soil depths during a rainy season, and found that while the top 5 cm of soil was most affected, bacteria up to 20 cm below were affected over time.

The extreme effects of burning at the surface can potentially release toxic compounds (e.g., lead, mercury, arsenic) from the soils which could negatively impact microorganisms, through mechanisms such as enzyme inactivation and cell damage (Certini et al., 2021; Terzano et al., 2021). It is well known that wildfires can cause microbial death (Hart et al., 2005; Terzano et al., 2021; Xiang et al., 2014), likely because most soil-dwelling microbes live in the uppermost soil layer which is most affected by fire (Certini et al., 2021). Less information exists on the effects of wildfire intensity and its relationship with nutrient cycling bacteria, however, so it is unknown whether reduced populations of nutrient cycling bacteria could slow ecosystem recovery post-fire.

1.2 Development of Antibiotic Resistance Genes in Microorganisms

In addition to changes in bacteria community structure, wildfires could contribute to the development and distribution of antibiotic resistance genes (ARGs) in soil microbes. While bacteria play key roles in maintaining ecological homeostasis, there is limited research around wildfire impacts on the

structure and function of soil bacteria or the distribution of ARGs in soils exposed to wildfire. There are currently 700,000 global deaths per year from antibiotic resistant infections and this number is expected to increase to 10 million deaths annually by 2050 (Patel & Williams, 2019). Antibiotics are used to treat bacterial infections and to raise healthy livestock (Polianciuc et al., 2020) and these antibiotics can find their way to the environment through both natural (e.g., gene transfer among bacterial communities) and anthropogenic (e.g., wastewater-derived biosolids) sources (Zhang et al., 2021). Wildfires also increase surface runoff, generally by decreasing infiltration capabilities of the soil (Ebel, 2020; Smith et al., 2008), providing a potential long-range transportation route of ARGs through runoff events (Zhang et al., 2021). This research gap surrounding wildfire impacts on ARG development has left society unprepared to deal with the potential rise in antibiotic resistant bacteria due to increasing wildfire activity.

Horizontal gene transfer (i.e., the transfer of genetic information among unrelated organisms) is main mechanism for inducing genetic modifications in bacterial communities and is known to play a significant role in the spread of antibiotic resistance (Zhang et al., 2021). Numerous studies have demonstrated that genetic adaptation rates are linked to both physicochemical and environmental factors (e.g., limited or altered nutrient availability) as well as internal microbial factors (e.g., metabolic stress), which directly correlate to the gene transfer potential of a microbial community (e.g., Hardiman et al., 2016; Sheppard et al., 2020). However, these studies have been conducted under steady-state environmental conditions and have completely ignored the influence of disruptive events such as wildfires. Post-fire landscapes are characterized by many conditions that could promote the spread of antibiotic resistance genes including limited resource availability, release of heavy metals, and altered fungal-bacterial community dynamics (Liodakis & Tsoukala, 2009; Nelson et al., 2021; Yogabaanu et al., 2017). Assessing the impacts of wildfires on the abundance of antibiotic resistance genes in environmental microbes is critical for evaluating the long-term impact of wildfires for human and environmental health.

It has also been demonstrated that wildfires result in an increase in heavy metal concentrations in the environment (Liodakis & Tsoukala, 2009). Wildfires result in the deposition of ash, a combination of minerals and burned organic material, onto surface soils (Bodí et al., 2014). Wood ash resulting from wildfires is a well-documented source of heavy metals such as zinc and copper, which are often released from burned plants and soils during and after combustion reactions (Bodí et al., 2014; Zhang et al., 2021). In addition, modern firefighting practices involve the use of ammonium sulfates, which affect heavy metal solubility (Liodakis & Tsoukala, 2009). When the sulfate ions in the fire retardants form sulfuric acid, soil pH is lowered, causing heavy metals in the soil and ash to become more soluble in water (Liodakis & Tsoukala, 2009). This increased solubility may result in more frequent contact of heavy metals in soils with bacteria (Plumlee et al., 2007). The release of heavy metals following wildfires may also be a major factor influencing the spread of antibiotic resistant bacteria. For example, increases in zinc concentrations in soil have resulted in the development of zinc resistance in bacteria populations. These increases in zinc resistant bacteria are also correlated with a rise in antibiotic resistance in the same bacteria (Dickinson et al., 2019). The elevated concentrations of heavy metals in soils after wildfires may trigger a similar increase in the abundance and diversity of antibiotic resistance in bacteria that remain in wildfire affected soils.

Bacteria and fungi appear to respond differently to wildfire exposure. A study by Vázquez and colleagues (1993) showed that one month after a simulated wildfire event, the bacteria population increased while the number of fungi decreased dramatically and was slow to rebound. The bacteria population likely rebounded quickly due to beneficial moisture and temperature levels as well as an increase in soil nutrients. While fungal populations could be expected to recover equally well, there were two likely reasons for the fungal population decline. The organic substrates used for energy and nutrition by the fungi were likely rendered unusable by the wildfire heat and fungal growth was also likely inhibited

by chemicals produced during combustion (Vázquez et al., 1993). Fungi are known to produce secondary metabolites in extreme environments for competitive and defensive purposes (Yogabaanu et al., 2017) and these secondary metabolites often have antibiotic properties (Fatima et al., 2019). Fungi may produce secondary metabolites due to environmental stressors from wildfires and some of these metabolites have antibiotic properties (Ebrahimi et al., 2021). This could further increase ARG abundance in microbes after wildfires as microorganisms are exposed to elevated antibiotic levels in the soil.

1.3 Statement of Research Goals and Objectives

Soil microbial communities are often negatively impacted by wildfire events and these impacts will likely be exacerbated as wildfires become more frequent. Nitrogen-fixing bacteria play a key role in nitrogen acquisition in plants (Nelson et al., 2021), so an impact on nitrogen fixing bacteria could have devastating consequences for ecosystem recovery. Additionally, the potential increase of ARGs from wildfires is a pressing concern, especially given the increase in antibiotic resistant infections worldwide. Thus, the research objectives for this thesis were to:

1. Assess soil microbiome structures following wildfire events and evaluate the association of wildfire events on the distribution of nutrient-cycling bacteria; and

2. Examine whether wildfire-exposed soils are associated with increased abundance and diversity of antibiotic resistance genes.

The underlying hypothesis for Objective 1 is that nutrient cycling bacteria will be most negatively affected by high intensity wildfires because high intensity wildfires are extremely damaging to soil bacteria (Bárcenas-Moreno & Bååth, 2009). The underlying hypothesis for Objective 2 is that antibiotic resistance gene abundance will be higher in soils exposed to high intensity wildfires.

To identify bacteria affected by wildfires, soil and ash samples were collected from fire-affected areas in California, Washington, and Idaho and bacterial DNA was isolated from each sample. To achieve Objective 1, the DNA samples underwent Illumina MiSeq Sequencing to identify the bacterial community structure in each sample. Spearman correlations and Jonckheere-Terpstra (JT) tests were then used to identify significant associations among soil microbiome structures, wildfire intensity, and soil depth. To achieve Objective 2, samples were also screened for the presence of ARGs using polymerase chain reaction (PCR) and gel electrophoresis. Selected genes included ARGs conferring microbial resistance to conventional antibiotics (i.e., *sul1*, *sul2*, *tetM*, *tetB*, *tetO*, *tetW*, and *ermF*) and last resort antibiotics (i.e., *mcr-1, optrA, fosA2*, and *cfr*). By comparing ARGs in controls (i.e., unburned and physically similar soils) versus fire-affected soils, wildfire impacts on ARG development in soil bacteria could be determined. The findings from this thesis will increase the knowledge of bacteria taxa affected by wildfires, enabling researchers to better understand the impacts of wildfires on ecosystem recovery. Furthermore, any increase of knowledge of wildfire effects on ARG development would be beneficial as antibiotic resistant bacteria are an ever-growing threat.

CHAPTER TWO: ASSESSING SOIL MICROBIOME RESPONSES TO WILDFIRES

2.1 Introduction

Wildfire frequency and acreage burned in the United States has been increasing since 1986 (*National Interagency Fire Center*, 2021), including more intense (i.e., higher heat) and frequent fires in the western United States (Keane & Karau, 2010). This is due to an increase in wildfire fuel (i.e., combustible organic material; Keane & Karau, 2010) and drought occurrence (Kountouris, 2020). The U.S. has experienced increases in the number of wildfires (Moritz et al., 2012), the length of the wildfire season (Westerling et al., 2006), and affected burn area (Dennison et al., 2014). In areas that are not wildfire dependent, these wildfires can have destructive consequences, resulting in the destruction of animal habitats (Rochelmeyer et al., 2019) and plant life by destroying bark, roots, and exposing bare soil (Smith, 2000). Large flora and fauna are not the only affected organisms in the environment. Soil bacteria are often affected by wildfires, both directly through microbial death (Kara & Bolat, 2009) and indirectly due to changes in soil characteristics post-fire that affect metabolic rates and viability (Whitman et al., 2019). Bacteria manage nutrient cycling in soils and sediments across the globe (Kara & Bolat, 2009) and a reduction in healthy soil microbial populations leads to a loss of soil nutrients and a decrease in ecosystem diversity in the soil microbiome. Soil bacteria also play an active role in plant development by providing plants with usable form of nutrients needed for growth (Moreira et al., 2020). As a result, when the structure (i.e., species diversity and abundance) or function (i.e., genetic diversity and resilience) of soil microbiomes is changed by wildfire, there can be long-lasting effects on the surrounding ecosystem.

Soil bacteria can have varying responses to wildfires depending on wildfire intensity. Wildfire intensity is the measure of heat transfer at the front of wildfires, while wildfire severity refers to the resulting ecosystem impact from wildfires (Keeley, 2008; Robichaud et al., 2000). Intensity was used in this study, rather than severity, because intensity measurements can be used to estimate fire heats and

the resulting impacts on soil bacteria abundance. Severity is not a direct measurement of fire heat, and accurately measuring fire heat is important because bacteria have varying responses to different heat levels (Moya et al., 2021; Terzano et al., 2021). Low intensity burning can mobilize and increase the bioavailability of soil nutrients and drive increases in the proliferation of some bacterial taxa (Moya et al., 2021). In contrast, higher intensity fires can result in increasingly negative effects on plant and soil properties such as nutrient levels (Gray & Dighton, 2009), pH, and porosity (Terzano et al., 2021), leading to the development of unfavorable environmental conditions and microbial death. However, the persistence of these changes are dependent on plant and soil characteristics such as the area occupied by tree trunks and the mass of litter on forest floors (Adkins et al., 2020). Although wildfires naturally regulate nutrients in ecosystems (Pausas & Bond, 2020; Pereira et al., 2021), it is likely that, due to the damaging effects of high intensity fires on microbes, fires that affect soil temperatures can have negative impacts on ecosystem recovery caused by the loss of nutrient cycling bacteria or diminished metabolic activity in post-fire recovery.

2.1.1 Impacts of Wildfire Intensity on Soil Chemistry

Wildfires can cause changes in soil physiochemical characteristics, such as soil pH, composition, porosity, and available nutrients, and these changes can persist for several years after initial wildfire events (Xu et al., 2012). Wildfires tend to increase soil pH and electrical conductivity and decrease permeability, porosity, hydraulic conductivity, and available nutrients. There are additional effects that have been observed in soils affected by wildfire, including changes in microbial biomass, soil structure stability, and water repellence (Terzano et al., 2021). Wildfires can also have different intensities, which is the measure of heat energy produced during a wildfire (Dellasala, 2018). Maximum ground temperatures during wildfires typically range from 200-300°C, although high-fuel areas can reach temperatures above 500°C (Bárcenas-Moreno & Bååth, 2009), while fire temperatures themselves can

reach 1,500°C (Knicker, 2007). As fuel accumulates (e.g., dead trees), wildfires are likely to have higher intensities (Wu et al., 2013). Wildfire intensity is measured in the rate of heat transfer over distance, typically represented in watts or joules per meter (Frangieh et al., 2020; Knicker, 2007). Measuring fire intensity with physical instruments can be difficult, however, thus remote-imaging methods are considered most effective. The Sentinel-2 mission, launched by the European Space Agency, has two satellites that use spectral bands to measure environmental conditions on the ground (Earth Resources Observation and Science Center, 2017). Sentinel-2 can be used for identifying and tracking wildfires, and the spectral bands used can identify different wildfire intensities in W/m^2 (Hu et al., 2021).

Low-intensity wildfires have not been found to drive significant alterations to many soil physiochemical properties because the temperatures associated with low-intensity burns are not high enough to create any major chemical reactions in the soil (Moya et al., 2021). For example, no changes in pH, soil moisture, or temperature were observed 14 months after a low intensity wildfire in northwest China (Xu et al., 2012). Kranz & Whitman (2019) found a similar result when they studied low temperature prescribed fires in Wisconsin, USA, and found no change in these soil properties post-fire compared to control samples. Low intensity burning has also been found to promote phosphorus availability (Gray & Dighton, 2009) and increase amount of positively charged nutrients (e.g., K^+, Mg^{2+}) in the soil (Francos et al., 2018). As phosphorous is a growth-limiting nutrient for many microbes, increasing its soil availability has positive effects on the growth of soil microbial communities (Moya et al., 2021). A study using experimental low-intensity wildfires found that microbial biomass and activity (e.g., respiration) were reduced after the wildfire but microbial diversity increased and the wildfire did not significantly change the soil (Fontúrbel et al., 2012). However, even low intensity wildfires have been found to strongly affect soil organic matter (SOM) (Terzano et al., 2021). SOM is partially decayed organic plant and animal matter found only in the soil (Fageria, 2012). SOM is easily destructible at low temperatures and it accumulates

in the topsoil (Terzano et al., 2021). As wildfires disproportionately affect surface soils (Fairbanks et al., 2020), SOM is at high risk of combustion during wildfires. This decrease in SOM is balanced, however, by the deposition of new organic matter from wildfires into the soils, which may cause a spike in SOM of up to 30% post-fire (Terzano et al., 2021). Heterotrophic bacteria consume organic carbon sources (Wang et al., 2021), so it is likely that they would benefit from low intensity wildfires.

Moderate intensity wildfires have been found to alter soil characteristics with effects persisting for years after the wildfire. Three years after a moderate intensity wildfire in a *P. ponderosa* forest, the burned soils had higher pH, less moisture content, and higher levels of extractable phosphorus and zinc than unburned soils (Xu et al., 2012). A moderate intensity shrubbery wildfire was studied over time in northwest China and research showed that soil pH increased immediately after the wildfire when compared to unburned soils and remained at elevated levels during the 15 month study post-fire (Xu et al., 2012). Soil pH increases are due to the release and deposition of cations onto surface soils (Smith et al., 2008). The study by Xu et al. (2012) also showed that available phosphorus increased sharply after four months and eventually reached a level eight-times higher than the unburned soil, while soil organic matter and total nitrogen decreased to lower levels than the unburned soils for the entire 15-month study. Available nitrogen was found to decrease two months after the wildfire, but began to increase once vegetation was restored one year after the wildfire (Xu et al., 2012).

High intensity wildfires decrease nitrogen-fixing gene abundance (Nelson et al., 2021) and this decrease in nitrogen fixation could prove detrimental for already struggling plant life post-fire. This decrease in nitrogen fixing genes is not unexpected, as nitrogen cycling bacteria are known to be highly sensitive to environmental changes and are quickly negatively impacted when these changes create less hospitable growing conditions (Nelson et al., 2021). One year and two months after a high intensity wildfire, soil increased in moisture and temperature and decrease in pH levels (Xu et al., 2012). One study

looked at differences between prescribed burns and high intensity wildfires and saw that within 15 years after a wildfire, percent total carbon remained relatively unchanged. After 15 years, however, the occurrence of high intensity wildfires increased soil carbon substantially, especially when the time since the wildfire was greater than 30 years (Sawyer et al., 2018). However, there are many confounding factors present in such a study because of the extended time scope and it is difficult to understand causative factors in both prescribed burns and high intensity fires over a long period of time.

Additionally, wildfires result in the deposition of ash, a combination of minerals (e.g., phosphates, sulfates, carbonates) and burned organic material (e.g., slightly burned vegetation, charcoal), onto surface soils (Bodí et al., 2014). Although complete combustion processes of vegetation releases gases and leaves behind mineral components, combustion during wildfires is often incomplete, thus some of the fuel remains behind as charred organic material (Bodí et al., 2014). The color of ash can vary based on the completeness of combustion, with low combustion completeness resulting in darker ash and high combustion completeness resulting in white ash, normally found on top of black ash (Bodí et al., 2014). Wood ash resulting from wildfires is a well-documented source of heavy metals such as zinc and copper, which are often released from burned plants and soils during and after combustion reactions (Bodí et al., 2014; Zhang et al., 2021). High concentrations of heavy metals can result in microbial death (Igiri et al., 2018), and ash from high temperature combustion processes has fewer nutrients than ash from low temperature combustion (Bodí et al., 2014).

2.1.2 Nutrient Cycling in Wildfire Affected Soils

Soil bacteria are responsible for a number of roles in the soil, including the cycling of key nutrients including nitrogen, sulfur, phosphorus, and carbon (Yadav et al., 2021; Xu et al., 2021). In the nitrogen cycle, bacteria can transform organic nitrogen from decomposed plant and animals into an inorganic form that plants can use. When the plants die, the nitrogen returns to the soil in an organic form and the cycle

continues (Killpack & Buchholz, 1993). The nitrogen cycle involves nitrogen fixation, nitrification, and denitrification (Too et al., 2021). Bacteria can fix atmospheric nitrogen, converting it to more reactive compounds such as ammonia or ammonium (Natwora & Sheik, 2021; Too et al., 2021) and plant growth is benefitted by this process (Yadav et al., 2021). Nitrogen fixation is mediated by the enzyme nitrogenase, which converts nitrogen gas to ammonia (Kuypers et al., 2018).

Nitrogen-fixing genera include *Beijerinckia*, *Azotobacter*, *Azospirillum*, *Herbaspirillum*, *Gluconacetobacter*, *Burkholderia*, *Clostridium*, *Methanosarcina*, and *Paenibacillus* (Aasfar et al., 2021). Bacteria also degrade organic nitrogen from plants in the process of ammonification, releasing ammonia in the process (Kuypers et al., 2018). Ammonia can undergo oxidation, where ammonia monooxygenase is used to oxidize the ammonia to hydroxylamine (Kuypers et al., 2018). Ammonia oxidizing bacteria include the genera *Nitrosospira* and *Nitrosomonas* (He et al., 2012). Ammonia oxidation requires energy, so energy is conserved by further oxidizing the hydroxylamine to nitric oxide by using hydroxylamine oxidoreductase and then even further oxidizing the nitric oxide to nitrite (Kuypers et al., 2018). Ammonia can also undergo nitrification, which is the conversion of ammonia to nitrite (e.g., by archaea such as *Nitrososphaera*) and then to nitrate through oxidation using nitrite oxidoreductase by bacteria such as *Nitrobacter* and *Nitrospira* (Kuypers et al., 2018; Norton & Ouyang, 2019; Too et al., 2021). Some species of *Nitrospira* have even been found to utilize the entire nitrification reaction from ammonia to nitrite by themselves (Norton & Ouyang, 2019).

Nitrogen can also be removed from the soil environment through denitrification, which is the reduction of nitrate back to nitrite by either a membrane-bound nitrate reductase or periplasmic nitrate reductase (Kuypers et al., 2018). The nitrite is then transformed to ammonium by a reductase enzyme (Kuypers et al., 2018). Alternatively, nitrite can be reduced to nitric oxide then further reduced to nitrous oxide before it undergoes a final reduction step to nitrogen gas (Kuypers et al., 2018; Too et al., 2021).

Examples of key denitrifying bacteria are the genera *Pseudomonas, Paracoccus*, *Microvirgula,* and *Thaurea* (Takaya et al., 2003). Too and colleagues (2021) identified several bacteria involved in the nitrogen cycle in a peat swamp forest, with two *Dyella* spp. and one *Paraburkholderia* sp. reducing nitrate to nitrite and one *Klebsiella* sp. reducing nitrate to ammonium. Additionally, nitrogen starts to volatilize at temperatures above 120°C. Although the total loss of nitrogen due to wildfires rarely exceeds 50%, long-term reduction in soil nitrogen may stunt microbial activity (Hart et al., 2005). Bacteria can have complicated metabolisms with some being able to accomplish multiple steps of the nitrogen cycle simultaneously (Kuypers et al., 2018). Thus, the reduction in one taxon can have negative effects on multiple steps in the nitrogen cycle.

During the sulfur cycle, sulfur is deposited into the soil from the atmosphere, mineral weathering, and organic material decomposition (Edwards, 1998). Bacteria are primarily responsible for sulfur mineralization, the transformation of organic sulfur, often bound to hydrocarbons, to inorganic sulfur (Gu et al., 2017). When the sulfur is transformed to an inorganic form, plants can utilize it and release it back into the soil in an organic form upon death (Edwards, 1998). Genera that can decompose organic sulfur include species of *Methylophaga* (Schäfer, 2007) and *Arthrobacter* (Yang et al., 2007). Sulfide-oxidizing bacteria and sulfate-reducing bacteria are heavily involved in the sulfur cycle (Aranda et al., 2015; Tang et al., 2009). Examples of sulfide-oxidizing bacteria include *Candidatus Isobeggiatoa, Candidatus Parabeggiatoa*, and *Arcobacter* (Aranda et al., 2015). Complete oxidizers include the genera *Desulfobacter* and *Desulfococcus* and incomplete oxidizers include *Desulfovibrio* and *Desulfofustis* (Tang et al., 2009).

The phosphorus cycle is similar to the sulfur cycle, where weathering breaks down minerals and causes phosphate ions to be released. Plants utilize these phosphate ions and phosphorus is released in a carbon-bonded organic form upon floral death. Bacteria can utilize this organic phosphorus, transforming it back to an inorganic form, continuing the cycle (Zeng et al., 2022). Most soil phosphorus (i.e., 95-99%)

is in a form that plants cannot use (i.e., insoluble organic and inorganic phosphorus, soluble organic phosphorus), so transformation of unusable phosphorus by microbes is essential for ecosystem health (Wan et al., 2020). Taxa that can degrade organic phosphorus include species of *Flavobacterium*, *Enterobacter*, and *Arthrobacter*, and *Pseudomonas* (Singh & Walker, 2006).

Finally, in the carbon cycle, plants use photosynthesis to fix atmospheric carbon (CO_2) and store it underground (Johnston et al., 2004). Microorganisms can convert soil carbon back to CO_2 and the cycle repeats (Ecoss, 2015). Some species involved in the conversion of soil to atmospheric carbon are *Bacteroides succinogenes, Clostridium butyricum,* and *Syntrophomonas* spp. (Abatenh et al., 2018). Additionally, when plants and animals die, soil microorganisms recycle the nutrients from the dead matter back into the environment so it can be reused by other species in the form of DNA, RNA, and amino acid construction (Yadav et al., 2021). Soil bacteria play a key role in nutrient cycling, but these roles are likely disrupted by wildfire events. The risk to nutrient cycling microbes by forest wildfires is corroborated by the Mittal and colleagues (2019), whose research suggests that forest wildfires reduce the population of nutrient cycling bacteria.

2.1.3 Post-Wildfire Microbe Recovery

Various studies have examined the effects of wildfires on microorganisms. Bárcenas-Moreno and Bååth (2009) designed experimental fires heating Mediterranean pine forest soil to observe microbe recovery. They found that bacteria growth was inhibited at first with temperatures above 50°C, but the communities recovered days after the wildfire to levels above the control. Above 200°C, growth was completely inhibited immediately post-fire, but these samples eventually displayed the most bacteria growth. Samples heated above 500°C had low growth with no change due to the destruction of the organic matter in the soil (Bárcenas-Moreno & Bååth, 2009). Another study found that in both low and moderate-

intensity prescribed wildfires, changes in microorganisms were short-lasting and moderate (Certini et al., 2021).

Acea and Carballas (1996) studied microbial responses one month and one year after an Atlantic European forest wildfire of unstated intensity. They found that there were high numbers of ammonium-producing microbes but found low amounts of nitrite and nitrate producers in the control and burned samples. One month after the wildfire, ammonifying microbes increased while there was no change for nitrifying groups. One year after the wildfire, the positive of effects of the wildfire had mostly disappeared for the ammonifiers and no change was noted for the nitrifiers (Acea & Carballas, 1996).

2.1.4 Wildfire-Induced Impacts to Soil Microbiome Structures

Previous studies have shown that bacteria taxa have varying responses to wildfire intensity and community recovery post-fire is not static. Bacteria after a moderate-intensity wildfire were found to change their community structure monthly throughout their recovery period during a 15-month study (Y. Xu et al., 2012).

Phyla that have been observed to increase post-fire are Actinobacteria, Proteobacteria, Eremiobacterota, and Dormibacterota (Nelson et al., 2021). Within Actinobacteria the families *Streptosporangiaceae*, *Solirubrobacteraceae*, *Frankiaceae*, and *Streptomycetaceae* increase post-fire (Nelson et al., 2021) and Actinobacteria genera *Arthrobacter*, *Modestobacter*, *Blastococcus*, and *Actinomadura* have also shown increases (Nelson et al., 2021). Additionally, the genera *Massilia*, part of the *Oxalobacteraceae* family, increases post-fire (Whitman et al., 2019). After a pine forest wildfire, gram-positive bacteria were the dominant bacteria type (Mittal et al., 2019). While 99% of the deaths of nitrate oxidizers occur between 80°C and 90°C, depending on the soil moisture content, nitrate oxidizing bacteria (e.g. *Nitrobacter* spp.) have been found to increase post-wildfire (Hart et al., 2005). Whitman and

colleagues (2019) identified significant wildfire responding genera (i.e., *Massilia* spp. and *Arthrobacter* spp.) by utilizing high-throughput amplicon sequencing and determined that wildfire occurrence, soil moisture, floral community, soil pH and texture, and total carbon all significantly predict microbial community composition in the soil. Additionally, as burn severity increases, the microbial soil community composition increasingly changes (Whitman et al., 2019).

Phyla that decrease with wildfire intensity are Bacteroidetes and Acidobacteria (Adkins et al., 2020), and the phyla Chlamydiae and Elusimicrobia dramatically decrease post-fire (Nelson et al., 2021). Gram-negative genera were reduced after a pine forest wildfire (i.e., *Pseudomonas, Serratia, Enterobacter, Burkholderia, Klebsiella*, and *Pantoea*) (Mittal et al., 2019).

Kranz and Whitman (2019) identified the major phyla (i.e., Actinobacteria, Proteobacteria, Acidobacteria, Planctomycetes, and Verrucomicrobia) in both visually burned and unburned soils after a low intensity wildfire through high-throughput amplicon sequencing but determined that there was no significant change in phyla distribution between burned and unburned soils. Furthermore, they suggest that the community shifts seen were likely from plant changes rather than heat or soil property effects (Kranz & Whitman, 2019).

Bacteria are likely to have varying responses to wildfires because wildfires can affect soil nutrients in different ways. As organic carbon is destroyed at higher temperatures (i.e., almost completely combusted at 460°C) (Knicker, 2007) and released as carbon dioxide (Bárcenas-Moreno & Bååth, 2009; Di Carlo et al., 2015), high intensity wildfires would likely have negative effects on heterotrophic bacteria that depend on soil organic matter. Nitrogen suffers a similar fate as it begins to volatilize at 200°C and above 500°C half of soil nitrogen is vaporized (Knicker, 2007). Low intensity wildfires can transform organic nitrogen into ammonium and nitrate (Knicker, 2007), however, which would benefit bacteria that utilize

these nutrients. Phosphorus is more temperature resistant, combusting at 760°C and sulfur combusts at 800°C into sulfur dioxide (King et al., 2013; Knicker, 2007), so it is likely that high intensity wildfires would result in the highest losses of phosphorus and sulfur. Burning can also convert organic phosphorus to orthophosphate, which is the form used by plants (Knicker, 2007).

2.1.5 Microbial Response to Wildfires Across Soil Depth

Wildfire effects on microorganisms are highest in the top layer of surface soil (Hart et al., 2005), although wildfires can affect soil physiochemical properties to a 15 cm depth in the soil (Terzano et al., 2021). Qin and Liu (2021) analyzed impacts from a severe (i.e., destructive) wildfire on soil depths ranging from 0-20 cm during a rainy season in a north China *Pinus tabulaeformis* forest and determined that most significant effects on microbes occurred in the first five centimeters of soil. Their results showed that soil pH was the driver in microbial diversity between unburned and burned samples, while organic matter and available potassium determined microbial diversity with depth and time since the wildfire (Qin & Liu, 2021).

Thus, the first research objective for this thesis was to assess soil microbiome structures following wildfire events and evaluate the association of wildfire events on the distribution of nutrient-cycling bacteria. Due to the negative impacts associated with high-intensity wildfire events, I hypothesized that nutrient cycling bacteria will be most negatively affected by high intensity wildfires.

2.2 Materials and Methods

Wildfire severity measures the impacts and damages of fires on ecosystems, while wildfire intensity refers to the heat transfer at the front of the wildfire (Frangieh et al., 2020; Keeley, 2008; Robichaud et al., 2000; Sousa, 1984). For this study, measurements of intensity were used to examine fire impacts on soils. Wildfire severity measurements were collected using Sentinel-2 satellite data,

while intensity readings were collected using both Moderate Resolution Imaging Spectroradiometer (MODIS) and fire radiative power data by the U.S. Forest Service. Wildfire intensity soil samples were taken from area affected by the Woolsey Fire in the Santa Monica Mountains National Recreation Area in California. The Woolsey Fire began on November 8, 2018 and by the time it was contained on January 4, 2019 it had burned 94,946 acres (*Woolsey Fire*, 2021). The area has a Mediterranean ecosystem, characterized by mild winters with precipitation and warm summers with little precipitation (Mulholl et al., 2015a). The geology of the site is varied, containing volcanic and ocean sediments, canyons, and mountainous terrain (Mulholl et al., 2015b).

Soils were collected approximately one year after the Woolsey Fire (Figure 2) in January 2020 from areas exposed to high intensity wildfire (i.e., sites Corral Canyon (H8) and Kanan Dume (H9)), moderate intensity wildfire (i.e., sites Arroyo Sequit (M4), Corral Canyon (M5), Kanan Dume (M6), and Paramount Ranch (M7)), and low intensity wildfire (i.e., sites Cheeseboro Canyon (L2) and Laskey Mesa (L3)). The area experienced multiple rain events before samples were collected with 26-31 inches of rain reported from weather stations in the area in the year after burning (*Climate Data Online*, 2021). Control soils with no exposure to wildfire were collected from Encinal Canyon Road (C1a), Saddle Peak (C1b), and Calabasas Peak (C1c). Intensity readings were calculated using Moderate Resolution Imaging Spectroradiometer (MODIS) and fire radiative power data, collected from spectral band readings from the Sentinel-2 satellites by the U.S. Forest Service. At each location, soils were sampled in triplicate using a soil corer of approximately 1 in. diameter. Soils were then separated based on the following depths: 0 in. to 3 in. (topsoils) and 3 in. to 6 in. (bottom soils). Sampling materials were sterilized with 70% ethanol in between samples to prevent cross-contamination of soil microbes. Samples were shipped overnight on dry ice to Washington State University. Once received at the Gardner lab, soils were homogenized and

stored at -80°C, a common temperature for the storage of biological wildfire-affected soil samples (Maquia et al., 2021; Nelson et al., 2021), until DNA could be extracted.

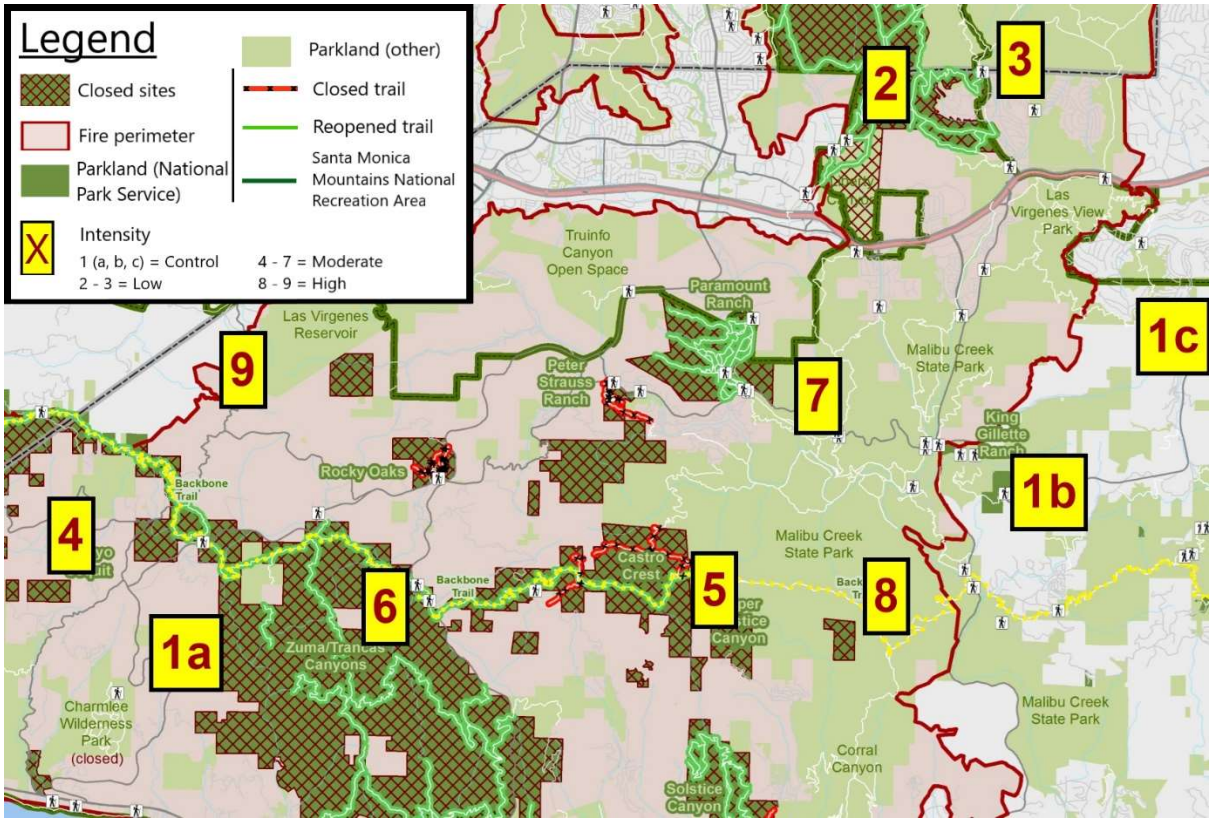

Figure 2. Map of sampling locations taken at the Woolsey wildfire in the Santa Monica Mountains National Recreation Area, California. Labels: C = control sample (i.e., no fire exposure), L = low intensity sample, M = moderate intensity sample, H = high intensity sample. Control – C1a: Encinal Canyon Road; C1b: Saddle Peak; C1c: Calabasas Peak. Low intensity – L2: Cheeseboro Canyon; L3: Laskey Mesa. Moderate intensity – M4: Arroyo Sequit; M5: Corral Canyon; M6: Kanan Dume; M7: Paramount Ranch; High intensity – H8: Corral Canyon; H9: Kanan Dume. The red border shows burn area, the green border shows the perimeter of the Santa Monica Mountains Natural Recreation Area, and the green area with red diamonds shows areas closed due to fire damage.

For Kincade, Tubbs, Williams Flats, Northstar, and Hayes wildfire sites, soil samples were collected immediately following wildfires. Intensity and severity measurements were determined as described for the Woolsey wildfire. The Kincade wildfire burned from October 23rd, 2019 to November 6th, 2019

northeast of Geyserville, CA, burning 77,758 acres (*Kincade Fire*, 2020). Six samples of unknown burn intensity were collected from the Kincade Fire: three topsoil and three bottom soil. After sampling, the soil samples were stored at -20°C until they were brought to WSU and kept for long-term storage at -80°C. The Tubbs wildfire burned from October 8th 2017 to February 9th, 2018 off of Highway 128 and Bennett Lane, CA, burning 36,807 acres (*Tubbs Fire*, 2019). Six high intensity burn samples were collected from the Tubbs Fire: three topsoil and three bottom soil. After sampling, the soil samples were stored at -20°C until they were brought to WSU and kept for long-term storage at -80°C. The Williams Flats wildfire burned from August 2nd, 2019 to August 25th, 2019, five miles southeast of Keller, WA, burning 44,446 acres (*Williams Flats Fire*, 2019). Fourteen samples were collected from the Williams Flats Fire in a high-burn intensity area as defined by Parson et al. (2010): three ash samples and 11 soil core samples, with depths ranging from 15 to 220 cm below ground. After sampling, the soil and ash samples were stored at -20°C until they were brought to WSU and kept for long-term storage at -80°C. The Mesa wildfire started on July 26th, 2018, 23.8 miles southwest of McCall, ID, and burned 34,719 acres. Five high intensity burn samples were collected from the Mesa Fire: three ash samples and two soil samples, one with a 15 cm depth and the other with a 50 cm depth. Ash is a layer of mineral and organic matter deposited on top of soil after the combustion of organic matter (Bodí et al., 2014). Black ash suggests lower combustion completeness of vegetation, while white ash suggests higher combustion completeness (Bodí et al., 2014). The ash samples collected for the Williams Flats and Mesa fires were white, indicating higher combustion completeness. After sampling, the soil and ash samples were stored at -20°C until they were brought to WSU and kept for long-term storage at -80°C.

The Northstar fire burned from August 13th, 2015 to October 9th, 2015 in the Colville Reservation, WA, burning 28,000 acres (Bureau of Indian Affairs, 2016). The Hayes Fire began on July 21st, 2016 and burned over 1,500 acres (Peninsula Daily News, 2016). One topsoil sample was taken from the both the

Northstar and Hayes wildfires. For each location, soils were again sampled using a soil corer of approximately 1 in. diameter. White ash samples collected from depositions above the surface soil, indicating higher combustion completeness (Bodí et al., 2014), were collected within two weeks using sterilized shovels following the suppression of active wildfire. After sampling, the soil samples were stored at -20°C until they were brought to WSU and kept for long-term storage at -80°C. No rainfall occurred between fires and sample collection. Following collection, all samples were immediately transported to the Gardner lab at Washington State University and stored at -80°C until DNA could be extracted. Full sample information is shown in Appendix One. Site maps for the Kincade, Tubbs, Williams Flats, and Mesa wildfires are shown in Figure 3.

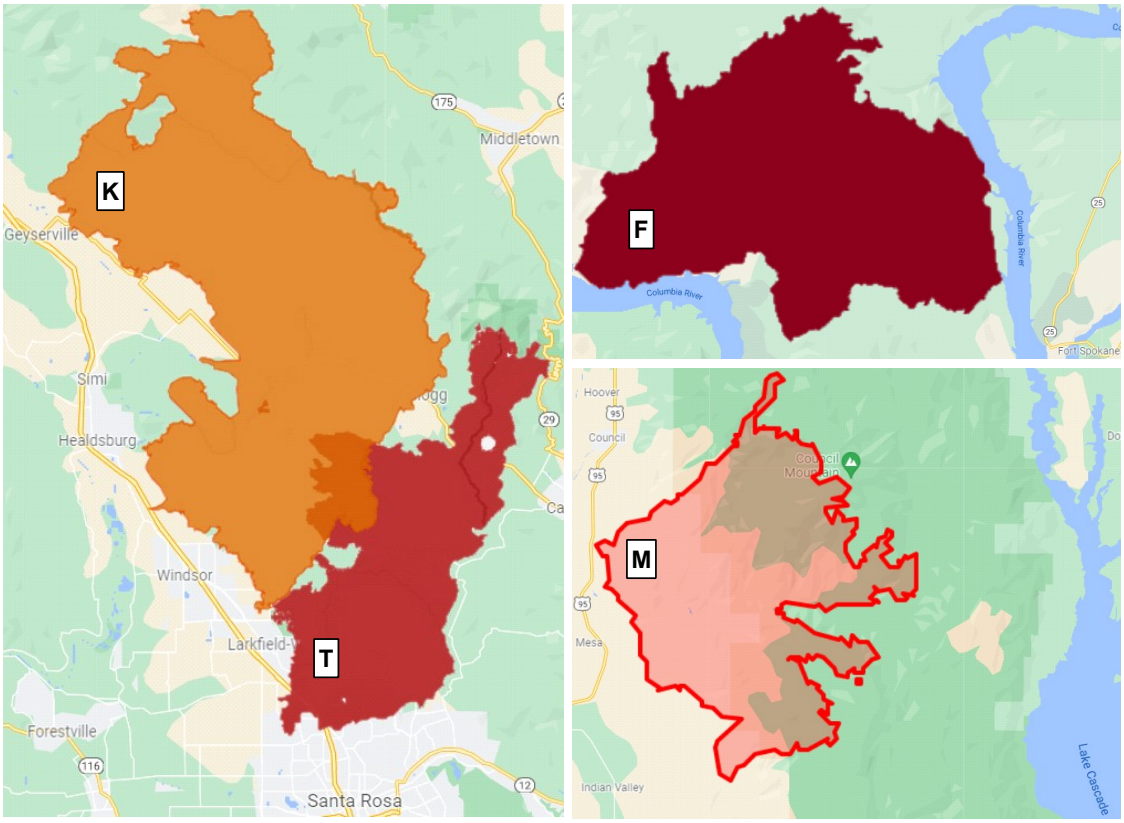

Figure 3. Burn areas for the Kincade (K), Tubbs (T) wildfires north of Santa Rosa, California, Williams Flats (F) wildfire northwest of Fort Spokane, Washington, and Mesa (M) wildfire west of Lake Cascade, Idaho.

Highlighted areas for each fire show burn area, with the Kincade fire area represented in orange and the Tubbs fire area represented in red, with the overlap between the two fires in dark orange.

DNA was extracted from 114 samples using the DNeasy PowerLyzer PowerSoil® Kit by QIAGEN. Approximately 0.25 grams of the soil or ash samples was used in each isolation. Each isolation followed the procedure outlined in the DNeasy PowerLyzer PowerSoil® Handbook, with some changes. First, the initial vortexing time of the PowerBead tube was increased from 10 to 20 minutes to increase DNA yields. Second, the final elution of the MB Spin Column membrane was done using 30 µL of solution C6 rather than the recommended 100 µL to avoid diluting eluted DNA. Resulting DNA concentrations were then analyzed using the Qubit 4.0 Fluorometer from Thermo Fisher Scientific. Extracted DNA was stored at -20°C for downstream analysis.

Illumina MiSeq *16S* Metagenomic Sequencing was performed on 112 soil and ash samples gathered from wildfire events. Two samples out of the total 114, the 180-200 cm and 200-220 cm soil core samples from Williams Flats, were omitted due to lack of high-quality DNA. The process performed followed the *16S* Metagenomic Sequencing Library Preparation guide. The DNA samples were normalized to 5 ng/µL and polymerase chain reaction (PCR) was performed to amplify the third and fourth regions of *16S* rRNA gene using specific primers (805R: 5‘-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTA CNVGGGTATCTAATCC-3‘; 341F:5‘-TCGTCGGCAGCGTCAGATGTCTATAAGAGACAGCCTACGGGNBGCAS CAG-3‘). The Amplicon PCR steps used were: 1) denaturation at 95°C for three minutes, 2) 25 cycles of denaturation at 95°C for 30 seconds, primer annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds, 3) final extension at 72°C for five minutes, 4) hold at 4°C. The amplicon then was brought up to room temperature and amplicon cleanup was performed using AMPure XP beads and fresh 80% ethanol to remove primers and primer dimer species. After cleanup, the amplicon was resuspended in 10 mM Tris pH 8.5. Index PCR was then performed to attach dual indices onto the ends of the amplified *16S* genes to associate each gene with the sample it originated from. The Index PCR was performed using

Nextera XT chemistry. Index PCR steps included: 1) Initial denaturation at 95°C for three minutes, 2) eight cycles of denaturation at 95°C for 30 seconds, primer annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds, 3) final extension at 72°C for five minutes, and 4) hold at 4°C. The final step was to clean the Index PCR amplicon by once again using AMPure XP beads and fresh 80% ethanol to remove excess primers and primer dimers from the indexed *16S* amplicon before resuspending the amplicon in 10 mM Tris pH 8.5. The index amplicon DNA concentration was then analyzed using the Qubit 4 Fluorometer and the samples were normalized to 30 nM and pooled together in a microfuge tube to create the *16S* DNA library. The final library was then sent to the Northwest Genomics Center at the University of Washington for sequencing. After sequencing, the demultiplexed raw data was trimmed, filtered, and bacteria taxa were assigned from the 2013 Green Genes database using the *16S* Metagenomics application in the Illumina BaseSpace program. The final data was imported into GraphPad Prism and IBM SPSS Statistics 26 for analyses.

Stacked bar graphs were made to visualize the distribution of taxa in Woolsey, Williams Flats, and other wildfire samples. To create the stacked bar graphs, the number of operational taxonomic units (OTUs) in any replicate samples for wildfire events were averaged together. The fraction of total analysis in GraphPad Prism was then used to obtain the average relative abundance values for both replicate and single samples. Stacked bar graphs were made for the top 30 most abundant taxa for families and top 63 more abundant taxa for genera.

The fraction of total analysis was also run on all non-averaged OTU values for the same wildfire samples to determine average relative abundance. The most abundant 30 families and 63 genera were then used for the following analytical tests. A non-parametric Spearman correlation in GraphPad prism was run on the average relative abundance values for the Woolsey fire samples to determine whether average relative abundance was correlated with wildfire intensity. A Jonckheere-Terpstra (JT) non-

parametric test was then run on the same samples using IBM SPSS Statistics 26 to verify the results of the Spearman correlation and to run post-hoc pairwise comparisons to determine significant differences in average relative abundance between fire intensities. The JT test has been used previously for sequencing analysis (Cordero et al., 2021; Wijetunga et al., 2016) and the JT test was chosen as opposed to a Kruskal-Wallis Test because the fire intensities are ordered and the JT test is designed for ordered independent variables while the Kruskal-Wallis Test is not (Bewick et al., 2004). Tukey box and whisker plots were then made for the Woolsey raw average relative abundance values using GraphPad Prism to visualize the distributions of taxa significantly associated with wildfire intensity.

Spearman correlations were also run on the soil depth samples from the Williams Flats wildfire to determine the association between taxa and soil depth in wildfire affected soils. Any significant taxa were then compared to significant taxa from the Woolsey intensity samples to identify any trends between fire intensity and taxa depth in the soil. A Kruskal-Wallis non-parametric test was then run on the moderate intensity Woolsey wildfire samples to determine whether sample physical location was a determining factor in community composition.

2.3 Illumina MiSeq Sequencing Results

2.3.1 Woolsey Fire: Wildfire Intensity Taxa Distribution

Vertically homogenized Woolsey wildfire samples were excluded from statistical analysis so that the distribution of soil taxa would be clearly defined across top and bottom soil boundaries. The samples are included in the appendices and were used for antibiotic resistance gene testing in Chapter Three.

Soil microbial communities were diverse across all Woolsey samples (Figure 4). Control soils without exposure to wildfires were largely dominated by *Chthoniobacteraceae* (average relative abundance = 5.48% ± 1.47%), *Bacillaceae* (average relative abundance = 4.32% ± 1.99%), and

Conexibacteraceae (average relative abundance = 5.59% ± 0.63%) families. Soils exposed to low intensity wildfire were largely dominated by *Conexibacteraceae* (average relative abundance = 4.46% ± 0.87%), *Nocardioidaceae* (average relative abundance = 5.26% ± 0.93%), and *Rubrobacteraceae* (average relative abundance = 4.75% ± 1.20%). Soils exposed to moderate intensity wildfire were largely dominated by *Chthoniobacteraceae* (average relative abundance = 7.44% ± 4.20%), *Micrococcacea* (average relative abundance = 3.93% ± 2.37%), and *Conexibacteraceae* (average relative abundance = 3.75% ± 1.46%). Soils exposed to high intensity wildfire were largely dominated by *Micrococcaceae* (average relative abundance = 6.37% ± 5.54%), *Bacillaceae* (average relative abundance = 5.13% ± %), and *Oxalobacteraceae* (average relative abundance = 5.32% ± 4.02%). The most abundant families for all categories (i.e., control, low, moderate, and high) averaged together are *Chthoniobacteraceae* (average relative abundance = 5.43% ± 3.84%), *Micrococcaceae* (average relative abundance = 3.73% ± 3.34%), and *Conexibacteraceae* (average relative abundance = 4.10% ± 1.34%).

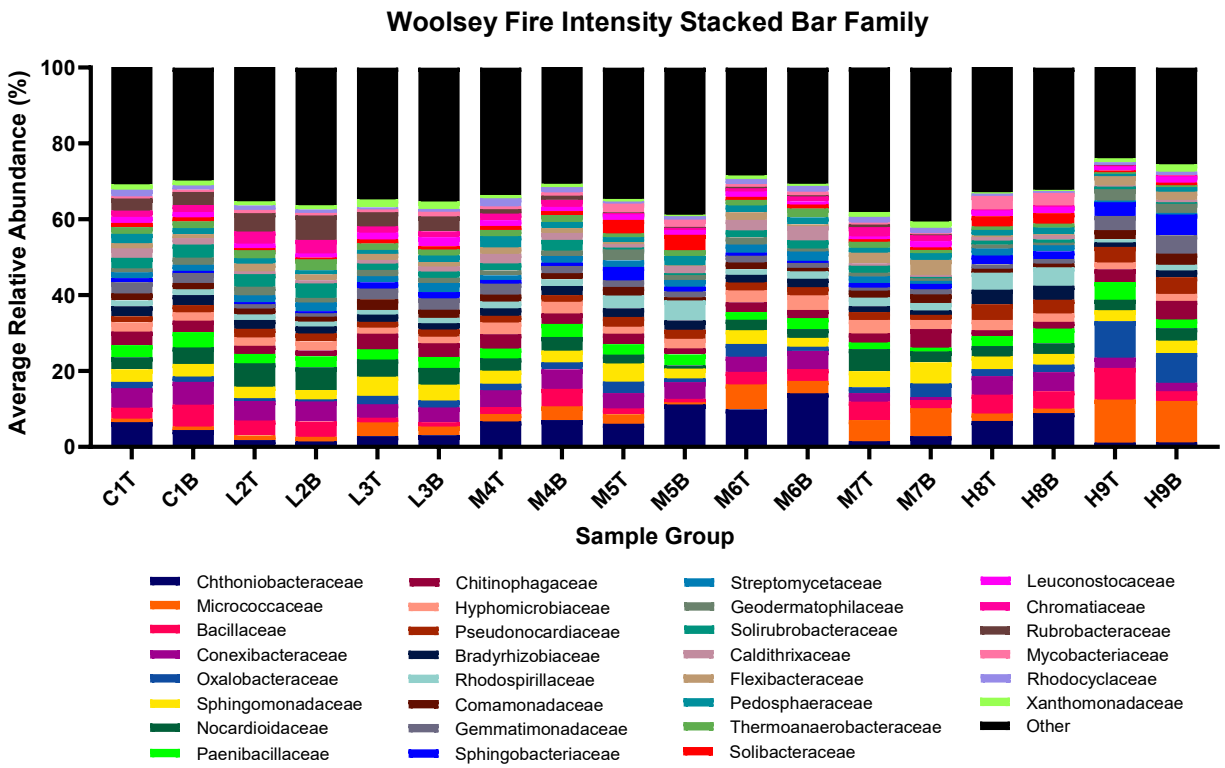

Figure 4. Sequencing data for family taxa for wildfire intensity. Sample names: C = control, L = low intensity, M = moderate intensity, H = high intensity. Sample numbers correspond to the map in Figure 2. Soil sample type is denoted by "T" (topsoil, 0-3 in) or "B" (bottom soil, 5-10 in).

Rubrobacteraceae is most abundant in control soils (3.25% ± 0.84%) and low intensity soils (4.42% ± 2.07%) and least abundant in high intensity soils (0.01% ± 0.02%). This suggests that *Rubrobacteraceae* may benefit from low-intensity fires and may be negatively associated with fire intensity overall. *Solirubrobacteraceae* is most abundant in control soils (3.05% ± 0.87%) and least abundant in high intensity soils (0.70% ± 0.31%), suggesting that *Solirubrobacteraceae* is negatively associated with fire intensity. *Micrococcaceae* is least abundant in control soils (0.86% ± 0.53%) and most abundant in high intensity soils (6.09% ± 5.58%), suggesting that *Micrococcaceae* may increase in relative abundance with higher fire intensities. *Chthoniobacteraceae* is least abundant in low intensity soils (2.32% ± 0.75%) and most abundant in moderate intensity soils (7.58% ± 6.45%), suggesting that *Chthoniobacteraceae* may be most benefitted by moderate intensity fires. While the relative abundance of *Comamonadaceae*

decreases at high intensities, a comparison of medians shows the average relative abundances at different intensities to be similar from the control soils (1.75% ± 0.29%) to high intensity soils (1.85% ± 1.09%), suggesting that the abundance of *Comamonadaceae* may not be associated with wildfire intensity.

For bacterial families that increase in abundance with increasing fire intensity (e.g., *Micrococcaceae*), it is possible that species within them are more suited to survive wildfire events and can fill the gap left behind by species negatively affected by fire. For those families that are negatively associated with fire intensity (e.g., *Rubrobacteraceae, Solirubrobacteraceae)*, it could be that the fire is killing off fragile species within those families or the nutrients that the bacteria rely on are being reduced by wildfire.

Figure 5 shows the sequencing data at the genus level for the top 63 most-populous results for wildfire intensity.

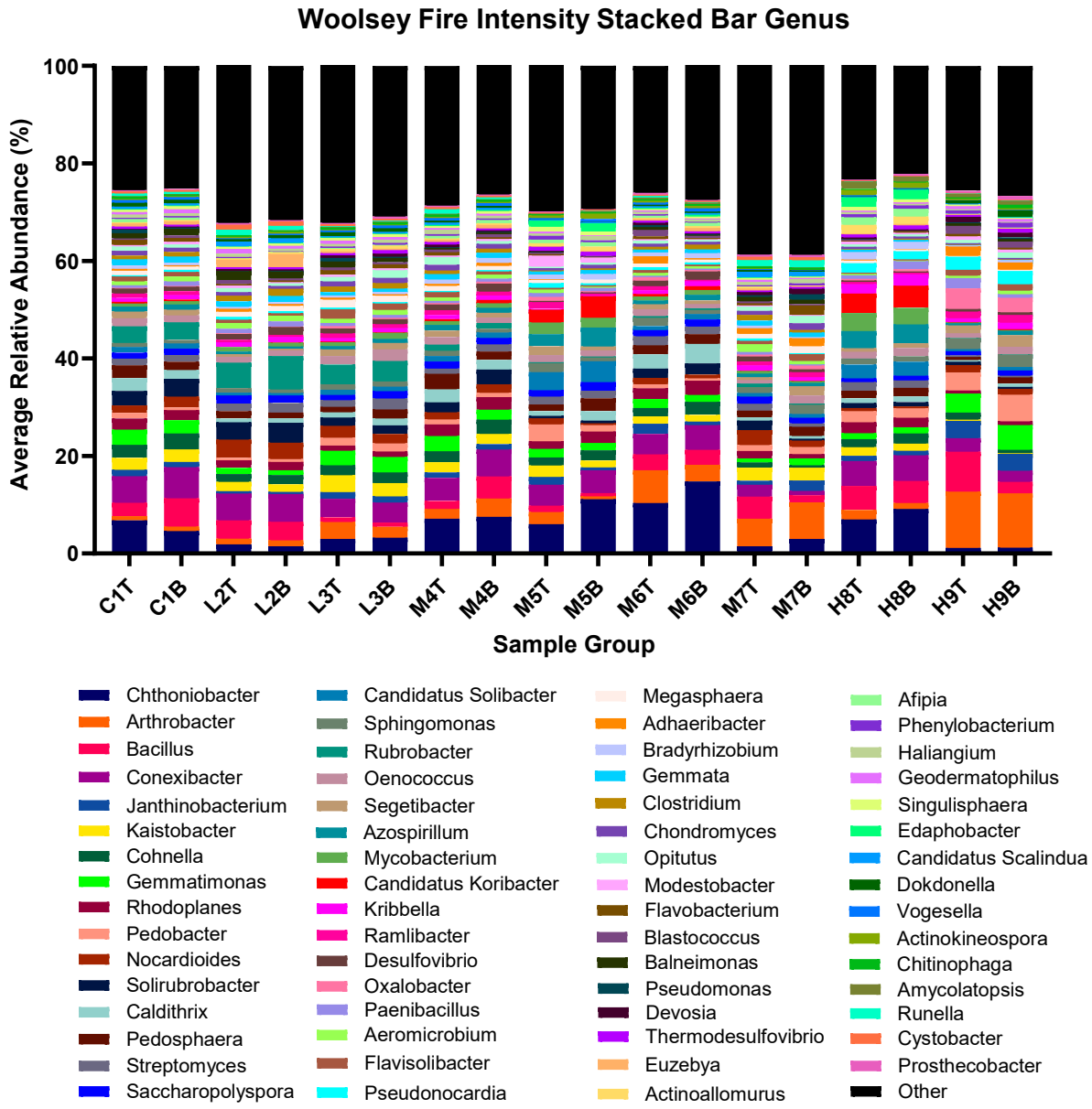

Figure 5. Sequencing data for genera taxa for wildfire intensity. Sample names: C = control, L = low intensity, M = moderate intensity, H = high intensity. Sample numbers correspond to the map in Figure 2. Soil sample type is denoted by "T" (topsoil, 0-3 in) or "B" (bottom soil, 5-10 in).

In Figure 5, the top six most abundant genera for each intensity category are as follows. Control soils without exposure to wildfires were largely dominated by *Chthoniobacter* (5.69% ± 1.55%), *Bacillus* (average relative abundance = 4.26% ± 2.16%), *Conexibacter* (average relative abundance = 5.93% ±

0.67%), *Gemmatimonas* (average relative abundance = 2.95% ± 0.26%), *Solirubrobacter* (average relative abundance = 3.29% ± 0.49%), and *Rubrobacter* (average relative abundance = 3.50% ± 0.15%). Soils exposed to low intensity wildfire were largely dominated by *Chthoniobacter* (average relative abundance = 2.40% ± 0.83%), *Conexibacter* (average relative abundance = 4.78% ± 0.97%), *Kaistobacter* (average relative abundance = 2.40% ± 0.85%), *Nocardioides* (average relative abundance = 2.86% ± 0.80%), *Solirubrobacter* (average relative abundance = 2.78% ± 1.23%), and *Rubrobacter* (average relative abundance = 5.08% ± 1.32%). Soils exposed to moderate intensity wildfire were largely dominated by Chthoniobacter (average relative abundance = 7.68% ± 4.36%), *Arthrobacter* (average relative abundance = 4.01% ± 2.41%), *Bacillus* (average relative abundance = 2.56% ± 1.49%), *Conexibacter* (average relative abundance = 3.98% ± 1.55%), *Kaistobacter* (average relative abundance = 2.02% ± 0.53%), and *Rhodoplanes* (average relative abundance = 2.05% ± 0.66%). Soils exposed to high intensity wildfire were largely dominated by *Chthoniobacter* (average relative abundance = 4.62% ± 4.09%), *Arthrobacter* (average relative abundance = 6.46% ± 5.66%), *Bacillus* (average relative abundance = 5.01% ± 2.43%), *Conexibacter* (average relative abundance = 3.85% ± 1.53%), *Gemmatimonas* (average relative abundance = 2.86% ± 1.92%), and *Pedobacter* (average relative abundance = 3.36% ± 1.60%). The most abundant genera for all categories (i.e., control, low, moderate, and high) averaged together are *Chthoniobacter* (average relative abundance = 5.60% ± 3.96%), *Arthrobacter* (average relative abundance = 3.77% ± 3.42%), *Bacillus* (average relative abundance = 3.24% ± 1.99%), *Conexibacter* (average relative abundance = 4.34% ± 1.43%), *Kaistobacter* (average relative abundance = 1.91% ± 0.85%), and *Gemmatimonas* (average relative abundance = 2.21% ± 1.15%).

From visual observation, *Arthrobacter* is least abundant in control soils (0.84% ± 0.51%) and is most abundant in high intensity soils (6.18% ± 5.68%), suggesting that *Arthrobacter* abundance is positively associated with wildfire intensity. *Chthoniobacter* is least abundant at low intensity soils (2.41%

± 0.75%) and high intensity soils (4.34% ± 3.79%) but more abundant than the control soils (5.66% ± 1.20%) in moderate intensity soils (7.81% ± 4.83%), suggesting that *Chthoniobacter* is most prevalent following moderate intensity wildfires. *Rubrobacter* is most abundant in control soils (3.45% ± 0.90%) and low intensity soils (4.73% ± 2.25%) and least abundant in high intensity soils (0.02% ± 0.02%). *Euzebya* is most abundant in control soils (0.67% ± 0.25%) and low intensity soils (1.14% ± 0.99%) and least abundant in high intensity soils (0.11% ± 0.13%). *Solirubrobacter* is most abundant in control soils (3.24% ± 0.93%) and least abundant in high intensity soils (0.72% ± 0.33%). *Rhodoplanes* is most abundant in control soils (2.13% ± 0.42%) and least abundant in low intensity soils (1.32% ± 0.36%) and high intensity soils (1.37% ± 0.75%). The data suggest that the abundance of *Rubrobacter, Euzebya, Solirubrobacter,* and *Rhodoplanes* is negatively associated with wildfire intensity. Similar to trends observed among family-level taxa, the genera that are positively associated with wildfire are likely to have increased survivability after wildfire events and can reproduce quickly after competition from other bacteria decreases, potentially due to bacterial death or decreases in available nutrients.

2.3.2 Influence of Wildfire Intensity on Soil Bacteria

Spearman correlations were used to detect significant positive or negative correlations between wildfire intensity and bacteria taxa in Woolsey samples (n = 54) at both the family and genus taxonomic level. Only samples from the Woolsey wildfire were included so all samples would be from the same fire to obtain accurate results. Soil samples include controls with no fire exposure (n = 6) and soils exposed to low (n = 12), moderate (n = 24), and high (n = 12) fire intensities.

A non-parametric JT test was then run on the same Woolsey wildfire samples for both family from Figure 4 and genera from Figure 5 to determine the difference between each wildfire intensity group and the control samples. Table 1 shows the Spearman correlation values and JT test values at the family and

genus level. For ease of interpretation, only taxa with significant and robust correlations ($p < .05$ and $|r_s| > 0.4$) are included in the table.

Table 1. Spearman correlation values and Jonckheere-Terpstra test values for control, low, moderate, and high intensity Woolsey wildfire samples. Statistical significance is assigned for p < 0.05.

	Spearman		Jonckheere-Terpstra	
Family	*p*	r_s	*p*	Standardized Test Statistic
Chromatiaceae	<0.0001	-0.704	<0.0001	-5.363
Micrococcaceae	0.0021	0.410	0.002	3.158
Nocardioidaceae	0.0003	-0.470	0.001	-3.253
Pseudonocardiaceae	<0.0001	0.557	<0.0001	4.205
Rubrobacteraceae	<0.0001	-0.899	<0.0001	-7.14
Solirubrobacteraceae	<0.0001	-0.651	<0.0001	-4.776
Sphingobacteriaceae	<0.0001	0.513	<0.0001	3.84
Thermoanaerobacteraceae	<0.0001	-0.608	<0.0001	-3.713
Xanthomonadaceae	0.0002	-0.481	<0.0001	-3.713
	Spearman		Jonckheere-Terpstra	
Genus	*p*	*r*	*p*	Standardized Test Statistic
Aeromicrobium	<0.0001	-0.612	<0.0001	-4.538
Amycolatopsis	<0.0001	0.578	<0.0001	4.395
Arthrobacter	0.0015	0.421	0.001	3.269
Balneimonas	<0.0001	-0.640	<0.0001	-4.617
Candidatus Scalindua	<0.0001	-0.615	<0.0001	-4.57
Chondromyces	<0.0001	-0.613	<0.0001	-4.57
Clostridium	<0.0001	-0.763	<0.0001	-5.871
Cystobacter	<0.0001	-0.692	<0.0001	-5.284
Desulfovibrio	0.0004	-0.461	0.001	-3.221
Dokdonella	<0.0001	-0.503	<0.0001	-4.189
Euzebya	<0.0001	-0.672	<0.0001	-5.062
Gemmata	<0.0001	-0.668	<0.0001	-5.141
Haliangium	0.0003	-0.477	0.001	-3.269
Kaistobacter	<0.0001	-0.540	<0.0001	-4.062
Megasphaera	<0.0001	-0.579	<0.0001	-4.284
Nocardioides	<0.0001	-0.590	<0.0001	-4.284
Oxalobacter	<0.0001	0.540	<0.0001	4.205
Pedobacter	<0.0001	0.513	<0.0001	3.824

Phenylobacterium	<0.0001	0.533	<0.0001	3.84
Pseudonocardia	<0.0001	0.555	<0.0001	4.11
Rubrobacter	<0.0001	-0.896	<0.0001	-7.124
Runella	<0.0001	-0.556	<0.0001	-3.951
Solirubrobacter	<0.0001	-0.653	<0.0001	-4.808

There were no differences in significant taxa identified by the Spearman or JT tests. In Table 2, an *r* value or standardized test statistic greater than zero indicates that the taxa are positively associated with wildfire intensity (i.e., increase in relative abundance as wildfire intensity increases). This increase may be due to decreases in competition from taxa damaged by wildfires or increases in nutrient availability due to the wildfire. The relative abundance of the following families was positively associated with wildfire intensity: *Micrococcaceae* (*r* = 0.410)*, Pseudonocardiaceae* (*r* = 0.557)*,* and *Sphingobacteriaceae* (*r* = 0.513). The abundance of the following genera was also positively associated with wildfire intensity: *Amycolatopsis* (*r* = 0.578), *Arthrobacter* (*r* = 0.421), *Oxalobacter* (*r* = 0.540), *Pedobacter* (*r* = 0.513), *Phenylobacterium* (*r* = 0.533), and *Pseudonocardia* (*r* = 0.555). If these taxa include nutrient cycling microorganisms, then an increase in average relative abundance from high intensity wildfires could result in greater recovery of nutrients that are destroyed in high intensity fires and likely a more rapid recovery of the ecosystem following the wildfire.

In contrast, an *r* value or standardized test statistic less than zero indicates that the abundance of the taxa are negatively associated with wildfire intensity (i.e., decrease in relative abundance as wildfire intensity increases). This could be because these taxa are sensitive to fire conditions or live near the surface where temperatures are unbearable during wildfires, or that the nutrients they depend on decrease post-fire. The abundances of the following families were negatively associated with wildfire intensity: *Chromatiaceae* (*r* = -0.704), *Nocardioidaceae* (*r* = -0.470), *Rubrobacteraceae* (*r* = -0.899), *Solirubrobacteraceae* (*r* = -0.651), *Thermoanaerobacteraceae* (*r* = -0.608), and *Xanthomonadaceae* (*r* = -

0.481). Additionally, the abundances of the following genera were negatively associated with wildfire intensity: *Aeromicrobium* (*r* = -0.612), *Balneimonas* (*r* = -0.640), *Candidatus Scalindua* (*r* = -0.615), *Chondromyces* (*r* = -0.613), *Clostridium* (*r* = -0.763), *Cystobacter* (*r* = -0.692), *Desulfovibrio* (*r* = -0.461), *Dokdonella* (*r* = -0.503), *Euzebya* (*r* = -0.672), *Gemmata* (*r* = -0.668), *Haliangium* (*r* = -0.477), *Kaistobacter* (*r* = -0.540), *Megasphaera* (*r* = -0.579), *Nocardioides* (*r* = -0.590), *Rubrobacter* (*r* = -0.896), *Runella* (*r* = -0.556), and *Solirubrobacter* (*r* = -0.653). Nutrient recovery in the soil post-fire could be slowed if nutrient cycling taxa decrease in relative abundance and a lack of soil nutrients would likely slow down ecosystem recovery.

Post-hoc pairwise comparison tests were performed on significant JT results. These post-hoc tests allow for comparisons of the effects of individual intensities on average relative abundance. This approach allows for the identification of potential optimal intensities (i.e., taxa which thrive in low or moderate intensities) or non-linear associations between intensity and abundance. Results of the pairwise comparisons are shown in Table 2.

Table 2. Pairwise comparisons for medians of a Jonckheere-Terpstra test comparing wildfire intensities. 0 = control, 1 = low intensity, 2 = moderate intensity, 3 = high intensity. Significance is defined as p < 0.05.

	Pairwise Comparison (*p*-value, **Standardized Test Statistic**)					
Family	0-1	0-2	0-3	1-2	1-3	2-3
Chromatiaceae	NS	NS	0.000, **-3.372**	0.004, **-2.685**	0.000, **-4.157**	0.000, **-4.497**
Micrococcaceae	0.010, **2.341**	0.004, **2.644**	0.004, **2.622**	NS	NS	NS
Nocardioidaceae	0.006, **2.529**	0.017, **-2.126**	0.046, **-1.686**	0.000, **-3.893**	0.000, **-3.291**	NS
Pseudonocardiaceae	NS	NS	0.001, **2.997**	NS	0.000, **3.695**	0.000, **3.993**
Rubrobacteraceae	0.131, **1.124**	0.000, **-3.681**	0.000, **-3.372**	0.000, **-4.698**	0.000, **-4.157**	0.000, **-4.799**
Solirubrobacteraceae	NS	0.002, **-2.903**	0.000, **-3.372**	0.006, **-2.517**	0.000, **-3.984**	0.032, **-1.846**
Sphingobacteriaceae	NS	NS	0.001, **3.091**	NS	0.001, **3.060**	0.000, **3.691**

Thermoanaerobacteraceae	NS	0.007, **-2.437**	0.046, **-1.686**	0.000, **-3.490**	0.008, **-2.425**	NS
Xanthomonadaceae	NS	0.007, **-2.437**	0.46, **-1.686**	0.000, **-3.490**	0.008, **-2.425**	NS
	Pairwise Comparison (*p*, **Standardized Test Statistic**)					
Genus	0-1	0-2	0-3	1-2	1-3	2-3
Aeromicrobium	0.038, **1.780**	NS	0.003, **-2.716**	0.000, **-3.423**	0.000, **-3.868**	0.004, **-2.618**
Amycolatopsis	NS	NS	0.002, **2.903**	0.047, **1.678**	0.000, **3.522**	0.000, **3.624**
Arthrobacter	0.007, **2.435**	0.003, **2.800**	0.004, **2.622**	NS	NS	NS
Balneimonas	NS	0.001, **-3.059**	0.001, **-3.278**	0.000, **-3.758**	0.000, **-3.637**	NS
Candidatus Scalindua	NS	NS	0.001, **-3.278**	0.040, **-1.745**	0.000, **-4.041**	0.000, **-4.262**
Chondromyces	NS	0.044, **-1.711**	0.000, **-3.372**	NS	0.000, **-3.753**	0.000, **-3.960**
Clostridium	0.025, **1.967**	0.031, **-1.867**	0.000, **-3.372**	0.000, **-3.591**	0.000, **-4.157**	0.000, **-4.698**
Cystobacter	NS	NS	0.000, **-3.372**	0.003, **-2.718**	0.000, **-4.157**	0.000, **-4.597**
Desulfovibrio	NS	NS	0.002, **-2.903**	NS	0.000, **-3.637**	0.001, **-3.154**
Dokdonella	NS	0.000, **-3.526**	NS	0.000, **-4.463**	0.047, **-1.674**	NS
Euzebya	NS	0.011, **-2.281**	0.001, **-3.278**	0.007, **-2.483**	0.000, **-3.811**	0.000, **-3.490**
Gemmata	0.016, **-2.154**	0.002, **-2.852**	0.000, **-3.372**	NS	0.000, **-3.753**	0.000, **-4.396**
Haliangium	NS	NS	0.000, **-3.372**	NS	0.000, **-3.522**	0.000, **-3.893**
Kaistobacter	NS	0.015, **-2.178**	0.001, **-3.091**	NS	0.002, **-2.944**	0.002, **-2.886**
Megasphaera	0.016, **2.154**	NS	0.002, **-2.903**	0.007, **-2.483**	0.000, **-4.157**	0.000, **-4.027**
Nocardioides	0.038, **1.780**	0.011, **-2.281**	0.004, **-2.622**	0.000, **-3.591**	0.000, **-3.580**	NS
Oxalobacter	0.046, **1.686**	0.002, **2.903**	0.004, **2.622**	0.040, **1.745**	0.005, **2.598**	0.013, **2.215**
Pedobacter	NS	NS	0.001, **3.091**	NS	0.001, **3.060**	0.000, **3.691**
Phenylobacterium	NS	NS	0.000, **3.372**	NS	0.000, **4.157**	0.000, **4.060**

Pseudonocardia	NS	NS	0.001, **3.278**	NS	0.000, **3.868**	0.000, **4.363**
Rubrobacter	NS	0.000, **-3.629**	0.000, **-3.372**	0.000, **-4.698**	0.000, **-4.157**	0.000, **-4.799**
Runella	NS	NS	0.000, **-3.372**	NS	0.000, **-4.157**	0.001, **-3.188**
Solirubrobacter	NS	0.002, **-2.903**	0.000, **-3.372**	0.006, **-2.517**	0.000, **-3.984**	0.028, **-1.913**

In Table 2, the sign of the standardized test statistic shows whether there is an increase or decrease in relative abundance from one group to the next. For most samples, the average relative abundance at high wildfire intensity differs significantly from control abundance, while low and moderate intensities are not often significantly different from the controls. This difference between high intensity versus low and moderate intensities suggests that high wildfire intensity results in the greatest change in average relative abundance of soil bacteria. The "0-1," "0-2," and "0-3" columns of Table 2 offer the most insight, as they indicate whether the control samples differ significantly from other fire intensities. In other words, if the pairwise comparison is not significant from the control to either the low, moderate, or high fire intensity, the taxa are likely unaffected by that wildfire intensity.

The abundance of *Chromatiaceae, Pseudonocardiaceae,* and *Sphingobacteriaceae* family-level taxa and *Amycolatopsis, Candidatus Scalindua, Cystobacter, Desulfovibrio, Haliangium, Pedobacter, Phenylobacterium, Pseudonocardia,* and *Runella* genera is only significantly different in the comparison from the control to high fire intensity. This suggests that they may not be significantly impacted by low and moderate intensity wildfires and are likely to be more resilient to wildfire-induced stress. The families *Micrococcaceae, Nocardioidaceae,* and *Rubrobacteraceae* and the genera *Arthrobacter, Clostridium, Gemmata, Nocardioides,* and *Oxalobacter* were significant at low, moderate, and high intensities compared to the control, suggesting that they are associated with all wildfire intensities. Additionally, most taxa that have significantly different distributions than the control at low intensities, except for

Gemmata, have greater abundance following low intensity fire than they do in the control. This positive association is supported by research showing that low wildfire intensities can be beneficial for microbial communities (Moya et al., 2021).

Additionally, the "1-2," "1-3," and "2-3" columns in Table 2 show the comparison of distributions between different fire intensities. *Micrococcaceae* and *Arthrobacter* have no significant differences between their distributions in the comparisons between fire intensities but are significant in all three control versus intensity comparisons, suggesting that the distributions of the two taxa are very similar once they are exposed to fire. As *Arthrobacter* belongs to the *Micrococcaceae* family, this may suggest that family wildfire trends are driven by the dominant genera in that family.

We identified the taxonomic ranks of the significant families and genera from both the Spearman and JT tests for bacteria taxa versus wildfire intensity and the significant families were compared to the families of the significant genera. Overlaps between family and family belonging to genera were *Nocardioidaceae* containing *Nocardioides*, *Pseudonocardiaceae* containing *Pseudonocardia*, *Solirubrobacteraceae* containing *Solirubrobacter*, *Rubrobacteraceae* containing *Rubrobacter*, *Xanthomonadaceae* containing *Dokdonella*, and *Micrococcaceae* containing *Arthrobacter*.

Figure 6 shows the statistically significant family taxa that decrease with increasing wildfire intensity.

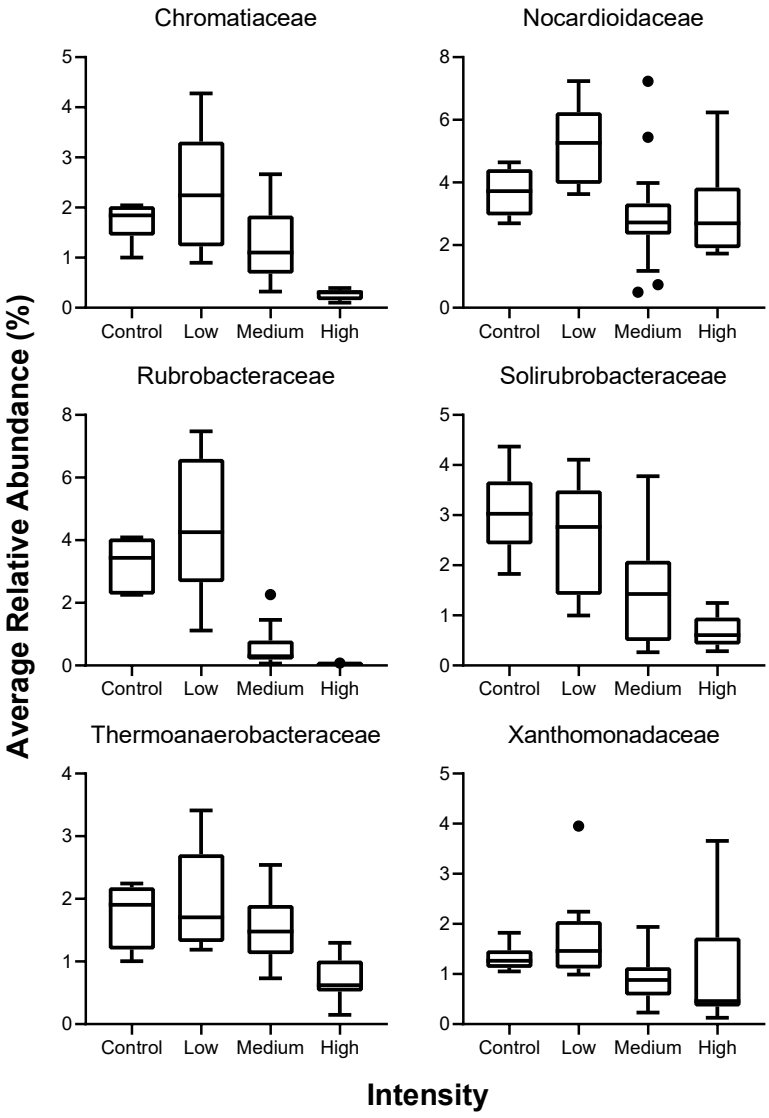

Figure 6. Tukey box and whisker plots for statistically significant family taxa that decrease with increasing wildfire intensity from the Woolsey fire.

In Figure 6, the relative abundance at high intensities of *Chromatiaceae* (0.26% ± 0.10%) and *Rubrobacteraceae* (0.01% ± 0.02%) are close to 0%, suggesting that both families are negatively affected at high wildfire intensities. This is also demonstrated in Table 2, where *Chromatiaceae's* only significant pairwise comparison is from the control to high wildfire intensity (std. test statistic = -3.372) and *Rubrobacteraceae* is significant at all intensities compared to the control. *Solirubrobacteraceae* shows a

clear downward trend in the Figure 6 plot, decreasing from the control (3.05% ± 0.87%) to high intensity (0.70% ± 0.32$), although the pairwise comparison does not show significance when comparing the control to low fire intensity. *Nocardioidaceae* increases from control (3.70% ± 0.75%) to low intensity (5.25% ± 1.20%) samples and *Rubrobacteraceae* also increases from control (3.26% ± 0.84%) to low intensity (4.42% ± 2.07%) samples. For both taxa this increase is significant in the pairwise comparisons.

Figure 7 shows the statistically significant family taxa that increase with increasing wildfire intensity.

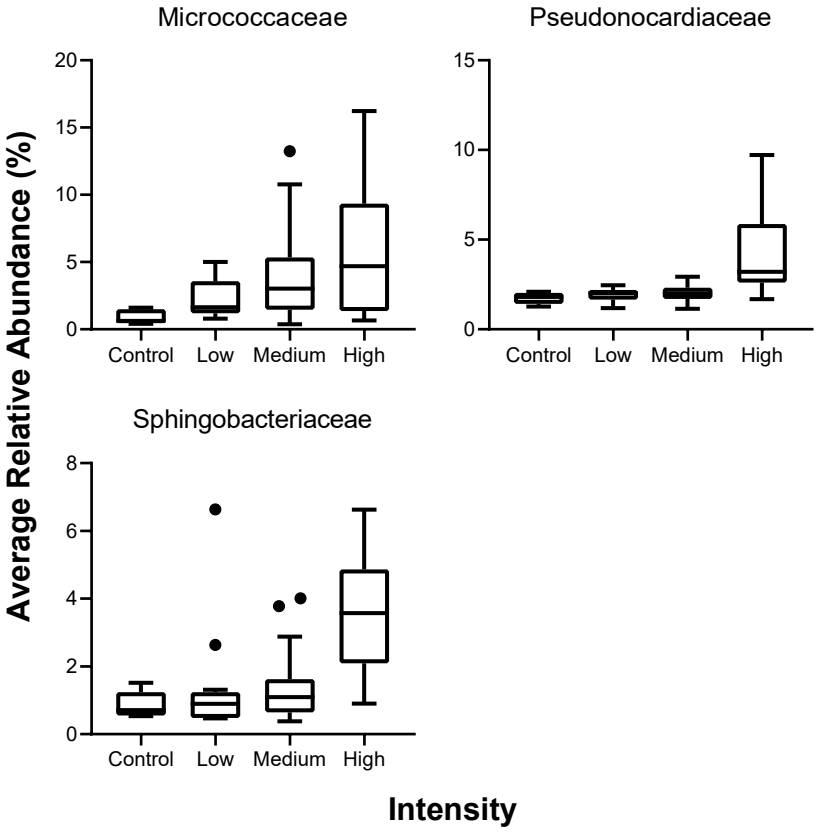

Figure 7. Tukey box and whisker plots for statistically significant family taxa that increase with increasing wildfire intensity from the Woolsey fire.

In Figure 7, *Pseudonocardiaceae* has very similar distributions for average relative abundance in the control (average relative abundance = 1.74% ± 0.32%, range = 0.83%), low (average relative abundance = 1.94% ± 0.38%, range = 1.29%), and moderate (average relative abundance = 2.00% ± 0.42%, range = 1.80%) intensities, with an increase in both mean and range at high wildfire intensity (average relative abundance = 4.11% ± 2.29%, range = 8.04%). In the pairwise comparisons in Table 2, this increase at high intensity is significant. The distributions for *Sphingobacteriaceae* show a similar trend as *Pseudonocardiaceae*, with control abundance values (average relative abundance = 0.86% ± 0.39%) being significantly less than high abundance values (average relative abundance = 3.53% ± 1.71%). The trends for *Sphingobacteriaceae* and *Pseudonocardiaceae* suggest that both taxa are benefited by high wildfire intensities. *Micrococcaceae* shows a steady increase in distributions from the control (average relative abundance = 0.86%, range = 1.19%) to high intensity (average relative abundance = 6.09% ± 5.58%, range = 16.56%), and this is reflected in the pairwise comparisons with all comparisons to the control being significant. This suggests that *Micrococcaceae* is benefitted by all wildfire intensities.

Figure 8 shows the statistically significant genera taxa that decrease with increasing wildfire intensity.

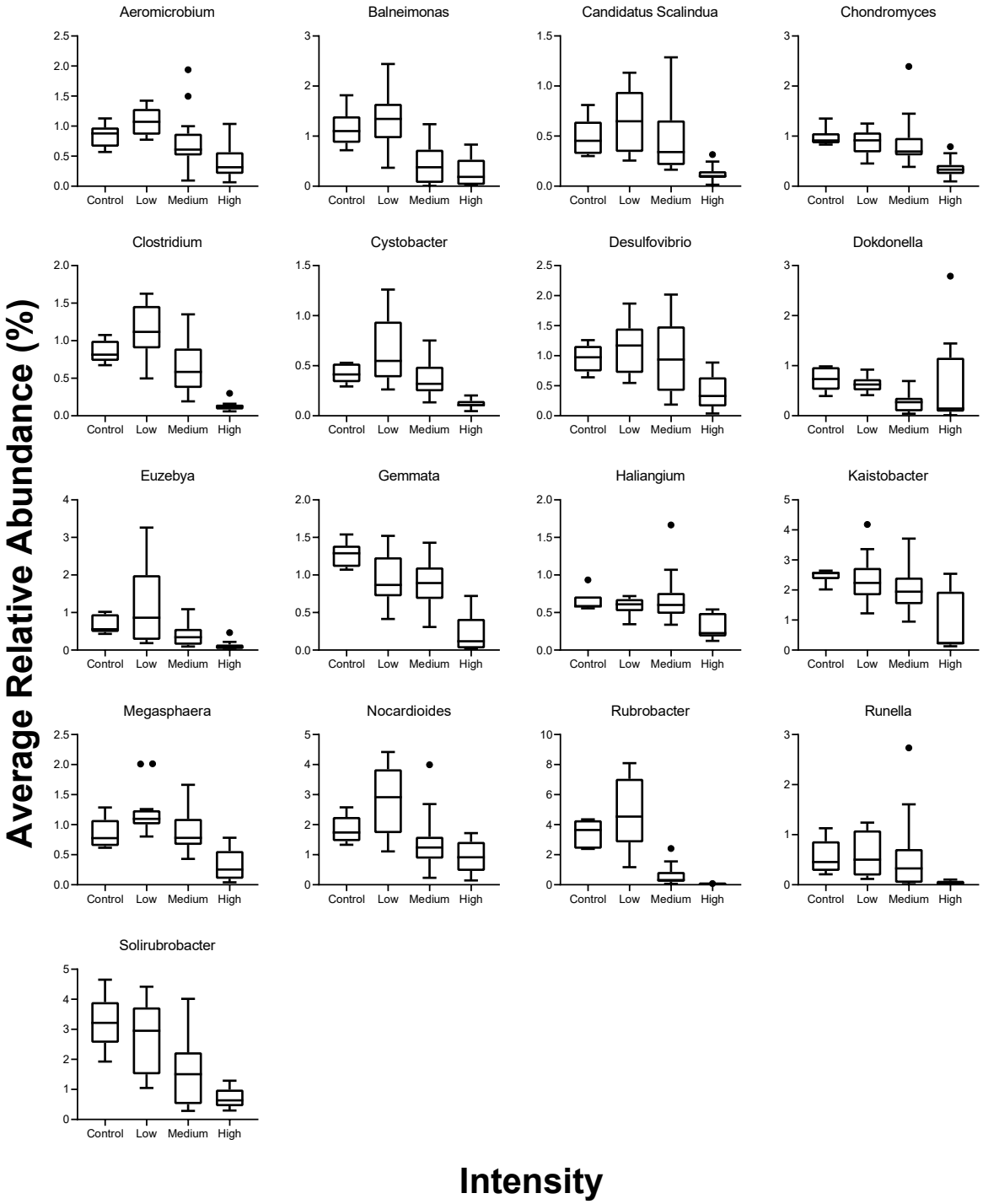

Figure 8. Tukey box and whisker plots of statistically significant genera taxa that decrease with increasing wildfire intensity from the Woolsey fire.

From Figure 8, the average relative abundance of many taxa decreases at high wildfire intensities, a trend that is also reflected in Table 2. These genera may rely on nutrients in the topsoil to survive or may be in higher soil depths that are readily affected by fire. *Dokdonella* is the only genera that is not significantly different from the control (average relative abundance = 0.73% ± 0.23%) than at high intensities (average relative abundance = 0.59% ± 0.85%). The ranges for *Dokdonella* are similar at control (range = 0.59%), low (range = 0.51%), and moderate (range = 0.65%) intensities while the range is much larger at high intensities (range = 2.78%). This may be because *Dokdonella* experiences negative effects from low to moderate intensity fire but in high intensity fire it is less affected than other taxa and thus is able to take the place of other bacteria in the soil that were more significantly hurt by the fire.

Figure 9 shows the statistically significant genera taxa that increase with increasing wildfire intensity.

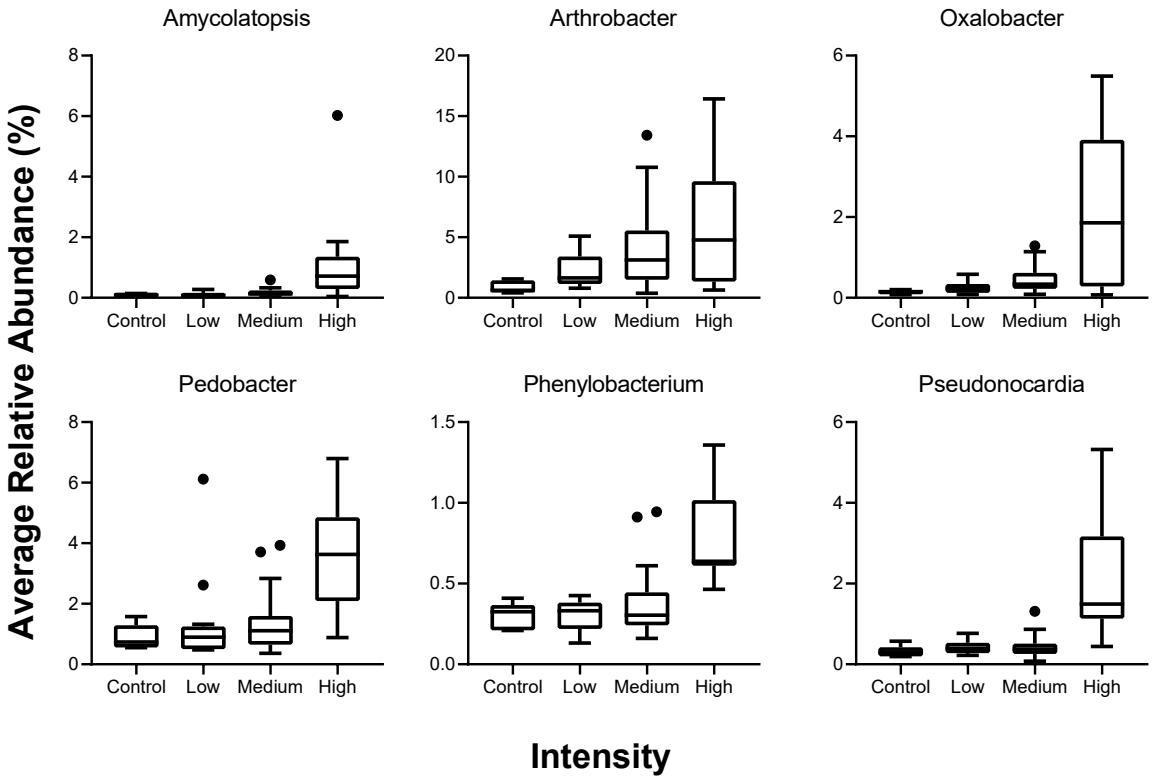

Figure 9. Tukey box and whisker plots of statistically significant genera taxa that increase with increasing wildfire intensity from the Woolsey fire.

Eight genera were found to increase with fire intensity in Figure 9, compared to the 17 that decrease with intensity in Figure 8, suggesting that the abundance of bacteria is generally negatively associated with increasing fire intensities. The ranges of the relative abundance also generally increase at high fire intensity, suggesting that high intensity fires can have varying results on relative abundance based on the geographical location. *Amycolatopsis* has low abundance values at control (0.32% ± 0.14%), low (0.43% ± 0.17%), and moderate (0.44% ± 0.26%) intensities and increases in abundance at high intensity (2.13% ± 1.59%). *Oxalobacter* follows a similar trend at control (0.14% ± 0.05%), low (0.25% ± 0.16%), moderate (0.47% ± 0.35%), and high intensities (2.15% ± 1.97%). *Pseudonocardia* behaves similarly at control (0.32% ± 0.14%), low (0.43% ± 0.17%), moderate (0.44% ± 0.26%), and high intensities

(2.13% ± 1.58%). The increase in *Amycolatopsis* and *Pseudonocardia* at high intensity is reflected in the pairwise comparison in Table 2, where these taxa only significantly increase at high intensity when compared to the control. *Oxalobacter* increases significantly at all fire intensities when compared to the control.

2.3.3 Statistical Significance of Physical Location on Soil Bacteria Communities

A Kruskal-Wallis nonparametric test was run on bacteria taxa versus physical location for all locations with a moderate wildfire intensity for the Woolsey fire. This test compared the distributions of each location group, and significant taxa were ones that had varying distributions across location. For family, all top 30 taxa were significant ($p < 0.05$) except for *Oxalobacteraceae*, *Comamonadaceae*, *Pedosphaeraceae*, and *Leuconostocaceae*. For genera, all top 63 genera were significant ($p < 0.05$) except for *Janthinobacterium, Pedosphaera, Saccharopolyspora, Sphingomonas, Oenococcus, Segetibacter, Ramlibacter, Oxalobacter,* and *Haliangium*. This suggests that the significant taxa had varying distributions due to changes in soil properties, since all locations were exposed to moderate intensity fire.

2.3.4 Williams Flats Fire: Soil Depth and Resulting Taxa Distribution

Soil samples from 180-220 cm were excluded from sequencing and statistical tests because the samples' DNA concentrations were too low to register during Qubit testing. Figure 10 shows the sequencing data at the family level for the top 30 most-populous results for soil depth.

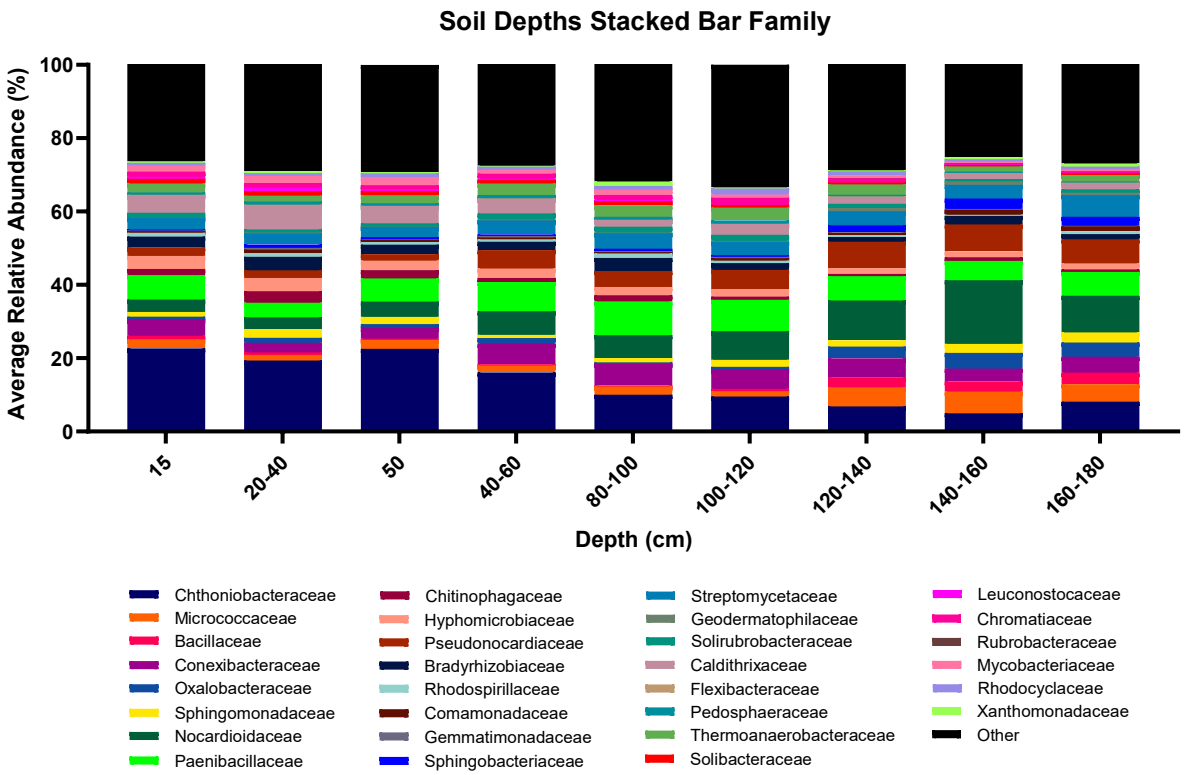

Figure 10. Sequencing data for family taxa for soil depth samples ranging from 15 cm to 180 cm below ground from the Williams Flats fire. Samples were taken from a high intensity burn area.

In Figure 10, the three most abundant families for the top third (i.e., 15 cm, 20-40 cm, and 50 cm), the middle third (i.e., 40-60 cm, 80-100 cm, and 100-120 cm), and the bottom third (i.e., 120-140 cm, 140-160 cm, and 160-180 cm) are as follows. Note that standard deviation is not given as replicates were not taken for each soil depth sample. For the top third of the soil core, samples were dominated by *Chthoniobacteraceae* (average relative abundance = 21.51% ± 1.88%), *Paenibacillaceae* (average relative abundance = 5.66% ± 1.46%), and *Caldithrixaceae* (average relative abundance = 5.27% ± 0.90%). For the middle third of the soil core, samples were dominated by *Chthoniobacteraceae* (average relative abundance = 11.89% ± 3.66%), *Nocardioidaceae* (average relative abundance = 6.83% ± 0.87%), and *Paenibacillaceae* (average relative abundance = 8.58% ± 0.60%). For the bottom third of the soil core, samples were dominated by *Chthoniobacteraceae* (average relative abundance = 6.65% ± 1.58%),

Nocardioidaceae (average relative abundance = 12.74% ± 4.04%), and *Pseudonocardiaceae* (average relative abundance = 7.01% ± 0.42%). The most abundant families for all soil depths averaged together are *Chthoniobacteraceae* (average relative abundance = 13.35% ± 6.89%), *Nocardioidaceae* (average relative abundance = 7.72% ± 4.53%), and *Paenibacillaceae* (average relative abundance = 6.80% ± 1.61%). Throughout all the soil depths, *Chthoniobacteraceae* is one of the most abundant taxa, while *Nocardioidaceae* appears to dominate at lower depths.

From visual observation, *Chthoniobacteraceae* is most abundant at shallow depths (15 cm, 22.65%) than deeper depths (140-160 cm, 4.99%). *Paenibacillaceae* has a lower average relative abundance at shallow depths (15 cm, 6.68%) than moderate depths (80-100 cm, 9.19%) and has the lowest relative abundance at deeper depths (140-160 cm, 5.26%). *Thermoanaerobacteraceae* is more abundant at shallow depths (15 cm, 2.48%) and less abundant at deeper depths (140-160 cm, 1.40%). These data indicate that the relative abundance of *Chthoniobacteraceae, Paenibacillaceae,* and *Thermoanaerobacteraceae* decreases at greater soil depths.

In contrast*, Bacillaceae* is more abundant at shallow depths (15 cm, 0.96%) than moderate depths (80-100 cm, 0.32%) and is most abundant at deeper depths (160-180 cm, 3.07%). *Micrococcaceae* is least abundant at shallow depths (15 cm, 2.50%) than deeper depths (140-160 cm, 5.90%). *Nocardioidaceae* is least abundant at shallow depths (20-40 cm, 3.19%) than deeper depths (140-160 cm, 17.38%). *Pseudonocardiaceae* is least abundant at shallow depths (20-40 cm, 2.10%) and most abundant at deeper depths (140-160 cm, 7.32%). This indicates that the relative abundance of *Bacillaceae*, *Micrococcaceae, Nocardioidaceae,* and *Pseudonocardiaceae* increases with soil depth.

Figure 11 shows the sequencing data at the genus level for the top 63 most-populous results for soil depth.

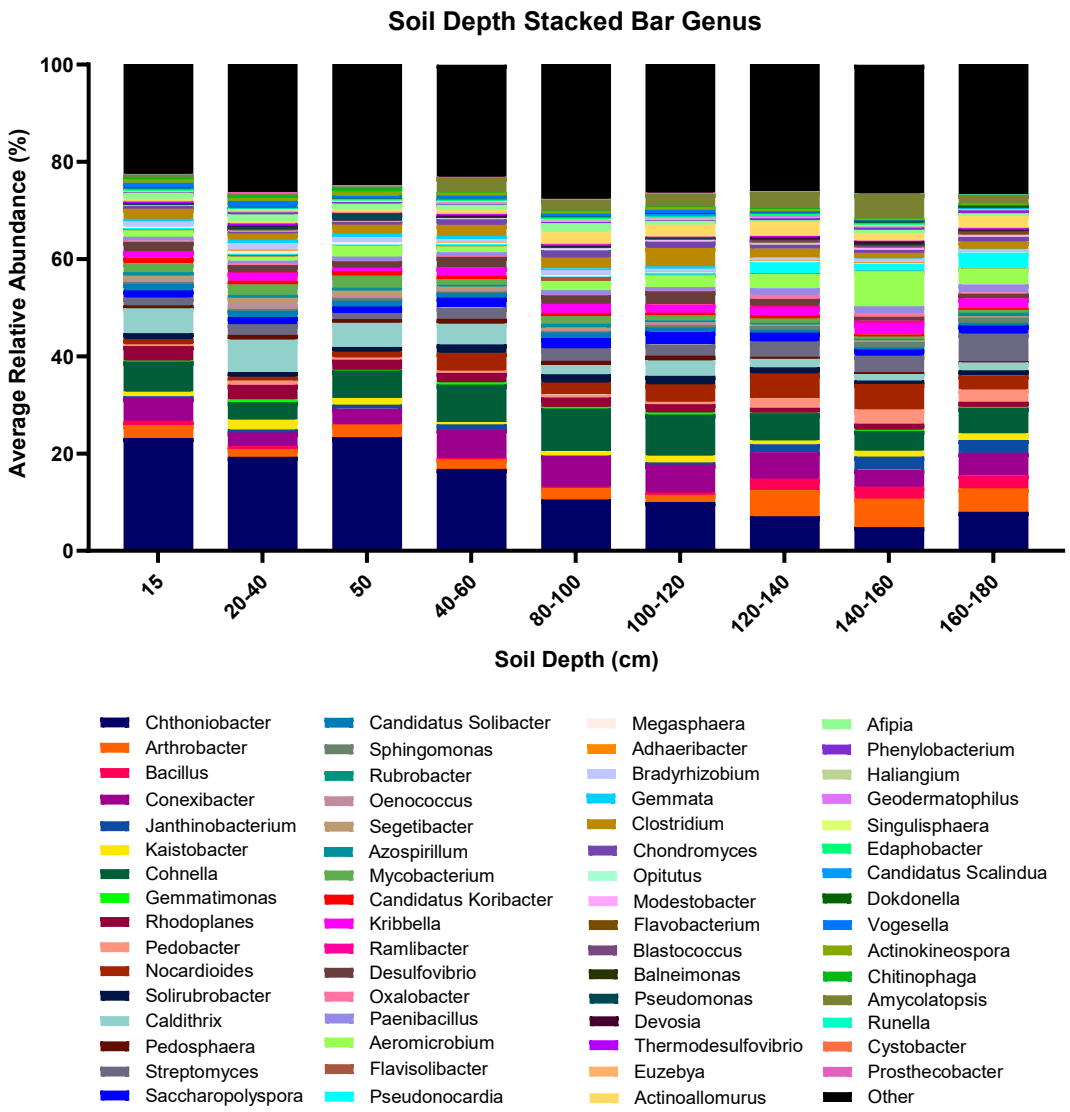

Figure 11. Sequencing data for genera taxa for soil depth samples ranging from 15 cm to 180 cm below ground from the Williams Flats fire. Samples were taken from a high intensity burn area.

In Figure 11, the top six most abundant genera for the top third (i.e., 15 cm, 20-40 cm, and 50 cm), the middle third (i.e., 40-60 cm, 80-100 cm, and 100-120 cm), and the bottom third (i.e., 120-140 cm, 140-160 cm, and 160-180 cm) are shown. Note that standard deviation is not given as replicates were not taken for each soil depth sample. For the top third of the soil core, samples were dominated by *Chthoniobacter* (average relative abundance = 21.99% ± 2.28%), Arthrobacter (average relative

abundance = 2.27% ± 0.57%), *Conexibacter* (average relative abundance = 3.55% ± 1.02%), *Cohnella* (average relative abundance = 5.16% ± 1.37%), *Rhodoplanes* (average relative abundance = 2.70% ± 0.54%), and *Caldithrix* (average relative abundance = 5.57% ± 0.96%). For the middle third of the soil core, samples were dominated by *Chthoniobacter* (average relative abundance = 12.49% ± 3.79%), *Conexibacter* (average relative abundance = 5.98% ± 0.27%), *Cohnella* (average relative abundance = 8.34% ± 0.55%), *Nocardioides* (average relative abundance = 3.25% ± 0.70%), *Caldithrix* (average relative abundance = 3.09% ± 1.17%), and *Clostridium* (average relative abundance = 2.75% ± 0.88%). For the bottom third of the soil core, samples were dominated by *Chthoniobacter* (average relative abundance = 6.68% ± 1.62%), *Arthrobacter* (average relative abundance = 5.35% ± 0.51%), *Conexibacter* (average relative abundance = 4.55% ± 0.95%), *Cohnella* (average relative abundance = 4.97% ± 0.78%), *Nocardioides* (average relative abundance = 4.42% ± 1.30%), and *Aeromicrobium* (average relative abundance = 4.47% ± 2.38%). The most abundant genera for all soil depths averaged together are *Chthoniobacter* (average relative abundance = 13.72% ± 7.10%), *Arthrobacter* (average relative abundance = 3.19% ± 1.69%), *Conexibacter* (average relative abundance = 4.70% ± 1.27%), *Cohnella* (average relative abundance = 6.16% ± 1.84%), *Nocardioides* (average relative abundance = 2.90% ± 1.67%), and *Caldithrix* (average relative abundance = 3.41% ± 1.91%).

C*hthoniobacter* is most abundant at shallow depths (15 cm, 23.2%) and least abundant at deeper depths (160-180 cm, 8.04%). *Rhodoplanes* is most abundant at shallow depths (15 cm, 2.99%) and least abundant at deeper depths (160-180 cm, 1.06%). *Caldithrix* is most abundant at shallow depths (15 cm, 5.10%) and least abundant at deeper depths (160-180 cm, 1.61%). *Mycobacterium* is also most abundant at shallow depths (15 cm, 1.84%) and least abundant at deeper depths (160-180 cm, 0.62%). These data indicate that the relative abundance of C*hthoniobacter, Rhodoplanes, Caldithrix,* and *Mycobacterium* decrease at deeper soil depths. In contrast, *Bacillus* is least abundant at shallow depth (15 cm, 0.88%) and

most abundant at deeper depths (160-180 cm, 2.68%). *Nocardioides* is least abundant at shallow depths (15 cm, 1.02%) and most abundant at deeper depths (140-160 cm, 5.24%). *Streptomyces* is least abundant at shallow depths (15 cm, 1.57%) and most abundant at deeper depths (160-180 cm, 5.65%). *Aeromicrobium* is also least abundant at shallow depths (15 cm, 1.31%) and most abundant at deeper depths (140-160 cm., 7.21%). *Pseudonocardia* is least abundant in shallow depths (15 cm, 0.30%) and most abundant in deeper depths (160-180 cm, 3.24%). These data indicate that the relative abundance of *Bacillus, Nocardioides, Streptomyces, Aeromicrobium,* and *Pseudonocardia* increases with soil depth.

Although these are wildfire affected soils, there is also the possibility that these vertical distributions of bacteria are how they are naturally distributed, as there were no control samples for the soil cores. Thus, we compared the taxa that increase with soil depth (i.e., having the lowest relative abundance at the surface) to the taxa that are negatively affected by wildfire intensity from Table 1. Any overlaps between the two data sets could suggest that wildfires are the cause of the decreased relative abundance in the surface soils. We also compared the taxa that decrease with soil depth (i.e., having the highest relative abundance at the surface) to the taxa that are positively affected by wildfire intensity.

2.3.4.1 Statistical Significance of Soil Depth on Soil Bacteria

Spearman correlations were used to determine whether average relative abundance changed with soil depth. All significant ($p < 0.05$) samples for both family and genus had r values greater than $|0.4|$. The results of the correlations are shown in Table 3.

Table 3. Spearman correlation values for bacteria taxa versus soil depth in wildfire affected soil samples.

Family	p	r_s
Bradyrhizobiaceae	0.0369	-0.72
Caldithrixaceae	0.0004	-0.95
Chitinophagaceae	0.0061	-0.85
Chthoniobacteraceae	0.0007	-0.93
Geodermatophilaceae	0.0083	0.83

Hyphomicrobiaceae	0.002	-0.90
Leuconostocaceae	0.0007	-0.93
Mycobacteriaceae	0.002	-0.90
Nocardioidaceae	0.0013	0.92
Pseudonocardiaceae	0.0045	0.87
Solibacteraceae	0.0002	-0.97
Sphingobacteriaceae	0.0311	0.73
Streptomycetaceae	0.0369	0.72

Genus	*p*	r_s
Actinoallomurus	0.0138	0.80
Actinokineospora	0.002	-0.90
Aeromicrobium	0.0045	0.87
Afipia	0.0214	-0.77
Blastococcus	0.0083	0.83
Bradyrhizobium	0.0061	-0.85
Caldithrix	0.0004	-0.95
Candidatus Koribacter	0.002	-0.90
Candidatus Solibacter	0.0002	-0.97
Chthoniobacter	0.002	-0.90
Gemmata	0.0061	-0.85
Kribbella	0.0369	0.72
Megasphaera	0.0172	-0.78
Modestobacter	0.0002	0.97
Mycobacterium	0.002	-0.90
Nocardioides	0.0255	0.75
Oenococcus	0.0045	-0.87
Paenibacillus	0.0369	0.72
Pedobacter	0.0172	0.78
Prosthecobacter	0.0138	-0.80
Pseudonocardia	0.0255	0.75
Rhodoplanes	0.0007	-0.93
Segetibacter	0.002	-0.90
Singulisphaera	0.0031	-0.88
Streptomyces	0.0007	0.93
Vogesella	0.0045	-0.87

An ANOVA or similar test was not run on the soil core data because the soil cores are individual samples rather than groups.

The families that increase relative abundance with soil depth are as follows: *Geodermatophilaceae* (r = 0.83), *Nocardioidaceae* (r = 0.92), *Pseudonocardiaceae* (r = 0.87), *Sphingobacteriaceae* (r = 0.73), and *Streptomycetaceae* (r = 0.72). The families that decrease relative abundance with soil depth are as follows: *Bradyrhizobiaceae* (r = -0.72), *Caldithrixaceae* (r = -0.95), *Chitinophagaceae* (r = -0.85), *Chthoniobacteraceae* (r = -0.85), *Hyphomicrobiaceae* (r = -0.90), *Leuconostocaceae* (r = -0.93), *Mycobacteriaceae* (r = -0.90), and *Solibacteraceae* (r = -0.97).

The genera that increase relative abundance with soil depth are as follows: *Actinoallomurus* (r = 0.80), *Aeromicrobium* (r = 0.87), *Blastococcus* (r = 0.83), *Kribbella* (r = 0.72), *Modestobacter* (r = 0.97), *Nocardioides* (r = 0.75), *Paenibacillus* (r = 0.72), *Pedobacter* (r = 0.78), *Pseudonocardia* (r = 0.75), and *Streptomyces* (r = 0.93). The genera that decrease relative abundance with soil depth are as follows: *Actinokineospora* (r = -0.90), *Afipia* (r = -0.77), *Bradyrhizobium* (r = -0.85), *Caldithrix* (r = -0.95), *Candidatus Koribacter* (r = -0.90), *Candidatus Solibacter* (r = -0.97), *Chthoniobacter* (r = -0.90), *Gemmata* (r = -0.85), *Megasphaera* (r = -0.78), *Mycobacterium* (r = -0.90), *Oenococcus* (r = -0.87), *Prosthecobacter* (r = -0.80), *Rhodoplanes* (r = -0.93), *Segetibacter* (r = -0.90), *Singulisphaera* (r = -0.88), and *Vogesella* (r = -0.87).

More taxa are observed to decrease with soil depth than increase with soil depth. This is logical as there are more nutrients in surface soils than deeper soils. These are relative abundance values, however, so increases or decreases seen in one taxon may be due to increases or decreases in another taxon. Furthermore, these significant results do not mean that these taxa increase or decrease with depth due to wildfire, only that these trends were evident in wildfire affected samples.

We compared the taxa associated with intensity to taxa associated with soil depth to ascertain if wildfires influenced the distribution of bacteria in the soil. No overlaps were found in the comparisons on taxa that increase with fire intensity and decrease with depth. There were overlaps, however, in taxa that

decrease in relative abundance with wildfire intensity and increase with depth. The family *Nocardioidaceae* and the genera *Aeromicrobium* and *Nocardioides* both decrease with wildfire intensity and increase with depth. These results could suggest that wildfires cause *Aeromicrobium* and members of the *Nocardioidaceae* family to have low relative abundances in surface soils, but further research would be needed to confirm that as there were no controls in the soil core samples.

2.3.5 Community Composition of Other Fire and Ash Samples

Results from the Kincade, Tubbs, Northstar, and Hayes wildfires were excluded from statistical analysis because they lacked controls and offered little value for comparisons to other fires with more abundant samples. The samples are still included in the appendices as they were used for antibiotic resistance gene testing in Chapter Three and they did undergo sequencing, thus their taxa distribution could be informative for future studies.

Figure 12 shows the sequencing data at the family level for the top 30 most-populous results for miscellaneous soil and ash samples.

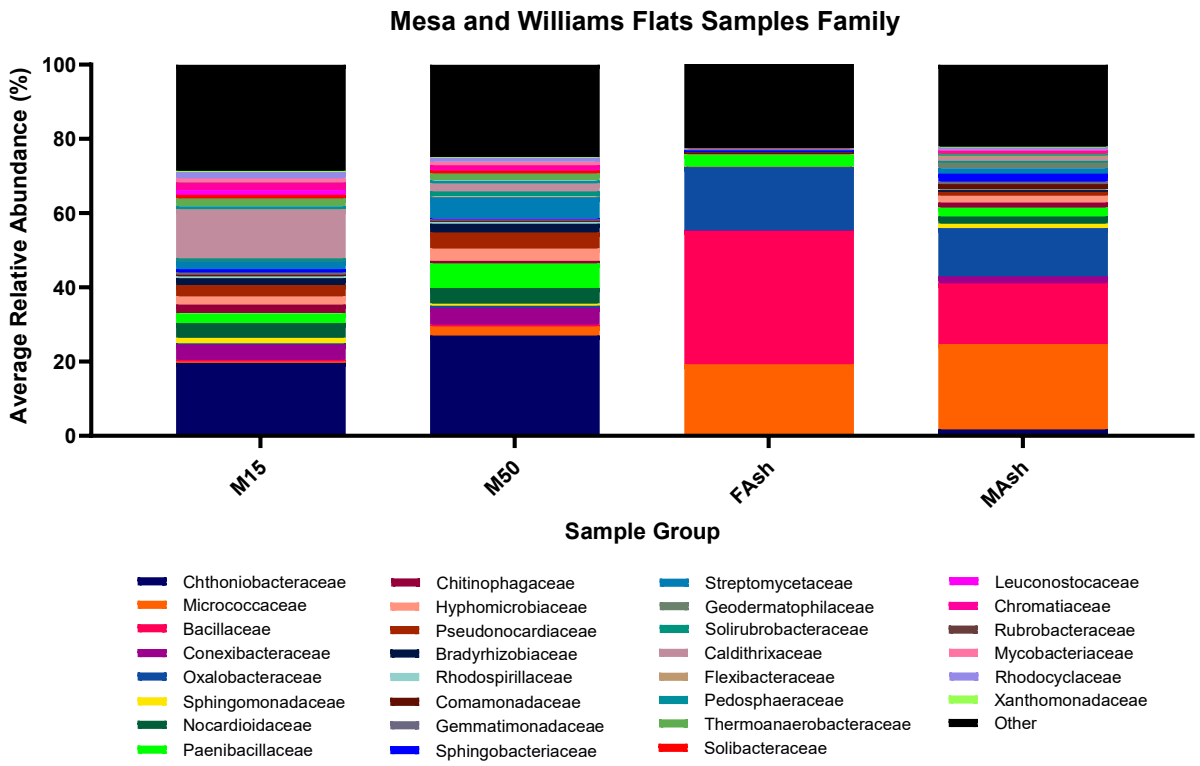

Figure 12. Sequencing data for family taxa for fire-exposed soils, including depth and ash samples. Sample names: first letter: M = Mesa, F = Williams Flats; M15 is 15 cm soil depth at Mesa, M50 is 50 cm soil depth at Mesa. "Ash" denotes an ash sample.

In Figure 12, the top three most abundant families for the miscellaneous samples are as follows. Note that standard deviations are not given as each category is composed of one sample. The Mesa 15 cm depth sample was largely dominated by *Chthoniobacteraceae* (average relative abundance = 19.64%), *Conexibacteraceae* (average relative abundance = 4.20%), and *Caldithrixaceae* (average relative abundance = 13.09%). These families in the Mesa 15 cm depth sample are similar to the Williams Flats families in Figure 10, with *Chthoniobacteraceae* and *Caldithrixaceae* being some of the most abundant families in both. This suggests that, in fire-affected soils, *Chthoniobacteraceae* and *Caldithrixaceae* are present in the soil layers close to the surface. The Mesa 50 cm depth sample was largely dominated by *Chthoniobacteraceae* (average relative abundance = 27.04%), *Paenibacillaceae* (average relative abundance = 6.62%), and *Streptomycetaceae* (average relative abundance = 5.58%). The Williams Flats

ash sample was largely dominated by *Micrococcaceae* (average relative abundance = 19.17%), *Bacillaceae* (average relative abundance = 35.96%), and *Oxalobacteraceae* (average relative abundance = 17.02%). The Mesa ash sample was largely dominated by *Micrococcaceae* (average relative abundance = 23.06%), *Bacillaceae* (average relative abundance = 16.32%), and *Oxalobacteraceae* (average relative abundance = 12.97%).

For two ash samples collected from Williams Flats and Mesa, three family-level taxa dominate microbial communities: *Micrococcaceae* (Williams Flat: 19.17%, Mesa: 23.06%), *Bacillaceae* (Williams Flats: 35.96%, Mesa 16.32%), and *Oxalobacteraceae* (Williams Flat: 17.02%, Mesa 12.97%). The large relative abundances of *Micrococcaceae, Bacillaceae,* and *Oxalobacteraceae* in the ash samples suggest that these taxa are benefitted by the occurrence of ash, possibly because ash is nutrient rich (Knicker, 2007). Ash samples have also previously been shown to have high concentrations of heavy metals (Liodakis & Tsoukala, 2009), so the bacteria that dominate these samples may be more resistant to heavy metals than other taxa.

Figure 13 shows the genera for the Mesa and Williams Flats soil and ash samples. Note that standard deviations are not given each category is composed of one sample. The Mesa 15 cm depth sample is largely dominated by *Chthoniobacter* (average relative abundance = 20.57%), *Conexibacter* (average relative abundance = 4.43%), *Cohnella* (average relative abundance = 2.44%), *Caldithrix* (average relative abundance = 13.82%), *Saccharopolyspora* (average relative abundance = 1.98%), and *Desulfovibrio* (average relative abundance = 2.01%). The Mesa 50 cm depth sample is largely dominated by *Chthoniobacter* (average relative abundance = 28.20%), *Arthrobacter* (average relative abundance = 2.50%), *Conexibacter* (average relative abundance = 4.76%), *Cohnella* (average relative abundance = 6.47%), *Rhodoplanes* (average relative abundance = 2.87%), and *Streptomyces* (average relative abundance = 3.71%).

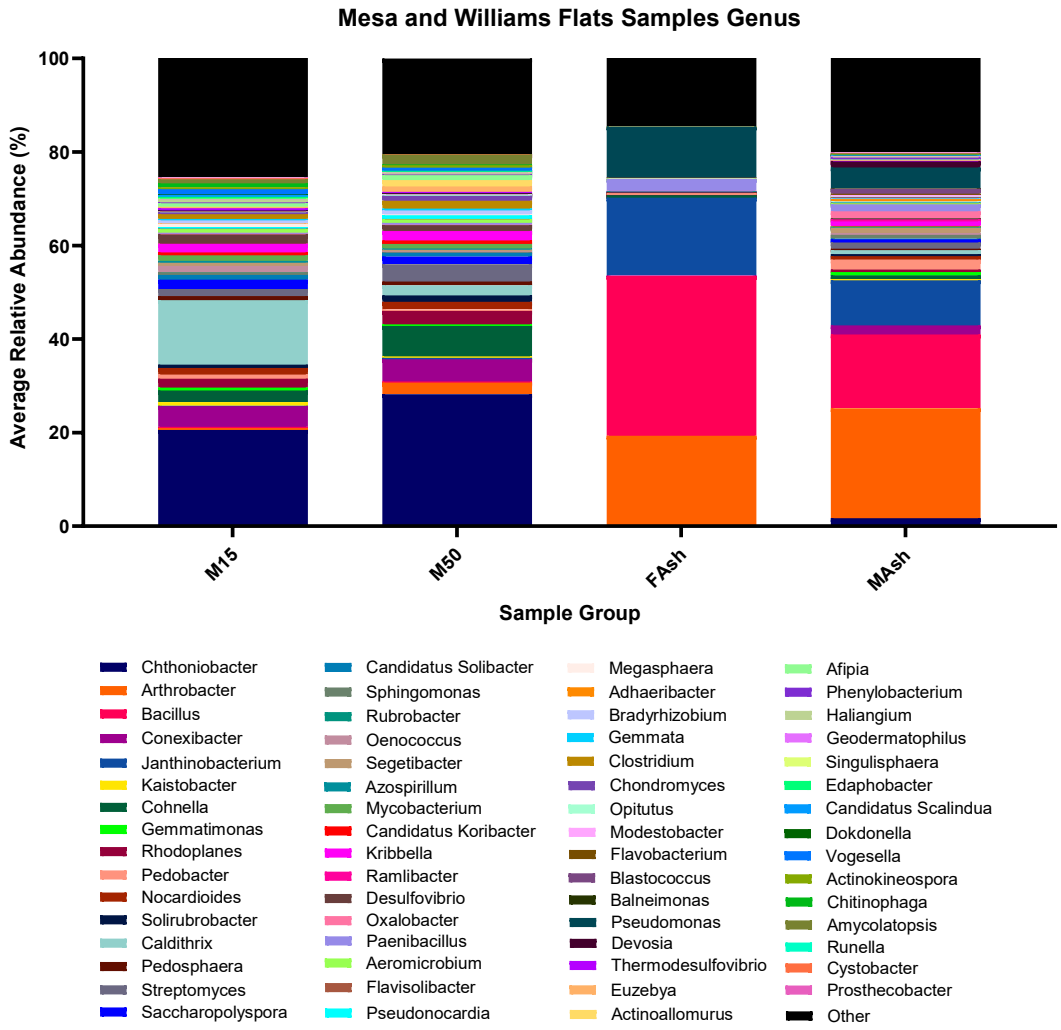

Figure 13. Sequencing data for genera taxa for fire-exposed soils, including depth and ash samples. Sample names: first letter: M = Mesa, F = Williams Flats; M15 is 15 cm soil depth at Mesa, M50 is 50 cm soil depth at Mesa. "Ash" denotes an ash sample.

Similar to the family ash trends, four taxa dominate the genera in the ash samples. *Arthrobacter* has a relative abundance of 19.23% at Williams Flats and 23.53% at Mesa, while *Bacillus* has a relative abundance of 34.15% at Williams Flats and 15.76% at Mesa. *Janthinobacterium* has a relative abundance of 16.62% at Williams Flats and 9.64% at Mesa, and *Pseudomonas* has a relative abundance of 10.94% at Williams Flats and 4.47% at Mesa. Like the family taxa, these genera may benefit from the nutrients in ash (Knicker, 2007) and may be able to resist the heavy metals found in ash (Liodakis & Tsoukala, 2009).

2.4 Discussion

2.4.1 Roles of Nutrient Cycling Bacteria

Twenty-two nitrogen cyclers were linked to wildfire intensity, including five positive relationships and 17 negative relationships. Among the taxa that increase with intensity, no families were found to have nitrogen cycling capabilities. For the nitrogen cycling genera that increase with intensity, *Arthrobacter* spp. have been found to fix atmospheric nitrogen and are involved in the soil-nitrogen cycle post-wildfire (Fernández-González et al., 2017). Although *Amycolatopsis* spp. are often found in nitrogen-poor environments, they metabolize nitrogen (Sánchez-Hidalgo et al., 2018) and *Amycolatopsis mediterranei* can metabolize inorganic nitrogen compounds (Zhao et al., 2010). *Phenylobacterium* spp. and *Pseudonocardia nantongensis* can reduce nitrate to nitrite (Chu et al., 2015; Thawai, 2018) and *Pseudonocardia dioxanivorans* can fix atmospheric nitrogen (Mahendra & Alvarez-Cohen, 2005).

For the nitrogen cycling families that decrease with wildfire intensity, ammonia is the preferred nitrogen source for *Chromatiaceae* (Imhoff, 2014). *Nocardioidaceae* uses a wide range of nitrogen sources (Evtushenko & Ariskina, 2015). Additionally, the *Xanthomonadaceae* family contains denitrifiers (Tang et al., 2020). For the nitrogen cycling genera that decrease with wildfire intensity, some species of *Aeromicrobium* can reduce nitrate (Evtushenko & Krausova, 2015). *Balneimonas* spp., *Clostridium* spp., and *Desulfovibrio* spp. can fix atmospheric nitrogen (Li et al., 2020; Chen et al., 2001; Sayavedra et al., 2021). *Candidatus Scalindua* spp. utilize aerobic ammonium oxidation and can contribute to nitrogen cycling (Schmid et al., 2003) and *Euzebya* spp. and *Solirubrobacter* spp. play roles in the nitrogen cycle (Xu et al., 2019; Maquia et al., 2021). A strain of *Dokdonella koreensis* reduces nitrate to nitrite (Li et al., 2013). Network analysis has shown that *Dokdonella* is positively correlated with nitrite reductase genes *nirS* and *nirK* (Wang et al., 2019). A strain of *Megasphaera elsdenii* can use ammonium as a nitrogen source and sulfate as a sulfur source (Forsberg, 1978). *Nocardioides* can use a variety of nitrogen sources (Evtushenko

et al., 2015) and *Rubrobacter xylanophilus* can reduce nitrate to nitrite (Carreto et al., 1996). Some *Haliangium* spp. are shown to be denitrifiers (McIlroy et al., 2016). *Runella* contains solid phase denitrifiers (J. Zhao et al., 2017). *Solirubrobacter* supports a role in the nitrogen cycle (Maquia et al., 2021) and one *Solirubrobacter* strain uses ammonium as its sole nitrogen source (Singleton et al., 2003).

Although five nitrogen cycling taxa are shown to increase with fire intensity, 17 nitrogen cyclers decrease with fire intensity. Nitrogen begins to volatilize at 200°C and at 500°C half the available nitrogen in the soil is vaporized (Knicker, 2007), thus high intensity fires would result in the loss of nitrogen needed for plant recovery. These results suggest that nitrogen availability after high intensity fires will be limited as the populations of nitrogen cycling bacteria decrease and nitrogen is destroyed in fire heat, resulting in slower plant growth and ecosystem recovery.

Six sulfur cyclers were linked to wildfire intensity, including two positive relationships and four negative relationships. For the taxa that increase with fire intensity, one family, *Pseudonocardiaceae*, is associated with the sulfur cycle (Cobo-Díaz et al., 2017). For the genera that increase with wildfire intensity, *Arthrobacter* spp. metabolize organic sulfur (Romaniuk et al., 2018). For the families that decrease with fire intensity, *Chromatiaceae* spp. can oxidize inorganic sulfur (Frigaard & Dahl, 2009). For sulfur cycling genera that decrease with wildfire intensity, species of *Desulfovibrio* and a *Clostridium* sp. have been shown to reduce sulfur (Johnson & Hug, 2019; Sallam & Steinbüchel, 2009). *Euzebya* spp. have also been shown to have roles in the sulfur cycle (Xu et al., 2019).

Although the comparison of sulfur cycling taxa that increase versus decrease in relative abundance with intensity may not be as numerous as the nitrogen cycling taxa, there are twice as many sulfur cyclers that are negatively associated with intensity than are positively associated. Similar to the nitrogen cyclers, a decrease in sulfur cycling bacteria post-wildfire could decrease nutrient availability.

Sulfur combusts at 800°C (King et al., 2013), however, so it is likely that only high intensity fires that impact surface soils would immediately decrease sulfur availability in the soil. High intensity fires can reach temperatures of up to 1000°C but, due to the insulating qualities of dry soil, soils below a depth of 5 cm are rarely likely to exceed 150°C (Smith et al., 2008; Terzano et al., 2021). Thus, sulfur is unlikely to be affected by wildfires in soils 5 cm below the surface.

Seven phosphorous cyclers were linked to wildfire intensity, including one positive relationship and six negative relationships. Among the taxa that increase with intensity, no families were found to have phosphorous cycling capabilities. For the genera that increase with fire intensity, a strain of *Arthrobacter* has shown to degrade glyphosate, an organophosphorus herbicide (Kehler et al., 2021). For the families that decrease with fire intensity, some *Xanthomonadaceae* spp. are phosphorous solubilizers, meaning those species can make phosphorous more soluble and thus more available for plant uptake. Among the genera that decrease with fire intensity, *Aeromicrobium* spp., *Haliangium* spp., and *Nocardioides* spp. are also phosphorous solubilizers (Vieira et al., 2020; Y. Wang et al., 2021). *Balneimonas* spp. can mobilize phosphorus, also making it available for plants (Ikoyi et al., 2018). Finally, *Euzebya* spp. are also involved in the phosphorus cycle (Xu et al., 2019). One phosphorus-cycling genus was found to be positively associated with fire intensity, while six taxa were negatively associated, suggesting that phosphorus availability will decrease after high intensity fires. Phosphorous combusts at 760°C (Knicker, 2007), so it is likely that phosphorus destruction from wildfires would likely only occur during high intensity surface soil fires up to 5 cm below the surface, similar to sulfur.

Some taxa (i.e., *Chromatiaceae*, *Xanthomonadaceae*, *Aeromicrobium*, *Balneimonas*, *Clostridium*, *Desulfovibrio*, *Euzebya*, *Haliangium*, and *Nocardioides*) are connected to more than one nutrient cycle, so a loss in these taxa after high intensity wildfires would be detrimental to multiple nutrient cycles, further damaging ecosystem recovery post-wildfire. Additionally, although some taxa were not found to have

strong roles in the nitrogen, sulfur, or phosphorous cycles, these bacteria play roles in the environment. *Micrococcaceae, Sphingobacteriaceae*, and *Oxalobacter* all increase with fire intensity and could play a role in organic matter decomposition, degrading organic molecules that were released by fire and thus releasing carbon-bound nutrients into the soil. *Rubrobacteraceae*, *Solirubrobacteraceae*, *Thermoanaerobacteraceae*, *Chondromyces*, *Cystobacter*, *Gemmata*, and *Kaistobacter* decrease with fire intensity, and they could play a similar role. Their decrease after high intensity fires could result in a lack of carbon nutrient cycling. Additionally, *Kaistobacter* has been shown to suppress disease in tobacco (Liu et al., 2016), and if the other taxa listed previously play a similar role in plant disease suppression, plant recovery after wildfires could be harmed by the population decrease in these taxa.

2.4.2 Overlap of Fire Intensity and Soil Depth

Nocardioidaceae and the genera *Aeromicrobium* and *Nocardioides* both decreased with wildfire intensity and increased with soil depth. *Nocardioidaceae* spp. and *Nocardioides* spp. are nitrogen cyclers and *Nocardioides* spp. are also phosphorus cyclers, while *Aeromicrobium* spp. are connected to both the phosphorus and nitrogen cycles. The overlap between wildfire intensity and soil depth could suggest that these taxa are both decreasing after high intensity wildfires and are losing abundance in surface soils. This could suggest that these taxa are not available to cycle nutrients in surface soils after high intensity wildfires, although further experiments would be needed to verify this as the Williams Flats soil core samples did not have control values for comparison.

2.4.3 Risk of Pathogenic Bacteria in Ash

Ash is easily transported by wind and water after wildfires (Bodí et al., 2014). Humans may be exposed to ash after wildfires, thus the taxa that dominated ash samples were examined to identify pathogen-containing species within them. The families that dominate the Mesa and Williams Flats ash samples are *Micrococcaceae*, *Bacillaceae*, and *Oxalobacteraceae*, while the genera are *Arthrobacter*,

Bacillus, *Janthinobacterium*, and *Pseudomonas*. Most of these taxa contain pathogenic species, such as *Micrococcaceae* containing *Staphylococcus aureus* and S. *haemolyticus* which cause mastitis (Batt & Tortorello, 2014). Species of *Bacillaceae* and *Bacillus* can cause food poisoning, and *Bacillus cereus* can cause lung infections (Taussig et al., 2008). Some *Oxalobacteraceae* spp. are plant pathogens (Baldani et al., 2014). *Arthobacter* spp. can be opportunistic pathogens, taking advantage of immunocompromised hosts (Huang et al., 2005). Species of *Janthinobacterium* are considered non-pathogenic (Oh et al., 2019), but *Pseudomonas aeruginosa* often causes infections in humans (CDC, 2019).

The microbiomes of each ash sample contained pathogen-containing taxa. Although we have identified these taxa, this does not necessarily mean that they contain active pathogens. The presence of pathogens must be determined by species-level identification and verification of pathogenicity (e.g., the expression of genes regulating infectivity). The risk of long-range spread of potential pathogens is also determined by the potential concentration of microbes on ash particles. Wildfire ash is likely to have lower concentrations of bacteria, particularly in higher combustion ash, than in soil samples due to the nutrient-limiting qualities of higher combustion ash. Thus, it is likely that pathogen-containing taxa are likely to be present in ash in fewer numbers, though enumeration analyses (e.g., qPCR or selective plating) are needed to verify these trends. However, these results are still noteworthy as no studies known to the author have yet evaluated wildfire ash microbiomes. These data also suggest there is a potential risk that pathogens may be transported across large distances due to the extreme mobility of ash via air, erosion, or runoff. However, additional work is needed to characterize potential pathogens at the species level and verify pathogenicity.

CHAPTER THREE: ANTIBIOTIC RESISTANCE GENE DISTRIBUTION IN WILDFIRE-AFFECTED SOIL SAMPLES

3.1 Introduction

Antibiotic resistant infections are an extremely dangerous phenomenon that is bound to grow more dangerous. Currently there are 700,000 deaths worldwide annually from antibiotic-resistant infections that death toll is projected to reach 10 million deaths annually by 2050 (Patel & Williams, 2019). With the number and severity of wildfires also on the rise (Dennison et al., 2014; Moritz et al., 2012), the relatively unexplored overlap between wildfires and antibiotic resistance gene development must be examined. Antibiotics are frequently used in healthcare and agriculture settings and when these antibiotics enter the soil through various processes (e.g., runoff, animal waste) the antibiotics that are not fully metabolized sorb to the soil (Cycoń et al., 2019). Concentrations of these sorbed antibiotics in the soil create conditions that encourage the proliferation of antibiotic resistance genes (ARGs) and among soil bacteria populations (Cycoń et al., 2019).

One pathway of ARG transfer between bacteria is through horizontal gene transfer (HGT). There are four ways that horizontal gene transfer occurs: conjugation, transformation, transduction, and gene transfer agents (von Wintersdorff et al., 2016). The first HGT pathway, conjugation, is the most common form of ARG transfer between microorganisms. Conjugation involves the use of pili or bacterial adhesins and requires direct contact between microbes. During contact, a plasmid containing ARGs is transferred between the host and a receiving microbe. While plasmid transfer during these conjugation events could also occur during transformation or transduction, conjugation is the most likely form of transfer due to the protection that it offers to the plasmid being transferred (von Wintersdorff et al., 2016). Choi et al. (2017) reported an increase in motility genes post-wildfire. This could increase the rate of conjugation ARG transfer, especially during the recovery period of microorganisms post-fire.

The second HGT pathway, transformation, is the intake and usage of DNA found outside of a bacterial cell. To successfully undergo transformation, there are a few requirements. The first is that there must be DNA in the environment surrounding the receiving cell. Secondly, the receiving cell must be capable of accepting DNA from its environment. Finally, the cell must be able to use the DNA, either by making it a part of its genome or by "recircularization" if the DNA received is from a plasmid (von Wintersdorff et al., 2016). Wildfires would most likely increase the rate of transformation ARG transfer due to greater amounts of environmental DNA found due to cell lysis from wildfire heat (Hart et al., 2005).

The third HGT pathway is transduction, in which bacteriophages, viruses that infect a bacterial cell and use it for replication (Clokie et al., 2011), transfer ARGs across cells. If a bacteriophage accidentally incorporates bacterial DNA with ARGs, this DNA can be transferred to a new uninfected cell. This receiving bacterium will then receive the ARGs in the genetic payload from the virus (von Wintersdorff et al., 2016). Transduction ARG transfer would most likely decrease after a wildfire because the process requires living bacterial cells to act as hosts for DNA assimilation.

The final HGT pathway is through gene transfer agents. Gene transfer agents resemble bacteriophages and are produced within the host cell. Rather than transferring the genetic content of a virus, however, the gene transfer agent will carry parts of the host cell's genetic makeup to a receiving cell (von Wintersdorff et al., 2016). Gene transfer agents are usually released during cell lysis and because they carry incomplete versions of the host DNA, they are unable to undergo self-replication like bacteriophages (von Wintersdorff et al., 2016). The process of ARG transfer through gene transfer agents would likely increase after wildfire events, due to cell lysis during fires, similar to transformation HGT.

Wildfires are also likely to increase antibiotic concentrations in the soil because fungi are known to produce secondary metabolites in extreme environments (e.g., post-wildfire) for competitive and

defensive purposes (Yogabaanu et al., 2017). These secondary metabolites often have antibiotic properties (Fatima et al., 2019). Environmental stresses from wildfires can cause fungi to produce secondary metabolites, some of which have antibiotic properties (Ebrahimi et al., 2021). These increased concentrations of soil antibiotics could further increase ARG abundance in microbes after wildfires as exposure to antibiotics increases.

Heavy metal concentrations are also likely to increase in the soil post-fire, as wood ash from fires is full of heavy metals and modern firefighting practices use ammonium sulfates that increase heavy metal solubility (Liodakis & Tsoukala, 2009). By increasing the concentrations of heavy metals in the soil, the likelihood of co-selecting for antibiotic resistance genes is increased, as heavy metals can cause antibiotic resistance to be co-selected (Seiler & Berendonk, 2012). This co-selection of ARGs on mobile genetic elements (e.g., plasmids) may cause ARGs to spread in a post-fire environment.

Some ARGs confer resistance to common antibiotics (i.e., *sul1*, *sul2*, *tetM*, *tetB*, *tetO*, *tetW*, and *ermF*) and last resort antibiotics (i.e., *mcr-1, optrA, fosA2*, *cfr*). For the common antibiotics, the *sul1* and *sul2* genes confer resistance to sulfonamides (Pei et al., 2006). *sul1* and *sul2* have been found to co-exist on chromosomes and plasmids (N. Wang et al., 2014) and have been found in strains of *Salmonella enterica* (Antunes et al., 2005). Sulfonamides work by inhibiting the production of folic acid by binding to the enzyme dihydropteroate synthase (DHPS) and the presence of *sul* genes result in the production of DHPS enzymes that resist sulfonamide binding (Sköld, 2000). Tetracyclines work by binding to ribosomes and preventing tRNA binding (Dönhöfer et al., 2012) and *tet* genes confer resistant to tetracyclines (Aminov et al., 2001) through one of three methods: efflux pumps (i.e., using transport proteins to remove the antibiotic from the cell), ribosomal protection by preventing antibiotic binding, or inactivation of the drug through enzymes (Bryan et al., 2004). *Tet* genes have been found in *Aeromonas* spp., *Citrobacter freundi, Pseudomonas putida,* and *Yersinia ruckeri* (Hedayatianfard et al., 2014). *ErmF,* which confers

resistance to erythromycin (J. Chen et al., 2007), works through inhibiting protein synthesis by binding to bacterial ribosomes (Weisblum, 1995) and has been found in *Bacteroides fragilis* (Jensen et al., 1999). The *erm* class of resistance genes can provide erythromycin resistance by altering the structure of the ribosomal proteins, preventing antibiotic binding to the ribosomes (Weisblum, 1995).

Some antibiotics are last resort antibiotics that are used when other common antibiotics have failed to treat bacterial infections (Osei Sekyere, 2018). As is common, however, bacteria are developing resistances to these last-resort antibiotics. The *mcr-1* gene is a variant of *mcr,* a gene that confers resistance to colistin (Osei Sekyere, 2018) and has been found in the *Enterobacteriaceae* family (Li et al., 2020). Colistin works by binding to lipids and initiating cell lysis and *mcr* codes for the transfer of phosphoethaloamine to the lipid binding site of colistin, reducing the lipid's anionic charge and preventing the cationic colistin from binding (Osei Sekyere, 2018). *OptrA* and *cfr* genes both confer resistance to the oxazolidinone class of antibiotics and have been found to co-exist on plasmids on *Enterococcus faecalis* (Almeida, Gaca, et al., 2020). O*ptrA* provides protection from oxazolidinones by coding for ATP-binding cassette proteins that give ribosomal protection (Almeida, Lebreton, et al., 2020) and *optrA* has been found to be transferred between *Enterococcus* spp. (Cui et al., 2016). *Cfr* codes for the addition of a methyl group to the binding site of oxazolidinones on rRNA, preventing antibiotic binding (Long et al., 2006). *FosA2* confers resistance to fosfomycin by coding for the production of an enzyme that inactivates fosfomycin (Benzerara et al., 2017) and has been found in *Enterobacter cloacae* (H. Xu et al., 2011). Due to the increasing risk of antibiotic resistant infections, the link between wildfires and ARGs must be explored. The hypothesis of this study was that wildfires would increase ARG abundance, possibly through a variety of mechanisms as listed previously.

3.2 Materials and Methods

Wildfire intensity samples were taken from area affected by the Woolsey Fire in the Santa Monica Mountains National Recreation Area in California. The Woolsey Fire began on November 8, 2018, and by the time it was contained on January 4, 2019 it had burned 94,946 acres (*Woolsey Fire*, 2021). The area has a Mediterranean ecosystem, characterized by mild winters with precipitation and warm summers with little precipitation (Mulholl et al., 2015a). The geology of the site is varied, containing volcanic and ocean sediments, canyons, and mountainous terrain (Mulholl et al., 2015b).

Soils were collected approximately one year after the Woolsey Fire in January 2020 from areas exposed to high intensity wildfire (i.e., sites Corral Canyon (H8) and Kanan Dume (H9)), moderate intensity wildfire (i.e., sites Arroyo Sequit (M4), Corral Canyon (M5), Kanan Dume (M6), and Paramount Ranch (M7)), and low intensity wildfire (i.e., sites Cheeseboro Canyon (L2) and Laskey Mesa (L3)). Controls soils with no exposure to wildfire were collected from Encinal Canyon Road (C1a), Saddle Peak (C1b), and Calabasas Peak (C1c). Intensity readings were collected using spectral band data from the Sentinel-2 satellites by the U.S. Forest Service. At each location, soils were sampled in triplicate using a soil corer of approximately 1 in. diameter. Soils were then separated based on the following depths: 0 in. to 3 in. (topsoils) and 3 in. to 6 in. (bottom soils). Sampling materials were sterilized with 70% ethanol in between samples to prevent cross-contamination of soil microbes. Samples were shipped overnight on dry ice to Washington State University. Once received, soils were homogenized and stored at -80°C until DNA could be extracted. Maps of sample locations are shown in Figure 2 and Figure 3.

For Kincade, Tubbs, and Williams Flats wildfire sites, soil samples were collected immediately following wildfires. The Kincade wildfire burned from October 23rd, 2019 to November 6th, 2019 northeast of Geyserville, CA, burning 77,758 acres (*Kincade Fire*, 2020). Six samples of unknown burn intensity were collected from the Kincade Fire: three topsoil and three bottom soil. The Tubbs wildfire burned from

October 8th 2017 to February 9th, 2018 off of Highway 128 and Bennett Lane, CA, burning 36,807 acres (*Tubbs Fire*, 2019). Six high intensity burn samples were collected from the Tubbs Fire: three topsoil and three bottom soil samples. The Williams Flats wildfire burned from August 2nd, 2019 to August 25th, 2019 five miles southeast of Keller, WA, burning 44,446 acres (*Williams Flats Fire*, 2019). Fourteen samples were collected from the Williams Flats Fire in a high-burn intensity area as defined by Parson et al. (2010): three ash samples and 11 soil core samples, with depths ranging from 15 to 220 cm below ground. The Mesa wildfire started on July 26th, 2018, 23.8 miles southwest of McCall, ID, and burned 34,719 acres. Five high intensity burn samples were collected from the Mesa Fire: 3 ash samples and two soil samples (Depth: 15 cm, 50 cm). Finally, one topsoil sample was taken from both the Northstar and Hayes Fires. For each location, soils were again sampled using a soil corer of approximately 1 in. diameter. Ash samples were collected using sterilized shovels. Following collection, all samples were immediately transported to Washington State University and stored at -80°C until DNA could be extracted.

DNA was extracted from 114 samples using the DNeasy PowerLyzer PowerSoil® Kit by QIAGEN. Approximately 0.25 grams of the soil or ash samples was used in each isolation. Each isolation followed the procedure outlined in the DNeasy PowerLyzer PowerSoil® Handbook, with some changes. First, the initial vortexing time of the PowerBead tube was increased from 10 to 20 minutes to increase DNA yields. Second, the final elution of the MB Spin Column membrane was done using 30 µL of solution C6 rather than the recommended 100 µL to avoid diluting eluted DNA. Resulting DNA concentrations were then analyzed using the Qubit 4.0 Fluorometer from Thermo Fisher Scientific. Extracted DNA was stored at -20°C for downstream analysis.

PCR was performed on the 114 wildfire soil DNA samples. Eleven antibiotic resistance genes were targeted that confer microbial resistance to conventional antibiotics (i.e., *sul1*, *sul2*, *tetM*, *tetB*, *tetO*, *tetW*,

and *ermF*) and last resort antibiotics (i.e., *mcr-1, optrA, fosA2*, *cfr*). The PCR was performed using 25 µL reactions using targeted primers and primer-specific annealing temperatures, shown in Table 4.

Table 4. Antibiotic resistance genes targeted, the forward and reverse primers, the amplicon size in base pairs (bp), the annealing temperature, the resistance target, and the primer source.

Gene	Forward Primer (5'→3')	Reverse Primer (5'→ 3')	Size (bp)	Annealing Temperature (°C)	Resistance Target	Source
mcr-1	AGTCCGTTTGTT-CTTGTGGC	AGATCCTTGGTC-TCGTCTTG	320	51.9	Colistin	Osei Sekyere, 2018
FOSA2	GCTGCAATCACT-CAACCATC	AAGCTGGAGCTG-CACGTC	712	52.3	Fosfomycin	H. Xu et al., 2011
optrA	CAGGTGGTCAGC-GAACTAAG	GCCACACCACCC-ATAAGTGTT	1000*	53.4	Oxazolidinone	Cui et al., 2016
cfr	TGAAGTATAAAG-CAGGTTGGGAGT-CA	ACCATATAATTG-ACCACAAGCAGC	746	53.6	Oxazolidinone	Kehrenberg & Schwarz, 2006
sul1	CGCACCGGAAAC-ATCGCTGCAC	TGAAGTTCCGCC-GCAAGGCTCG	163	61.8	Sulfonamide	Pei et al., 2006
sul2	TCCGGTGGAGGC-CGGTATCTGG	CGGGAATGCCAT-CTGCCTTGAG	191	58.6	Sulfonamide	Pei et al., 2006
tet(M)	ACAGAAAGCTTA-TTATATAAC	TGGCGTGTCTAT-GATGTTCAC	171	72.0	Tetracycline	Aminov et al., 2001
tet(B)	AAAACTTATTAT-ATTATAGTG	TGGAGTATCAAT-AATATTCAC	169	35	Tetracycline	Aminov et al., 2001

tet(O)	ACGGARAGTTTA-TTGTATACC	TGGCGTATCTAT-AATGTTGAC	171	45.5	Tetracycline	Aminov et al., 2001
tet(W)	GAGAGCCTGCTA-TATGCCAGC	GGGCGTATCCAC-AATGTTAAC	168	50.9	Tetracycline	Aminov et al., 2001
ermF	CGACACAGCTTT-GGTTGAAC	GGACCTACCTCA-TAGACAAG	309	49.4	Erythromycin	J. Chen et al., 2007

Thermocycling conditions for PCR were as follows: 1) denaturation at 95°C for three minutes, 2) 35 cycles of denaturation at 95°C for 30 seconds, annealing at the temperature listed in Table 4 for 30 seconds, and extension at 72°C for one minute, 3) a final extension step at 72°C for five minutes, and 4) holding at 4°C until use. After PCR, the samples were stored in a -20°C freezer until use. Agarose gel electrophoresis was then performed on the PCR product to visually confirm the presence of gene fragments.

3.3 Results

Of the 11 genes targeted in PCR, *mcr-1*, *fosA2*, *tetM*, *tetB*, *tetO*, *cfr*, and *ermF* did not show any positive results for the 114 samples tested. There were, however, positive results for *sul1, sul2, tetW*, and potentially *optrA*. Full results are shown in Appendix Two.

PCR and gel electrophoresis were run using primers for *optrA.* However, the paper by Cui et al. (2016) that the primers were sourced from failed to include any gel photos or amplicon lengths, so any positive results on this study's optr*A* gels will have to be treated with uncertainty. There were three total potential positive results in the gel at 1000 bp from the Williams Flats samples with soil depths of 20-40

cm, 15 cm, and 40-60 cm from right to left shown in Figure 14. There were 10 positive results for *tetW* in the 114 samples tested. Two of the positive results, samples M5T and M7H, are shown in Figure 14.

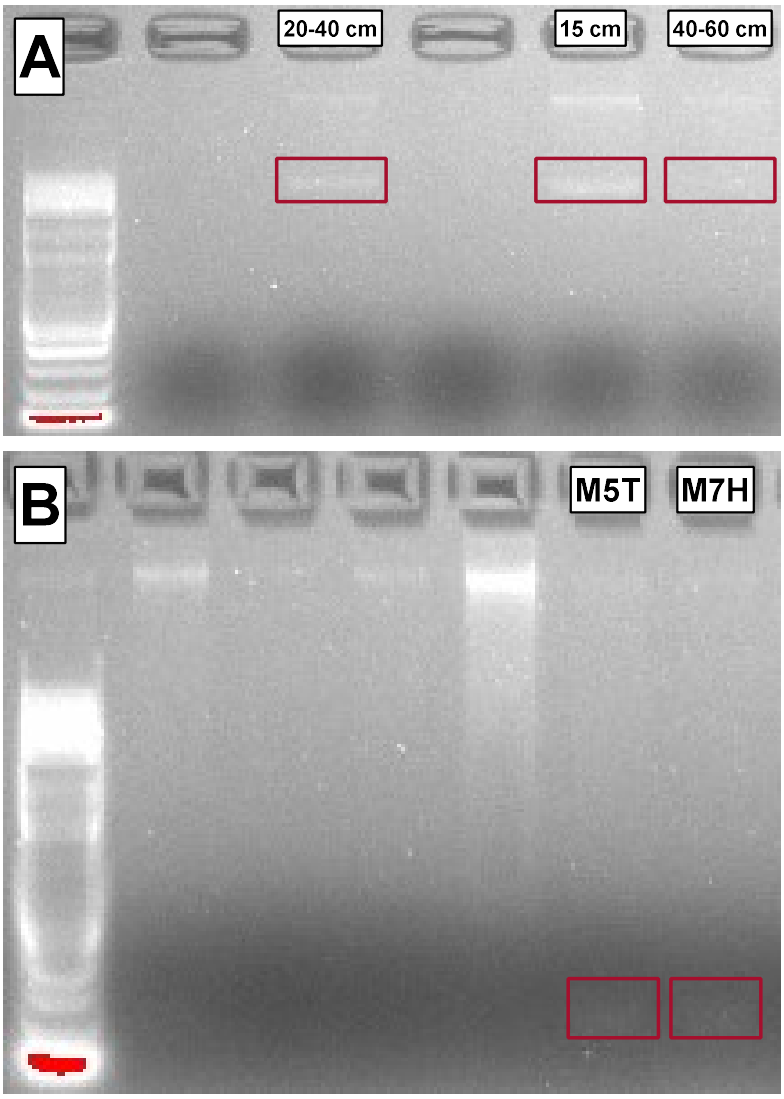

Figure 14. (A): Potentially positive results for the optrA *amplicon in Williams Flats soil depth samples at 1000 bp. The soil depths from left to right are as follows: 20-40 cm, 15 cm, and 40-60 cm depths. (B): Positive results for the* tetW *amplicon in samples M5T (Moderate, Corral Canyon, topsoil) and M7H (Moderate, Paramount Ranch, vertically homogenized) at 168 bp. Sample names have an "M" for moderate intensity wildfire samples from the Woolsey fire, while the letter at the end denotes sample depth by a "T" (topsoil, 0-5 cm) or "H" (vertically homogenized). The letters in the middle in (B) correspond to the map in Figure 2.*

As shown in Figure 14, the positive results for *optrA* are faint, suggesting low concentrations. Additionally, the bands grow fainter as soil depth increases, suggesting that ARGs have highest concentrations in wildfire-affected soils in the top layers. This increased surface concentration is likely due to higher bacteria concentrations in samples closest to the surface, where nutrients are plentiful. Higher concentrations near the surface could also lead to increased risk of ARG transport (e.g., surface runoff, foot traffic, etc.) which could increase ARG concentration in areas where there previously was not a high prevalence of antibiotic resistant bacteria. Both samples in Figure 14 have very faint bands, suggesting a low concentration in both samples. These samples were from the moderate intensity Woolsey fire, although they were from different locations, showing that ARGs are found in multiple locations post-wildfire. The vertically homogenized sample in M7H has a slightly fainter band than M5T, suggesting that the topsoil in M5T has higher ARG concentrations than the combined top and bottom soil in M7H. Similar to the results in Figure 14A, this indicates that ARGs are more prevalent in samples closer to the surface, likely due to higher bacteria concentrations.

There were 27 positive results for *sul1* in the 114 samples tested. Four of the positive results in samples M6T and M6T are shown in Figure 15. There were 10 positive results for *sul2* in the 114 samples tested. Two of the positive results, samples KB and KT, are shown in Figure 15.

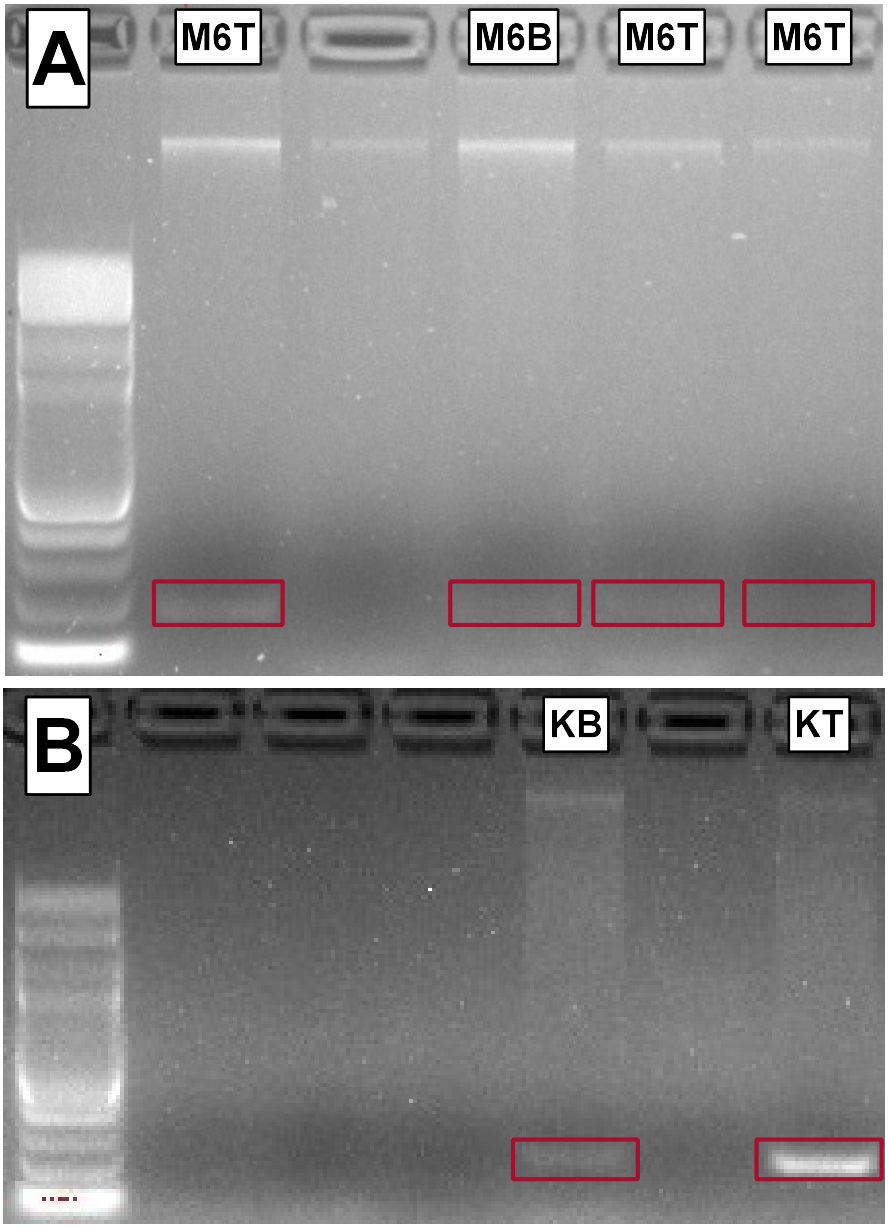

Figure 15. (A): - Positive results for the sul1 *amplicon in samples M6T and M6B at 163 bp. Samples are moderate intensity samples from Kanan Dume. For the last letter: T = "topsoil" (0-3 inches below the surface), B = "bottom soil" (3-6 inches below the surface). (B): Positive results for the* sul2 *amplicon in samples KB and KT with at 191 bp. Samples are of unknown intensity from the Kincade wildfire. Sample names begin with an "M" to denote moderate intensity samples from the Woolsey wildfire or with a "K" for samples from the Kincade fire. The letters at the end denote sample depth, a "T" (topsoil, 0-3 in) or "B" (bottom soil, 3-6 in). In (A), the numbers in the sample name correspond to the location in Figure 2.*

In Figure 15A, the far-left sample M6T shows a higher concentration than the other due to its brighter band. In Figure 15, sample KT shows a higher concentration than sample KB due to sample KT's much brighter band. Notably, both samples are from the Kincade fire, but sample KB is from bottom soil while sample KT is from the topsoil. These results, combined with the results from the depth samples in Figure 14, suggest a higher concentration of antibiotic resistance genes in surface soils than in deeper soils. This would be a reasonable assumption, as there are higher concentrations of microorganisms in surface soils than subsurface soils.

3.3.1 Woolsey Wildfire Intensity ARGs

The percentage of positive ARG results for the Woolsey wildfire samples are shown Table 5.

Table 5. Percentage of positive antibiotic resistance gene results for sul1*,* sul2*, and* tetW *genes from the Woolsey wildfire in California with varying wildfire intensities. "n" is the number of total samples in each intensity category.*

Intensity	*sul1*	*sul2*	*tetW*
Control (*n* = 9)	0%	0%	0%
Low (*n* = 18)	**28%**	**22%**	**6%**
Moderate (*n* = 36)	**33%**	**8%**	**19%**
High (*n* = 18)	**22%**	**6%**	**6%**

As shown in Table 5, there were no samples with ARGs identified in the control category, and positive results were found in both low, moderate, and high samples for *sul1*, *sul2*, and *tetW*. *sul1* remains relatively elevated in all wildfire intensities, ranging from the lowest at high intensity at 22% to the highest at 33% at moderate intensity, which is the greatest percentage of all amplicon abundance in the samples. *TetW* also peaks in the moderate intensity samples at 19% while *sul2* peaks in low intensity samples at 22% and decreases in moderate and high samples at 8% and 6%, respectively. The results for the *sul1* gene suggest that it is most prominent in low and moderate fire intensity soils, while *sul2* has higher

concentrations in low intensity soils. *TetW* appears to have the highest concentration in moderate fire intensity soils.

3.4 Discussion

Out of the 11 genes tested for ARGs, three positive results were found for *optrA,* 27 for *sul1*, 10 for *sul2* and 10 for *tetW*. The results for the *optrA* gene are potential hits, however, since the size of the optrA gene was not described in the source paper (Cui et al., 2016) and the listed size of 1000 bp is an inference from gel results. The results from Appendix Two suggest that *sul1* may be a common antibiotic resistance gene found after wildfires, as it appears in 23% of the 114 total samples tested. For control samples with no fire exposure, no ARGs were found. For low fire intensity samples, *sul1* was the most abundant ARG (abundance = 28%), followed by *sul2* (abundance = 22%) and *tetW* (abundance = 6%). For moderate intensity samples, *sul1* was also the most abundant ARG (abundance = 33%), followed by *tetW* (abundance = 19%), and *sul2* (abundance = 8%). For high intensity samples, *sul1* was again the most abundant ARG (abundance = 22%), followed by a tie in abundance between *sul2* (abundance = 6%), and *tetW* (abundance = 6%). These results suggest that *sul1* and *sul2* are abundant in low intensity wildfires, *sul1* and *tetW* are abundant in moderate intensity wildfires, and *sul1* is abundant in high intensity wildfires. ARGs are shown to be abundant at all fire intensities and absent in control samples, suggesting that wildfires are encouraging the development of ARGs in soil bacteria.

The results from Figure 14A and Figure 15B may suggest that ARG concentrations are higher in surface soil samples, as the *optrA* and *sul2* amplicon bands are brighter in surface soils. This is not corroborated by Figure 15A, however. Although the far-left sample M6T in Figure 15A is a topsoil sample and has a brighter band than the other samples, the two samples from the right are also from M6T and have fainter bands, similar to the bottom soil samples. The results in Appendix Two show that *sul1* has ten positive results in surface soils and five positive results in bottom soils. *Sul2* has four positive results

in surface soils and also four positive results in bottom soils. *TetW* has one positive result in surface soils and three positive results in bottom soils. From the distribution of ARGs in top and bottom soils, no clear conclusions can be made about ARG abundance versus depth. These results may indicate that soil depth plays a role in ARG concentrations and abundance but that ARGs are also location dependent. Perhaps the site topography plays a role in determining ARG concentration. It could be that areas with lower elevations than the surrounding terrain are more likely to have higher concentrations of ARGs as runoff accumulates in those areas.

The results from Table 5 suggest that ARGs do increase in wildfire affected soil. There were three possible mechanisms proposed to explain the increase of ARGs in wildfire soil. First, ARGs may be released during cell lysis from wildfire heat. For cell lysis to explain ARG development, ARGs would be present in the control samples, albeit at lower levels, due to the natural presence of ARGs in the environment. ARGs only appear in fire-affected soil, however, indicating that cell lysis does not fully explain the results in Table 5. Secondly, ARG development may occur as a natural reaction to fungi releasing secondary metabolites with antibiotic properties due to stress. However, only *sul1*¸ *sul2*, and *tetW* were found. The genes *sul1* and *sul2* encode for sulfonamide resistance (Antunes et al., 2005), and sulfonamide is created synthetically (van Miert, 1994). *TetW* encodes for tetracycline resistance (Cheng et al., 2013), and tetracycline is either derived from bacteria (i.e., streptomycetes) or produced synthetically (Grossman, 2016). Thus, none of the ARGs found in the samples would have developed as an evolutionary response to fungi. The final and most likely possibility for ARG development in fire-affected samples is that a post-fire environment enriched in heavy metals resulted in the co-selection of ARGs along with heavy metal resistance genes in mobile plasmids. These plasmids likely passed between bacteria through horizontal gene transfer and to new generations of bacteria during reproduction to improve survivability.

CHAPTER FOUR: CONCLUSIONS AND FUTURE DIRECTIONS

4.1 Conclusions

Wildfire frequency and severity has increased in recent years and additional research is needed to understand the holistic impacts of wildfire on environmental health and sustainability. While large-scale effects of wildfires are well-studied (e.g., property destruction, loss of animal habitats), the more subtle effects of wildfires have yet to be fully evaluated. Because of the importance microbes play in maintaining healthy ecosystems, any impact that wildfires may have on microbial communities has the potential to pose a serious threat to post-fire recovery efforts and human health for years to come. Wildfires are most likely to negatively impact microbial communities when burns reach higher intensities by destroying available nutrients and causing cell death through fire heat. Wildfires may also play an indirect role in antibiotic resistance gene (ARG) development and proliferation. Thus, the present research addressed two primary goals: 1) to identify changes in families and genera that were mediated by wildfire intensity and 2) to determine whether ARG abundance and distribution in soil microbial communities is affected by wildfire.

To achieve these goals, first, wildfire-affected soil and ash samples were collected from wildfires in Washington, Idaho, and California. Genomic DNA was isolated from each sample and was used to determine bacterial community structures in wildfire-affected soils using Illumina MiSeq sequencing. Statistical tests (i.e., Spearman correlations and Jonckheere-Terpstra tests) were then used to identify taxa significantly associated with wildfire burn intensity. Several taxa and many genera were identified with their abundances being significantly mediated by wildfire intensity, with six families and 17 genera negatively associated with wildfire intensity and three families and six genera positively associated with wildfire intensity. Families found to be significantly ($p < 0.05$) positively associated with wildfire intensity are *Micrococcaceae* spp. ($r = 0.410$), *Pseudonocardiaceae* spp. ($r = 0.557$), and *Sphingobacteriaceae* spp.

(r = 0.513), while genera found to be significantly (p < 0.05) positively associated with wildfire intensity are *Amycolatopsis* spp. (r = 0.578), *Arthrobacter* spp. (r = 0.421), *Oxalobacter* spp. (r = 0.540), *Pedobacter* spp. (r = 0.513), *Phenylobacterium* spp. (r = 0.533), and *Pseudonocardia* spp. (r = 0.555). These taxa may increase in relative abundance post-fire due to a decrease in competition from taxa that are more susceptible to wildfire impacts.

Families found to be significantly (p < 0.05) negatively associated with wildfire intensity are *Chromatiaceae* spp. (r = -0.704), *Nocardioidaceae* spp. (r = -0.470), *Rubrobacteraceae* spp. (r = -0.899), *Solirubrobacteraceae* spp. (r = -0.651), *Thermoanaerobacteraceae* spp. (r = -0.608), and *Xanthomonadaceae* spp. (r = -0.481). Genera found to be significantly (p < 0.05) negatively associated with wildfire intensity are *Aeromicrobium* spp. (r = -0.612), *Balneimonas* spp. (r = -0.640), *Candidatus Scalindua* spp. (r = -0.615), *Chondromyces* spp. (r = -0.613), *Clostridium* spp. (r = -0.763), *Cystobacter* spp. (r = -0.692), *Desulfovibrio* spp. (r = -0.461), *Dokdonella* spp. (r = -0.503), *Euzebya* spp. (r = -0.672), *Gemmata* spp. (r = -0.668), *Haliangium* spp. (r = -0.477), *Kaistobacter* spp. (r = -0.540), *Megasphaera* spp. (r = -0.579), *Nocardioides* spp. (r = -0.590), *Rubrobacter* spp. (r = -0.896), *Runella* spp. (r = -0.556), and *Solirubrobacter* spp. (r = -0.653). Many as many of these genera are known to be critical contributors to maintaining global nutrient cycles. For example, *Balneimonas* spp., *Clostridium* spp., and *Desulfovibrio* spp. are capable of fixing atmospheric nitrogen. Decreases in nitrogen fixing species could result in decreased nutrients for struggling plant communities in a post-wildfire environment.

Additionally, the community profiles of two ash samples were obtained and both samples from different locations featured the same top three taxa for families (i.e., *Micrococcaceae, Bacillaceae,* and *Oxalobacteraceae*) and top four taxa for genera (i.e., *Arthrobacter, Bacillus, Janthinobacterium,* and *Pseudomonas*). Ash that is transported during wildfire events could transport bacteria and any ARGs they

harbor to new areas and spreading ARGs through horizontal gene transfer. Additionally, if any pathogenic bacteria are contained in the ash, individuals that breathe in the ash may be introducing pathogens to their bodies.

To examine the effects of wildfires on environmental antibiotic resistance, extracted genomic DNA was also screened for the presence of several ARGs including those targeted that confer microbial resistance to conventional antibiotics (i.e., *sul1*, *sul2*, *tetM*, *tetB*, *tetO*, *tetW*, and *ermF*) and last resort antibiotics (i.e., *mcr-1, optrA, fosA2*, *cfr*). While no ARGs were found in the control samples for the Woolsey fire, *sul1*, *sul2*, and *tetW* ARGs were frequently in Woolsey fire soil samples, with *sul1* and *tetW* being most prominent in moderate intensity samples, *sul2* being prominent in low intensity samples. These data suggest that there may be a link between wildfire occurrence, intensity, and antibiotic resistance in soil microbial communities. This may arise through a number of mechanisms such as cell lysis from wildfire heat, co-selection on mobile genetic elements, and increased exposure to antibiotic fungal secondary metabolites. Increases in wildfire occurrence will likely result in increases in ARG soil concentrations, which presents a human health concern. Runoff and foot traffic through wildfire affected areas could spread ARGs to new areas and increase the threat of antibiotic resistant infections. The concentrations of antibiotics in the environment can be limited by reducing antibiotics used in agriculture and the prescription of antibiotics for illnesses when a bacterial infection is not necessarily present. Additionally, using ammonium sulfate-based fire retardants can increase heavy metals concentrations in the soil which can result in the co-selection for antibiotic resistance genes. Using alternative fire retardants that do not increase heavy metal solubility may slow the rate of ARG transfer between microorganisms.

4.2 Limitations and Future Research Directions

While the data presented here offer novel insights into the dynamics between soil microbes and wildfires, there are several study limitations that affect their potential impact. One limitation for this study was that it was conducted during the COVID-19 pandemic. Samples were initially collected for the Woolsey fire in January 2020, at the start of the pandemic. By the time the study was underway, additional samples could not be collected due to health concerns. Another limitation for the ARG portion of the study was that gradient tests could not be conducted to determine perfect primer annealing temperatures due to time constraints, as completing both the Illumina MiSeq sequencing and ARG tests were prioritized over gradient testing. This may have resulted in antibiotic resistance genes that were not discovered due to incorrect primer annealing temperature selection.

This study utilized intensity readings from satellite measurements for the Woolsey fire. While this method results in accurate measurements of heat transfer, it does not measure temperatures of the soil. Additionally, the fire characteristics (i.e., crown or surface) were not recorded, thus it is unknown whether the fire was burning near the surface or in the treetops for each geographical location with the Woolsey fire. This means that there could have been low intensity surface fires in the Woolsey fire that lingered, resulting in greater soil temperatures and soil property changes than high intensity crown fires. However, the pairwise comparisons for the Woolsey fire only showed one taxon, Gemmata, that significantly decreased from control to low intensity. Thus, it is unlikely that the Woolsey wildfire had low intensity fires that resulted in high soil temperatures, as this would likely cause a reduction in taxa abundance in the pairwise comparisons from control to low intensity. This was not generally seen, as three families and six genera increased relative abundance from control to low intensity, suggesting a benefit from low intensity fires.

While the Mesa and Williams Flats ash samples provided new insights into community composition of ash at the family and genus level, only one sample was able to be taken from each location and thus replicate samples could not be used to obtain average values. Additionally, while the Williams Flats soil samples were taken from a high intensity burn area, it would have been beneficial to take depth samples from areas with varying wildfire intensities. This would have allowed for an informative comparison between depth and wildfire intensity. Finally, the Mesa depth samples were only at 15 and 50 centimeters and a wider range of depth samples would have been beneficial to compare to the Williams Flats depth samples.

An additional limitation for both the community characterization and ARG goals of this thesis were that Woolsey wildfire control samples were not obtained before the fire and unburned locations were selected instead after the fire. While these unburned locations did not experience interaction with fire, there could still be transport of microorganisms between burned and unburned locations through runoff, air transport (e.g., ash, wind, etc.), and foot traffic. This could allow the microbial communities from burned and unburned locations to mix, affecting the sequencing results. While it is difficult to predict where a wildfire will occur, control samples collected before the wildfire would offer an even greater understanding in the change of microbial community composition and would be able to show a clear picture of change in the pre- and post-fire landscapes. Despite this limitation of control samples, this study still presents valuable data.

While the findings from this thesis expand upon the current knowledge of impacted taxa from wildfire events and offer new insights into ARG development post-fire, further research is needed. Woolsey samples were collected one year after the wildfire where microbial communities could have a chance to recover. Future studies should examine microbial community over time with exposure to a

variety of wildfire intensities, to further categorize microbial community response over time to wildfire intensity.

Future research for ARG development could involve using quantitative PCR (qPCR) to not only identify the presence of ARGs post-fire but quantify them. This would allow researchers to examine whether certain ARGs are selected for in greater numbers after a wildfire. Additionally, researching the specific mechanisms of ARG transfer would be ideal, as understanding the cause behind ARG development could allow future methods to combat the rise of antibiotic resistant infections.

A study involving experimental fire conditions at different temperatures (e.g., 100-500°C) and examining microbe recovery over time would provide insights into the change in community composition after wildfires with varying burn intensity. The samples from the Woolsey fire for intensity were taken one year after the fire and examining changes between the time of burning and one year after the wildfire would better categorize the effect on nutrient cycling microorganisms from wildfires over time. The current study for community sequencing could be improved on by selecting control samples from an area before it was burned by a wildfire and comparing those control samples to the post-fire soil samples to examine community changes in one location pre- and post-fire.

REFERENCES

Aasfar, A., Bargaz, A., Yaakoubi, K., Hilali, A., Bennis, I., Zeroual, Y., & Meftah Kadmiri, I. (2021). Nitrogen Fixing Azotobacter Species as Potential Soil Biological Enhancers for Crop Nutrition and Yield Stability. *Frontiers in Microbiology*, *12*, 354. https://doi.org/10.3389/fmicb.2021.628379

Abatenh, E., Gizaw, B., Tsegaye, Z., & Genene, T. (2018). Microbial Function on Climate Change—A Review. *Environment Pollution and Climate Change*, *02*. https://doi.org/10.4172/2573-458X.1000147

Abatzoglou, J. T., & Williams, A. P. (2016). Impact of anthropogenic climate change on wildfire across western US forests. *Proceedings of the National Academy of Sciences*, *113*(42), 11770–11775. https://doi.org/10.1073/pnas.1607171113

Acea, M. J., & Carballas, T. (1996). Changes in physiological groups of microorganisms in soil following wildfire. *FEMS Microbiology Ecology*, *20*(1), 33–39. https://doi.org/10.1016/0168-6496(96)00012-8

Adkins, J., Docherty, K. M., Gutknecht, J. L. M., & Miesel, J. R. (2020). How do soil microbial communities respond to fire in the intermediate term? Investigating direct and indirect effects associated with fire occurrence and burn severity. *Science of The Total Environment*, *745*, 140957. https://doi.org/10.1016/j.scitotenv.2020.140957

Almeida, L. M., Gaca, A., Bispo, P. M., Lebreton, F., Saavedra, J. T., Silva, R. A., Basílio-Júnior, I. D., Zorzi, F. M., Filsner, P. H., Moreno, A. M., & Gilmore, M. S. (2020). Coexistence of the Oxazolidinone Resistance–Associated Genes cfr and optrA in Enterococcus faecalis From a Healthy Piglet in Brazil. *Frontiers in Public Health*, *8*, 518. https://doi.org/10.3389/fpubh.2020.00518

Almeida, L. M., Lebreton, F., Gaca, A., Bispo, P. M., Saavedra, J. T., Calumby, R. N., Grillo, L. M., Nascimento, T. G., Filsner, P. H., Moreno, A. M., & Gilmore, M. S. (2020). Transferable Resistance

Gene *optrA* in Enterococcus faecalis from Swine in Brazil. *Antimicrobial Agents and Chemotherapy*, *64*(6). https://doi.org/10.1128/AAC.00142-20

Aminov, R. I., Garrigues-Jeanjean, N., & Mackie, R. I. (2001). Molecular Ecology of Tetracycline Resistance: Development and Validation of Primers for Detection of Tetracycline Resistance Genes Encoding Ribosomal Protection Proteins. *Applied and Environmental Microbiology*, *67*(1), 22–32. https://doi.org/10.1128/AEM.67.1.22-32.2001

Antunes, P., Machado, J., Sousa, J. C., & Peixe, L. (2005). Dissemination of Sulfonamide Resistance Genes (sul1, sul2, and sul3) in Portuguese Salmonella enterica Strains and Relation with Integrons. *Antimicrobial Agents and Chemotherapy*, *49*(2), 836–839. https://doi.org/10.1128/AAC.49.2.836-839.2005

Aranda, C. P., Valenzuela, C., Matamala, Y., Godoy, F. A., & Aranda, N. (2015). Sulphur-cycling bacteria and ciliated protozoans in a Beggiatoaceae mat covering organically enriched sediments beneath a salmon farm in a southern Chilean fjord. *Marine Pollution Bulletin*, *100*(1), 270–278. https://doi.org/10.1016/j.marpolbul.2015.08.040

Arnold, J. D., Brewer, S. C., & Dennison, P. E. (2014). Modeling Climate-Fire Connections within the Great Basin and Upper Colorado River Basin, Western United States. *Fire Ecology*, *10*(2), 64–75. https://doi.org/10.4996/fireecology.1002064

Baldani, J. I., Rouws, L., Cruz, L. M., Olivares, F. L., Schmid, M., & Hartmann, A. (2014). The Family Oxalobacteraceae. In E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, & F. Thompson (Eds.), *The Prokaryotes: Alphaproteobacteria and Betaproteobacteria* (pp. 919–974). Springer. https://doi.org/10.1007/978-3-642-30197-1_291

Bárcenas-Moreno, G., & Bååth, E. (2009). Bacterial and fungal growth in soil heated at different temperatures to simulate a range of fire intensities. *Soil Biology and Biochemistry*, *41*(12), 2517–2526. https://doi.org/10.1016/j.soilbio.2009.09.010

Batt, C. A., & Tortorello, M. L. (Eds.). (2014). *Encyclopedia of food microbiology* (2. ed). AP, Academic Press/Elsevier.

Benzerara, Y., Gallah, S., Hommeril, B., Genel, N., Decré, D., Rottman, M., & Arlet, G. (2017). Emergence of Plasmid-Mediated Fosfomycin-Resistance Genes among Escherichia coli Isolates, France. *Emerging Infectious Diseases*, *23*(9), 1564–1567. https://doi.org/10.3201/eid2309.170560

Bewick, V., Cheek, L., & Ball, J. (2004). Statistics review 10: Further nonparametric methods. *Critical Care*, *8*(3), 196–199. https://doi.org/10.1186/cc2857

Bladon, K. D., Emelko, M. B., Silins, U., & Stone, M. (2014). Wildfire and the Future of Water Supply. *Environmental Science & Technology*, *48*(16), 8936–8943. https://doi.org/10.1021/es500130g

Bodí, M. B., Martin, D. A., Balfour, V. N., Santín, C., Doerr, S. H., Pereira, P., Cerdà, A., & Mataix-Solera, J. (2014). Wildland fire ash: Production, composition and eco-hydro-geomorphic effects. *Earth-Science Reviews*, *130*, 103–127. https://doi.org/10.1016/j.earscirev.2013.12.007

Bormann, B. T., Homann, P. S., Darbyshire, R. L., & Morrissette, B. A. (2008). Intense forest wildfire sharply reduces mineral soil C and N: The first direct evidence. *Canadian Journal of Forest Research*, *38*(11), 2771–2783. https://doi.org/10.1139/X08-136

Bryan, A., Shapir, N., & Sadowsky, M. J. (2004). Frequency and Distribution of Tetracycline Resistance Genes in Genetically Diverse, Nonselected, and Nonclinical Escherichia coli Strains Isolated from Diverse Human and Animal Sources. *Applied and Environmental Microbiology*, *70*(4), 2503–2507. https://doi.org/10.1128/AEM.70.4.2503-2507.2004

Bureau of Indian Affairs. (2016, May). *Colville Reservation—North Nespelem Fuels Treatment | Indian Affairs*. U.S. Department of the Interior. https://www.bia.gov/bia/ots/dfwfm/bwfm/forestry-fire-management-stories/colville-reservation-north-nespelem-fuels

Carreto, L., Moore, E., Nobre, M. F., Wait, R., Riley, P. W., Sharp, R. J., & Da Costa, M. S. (1996). Rubrobacter xylanophilus sp. Nov., a New Thermophilic Species Isolated from a Thermally Polluted Effluent. *International Journal of Systematic Bacteriology*, *46*(2), 460–465. https://doi.org/10.1099/00207713-46-2-460

CDC. (2019, November 6). *Pseudomonas aeruginosa Infection*. Centers for Disease Control and Prevention. https://www.cdc.gov/hai/organisms/pseudomonas.html

Certini, G., Moya, D., Lucas-Borja, M. E., & Mastrolonardo, G. (2021). The impact of fire on soil-dwelling biota: A review. *Forest Ecology and Management*, *488*, 118989. https://doi.org/10.1016/j.foreco.2021.118989

Chen, J. S., Toth, J., & Kasap, M. (2001). Nitrogen-fixation genes and nitrogenase activity in Clostridium acetobutylicum and Clostridium beijerinckii. *Journal of Industrial Microbiology & Biotechnology*, *27*(5), 281–286. https://doi.org/10.1038/sj.jim.7000083

Chen, J., Yu, Z., Michel, F. C., Wittum, T., & Morrison, M. (2007). Development and Application of Real-Time PCR Assays for Quantification of erm Genes Conferring Resistance to Macrolides-Lincosamides-Streptogramin B in Livestock Manure and Manure Management Systems. *Applied and Environmental Microbiology*, *73*(14), 4407–4416. https://doi.org/10.1128/AEM.02799-06

Cheng, W., Chen, H., Su, C., & Yan, S. (2013). Abundance and persistence of antibiotic resistance genes in livestock farms: A comprehensive investigation in eastern China. *Environment International*, *61*, 1–7. https://doi.org/10.1016/j.envint.2013.08.023

Choi, S., Song, H., Tripathi, B. M., Kerfahi, D., Kim, H., & Adams, J. M. (2017). Effect of experimental soil disturbance and recovery on structure and function of soil community: A metagenomic and metagenetic approach. *Scientific Reports*, *7*(1), 2260. https://doi.org/10.1038/s41598-017-02262-6

Chu, C., Yuan, C., Liu, X., Yao, L., Zhu, J., He, J., Kwon, S.-W., & Huang, X. (2015). Phenylobacterium kunshanense sp. Nov., isolated from the sludge of a pesticide manufacturing factory. *International Journal of Systematic and Evolutionary Microbiology*, *65*(Pt 2), 325–330. https://doi.org/10.1099/ijs.0.063644-0

Climate Data Online. (2021, October 26). National Climatic Data Center. https://www.ncdc.noaa.gov/cdo-web/

Cobo-Díaz, J. F., Fernández-González, A. J., Villadas, P. J., Toro, N., Tringe, S. G., & Fernández-López, M. (2017). Taxonomic and Functional Diversity of a Quercus pyrenaica Willd. Rhizospheric Microbiome in the Mediterranean Mountains. *Forests*, *8*(10), 390. https://doi.org/10.3390/f8100390

Cordero, J. M., Núñez, A., García, A. M., & Borge, R. (2021). Assessment and statistical modelling of airborne microorganisms in Madrid. *Environmental Pollution*, *269*, 116124. https://doi.org/10.1016/j.envpol.2020.116124

Cui, L., Wang, Y., Lv, Y., Wang, S., Song, Y., Li, Y., Liu, J., Xue, F., Yang, W., & Zhang, J. (2016). Nationwide Surveillance of Novel Oxazolidinone Resistance Gene optrA in Enterococcus Isolates in China from 2004 to 2014. *Antimicrobial Agents and Chemotherapy*, *60*(12), 7490–7493. https://doi.org/10.1128/AAC.01256-16

Cycoń, M., Mrozik, A., & Piotrowska-Seget, Z. (2019). Antibiotics in the Soil Environment—Degradation and Their Impact on Microbial Activity and Diversity. *Frontiers in Microbiology*, *10*, 338. https://doi.org/10.3389/fmicb.2019.00338

Dellasala, D. (2018). *Emergence of a New Climate and Human-Caused Wildfire Era for Western USA Forests*. https://doi.org/10.1016/B978-0-12-409548-9.10999-6

Dennison, P. E., Brewer, S. C., Arnold, J. D., & Moritz, M. A. (2014). Large wildfire trends in the western United States, 1984–2011. *Geophysical Research Letters*, *41*(8), 2928–2933. https://doi.org/10.1002/2014GL059576

Di Carlo, P., Aruffo, E., Biancofiore, F., Busilacchio, M., Pitari, G., Dari-Salisburgo, C., Tuccella, P., & Kajii, Y. (2015). Wildfires impact on surface nitrogen oxides and ozone in Central Italy. *Atmospheric Pollution Research*, *6*(1), 29–35. https://doi.org/10.5094/APR.2015.004

Dickinson, A. W., Power, A., Hansen, M. G., Brandt, K. K., Piliposian, G., Appleby, P., O'Neill, P. A., Jones, R. T., Sierocinski, P., Koskella, B., & Vos, M. (2019). Heavy metal pollution and co-selection for antibiotic resistance: A microbial palaeontology approach. *Environment International*, *132*, 105117. https://doi.org/10.1016/j.envint.2019.105117

Dönhöfer, A., Franckenberg, S., Wickles, S., Berninghausen, O., Beckmann, R., & Wilson, D. N. (2012). Structural basis for TetM-mediated tetracycline resistance. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(42), 16900–16905. https://doi.org/10.1073/pnas.1208037109

Earth Resources Observation and Science Center. (2017). *Sentinel*. U.S. Geological Survey. https://www.usgs.gov/centers/eros/science/usgs-eros-archive-sentinel-2?qt-science_center_objects=0#qt-science_center_objects

Ebel, B. A. (2020). Temporal evolution of measured and simulated infiltration following wildfire in the Colorado Front Range, USA: Shifting thresholds of runoff generation and hydrologic hazards. *Journal of Hydrology*, *585*, 124765. https://doi.org/10.1016/j.jhydrol.2020.124765

Ebrahimi, K. S., Ansari, M., Hosseyni Moghaddam, M. S., Ebrahimi, Z., salehi, Z., Shahlaei, M., & Moradi, S. (2021). In silico investigation on the inhibitory effect of fungal secondary metabolites on RNA dependent RNA polymerase of SARS-CoV-II: A docking and molecular dynamic simulation study. *Computers in Biology and Medicine*, *135*, 104613. https://doi.org/10.1016/j.compbiomed.2021.104613

Ecoss. (2015). *The Role of Soil Processes in the Global Carbon Cycle – Ecoss*. https://ecoss.nau.edu/new-frontiers/

Edwards, P. J. (1998). *Sulfur cycling, retention, and mobility in soils: A review* (NE-GTR-250; p. NE-GTR-250). U.S. Department of Agriculture, Forest Service, Northeastern Research Station. https://doi.org/10.2737/NE-GTR-250

EPA. (2019, August 13). *Wildfires: How Do They Affect Our Water Supplies?* [Overviews and Factsheets]. United States Environmental Protection Agency. https://www.epa.gov/sciencematters/wildfires-how-do-they-affect-our-water-supplies

Evtushenko, L., & Ariskina, E. (2015). *Nocardioidaceae*. https://doi.org/10.1002/9781118960608.fbm00042

Evtushenko, L. I., & Krausova, V. I. (2015). Aeromicrobium. In *Bergey's Manual of Systematics of Archaea and Bacteria* (pp. 1–22). American Cancer Society. https://doi.org/10.1002/9781118960608.gbm00156

Evtushenko, L. I., Krausova, V. I., & Yoon, J.-H. (2015). Nocardioides. In *Bergey's Manual of Systematics of Archaea and Bacteria* (pp. 1–81). American Cancer Society. https://doi.org/10.1002/9781118960608.gbm00159

Fageria, N. K. (2012). Role of Soil Organic Matter in Maintaining Sustainability of Cropping Systems. *Communications in Soil Science and Plant Analysis*, *43*(16), 2063–2113. https://doi.org/10.1080/00103624.2012.697234

Fairbanks, D., Shepard, C., Murphy, M., Rasmussen, C., Chorover, J., Rich, V., & Gallery, R. (2020). Depth and topographic controls on microbial activity in a recently burned sub-alpine catchment. *Soil Biology and Biochemistry*, *148*, 107844. https://doi.org/10.1016/j.soilbio.2020.107844

Fernández-González, A. J., Martínez-Hidalgo, P., Cobo-Díaz, J. F., Villadas, P. J., Martínez-Molina, E., Toro, N., Tringe, S. G., & Fernández-López, M. (2017). The rhizosphere microbiome of burned holm-oak: Potential role of the genus Arthrobacter in the recovery of burned soils. *Scientific Reports*, *7*. https://www.nature.com/articles/s41598-017-06112-3

Flicoteaux, R., & Lucas, J. (1984). Weathering Of Phosphate Minerals. In J. O. Nriagu & P. B. Moore (Eds.), *Phosphate Minerals* (pp. 292–317). Springer. https://doi.org/10.1007/978-3-642-61736-2_9

Fontúrbel, M. T., Barreiro, A., Vega, J. A., Martín, A., Jiménez, E., Carballas, T., Fernández, C., & Díaz-Raviña, M. (2012). Effects of an experimental fire and post-fire stabilization treatments on soil microbial communities. *Geoderma*, *191*, 51–60. https://doi.org/10.1016/j.geoderma.2012.01.037

Forsberg, C. W. (1978). Nutritional characteristics of Megasphaera elsdenii. *Canadian Journal of Microbiology*, *24*(8), 981–985. https://doi.org/10.1139/m78-161

Francos, M., Úbeda, X., Pereira, P., & Alcañiz, M. (2018). Long-term impact of wildfire on soils exposed to different fire severities. A case study in Cadiretes Massif (NE Iberian Peninsula). *Science of The Total Environment*, *615*, 664–671. https://doi.org/10.1016/j.scitotenv.2017.09.311

Frangieh, N., Accary, G., Morvan, D., Méradji, S., & Bessonov, O. (2020). Wildfires front dynamics: 3D structures and intensity at small and large scales. *Combustion and Flame*, *211*, 54–67. https://doi.org/10.1016/j.combustflame.2019.09.017

Frigaard, N.-U., & Dahl, C. (2009). Sulfur metabolism in phototrophic sulfur bacteria. *Advances in Microbial Physiology*, *54*, 103–200. https://doi.org/10.1016/S0065-2911(08)00002-7

Gray, D. A., Dugar, G., Gamba, P., Strahl, H., Jonker, M. J., & Hamoen, L. W. (2019). Extreme slow growth as alternative strategy to survive deep starvation in bacteria. *Nature Communications*, *10*(1), 890. https://doi.org/10.1038/s41467-019-08719-8

Gray, D. M., & Dighton, J. (2009). Nutrient utilization by pine seedlings and soil microbes in oligotrophic pine barrens forest soils subjected to prescribed fire treatment. *Soil Biology and Biochemistry*, *41*(9), 1957–1965. https://doi.org/10.1016/j.soilbio.2009.06.021

Grossman, T. H. (2016). Tetracycline Antibiotics and Resistance. *Cold Spring Harbor Perspectives in Medicine*, *6*(4), a025387. https://doi.org/10.1101/cshperspect.a025387

Gu, Y., Yperman, J., Vandewijngaarden, J., Reggers, G., & Carleer, R. (2017). Organic and inorganic sulphur compounds releases from high-pyrite coal pyrolysis in H2, N2 and CO2: Test case Chinese LZ coal. *Fuel*, *202*, 494–502. https://doi.org/10.1016/j.fuel.2017.04.068

Guo, M., Li, J., Xu, J., Wang, X., He, H., & Wu, L. (2017). CO2 emissions from the 2010 Russian wildfires using GOSAT data. *Environmental Pollution (Barking, Essex: 1987)*, *226*, 60–68. https://doi.org/10.1016/j.envpol.2017.04.014

Hardiman, C. A., Weingarten, R. A., Conlan, S., Khil, P., Dekker, J. P., Mathers, A. J., Sheppard, A. E., Segre, J. A., & Frank, K. M. (2016). Horizontal Transfer of Carbapenemase-Encoding Plasmids and Comparison with Hospital Epidemiology Data. *Antimicrobial Agents and Chemotherapy*, *60*(8), 4910–4919. https://doi.org/10.1128/AAC.00014-16

Hart, S. C., DeLuca, T. H., Newman, G. S., MacKenzie, M. D., & Boyle, S. I. (2005). Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. *Forest Ecology and Management*, *220*(1), 166–184. https://doi.org/10.1016/j.foreco.2005.08.012

He, J.-Z., Shen, J.-P., Zhang, L.-M., & Di, H. J. (2012). A review of ammonia-oxidizing bacteria and archaea in Chinese soils. *Frontiers in Microbiology*, *3*, 296. https://doi.org/10.3389/fmicb.2012.00296

Hedayatianfard, K., Akhlaghi, M., & Sharifiyazdi, H. (2014). Detection of tetracycline resistance genes in bacteria isolated from fish farms using polymerase chain reaction. *Veterinary Research Forum*, *5*(4), 269–275.

Hu, X., Ban, Y., & Nascetti, A. (2021). Sentinel-2 MSI data for active fire detection in major fire-prone biomes: A multi-criteria approach. *International Journal of Applied Earth Observation and Geoinformation*, *101*, 102347. https://doi.org/10.1016/j.jag.2021.102347

Huang, Y., Zhao, N., He, L., Wang, L., Liu, Z., You, M., & Guan, F. (2005). Arthrobacter scleromae sp. Nov. Isolated from Human Clinical Specimens. *Journal of Clinical Microbiology*, *43*(3), 1451–1455. https://doi.org/10.1128/JCM.43.3.1451-1455.2005

Ice, G. G., Neary, D. G., & Adams, P. W. (2004). Effects of Wildfire on Soils and Watershed Processes. *Journal of Forestry*, *102*(6), 16–20. https://doi.org/10.1093/jof/102.6.16

Igiri, B. E., Okoduwa, S. I. R., Idoko, G. O., Akabuogu, E. P., Adeyi, A. O., & Ejiogu, I. K. (2018). Toxicity and Bioremediation of Heavy Metals Contaminated Ecosystem from Tannery Wastewater: A Review. *Journal of Toxicology*, *2018*, e2568038. https://doi.org/10.1155/2018/2568038

Ikoyi, I., Fowler, A., & Schmalenberger, A. (2018). One-time phosphate fertilizer application to grassland columns modifies the soil microbiota and limits its role in ecosystem services. *The Science of the Total Environment*, *630*, 849–858. https://doi.org/10.1016/j.scitotenv.2018.02.263

Imhoff, J. F. (2014). The Family Chromatiaceae. In E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, & F. Thompson (Eds.), *The Prokaryotes: Gammaproteobacteria* (pp. 151–178). Springer. https://doi.org/10.1007/978-3-642-38922-1_295

Jansson, J. K., & Hofmockel, K. S. (2020). Soil microbiomes and climate change. *Nature Reviews Microbiology*, *18*(1), 35–46. https://doi.org/10.1038/s41579-019-0265-7

Jensen, L. B., Frimodt-Møller, N., & Aarestrup, F. M. (1999). Presence of erm gene classes in Gram-positive bacteria of animal and human origin in Denmark. *FEMS Microbiology Letters*, *170*(1), 151–158. https://doi.org/10.1111/j.1574-6968.1999.tb13368.x

Johnson, L. A., & Hug, L. A. (2019). Distribution of reactive oxygen species defense mechanisms across domain bacteria. *Free Radical Biology and Medicine*, *140*, 93–102. https://doi.org/10.1016/j.freeradbiomed.2019.03.032

Johnston, C. A., Breshears, D. D., Cardon, Z. G., Currie, W., Emanuel, W., Gaudinski, J., Jackson, R. B., Lajtha, K., Nadelhoffer, K., Nelson Jr., D., Post, W. M., Retallack, G., & Wielopolski, L. (2004). *Carbon cycling in soil*. https://esajournals.onlinelibrary.wiley.com/doi/abs/10.1890/1540-9295%282004%29002%5B0522%3ACCIS%5D2.0.CO%3B2

Kara, O., & Bolat, I. (2009). Short-term effects of wildfire on microbial biomass and abundance in black pine plantation soils in Turkey. *Ecological Indicators*, *9*(6), 1151–1155. https://doi.org/10.1016/j.ecolind.2009.01.002

Keane, R. E., & Karau, E. (2010). Evaluating the ecological benefits of wildfire by integrating fire and ecosystem simulation models. *Ecological Modelling*, *221*(8), 1162–1172. https://doi.org/10.1016/j.ecolmodel.2010.01.008

Keeley, J. E. (2008). Fire. In S. E. Jørgensen & B. D. Fath (Eds.), *Encyclopedia of Ecology* (pp. 1557–1564). Academic Press. https://doi.org/10.1016/B978-008045405-4.00496-1

Kehler, A., Haygarth, P., Tamburini, F., & Blackwell, M. (2021). Cycling of reduced phosphorus compounds in soil and potential impacts of climate change. *European Journal of Soil Science*, *n/a*(n/a). https://doi.org/10.1111/ejss.13121

Kehrenberg, C., & Schwarz, S. (2006). Distribution of Florfenicol Resistance Genes fexA and cfr among Chloramphenicol-Resistant Staphylococcus Isolates. *Antimicrobial Agents and Chemotherapy*, *50*(4), 1156–1163. https://doi.org/10.1128/AAC.50.4.1156-1163.2006

Killpack, S. C., & Buchholz, D. (1993). *Nitrogen in the Environment: Nitrogen Cycle*. Extension - University of Missouri. https://extension.missouri.edu/publications/wq252

Kincade Fire. (2020, March 3). CAL FIRE. https://www.fire.ca.gov/incidents/2019/10/23/kincade-fire/

King, M. J., Davenport, W. G., & Moats, M. S. (2013). 3—Sulfur burning. In M. J. King, W. G. Davenport, & M. S. Moats (Eds.), *Sulfuric Acid Manufacture (Second Edition)* (pp. 19–29). Elsevier. https://doi.org/10.1016/B978-0-08-098220-5.00003-4

Knicker, H. (2007). How does fire affect the nature and stability of soil organic nitrogen and carbon? A review. *Biogeochemistry*, *85*(1), 91–118. https://doi.org/10.1007/s10533-007-9104-4

Köster, K., Aaltonen, H., Berninger, F., Heinonsalo, J., Köster, E., Ribeiro-Kumara, C., Sun, H., Tedersoo, L., Zhou, X., & Pumpanen, J. (2021). Impacts of wildfire on soil microbiome in Boreal environments. *Current Opinion in Environmental Science & Health*, *22*, 100258. https://doi.org/10.1016/j.coesh.2021.100258

Kountouris, Y. (2020). Human activity, daylight saving time and wildfire occurrence. *The Science of the Total Environment*, *727*, 138044. https://doi.org/10.1016/j.scitotenv.2020.138044

Kranz, C., & Whitman, T. (2019). Short communication: Surface charring from prescribed burning has minimal effects on soil bacterial community composition two weeks post-fire in jack pine barrens. *Applied Soil Ecology*, *144*, 134–138. https://doi.org/10.1016/j.apsoil.2019.07.004

Kuypers, M. M. M., Marchant, H. K., & Kartal, B. (2018). The microbial nitrogen-cycling network. *Nature Reviews Microbiology*, *16*(5), 263–276. https://doi.org/10.1038/nrmicro.2018.9

Li, B., Yin, F., Zhao, X., Guo, Y., Wang, W., Wang, P., Zhu, H., Yin, Y., & Wang, X. (2020). Colistin Resistance Gene mcr-1 Mediates Cell Permeability and Resistance to Hydrophobic Antibiotics. *Frontiers in Microbiology*, *10*, 3015. https://doi.org/10.3389/fmicb.2019.03015

Li, J., Gao, R., Chen, Y., Xue, D., Han, J., Wang, J., Dai, Q., Lin, M., Ke, X., & Zhang, W. (2020). Isolation and Identification of Microvirga thermotolerans HR1, a Novel Thermo-Tolerant Bacterium, and Comparative Genomics among Microvirga Species. *Microorganisms*, *8*(1), 101. https://doi.org/10.3390/microorganisms8010101

Li, Y., Zhang, J., Chen, Q., Yang, G., Cai, S., He, J., Zhou, S., & Li, S.-P. (2013). Dokdonella kunshanensis sp. Nov., isolated from activated sludge, and emended description of the genus Dokdonella. *International Journal of Systematic and Evolutionary Microbiology*, *63*(Pt 4), 1519–1523. https://doi.org/10.1099/ijs.0.041798-0

Liodakis, S., & Tsoukala, M. (2009). Ash Leaching of Forest Species Treated with Phosphate Fire Retardants. *Water, Air, and Soil Pollution*, *199*(1–4), 171–182. https://doi.org/10.1007/s11270-008-9869-7

Liu, X., Zhang, S., Jiang, Q., Bai, Y., Shen, G., Li, S., & Ding, W. (2016). Using community analysis to explore bacterial indicators for disease suppression of tobacco bacterial wilt. *Scientific Reports*, *6*(1), 36773. https://doi.org/10.1038/srep36773

Long, K. S., Poehlsgaard, J., Kehrenberg, C., Schwarz, S., & Vester, B. (2006). The Cfr rRNA Methyltransferase Confers Resistance to Phenicols, Lincosamides, Oxazolidinones, Pleuromutilins, and Streptogramin A Antibiotics. *Antimicrobial Agents and Chemotherapy*, *50*(7), 2500–2505. https://doi.org/10.1128/AAC.00131-06

Mahendra, S., & Alvarez-Cohen, L. (2005). Pseudonocardia dioxanivorans sp. Nov., a novel actinomycete that grows on 1,4-dioxane. *International Journal of Systematic and Evolutionary Microbiology*, *55*(2), 593–598. https://doi.org/10.1099/ijs.0.63085-0

Maquia, I. S. A., Fareleira, P., Castro, I. F. e., Soares, R., Brito, D. R. A., Mbanze, A. A., Chaúque, A., Máguas, C., Ezeokoli, O. T., Ribeiro, N. S., Marques, I., & Ribeiro-Barros, A. I. (2021). The Nexus between Fire and Soil Bacterial Diversity in the African Miombo Woodlands of Niassa Special Reserve, Mozambique. *Microorganisms*, *9*(8). https://www.mdpi.com/2076-2607/9/8/1562/htm

McIlroy, S. J., Starnawska, A., Starnawski, P., Saunders, A. M., Nierychlo, M., Nielsen, P. H., & Nielsen, J. L. (2016). Identification of active denitrifiers in full-scale nutrient removal wastewater treatment systems. *Environmental Microbiology*, *18*(1), 50–64. https://doi.org/10.1111/1462-2920.12614

Mittal, D., Shukla, R., Verma, S., Sagar, A., Verma, K. S., Pandey, A., Negi, Y. S., Saini, R. V., & Saini, A. K. (2019). Fire in pine grown regions of Himalayas depletes cultivable plant growth promoting beneficial microbes in the soil. *Applied Soil Ecology*, *139*, 117–124. https://doi.org/10.1016/j.apsoil.2019.03.020

Moreira, H., Pereira, S. I. A., Vega, A., Castro, P. M. L., & Marques, A. P. G. C. (2020). Synergistic effects of arbuscular mycorrhizal fungi and plant growth-promoting bacteria benefit maize growth under increasing soil salinity. *Journal of Environmental Management*, *257*, 109982. https://doi.org/10.1016/j.jenvman.2019.109982

Moritz, M. A., Parisien, M.-A., Batllori, E., Krawchuk, M. A., Dorn, J. V., Ganz, D. J., & Hayhoe, K. (2012). Climate change and disruptions to global fire activity. *Ecosphere*, *3*(6), art49. https://doi.org/10.1890/ES11-00345.1

Moya, D., Fonturbel, M. T., Lucas-Borja, M. E., Peña, E., Alfaro-Sanchez, R., Plaza-Álvarez, P. A., González-Romero, J., & de Las Heras, J. (2021). Burning season and vegetation coverage influenced the community-level physiological profile of Mediterranean mixed-mesogean pine forest soils. *Journal of Environmental Management*, *277*, 111405. https://doi.org/10.1016/j.jenvman.2020.111405

Mulholl, M. A. 26876, Calabasas, H., & Us, C. 91302 P.-2301 C. (2015a, August 7). *Mediterranean Ecosystem—Santa Monica Mountains National Recreation Area (U.S. National Park Service)*. https://www.nps.gov/samo/learn/nature/mediterraneanecosystem.htm

Mulholl, M. A. 26876, Calabasas, H., & Us, C. 91302 P.-2301 C. (2015b, August 13). *Geologic Formations—Santa Monica Mountains National Recreation Area (U.S. National Park Service)*. https://www.nps.gov/samo/learn/nature/geologicformations.htm

National Interagency Fire Center. (2021). https://www.nifc.gov/fireInfo/fireInfo_stats_totalFires.html

Natwora, K. E., & Sheik, C. S. (2021). Assessment of nitrogen fixation rates in the Laurentian Great Lakes. *Journal of Great Lakes Research*. https://doi.org/10.1016/j.jglr.2021.07.005

Neff, J. C., Harden, J. W., & Gleixner, G. (2005). Fire effects on soil organic matter content, composition, and nutrients in boreal interior Alaska. *Canadian Journal of Forest Research*, *35*(9), 2178–2187. https://doi.org/10.1139/x05-154

Nelson, A. R., Narrowe, A. B., Rhoades, C. C., Fegel, T. S., Daly, R. A., Roth, H. K., Chu, R. K., Amundson, K. K., Geonczy, S. E., Emerson, J. B., Young, R. B., Steindorff, A. S., Mondo, S. J., Grigoriev, I. V., Salamov, A., Borch, T., & Wilkins, M. J. (2021). *Playing with FiRE: A genome resolved view of the soil microbiome responses to high severity forest wildfire* (p. 2021.08.17.456416). https://doi.org/10.1101/2021.08.17.456416

Normand, P. (2006). The Families Frankiaceae, Geodermatophilaceae, Acidothermaceae and Sporichthyaceae. In M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, & E. Stackebrandt (Eds.), *The Prokaryotes: Volume 3: Archaea. Bacteria: Firmicutes, Actinomycetes* (pp. 669–681). Springer. https://doi.org/10.1007/0-387-30743-5_26

Norton, J., & Ouyang, Y. (2019). Controls and Adaptive Management of Nitrification in Agricultural Soils. *Frontiers in Microbiology*, *10*, 1931. https://doi.org/10.3389/fmicb.2019.01931

Oh, W. T., Giri, S. S., Yun, S., Kim, H. J., Kim, S. G., Kim, S. W., Kang, J. W., Han, S. J., Kwon, J., Jun, J. W., & Park, S. C. (2019). Janthinobacterium lividum as An Emerging Pathogenic Bacterium Affecting Rainbow Trout (Oncorhynchus mykiss) Fisheries in Korea. *Pathogens*, *8*(3), 146. https://doi.org/10.3390/pathogens8030146

Osei Sekyere, J. (2018). Mcr colistin resistance gene: A systematic review of current diagnostics and detection methods. *MicrobiologyOpen*, *8*(4), e00682. https://doi.org/10.1002/mbo3.682

Parson, A., Robichaud, P. R., Lewis, S. A., Napper, C., & Clark, J. T. (2010). *Field guide for mapping post-fire soil burn severity* (RMRS-GTR-243; p. RMRS-GTR-243). U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. https://doi.org/10.2737/RMRS-GTR-243

Patel, K. G., & Pyrsopoulos, N. T. (2019). Chapter 28—The Microbiome and Metabolome in Alcoholic Liver Disease. In J. Faintuch & S. Faintuch (Eds.), *Microbiome and Metabolome in Diagnosis, Therapy, and other Strategic Applications* (pp. 271–277). Academic Press. https://doi.org/10.1016/B978-0-12-815249-2.00028-2

Patel, V. C., & Williams, R. (2019). Antimicrobial resistance in chronic liver disease. *Hepatology International*, *14*(1), 24–34. https://doi.org/10.1007/s12072-019-10004-1

Pausas, J. G., & Bond, W. J. (2020). On the Three Major Recycling Pathways in Terrestrial Ecosystems. *Trends in Ecology & Evolution*, *35*(9), 767–775. https://doi.org/10.1016/j.tree.2020.04.004

Pei, R., Kim, S.-C., Carlson, K. H., & Pruden, A. (2006). Effect of River Landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). *Water Research*, *40*(12), 2427–2435. https://doi.org/10.1016/j.watres.2006.04.017

Peninsula Daily News. (2016, August 26). *Hayes Fire eats another 1,500 acres of Olympic National Park*. Peninsula Daily News. https://www.peninsuladailynews.com/news/hayes-fire-eats-another-1500-acres-of-olympic-national-park/

Pereira, P., Bogunovic, I., Zhao, W., & Barcelo, D. (2021). Short-term effect of wildfires and prescribed fires on ecosystem services. *Current Opinion in Environmental Science & Health*, *22*, 100266. https://doi.org/10.1016/j.coesh.2021.100266

Plumlee, G. S., Martin, D. A., Hoefen, T., Kokaly, R., Hageman, P., Eckberg, A., Meeker, G. P., Adams, M., Anthony, M., & Lamothe, P. J. (2007). *Open-File Report* (Open-File Report) [Open-File Report]. https://pubs.usgs.gov/of/2007/1407/pdf/OF07-1407_508.pdf

Polianciuc, S. I., Gurzău, A. E., Kiss, B., Ştefan, M. G., & Loghin, F. (2020). Antibiotics in the environment: Causes and consequences. *Medicine and Pharmacy Reports*, *93*(3), 231–240. https://doi.org/10.15386/mpr-1742

Qin, Q., & Liu, Y. (2021). Changes in microbial communities at different soil depths through the first rainy season following severe wildfire in North China artificial Pinus tabulaeformis forest. *Journal of Environmental Management*, *280*, 111865. https://doi.org/10.1016/j.jenvman.2020.111865

Robichaud, P. R., Beyers, J. L., & Neary, D. G. (2000). *Evaluating the effectiveness of postfire rehabilitation treatments* (RMRS-GTR-63; p. RMRS-GTR-63). U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. https://doi.org/10.2737/RMRS-GTR-63

Rochelmeyer, E., Stefano, J., Dorph, A., & Swan, M. (2019). Linking fuel, habitat and ground-dwelling mammals in flammable landscapes. *Forest Ecology and Management*, *441*, 215–228. https://doi.org/10.1016/j.foreco.2019.03.021

Romaniuk, K., Golec, P., & Dziewit, L. (2018). Insight Into the Diversity and Possible Role of Plasmids in the Adaptation of Psychrotolerant and Metalotolerant Arthrobacter spp. To Extreme Antarctic Environments. *Frontiers in Microbiology*, *9*, 3144. https://doi.org/10.3389/fmicb.2018.03144

Rubin-Blum, M., Dubilier, N., & Kleiner, M. (2019). Genetic Evidence for Two Carbon Fixation Pathways (the Calvin-Benson-Bassham Cycle and the Reverse Tricarboxylic Acid Cycle) in Symbiotic and Free-Living Bacteria. *MSphere*, *4*(1), e00394-18. https://doi.org/10.1128/mSphere.00394-18

Sallam, A., & Steinbüchel, A. (2009). Clostridium sulfidigenes sp. Nov., a mesophilic, proteolytic, thiosulfate- and sulfur-reducing bacterium isolated from pond sediment. *International Journal of Systematic and Evolutionary Microbiology*, *59*(Pt 7), 1661–1665. https://doi.org/10.1099/ijs.0.004986-0

Sánchez-Hidalgo, M., González, I., Díaz-Muñoz, C., Martínez, G., & Genilloud, O. (2018). Comparative Genomics and Biosynthetic Potential Analysis of Two Lichen-Isolated Amycolatopsis Strains. *Frontiers in Microbiology*, *9*, 369. https://doi.org/10.3389/fmicb.2018.00369

Santín, C., & Doerr, S. H. (2016). Fire effects on soils: The human dimension. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1696), 20150171. https://doi.org/10.1098/rstb.2015.0171

Sawyer, R., Bradstock, R., Bedward, M., & Morrison, R. J. (2018). Fire intensity drives post-fire temporal pattern of soil carbon accumulation in Australian fire-prone forests. *Science of The Total Environment*, *610–611*, 1113–1124. https://doi.org/10.1016/j.scitotenv.2017.08.165

Sayavedra, L., Li, T., Bueno Batista, M., Seah, B. K. B., Booth, C., Zhai, Q., Chen, W., & Narbad, A. (2021). Desulfovibrio diazotrophicus sp. Nov., a sulfate-reducing bacterium from the human gut capable of nitrogen fixation. *Environmental Microbiology*, *23*(6), 3164–3181. https://doi.org/10.1111/1462-2920.15538

Schäfer, H. (2007). Isolation of Methylophaga spp. From Marine Dimethylsulfide-Degrading Enrichment Cultures and Identification of Polypeptides Induced during Growth on Dimethylsulfide. *Applied and Environmental Microbiology*, *73*(8), 2580–2591. https://doi.org/10.1128/AEM.02074-06

Schmid, M., Walsh, K., Webb, R., Rijpstra, W. I., van de Pas-Schoonen, K., Verbruggen, M. J., Hill, T., Moffett, B., Fuerst, J., Schouten, S., Sinninghe Damsté, J. S., Harris, J., Shaw, P., Jetten, M., & Strous, M. (2003). Candidatus "Scalindua brodae", sp. Nov., Candidatus "Scalindua wagneri", sp. Nov., Two New Species of Anaerobic Ammonium Oxidizing Bacteria. *Systematic and Applied Microbiology*, *26*(4), 529–538. https://doi.org/10.1078/072320203770865837

Seiler, C., & Berendonk, T. (2012). Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Frontiers in Microbiology*, *3*, 399. https://doi.org/10.3389/fmicb.2012.00399

Shakesby, R. A. (2011). Post-wildfire soil erosion in the Mediterranean: Review and future research directions. *Earth-Science Reviews*, *105*(3), 71–100. https://doi.org/10.1016/j.earscirev.2011.01.001

Sheppard, R. J., Beddis, A. E., & Barraclough, T. G. (2020). The role of hosts, plasmids and environment in determining plasmid transfer rates: A meta-analysis. *Plasmid*, *108*, 102489. https://doi.org/10.1016/j.plasmid.2020.102489

Singh, B. K., & Walker, A. (2006, May 1). *Microbial degradation of organophosphorus compounds*. https://academic.oup.com/femsre/article/30/3/428/548351

Singleton, D. R., Furlong, M. A., Peacock, A. D., White, D. C., Coleman, D. C., & Whitman, W. B. (2003). Solirubrobacter pauli gen. Nov., sp. Nov., a mesophilic bacterium within the Rubrobacteridae related to common soil clones. *International Journal of Systematic and Evolutionary Microbiology*, *53*(2), 485–490. https://doi.org/10.1099/ijs.0.02438-0

Sköld, O. (2000). Sulfonamide resistance: Mechanisms and trends | Elsevier Enhanced Reader. *Drug Resistance Updates*, *3*(3), 155–160. https://doi.org/10.1054/drup.2000.0146

Smith, J. K. (2000). *Wildland fire in ecosystems: Effects of fire on fauna* (RMRS-GTR-42-V1; p. RMRS-GTR-42-V1). U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. https://doi.org/10.2737/RMRS-GTR-42-V1

Smith, N. R., Kishchuk, B. E., & Mohn, W. W. (2008). Effects of Wildfire and Harvest Disturbances on Forest Soil Bacterial Communities. *Applied and Environmental Microbiology*, *74*(1), 216–224. https://doi.org/10.1128/AEM.01355-07

Sousa, W. P. (1984). The Role of Disturbance in Natural Communities. *Annual Review of Ecology and Systematics*, *15*, 353–391.

Takaya, N., Catalan-Sakairi, M. A. B., Sakaguchi, Y., Kato, I., Zhou, Z., & Shoun, H. (2003). Aerobic Denitrifying Bacteria That Produce Low Levels of Nitrous Oxide. *Applied and Environmental Microbiology*, *69*(6), 3152–3157. https://doi.org/10.1128/AEM.69.6.3152-3157.2003

Tang, K., Baskaran, V., & Nemati, M. (2009). Bacteria of the sulphur cycle: An overview of microbiology, biokinetics and their role in petroleum and mining industries. *Biochemical Engineering Journal*, *44*(1), 73–94. https://doi.org/10.1016/j.bej.2008.12.011

Tang, S., Liao, Y., Xu, Y., Dang, Z., Zhu, X., & Ji, G. (2020). Microbial coupling mechanisms of nitrogen removal in constructed wetlands: A review. *Bioresource Technology*, *314*, 123759. https://doi.org/10.1016/j.biortech.2020.123759

Taussig, L. M., Landau, L., Le Souëf, P., Morgan, W. J., Martinez, F., & Sly, P. D. (Eds.). (2008). *Pediatric respiratory medicine* (Second Edition). Mosby, Elsevier.

Terzano, R., Rascio, I., Allegretta, I., Porfido, C., Spagnuolo, M., Khanghahi, M. Y., Crecchio, C., Sakellariadou, F., & Gattullo, C. E. (2021). Fire effects on the distribution and bioavailability of potentially toxic elements (PTEs) in agricultural soils. *Chemosphere*, *281*, 130752. https://doi.org/10.1016/j.chemosphere.2021.130752

Thawai, C. 2018. (2018). Pseudonocardia soli sp. Nov., isolated from mountain soil. *International Journal of Systematic and Evolutionary Microbiology*, *68*(4), 1307–1312. https://doi.org/10.1099/ijsem.0.002672

Thomas, D. (2017). The Costs and Losses of Wildfires: A Literature Survey. *NIST Special Publication*, 72.

Too, C. C., Ong, K. S., Yule, C. M., & Keller, A. (2021). Putative roles of bacteria in the carbon and nitrogen cycles in a tropical peat swamp forest. *Basic and Applied Ecology*, *52*, 109–123. https://doi.org/10.1016/j.baae.2020.10.004

Tubbs Fire. (2019, October 25). CAL FIRE. https://www.fire.ca.gov/incidents/2017/10/8/tubbs-fire-central-lnu-complex/

Ul-Hassan, A. (2009). *Encyclopedia of Microbiology* (Third).

van Miert, A. S. (1994). The sulfonamide-diaminopyrimidine story. *Journal of Veterinary Pharmacology and Therapeutics*, *17*(4), 309–316. https://doi.org/10.1111/j.1365-2885.1994.tb00251.x

Vázquez, F. J., Acea, M. J., & Carballas, T. (1993). Soil microbial populations after wildfire. *FEMS Microbiology Ecology*, *13*(2), 93–103. https://doi.org/10.1016/0168-6496(93)90027-5

Vieira, S., Sikorski, J., Dietz, S., Herz, K., Schrumpf, M., Bruelheide, H., Scheel, D., Friedrich, M. W., & Overmann, J. (2020). Drivers of the composition of active rhizosphere bacterial communities in temperate grasslands. *The ISME Journal*, *14*(2), 463–475. https://doi.org/10.1038/s41396-019-0543-4

von Wintersdorff, C. J. H., Penders, J., van Niekerk, J. M., Mills, N. D., Majumder, S., van Alphen, L. B., Savelkoul, P. H. M., & Wolffs, P. F. G. (2016). (PDF) Dissemination of Antimicrobial Resistance in Microbial Ecosystems through Horizontal Gene Transfer. *Frontiers in Microbiology*, *7*(173), 1–10. https://doi.org/10.3389/fmicb.2016.00173

Wan, W., Li, X., Han, S., Wang, L., Luo, X., Chen, W., & Huang, Q. (2020). Soil aggregate fractionation and phosphorus fraction driven by long-term fertilization regimes affect the abundance and composition of P-cycling-related bacteria. *Soil and Tillage Research*, *196*, 104475. https://doi.org/10.1016/j.still.2019.104475

Wang, D., Li, T., Huang, K., He, X., & Zhang, X.-X. (2019). Roles and correlations of functional bacteria and genes in the start-up of simultaneous anammox and denitrification system for enhanced nitrogen removal. *The Science of the Total Environment*, *655*, 1355–1363. https://doi.org/10.1016/j.scitotenv.2018.11.321

Wang, N., Yang, X., Jiao, S., Zhang, J., Ye, B., & Gao, S. (2014). Sulfonamide-Resistant Bacteria and Their Resistance Genes in Soils Fertilized with Manures from Jiangsu Province, Southeastern China. *PLOS ONE*, *9*(11), e112626. https://doi.org/10.1371/journal.pone.0112626

Wang, S., Yu, H., Su, Q., & Zuo, J. (2021). Exploring the role of heterotrophs in partial nitritation-anammox process treating thermal hydrolysis process—Anaerobic digestion reject water. *Bioresource Technology*, *341*, 125762. https://doi.org/10.1016/j.biortech.2021.125762

Wang, Y., Huang, Q., Gao, H., Zhang, R., Yang, L., Guo, Y., Li, H., Awasthi, M. K., & Li, G. (2021). Long-term cover crops improved soil phosphorus availability in a rain-fed apple orchard. *Chemosphere*, *275*, 130093. https://doi.org/10.1016/j.chemosphere.2021.130093

Weisblum, B. (1995). Erythromycin resistance by ribosome modification. *Antimicrobial Agents and Chemotherapy*, *39*(3), 557–585. https://doi.org/10.1128/AAC.39.3.577

Westerling, A. L., Hidalgo, H. G., Cayan, D. R., & Swetnam, T. W. (2006). Warming and Earlier Spring Increase Western U.S. Forest Wildfire Activity. *Science*, *313*(5789), 940–943. https://doi.org/10.1126/science.1128834

Whitman, T., Whitman, E., Woolet, J., Flannigan, M. D., Thompson, D. K., & Parisien, M.-A. (2019). Soil bacterial and fungal response to wildfires in the Canadian boreal forest across a burn severity gradient. *Soil Biology and Biochemistry*, *138*, 107571. https://doi.org/10.1016/j.soilbio.2019.107571

Wijetunga, N. A., Belbin, T. J., Burk, R. D., Whitney, K., Abadi, M., Greally, J. M., Einstein, M. H., & Schlecht, N. F. (2016). Novel epigenetic changes in CDKN2A are associated with progression of cervical intraepithelial neoplasia. *Gynecologic Oncology*, *142*(3), 566–573. https://doi.org/10.1016/j.ygyno.2016.07.006

Williams Flats Fire. (2019, August 25). Statesman Journal. https://data.statesmanjournal.com/fires/incident/6493/williams-flats-fire/

Woolsey Fire. (2021). https://www.fire.ca.gov/incidents/2018/11/8/woolsey-fire/

Wu, Z., He, H. S., Liu, Z., & Liang, Y. (2013). Comparing fuel reduction treatments for reducing wildfire size and intensity in a boreal forest landscape of northeastern China. *Science of The Total Environment*, *454–455*, 30–39. https://doi.org/10.1016/j.scitotenv.2013.02.058

Xiang, X., Shi, Y., Yang, J., Kong, J., Lin, X., Zhang, H., Zeng, J., & Chu, H. (2014). Rapid recovery of soil bacterial communities after wildfire in a Chinese boreal forest. *Scientific Reports*, *4*, 3829. https://doi.org/10.1038/srep03829

Xu, H., Miao, V., Kwong, R., & Xia, J. D. (2011). Identification of a novel fosfomycin resistance gene (fosA2) in Enterobacter cloacae from the Salmon River, Canada. *Letters in Applied Microbiology*. https://sfamjournals.onlinelibrary.wiley.com/doi/full/10.1111/j.1472-765X.2011.03016.x

Xu, H., Yu, M., & Cheng, X. (2021). Abundant fungal and rare bacterial taxa jointly reveal soil nutrient cycling and multifunctionality in uneven-aged mixed plantations. *Ecological Indicators*, *129*, 107932. https://doi.org/10.1016/j.ecolind.2021.107932

Xu, L., Sun, C., Huang, M., Wu, Y.-H., Yuan, C.-Q., Dai, W.-H., Ye, K., Han, B., & Xu, X.-W. (2019). Complete genome sequence of Euzebya sp. DY32-46, a marine Actinobacteria isolated from the Pacific Ocean. *Marine Genomics*, *44*, 65–69. https://doi.org/10.1016/j.margen.2018.09.008

Xu, Y., Sun, J., Lin, Q., Ma, J., Shi, Y., & Lou, K. (2012). Effects of a surface wildfire on soil nutrient and microbial functional diversity in a shrubbery. *Acta Ecologica Sinica*, *32*(5), 258–264. https://doi.org/10.1016/j.chnaes.2012.07.007

Yadav, A. N., Kour, D., Kaur, T., Devi, R., Yadav, A., Dikilitas, M., Abdel-Azeem, A. M., Ahluwalia, A. S., & Saxena, A. K. (2021). Biodiversity, and biotechnological contribution of beneficial soil

microbiomes for nutrient cycling, plant growth improvement and nutrient uptake. *Biocatalysis and Agricultural Biotechnology*, *33*, 102009. https://doi.org/10.1016/j.bcab.2021.102009

Yang, Y., Diao, M., Shi, W., Li, L., Dai, Q., & Qiu, G. (2007). Isolation and characterization of organic-sulfur degradation bacterial strain. *Journal of Central South University of Technology*, *14*(3), 324–329. https://doi.org/10.1007/s11771-007-0064-6

Yogabaanu, U., Weber, J.-F. F., Convey, P., Rizman-Idid, M., & Alias, S. A. (2017). Antimicrobial properties and the influence of temperature on secondary metabolite production in cold environment soil fungi. *Polar Science*, *14*, 60–67. https://doi.org/10.1016/j.polar.2017.09.005

Yu, P., He, X., Baer, M., Beirinckx, S., Tian, T., Moya, Y. A. T., Zhang, X., Deichmann, M., Frey, F. P., Bresgen, V., Li, C., Razavi, B. S., Schaaf, G., von Wirén, N., Su, Z., Bucher, M., Tsuda, K., Goormachtig, S., Chen, X., & Hochholdinger, F. (2021). Plant flavones enrich rhizosphere Oxalobacteraceae to improve maize performance under nitrogen deprivation. *Nature Plants*, *7*(4), 481–499. https://doi.org/10.1038/s41477-021-00897-y

Zeng, Q., Mei, T., Delgado-Baquerizo, M., Wang, M., & Tan, W. (2022). Suppressed phosphorus-mineralizing bacteria after three decades of fertilization. *Agriculture, Ecosystems & Environment*, *323*, 107679. https://doi.org/10.1016/j.agee.2021.107679

Zhang, M.-S., Li, W., Zhang, W.-G., Li, Y.-T., Li, J.-Y., & Gao, Y. (2021). Agricultural land-use change exacerbates the dissemination of antibiotic resistance genes via surface runoffs in Lake Tai Basin, China. *Ecotoxicology and Environmental Safety*, *220*, 112328. https://doi.org/10.1016/j.ecoenv.2021.112328

Zhang, Y., Yan, C., Liu, H., Pu, S., Chen, H., Zhou, B., Yuan, R., & Wang, F. (2021). Bacterial response to soil property changes caused by wood ash from wildfire in forest soils around mining areas:

Relevance of bacterial community composition, carbon and nitrogen cycling. *Journal of Hazardous Materials*, *412*, 125264. https://doi.org/10.1016/j.jhazmat.2021.125264

Zhao, J., Feng, L., Dai, J., Yang, G., & Mu, J. (2017). Characteristics of nitrogen removal and microbial community in biofilm system via combination of pretreated lignocellulosic carriers and various conventional fillers. *Biodegradation*, *28*(5–6), 337–349. https://doi.org/10.1007/s10532-017-9800-2

Zhao, W., Zhong, Y., Yuan, H., Wang, J., Zheng, H., Wang, Y., Cen, X., Xu, F., Bai, J., Han, X., Lu, G., Zhu, Y., Shao, Z., Yan, H., Li, C., Peng, N., Zhang, Z., Zhang, Y., Lin, W., … Zhao, G.-P. (2010). Complete genome sequence of the rifamycin SV-producing Amycolatopsis mediterranei U32 revealed its genetic characteristics in phylogeny and metabolism. *Cell Research*, *20*(10), 1096–1108. https://doi.org/10.1038/cr.2010.87

Zhou, X., Sun, H., Sietiö, O.-M., Pumpanen, J., Heinonsalo, J., Köster, K., & Berninger, F. (2020). Wildfire effects on soil bacterial community and its potential functions in a permafrost region of Canada. *Applied Soil Ecology*, *156*, 103713. https://doi.org/10.1016/j.apsoil.2020.103713

APPENDIX

APPENDIX ONE: “DNA CONCENTRATIONS”

Table 6. DNA concentrations from wildfire affected soils. Mixed samples are vertically homogenized.

Fire	Burn Intensity	Sample Location	Sample Type	Sample ID	DNA Concentration (Qubit; ng/ul)	Average Concentration (ng/ul)
Woolsey	Control	Saddle Peak	Mixed	40	142	55.6
		Calabassas Peak		41	13.6	
		Encinal Canyon Road		42	11.1	
		Calabassas Peak	Topsoil	34	39	32.4
		Saddle Peak		38	15.1	
		Encinal Canyon Road		39	43.2	
		Encinal Canyon Road	Bottom soil	35	51.4	77.2
		Saddle Peak		36	106	
		Calabassas Peak		37	74.2	
	Low	Cheeseboro Canyon	Mixed	94	102	120
				102	160	
				108	98.2	
			Topsoil	95	154	131.47
				97	159	
				101	81.4	
			Bottom soil	93	98	117
				98	149	
				107	104	
		Laskey Mesa	Mixed	91	13.1	12.3
				100	13.6	
				106	10.2	
			Topsoil	92	10.5	31.9
				96	39.6	
				99	45.6	

			Bottom soil	103	9.76	27.1
				104	40.2	
				105	31.2	
	Moderate	Arroyo Sequit	Mixed	44	34.2	93.6
				45	160	
				47	86.6	
			Topsoil	48	132	65.1
				50	36.6	
				51	26.8	
			Bottom soil	43	128	101
				46	69.2	
				49	106	
		Corral Canyon	Mixed	70	38.6	23.6
				71	9.82	
				72	22.4	
			Topsoil	54	62.8	40.9
				56	39	
				63	21	
			Bottom soil	58	28.4	17.7
				61	10.4	
				67	14.2	
		Kanan Dume	Mixed	62	123	72.2
				66	25.6	
				69	68	
			Topsoil	109	56.2	42.8
				112	36	
				113	36.2	
			Bottom soil	110	49.8	62.9
				111	57.6	
				114	81.4	
		Paramount Ranch	Mixed	57	11.5	12.1
				64	4.04	
				65	20.8	
			Topsoil	53	174	87.7
				59	79.4	
				60	9.62	
			Bottom soil	52	33.6	16.9
				55	7.44	

				68	9.74	
	High	Corral Canyon	Mixed	75	40.6	55.6
				85	91.2	
				86	35	
			Topsoil	74	57.8	38.4
				76	11.4	
				80	46	
			Bottom soil	77	43.2	29.0
				78	33	
				79	10.8	
		Kanan Dume	Mixed	82	34.4	44.7
				88	56.6	
				90	43	
			Topsoil	84	19.8	47.9
				87	68.4	
				89	55.4	
			Bottom soil	73	3.98	10.8
				81	4.76	
				83	23.8	
Kincade	Unknown	Unknown	Topsoil	1	5.14	34.7
				3	6.26	
				6	92.8	
			Bottom soil	2	2.28	37.2
				4	97.4	
				5	11.9	
Tubbs	High	Unknown	Topsoil	9	20.2	20.4
				10	7.66	
				12	33.4	
			Bottom soil	7	4.68	19.4
				8	17.2	
				11	36.4	
Williams Flats	High	Unknown	Ash	14	1.98	2.12
				17	2.18	
				18	2.2	
			soil: 15 cm	23	30	30.00
			soil: 20-40 cm	21	18.9	18.90

			soil: 50 cm	25	7.02	7.02
			soil: 40-60 cm	24	11.4	11.40
			soil: 80-100 cm	26	7.88	7.88
			soil: 100-120 cm	22	4.08	4.08
			soil: 120-140 cm	29	0.496	0.50
			soil: 140-160 cm	30	0.44	0.44
			soil: 160-180 cm	31	0.328	0.33
			soil: 180-200	32	out of range	out of range
			soil: 200-220	33	out of range	out of range
Mesa	High	Unknown	Ash	13	26	30.4
				15	26	
				16	39.2	
			soil: 15 cm	27	18.6	18.60
			soil: 50 cm	28	2.98	2.98
Northstar	Unknown	Unknown	topsoil	19	10.1	10.10
Hayes	Unknown	Unknown	topsoil	20	2.1	2.10

APPENDIX TWO: "ANTIBIOTIC RESISTANCE GENE DATA"

Table 7. Antibiotic resistance gene (ARG) results for wildfire samples. A "+" indicates ARG presence, while a "+" indicates ARG presence with a low concentration. A "- "indicates no ARG presence in the sample. For samples 1-12, the first letter "K" = Kincade Fire, "T" = Tubbs Fire and the last letter "T" = topsoil, "B" = bottom soil. For samples 13-18, the first letter "M" = Mesa Fire, "F" = Williams Flats Fire and the "Ash" denotes an ash sample. Sample 19 and 20 are topsoil samples from the Northstar (NT) and Hayes (JT) fires, respectively. Samples 21-26 and 29-33 are Williams Flats soil depth samples, with the depths displayed in the sample ID. Samples 27 and 28 are depth samples from the Mesa Fire with depths of 15 and 50 cm, respectively. Samples 34-114 are from the Woolsey Fire. Sample IDs correspond to the map in Figure 2. Letter at end: T = topsoil B = bottom soil, H = vertically homogenized.*

Sample Number	Sample ID	*mcr-1*	*fosA2*	*optrA*	*cfr*	*sul1*	*sul2*	*tetM*	*tetB*	*tetO*	*tetW*	*ermF*
1	KT	-	-	-	-	-	-	-	-	-	-	-
2	KB	-	-	-	-	-	-	-	-	-	-	-
3	KT	-	-	-	-	-	-	-	-	-	-	-
4	KB	-	-	-	-	+	+	-	-	-	-	-
5	KB	-	-	-	-	-	-	-	-	-	-	-
6	KT	-	-	-	-	+	+	-	-	-	-	-
7	TB	-	-	-	-	-	-	-	-	-	-	-
8	TB	-	-	-	-	-	-	-	-	-	-	-
9	TT	-	-	-	-	-	-	-	-	-	-	-
10	TT	-	-	-	-	-	-	-	-	-	-	-
11	TB	-	-	-	-	-	-	-	-	-	-	-
12	TT	-	-	-	-	-	-	-	-	-	-	-
13	MAsh	-	-	-	-	+*	-	-	-	-	-	-
14	FAsh	-	-	-	-	-	-	-	-	-	-	-
15	MAsh	-	-	-	-	+*	-	-	-	-	-	-
16	MAsh	-	-	-	-	+*	-	-	-	-	-	-
17	FAsh	-	-	-	-	-	-	-	-	-	+*	-
18	FAsh	-	-	-	-	-	-	-	-	-	-	-
19	NT	-	-	-	-	-	-	-	-	-	-	-
20	JT	-	-	-	-	-	-	-	-	-	-	-
21	20-40 cm	-	-	+	-	-	-	-	-	-	-	-
22	100-120 cm	-	-	-	-	-	-	-	-	-	-	-
23	15 cm	-	-	+	-	-	-	-	-	-	-	-
24	40-60 cm	-	-	+*	-	-	-	-	-	-	-	-
25	50 cm	-	-	-	-	-	-	-	-	-	-	-
26	80-100 cm	-	-	-	-	+	-	-	-	-	-	-
27	M15	-	-	-	-	-	-	-	-	-	-	-
28	M50	-	-	-	-	-	-	-	-	-	-	-

29	120-140 cm	-	-	-	-	-	-	-	-	-	-	-
30	140-160 cm	-	-	-	-	-	-	-	-	-	-	-
31	160-180 cm	-	-	-	-	-	-	-	-	-	-	-
32	180-200 cm	-	-	-	-	-	-	-	-	-	-	-
33	200-220 cm	-	-	-	-	-	-	-	-	-	-	-
34	C1cT	-	-	-	-	-	-	-	-	-	-	-
35	C1aB	-	-	-	-	-	-	-	-	-	-	-
36	C1bB	-	-	-	-	-	-	-	-	-	-	-
37	C1cB	-	-	-	-	-	-	-	-	-	-	-
38	C1bT	-	-	-	-	-	-	-	-	-	-	-
39	C1aT	-	-	-	-	-	-	-	-	-	-	-
40	C1bH	-	-	-	-	-	-	-	-	-	-	-
41	C1cH	-	-	-	-	-	-	-	-	-	-	-
42	C1aH	-	-	-	-	-	-	-	-	-	-	-
43	M4B	-	-	-	-	-	-	-	-	-	-	-
44	M4H	-	-	-	-	-	-	-	-	-	-	-
45	M4H	-	-	-	-	-	-	-	-	-	-	-
46	M4B	-	-	-	-	-	-	-	-	-	-	-
47	M4H	-	-	-	-	+*	-	-	-	-	-	-
48	M4T	-	-	-	-	-	-	-	-	-	-	-
49	M4B	-	-	-	-	-	-	-	-	-	-	-
50	M4T	-	-	-	-	-	-	-	-	-	-	-
51	M4T	-	-	-	-	-	-	-	-	-	-	-
52	M7B	-	-	-	-	-	-	-	-	-	-	-
53	M7T	-	-	-	-	-	-	-	-	-	-	-
54	M5T	-	-	-	-	-	-	-	-	-	-	-
55	M7B	-	-	-	-	+*	+	-	-	-	-	-
56	M5T	-	-	-	-	-	-	-	-	-	-	-
57	M7H	-	-	-	-	-	+	-	-	-	-	-
58	M5B	-	-	-	-	-	-	-	-	-	+	-
59	M7T	-	-	-	-	+	+	-	-	-	-	-
60	M7T	-	-	-	-	-	-	-	-	-	-	-
61	M5B	-	-	-	-	-	-	-	-	-	-	-
62	M6H	-	-	-	-	+	-	-	-	-	-	-
63	M5T	-	-	-	-	-	-	-	-	-	+	-
64	M7H	-	-	-	-	-	-	-	-	-	+	-
65	M7H	-	-	-	-	+	-	-	-	-	+*	-

66	M6H	-	-	-	-	+	-	-	-	-	-	-
67	M5B	-	-	-	-	-	-	-	-	-	+*	-
68	M7B	-	-	-	-	+*	-	-	-	-	+*	-
69	M6H	-	-	-	-	+*	-	-	-	-	-	-
70	M5H	-	-	-	-	-	-	-	-	-	+*	-
71	M5H	-	-	-	-	-	-	-	-	-	-	-
72	M5H	-	-	-	-	-	-	-	-	-	-	-
73	H9B	-	-	-	-	-	-	-	-	-	-	-
74	H8T	-	-	-	-	-	-	-	-	-	-	-
75	H8H	-	-	-	-	-	-	-	-	-	-	-
76	H8T	-	-	-	-	-	-	-	-	-	-	-
77	H8B	-	-	-	-	-	-	-	-	-	-	-
78	H8B	-	-	-	-	-	-	-	-	-	-	-
79	H8B	-	-	-	-	-	-	-	-	-	-	-
80	H8T	-	-	-	-	+*	-	-	-	-	-	-
81	H9B	-	-	-	-	-	-	-	-	-	-	-
82	H9H	-	-	-	-	-	-	-	-	-	-	-
83	H9B	-	-	-	-	-	-	-	-	-	-	-
84	H9T	-	-	-	-	-	-	-	-	-	-	-
85	H8H	-	-	-	-	-	-	-	-	-	+*	-
86	H8H	-	-	-	-	-	-	-	-	-	-	-
87	H9T	-	-	-	-	+	-	-	-	-	-	-
88	H9H	-	-	-	-	+	+	-	-	-	-	-
89	H9T	-	-	-	-	+*	-	-	-	-	-	-
90	H9H	-	-	-	-	-	-	-	-	-	-	-
91	L3H	-	-	-	-	-	-	-	-	-	+*	-
92	L3T	-	-	-	-	-	+*	-	-	-	-	-
93	L2B	-	-	-	-	-	-	-	-	-	-	-
94	L2H	-	-	-	-	-	-	-	-	-	-	-
95	L2T	-	-	-	-	+*	-	-	-	-	-	-
96	L3T	-	-	-	-	-	-	-	-	-	-	-
97	L2T	-	-	-	-	+*	-	-	-	-	-	-
98	L2B	-	-	-	-	+*	-	-	-	-	-	-
99	L3T	-	-	-	-	-	+*	-	-	-	-	-
100	L3H	-	-	-	-	-	-	-	-	-	-	-
101	L2T	-	-	-	-	-	-	-	-	-	-	-
102	L2H	-	-	-	-	-	-	-	-	-	-	-
103	L3B	-	-	-	-	-	-	-	-	-	-	-
104	L3B	-	-	-	-	-	+*	-	-	-	-	-
105	L3B	-	-	-	-	-	+*	-	-	-	-	-
106	L3H	-	-	-	-	+*	-	-	-	-	-	-
107	L2B	-	-	-	-	-	-	-	-	-	-	-

108	L2H	-	-	-	-	+	-	-	-	-	-	-
109	M6T	-	-	-	-	+	-	-	-	-	-	-
110	M6B	-	-	-	-	-	-	-	-	-	-	-
111	M6B	-	-	-	-	+*	-	-	-	-	-	-
112	M6T	-	-	-	-	+*	-	-	-	-	-	-
113	M6T	-	-	-	-	+*	-	-	-	-	-	-
114	M6B	-	-	-	-	-	-	-	-	-	-	-

APPENDIX THREE: “COMMUNITY COMPOSITION OF MISCELLANIOUS FIRE SAMPLES”

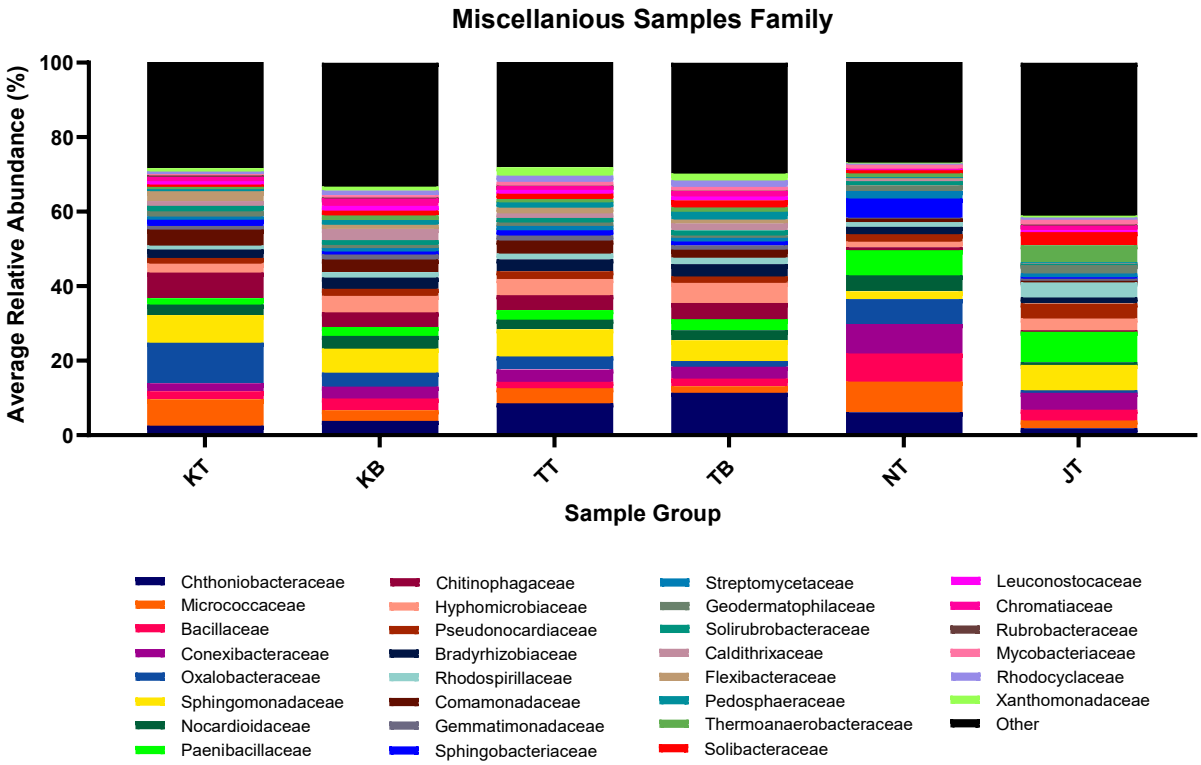

Figure 16. Soil family composition in wildfire affected soils. Naming scheme: First letter: “K” = Kincade Fire, “T” = Tubbs Fire, “N” = Northstar Fire, “J” = Hayes Fire; Last letter: “T” = topsoil (0-3 inches below surface), “B” = bottom soil (3-6 inches below surface).

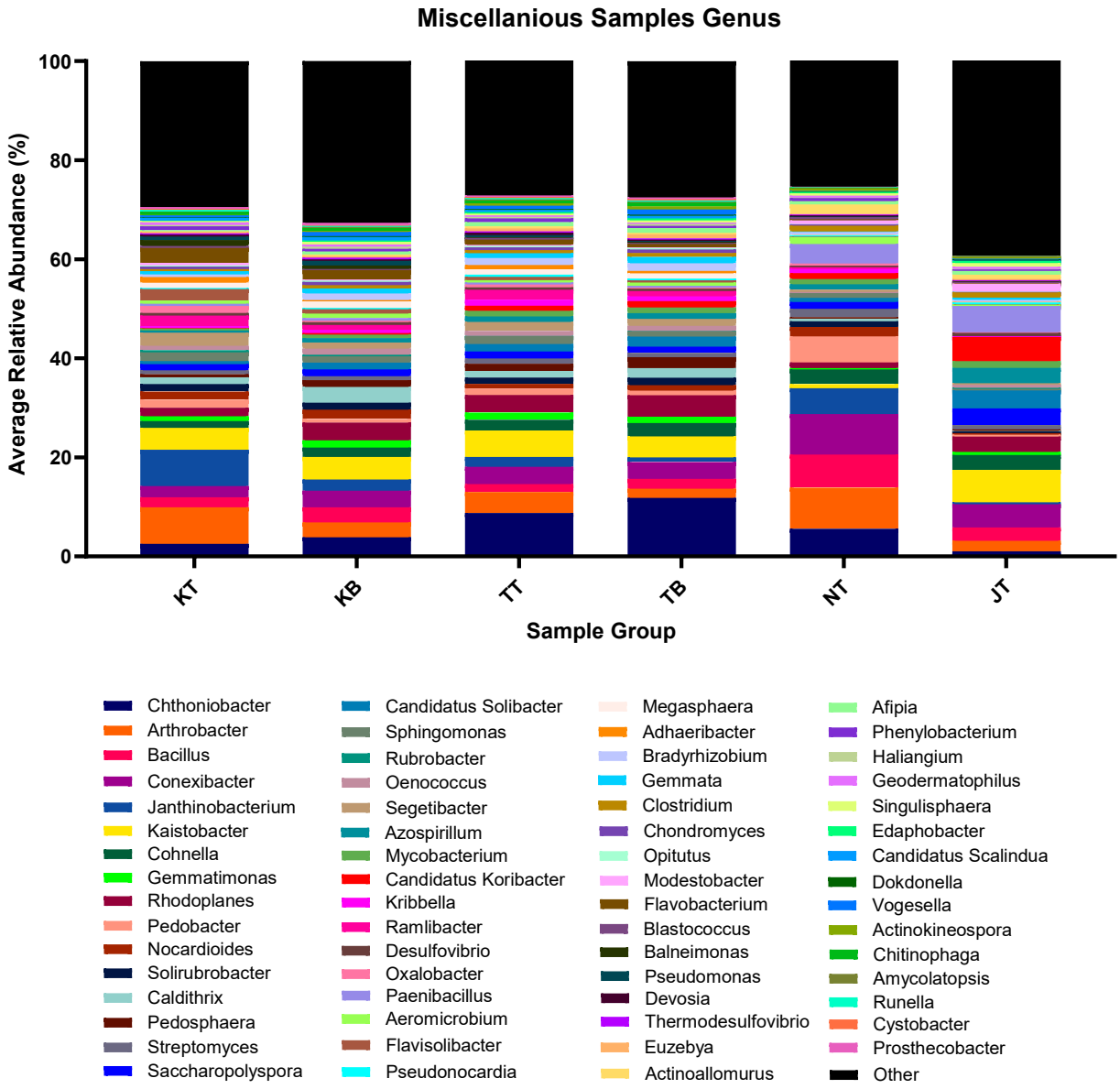

Figure 17. Soil genus composition in wildfire affected soils. Naming scheme: First letter: "K" = Kincade Fire, "T" = Tubbs Fire, "N" = Northstar Fire, "J" = Hayes Fire; Last letter: "T" = topsoil (0-3 inches below surface), "B" = bottom soil (3-6 inches below surface).