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Flowering Dogwoods under Fire: Responses of the Microbiome under Prescribed Burn Management

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DEDICATION

I dedicate this manuscript to my loving parents, Mr. Vivek Kapoor and Mrs. Neelam Kapoor, and some of my great friends and colleagues who have been my emotional support throughout this journey. I really appreciate their patience and invested time.

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ABSTRACT

Prescribed fire is a critical management tool that influences forest physical structure and biological composition. Management via prescribed burning reduces fuel accumulation and the probability of wildfire, recycles nutrients to soil, and minimizes the spread of insect pests and diseases. The plant microbiome also plays an important role in reducing the occurrence of plant disease and increasing nutrient availability under stressful environmental conditions. How prescribed fire can affect the microbiome of regionally native *Cornus florida*, which is economically and ecologically valued, is not well understood. The objective of this study was to evaluate shifts in fungal and bacterial communities of *C. florida* in five different niches that occur following a prescribed fire event. Bacterial and fungal communities across five niches from 20 *C. florida* trees were characterized using 16S and ITS2 rRNA gene amplicon analyses. Our results indicate that prescribed burn had variable effects on bacterial and fungal species richness or diversity of different niches as these niches are located at different proximities in respect to the burn treatment. However, these metrics did differ significantly between our two study years (2018 and 2019), likely due to the differences in the environmental factors between these years. The relative abundance of ectomycorrhizal species decreased while that of saprotrophic fungi increased in root niche following prescribed burn event. Further studies will be required to determine if this would have any consequences on the stability of mycorrhizal symbioses in *C. florida* trees.

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1 Chapter One: Literature Review

1.1 Abstract

Cornus florida, commonly known as flowering dogwood, is a deciduous understory tree native to the eastern United States. The tree is highly prized for its ornamental characteristics and is a valuable food source for wildlife. Fruits of *C. florida* contain one of the highest available levels of calcium and fat content among forest plant resources in the eastern United States. *Cornus florida* trees contribute about $30 million in revenue to the United States annually. *Cornus florida* is also a staple nursery crop in Tennessee, with more than 70% of trees and cultivars sold commercially having their origins as seedlings and liners started in the state. Throughout its native range, *C. florida* trees can be affected by different diseases including dogwood anthracnose and powdery mildew. Despite tree losses to these pathogens in the past decades, high genetic diversity in the native populations of *C. florida* trees has been sustained. The microbiome of *Cornus florida* trees that plays a crucial role in nutrient availability as well as protection against biotic and abiotic diseases have not been documented yet. Moreover, knowledge regarding the influence of important management activities such as prescribed burn on the microbiome of *C. florida* is limited.

1.2 General Description

Cornus florida L., commonly known as flowering dogwood, is a small, shade-tolerant, self-incompatible (outcrossing), insect-pollinated tree species that belongs to the family Cornaceae (Sharma et al. 2005; Sork et al. 2005; Harrar and Harrar 1962). Some of the distinguishing physical features of *C. florida* include large showy bracts, alligator bark on mature trees, onion-shaped terminal buds and opposite leaf arrangement with arcuate venation (Niemiera 2010; Owens 1941; Chase 1961; Wadl et al. 2011). A horticultural characteristic that helps *C. florida* stand out from other deciduous shade tree species is its inflorescence, which are surrounded by four showy large, petaloid, white, pink or reddish bracts (Zhuang et al. 2008). Leaves of *C. florida* contain pigments that exhibits pinkish-red colors, therefore contributing to the beautiful fall colors in the native distribution of the species (Holzmueller et al. 2006; Zhuang et al. 2008). Indigenous to the eastern United States (USA) (Kaveriappa, Phillips, and Trigiano 1997), *C. florida* is distributed from southern Ontario (Canada) and southern Michigan (USA) in the north, to northern Florida in the south, and as far west as eastern Texas (Mitchell, Gibbs, and Martin 1988). The species can survive a broad range of climatic conditions, tolerating summer temperatures as high as 46ºC in northern Florida (USA) and winter temperatures as low as -34ºC in New England (USA) and the northern states (Mitchell, Gibbs, and Martin 1988). *Cornus florida* is endangered in Maine (USA), vulnerable in New York (USA), and endangered in Vermont (USA) (Lynch and Ciesla 2012).

The genus *Cornus* consists of approximately 65 species and most of them are cultivated as ornamentals (Brockman 1968; Dirr 1998; Eyde 1988). The most commonly used species in the landscape are *C. florida* and *C. kousa* (Kousa dogwood) (Mmbaga and Sauve 2004). *Cornus kousa*, in comparison to *C. florida*, is more resistant to diseases like dogwood anthracnose and

powdery mildew caused by *Discula destructiva* Redlin (Redlin 1991) and *Erysiphe pulchra* (Cooke & Peck) (Braun and Takamatsu 2000) respectively (Wadl et al. 2008; Ranney, Grand, and Knighten 1995). *Cornus kousa* flowers about a month after the *C. florida* in the southern USA (Hadziabdic et al. 2005). It is suspected that dogwood anthracnose was introduced to the USA on *C. kousa* which did not exhibit any symptoms (Britton 1994; Mantooth et al. 2017). The native range of *C. kousa* is East Asia and it is considered as an ecological Asian replacement to *C. florida* (Cappiello and Shadow 2005). The genus exhibits a heterogeneous morphology as it greatly varies in inflorescence, fruits, and as well as the number of chromosomes (Xiang et al. 2005; Ma et al. 2017).

1.3 Morphology – General Botanical Characteristics

Cornus florida is highly valued for its appealing ornamental features (Schopmeyer 1974). It is an attractive understory tree because of the yellowish cluster of flowers that resides in the center of four showy bracts which are either white or pink in color (Niemiera 2010). Flowering period ranges from mid-March in the South to late May in the north (McLemore 1990). In addition to showy blossoms, *C. florida* also exhibits attractive fall foliage (Tirmenstein 1991). The leaves of *C. florida* are simple, opposite, oval, with a rounded base that tapers to a point at the leaf tip (Dirr 1977). The upper leaf surface is dark green while lower is glaucous during the growing season (Mitchell, Gibbs, and Martin 1988). Leaves of *C. florida* are high in fat, calcium, and fluorine content (McLemore 1990; Gill and Healy 1974).

The clustered fruits of *C. florida* are small, bright red drupes that are usually about 1.3 cm long and 0.6 cm in diameter with thin flesh (McLemore 1990). The ripening stage of fruits falls between September to late October (Lesser and Wistendahl 1974). Fruits of *C. florida* contain high levels of available calcium (Ca) (Thomas 1969), as well as a nutritionally highest

fat content (approximately 18%) that is among the highest available from forest plant resources in the eastern USA (Hadziabdic et al. 2005; McLemore 1990).

The bark of *C. florida* is among the thinnest of all eastern USA tress and can be easily identified as it closely resembles the skin of the alligator (Harmon 1984). The hard, heavy, strong, and shock-resistant wood of the tree species has been used to manufacture mallet heads, spools, and other special products (Duncan and Duncan 2000; Halls and Ripley 1961). Due to its thin bark, *C. florida* is sensitive to fire, however, due to its prolific sprouting ability, the number of stems may actually increase in burned regions of the forest (McLemore 1990).

1.4 Propagation – Regeneration Processes

Cornus florida can be propagated through seed (Hartmann and Kester 1975), hardwood and greenwood cuttings in summer and spring season respectively, layering, and micropropagation (Hadziabdic 2005). *Cornus florida* naturally germinates in the forest in spring following seed fall, but some seeds are not able to germinate until the second spring (McLemore 1990). *Cornus florida* is propagated through seeds and exhibit a broad range of desirable attributes for use in horticulture (Kaveriappa, Phillips, and Trigiano 1997). In order to overcome embryo dormancy, fresh collected seeds have to be stratified at 5˚C for 120 days (Brinkman 1974). Plants propagated through seeds initiate seed production by the age of 6 years (McLemore 1990). The dispersal of seeds is done by birds, mammals, and gravitational force (McLemore 1990). *Cornus florida* is mainly pollinated by andrenid and halictid bees, but also by beetles, and butterflies (Eyde 1988).

Several cultivars have been selected and commercialized on the basis of bract size and color, growth pattern and foliage aspects (Kaveriappa, Phillips, and Trigiano 1997). *Cornus florida* can be vegetatively propagated by root cuttings or by grafting buds onto the native

rootstocks in July and August (Witte 1995). A successful micropropagation protocol such as regeneration of whole plants from nodal (axillary bud) cultures of seedlings has also been developed for *C. florida* (Hadziabdic et al. 2005; Kaveriappa, Phillips, and Trigiano 1997).

1.5 Importance

Leaves of *C. florida* decompose at a faster rate than most of other tree species and its litter has high calcium (Ca) content, ~2–3.5% (Blair 1988; NASS 2012; Thomas 1969). Additionally, multiple studies have found that *C. florida* contains high levels of potassium (K) and magnesium (Mg) as compared to other tree species in oak hardwood forests (Holzmueller, Jose, and Jenkins 2007; Day Jr and Monk 1977; Elliott, Boring, and Swank 2002). Therefore, a significant ecological purpose is served by *C. florida* trees by acting not only as a source of Ca but of K and Mg as well for the corresponding plant species and woodland floor biota (Thomas 1969; Holzmueller, Jose, and Jenkins 2007).

Cornus florida is remarkably valuable for wildlife as the leaves, seed, fruit, twigs, and bark are utilized as a food source by various animals (McLemore 1990; Lynch and Ciesla 1981). Numerous song birds such as hermit, and gray-cheeked thrushes, white-throated sparrows, thrashers, bluebirds, and Eastern gray squirrels are attracted to dogwood berries, whereas American beavers prefer bark of *C. florida* (Linzey and Brecht 2003; Baird 1980; Hardin and Evans 1977). Deer and rabbits heavily feed upon the foliage and twigs of *C. florida* (McLemore 1990). *Cornus florida* have also been utilized to produce traditional medicine and as a food preservative (Jianrong 2003; Hwang 2002).

Cornus florida has a great economic importance, owing over $30 million in revenue in 2009 in USA (NASS 2012). It is also a primary nursery crop for the Tennessee economy (NASS 2012). Tennessee is the significant producer of *C. florida* with approximately 75% of the sales in

the USA, and numerous rural communities rely upon dogwood production for income (Mmbaga, Mackasmiel, and Mrema 2018).

1.6 Pathogens and Pests

Cornus florida trees are widely used in the landscape as an ornamental tree but they are prone to several major diseases and pests that can result in tree mortality (Holzmueller et al. 2006). Dogwood anthracnose, caused by *D. destructiva*, is the most devastating disease of *C. florida* in the eastern USA (Carr and Banas 2000). It was first discovered on *C. nuttallii* from Clark County, Washington, USA in 1976 (Davidson Jr and Byther 1979) and later reported on *C. florida* in eastern North America in the mid-1970s (Hibben and Daughtrey 1988). The fungus in the eastern USA was initially identified as *Colletotrichum gloeosporioides* (Penz.) (Pirone 1980) and later recognized as *Discula* spp. (Redlin 1991; Mantooth et al. 2017) The symptoms caused by this disease include leaf blotch, blight, leaf spot, bract necrosis and twig die-back (Redlin 1991). Lesions surrounded by purple margins first appear on the foliage and the infection spreads through the petiole, into the branch and finally into the trunk (Carr and Banas 2000). The developing cankers have the potential to girdle and eventually kill the tree (Carr and Banas 2000). Conidia produced on leaves and stem acts as primary inoculum (Hibben and Daughtrey 1988). A recent study focused on genetic diversity and population structure of *D. destructiva* isolates using microsatellite loci revealed low genetic diversity in pathogen populations (Mantooth et al. 2017). The authors identified the existence of four genetic clusters, which corresponded to two major geographic areas, the eastern USA, the Pacific Northwest, and to the two collection time periods when the isolates were collected (pre- and post-1993) (Mantooth et al. 2017).

The second major disease problem in *C. florida* is powdery mildew which is caused by *E. pulchra*. The disease initially appears on the young *C. florida* leaves as raised circular spots with a powdery white hyphal mass (McRitchie 1994). Infected leaves become distorted as they enlarge (Li et al. 2009). Leaf lesions may finally become necrotic and plant parts may get twisted or deformed (McRitchie 1994; Li et al. 2009). Conventional breeding techniques for powdery mildew resistance are cumbersome due to the long generation of the tree species (Parikh et al. 2017). The identification of quantitative trait loci associated with powdery mildew resistance in *C. florida* has also been done to improve resistance against this disease (Parikh et al. 2017). In addition to powdery mildew and dogwood anthracnose, it has been recently reported that a fungal pathogen *Macrophomina phaseolina* has the capability to cause cankers or stem lesions on *C. florida* (Mmbaga, Mackasmiel, and Mrema 2018), which is a highly devastating pathogen within its host range (Islam et al. 2012). Although very uncommon, virus incidence in *C. florida* has been reported by Reddick (1989) on trees used as a propagation material in Tennessee. The newly identified ToMV-DW virus was isolated from a single tree and was found to have tobamovirus-like particles, representing a distinct isolate of tomato mosaic virus (Reddick 1989). Ambrosia beetles (*Xylosandra* spp.), dogwood twig borer (*Oberea tripunctata*), scurfy scale (*Chionaspis lintneri*), and flatheaded borers (*Chrysobothris azurea* and *Agrilus cephalicus*) are several insect pest species reported to attack *C. florida* (Baker 1972; Reding et al. 2010). The exotic species such as *X. crassiusculus* (Granulate Ambrosia Beetle) and *X. germanus* (Blandford) have been found to attack healthy individuals of *C. florida* (Kühnholz, Borden, and Uzunovic 2001; Oliver and Mannion 2001), and they are readily distributed in the native, eastern USA distribution of this tree (Solomon 1995). When exposed to biotic or abiotic stressors, trees are known to release volatile compounds with low molecular weight such as acetone,

acetaldehyde, acetic acid, ethane, ethylene, ethanol, and methanol (Rottenberger et al. 2008; Holzinger et al. 2000). Certain species such as *X. germanus* are highly attracted to ethanol and hence, ethanol becomes an essential host-selection cue (Ranger, Tobin, and Reding 2015; Ranger et al. 2012). Reding et al. (2010) study revealed that more than 90% of *X. germanus* attacks on *C. florida* were restrained to the main trunk of the tree within 1 m of the soil surface (Reding et al. 2010).

1.7 Microbiome

A plant's microbiome is comprised of the community of the microorganisms that reside in the plant's internal tissues as well as on the external surfaces (Rout 2014). Tree-microbe interactions are complex and the resulting multi-organism networks play an important role in influencing plant growth and productivity (Bonfante and Anca 2009; Hacquard and Schadt 2015). Plant-associated microorganisms help in various activities such as water and nutrient absorption (Pii et al. 2015), combating plant pathogens (Berendsen, Pieterse, and Bakker 2012), and triggering host plant defenses under stress situations (Cregger et al. 2018). Although there are number of studies associated with plant-microbe interactions (Osono and Mori 2005; Osono 2007; Osono and Mori 2004), there is very limited research on the role of microbiome in *C. florida* populations. The microbiome of other species of dogwoods have been studied to some extent. For example, the distribution of phyllosphere fungi in giant dogwood (*Swida controversa*) (Osono, Bhatta, and Takeda 2004) and in red-osier dogwood (*C. stolonifera*) (Osono 2007) have been examined. The temporal patterns of phyllosphere fungi in giant dogwood during the growing season from May to October has also been analyzed (Osono and Mori 2005). Fungal species such as *Phomopsis* sp., *Pestalotiopsis* sp., *Trichoderma viride*, *Colletotrichum*

gloeosporioides, *Clonostachys rosea*, *Cladosporium cladosporioides*, and *Phoma* sp. were observed frequently to be associated with *S. controversa* (Osono and Mori 2005).

Although *C. florida* is of great ecological and economical value, little is known about its fungal and bacterial microbiome especially in its native environment. Previously, phyllosphere fungi associated with *C. stolonifera* and *S. controversa* were cataloged based on morphological identification (Osono and Mori 2005; Osono 2007; Osono and Mori 2004) but offer little insight into the biodiversity in other *Cornus* species, particularly those affected by *D. destructiva*. In addition, there is limited information on the effect of prescribed fire on the microbiome and overall tree health of *C. florida*.

1.8 Prescribed Fire and Wildfire

Prescribed fire is the intentional application of fire under particular conditions to attain certain management objectives (Wade and Lunsford 1989). Prescribed fire has been a crucial management tool frequently used in the southeastern USA (Brown et al. 2013). Fire plays an integral role in influencing the forest structure and composition (Wright, Wright, and Bailey 1982; Heinselman 1973). A wide range of objectives can be fulfilled through prescribed fire such as reducing wildlife-managing hazards by checking fuel accumulation, horticultural improvements, control of weeds, insects, and diseases (Wade and Lunsford 1989; Fernandes and Botelho 2003; Kilgore and Curtis 1987). The fire behavior is determined by various factors including fire type; length, height, depth of flame, and fire intensity (Weir 2009). A number of studies have reported a shift in soil- (Anderson et al. 2007; Bastias et al. 2006; Bastias, Xu, and Cairney 2006) and root-associated (Buscardo et al. 2010; Cairney 2002) fungal communities due to fire, particularly if the fire intervals are short.

Fairman et al. 2019 investigated the effects of short-interval wildfires on the resilience as well as resistant traits of the fire-tolerant forests in *Eucalyptus* trees (Fairman, Bennett, and Nitschke 2019). They concluded that in order to rescue themselves effectively from the adverse effects of high severity fires, the stems of *Eucalyptus* trees have to reach a minimum stem diameter known as escape size (Fairman, Bennett, and Nitschke 2019). Mikita-Barbato et al. (2015) evaluated the impacts of wildfire which burned over 7000 ha of New Jersey Pinelands, by associating soil properties to changes in microbial communities in organic horizon soils. Significant changes were observed in the physical and chemical characteristics in the organic horizon of the soils shortly after the fire (Mikita-Barbato, Kelly, and Tate III 2015). These changes occurred along with shifts in the bacterial, fungal, and archaeal communities that were most altered compared to unburned plots during the first year after fire (Mikita-Barbato, Kelly, and Tate III 2015). The microbial communities changed more slowly during the second year after fire and were still distinct from communities in the non-burned soils 25 months post-fire (Mikita-Barbato, Kelly, and Tate III 2015). The dynamics of soil carbon (C) and nitrogen (N) is governed by wildfire severity to a great extent (Adkins, Sanderman, and Miesel 2019). High-severity wildfires can even convert forests from C sinks to C sources when C losses via decomposition surpass photosynthetic C accumulation during post-fire forest recovery (Kashian et al. 2006; Adkins, Sanderman, and Miesel 2019).

Although prescribed fire has become an important forest management tool, a lot of our knowledge of fire effects on soil properties comes from wildfires (Oliver, Callaham Jr, and Jumpponen 2015). It is still uncertain whether the impacts of wildfires on plant microbial communities are comparable to those of prescribed burning as wildfires frequently burn hotter, consume more organic matter, and result in greater nutrient volatilization than prescribed burns

(Hatten and Zabowski 2010; Nilsson et al. 2019; Oliver, Callaham Jr, and Jumpponen 2015). Prescribed fires are usually applied under low fire intense and severe conditions, and are generally outside of the high risk conditions that are most conducive for wildfires (Oliver, Callaham Jr, and Jumpponen 2015). A study compared fire severity between prescribed fires and wildfires in a temperate ponderosa pine forest (Choromanska and DeLuca 2001). They reported that prescribed fire consumed 42% of the fine fuel and resulted in no overstory tree mortality, whereas wildfire consumed all fine fuel and led to complete stand mortality highlighting the substantial difference between wildfires and management through prescribed fire (Choromanska and DeLuca 2001). Therefore, the effects of prescribed burn on microbial communities might be expected to have less dramatic effects than those of wildfires (Neary et al. 1999).

Cornus florida is well-suited to periodic prescribed fires (Gill and Healy 1974). The tree species generally sprouts adequately from the root crown after the plants are top-killed or damaged by fire (Tirmenstein 1991; Gill and Healy 1974). Specific response after prescribed fire depends upon various factors such as intensity of fire, season of burn, site factors, fire frequency (Tirmenstein 1991). A study conducted in George National Forest near Brandywine, West Virginia reported that average seedling densities of *C. florida* before and after 5 years of prescribed fire were 605 and 737 seedlings/acre respectively; and sprout densities were 1,158 sprouts/acre before and 1,553 sprouts/acre 5 years after the fire (Wendel and Smith 1986).

1.9 Research Gap

Microbiome-mediated defenses can be utilized to reduce pathogen establishment or to interact with the immune response of host plants to reduce pathogen colonization success. The extent to which microbial communities of *C. florida* influence resistance or susceptibility to pressures like plant disease is largely unknown, especially in post-burn areas of the forest. Knowledge

regarding the influence of management activities such as prescribed burn on the microbiome of *C. florida* also is limited. This report provides a baseline evaluation of the responses of fungal and bacterial communities associated with *C. florida* before and after a prescribed burn management by evaluating the microbiomes associated with pre- and post-burn exposed *C. florida* trees. The main objective of this study was to estimate changes in fungal and bacterial microbiome across five different niches of *C. florida* in response to a prescribed forest fire. The niches included in this study were soil, roots, bark, stem, and leaves. We hypothesized that alpha diversity of microbial communities would decrease in burned plots after 152 days of the prescribed burn treatment and the extent of reduction in the alpha diversity would vary according to the niche. We also hypothesized that the prescribed burn treatment would have significant impacts on microbial community composition in all five niches. We expected different niches to respond differently to prescribed burn treatment and also expected to have differences in the microbial diversity and community composition due to different environment factors in years 2018 and 2019.

2 Chapter Two: Flowering Dogwoods under Fire: Responses of the Microbiome under Prescribed Burn Management

2.1 Abstract

Prescribed fire is a critical management tool that influences forest physical structure and biological composition. Management via prescribed burning reduces fuel accumulation and the probability of wildfire, recycles nutrients to soil, and minimizes the spread of insect pests and diseases. The plant microbiome plays an important role in reducing the occurrence of plant disease and increasing nutrient availability under stressful environmental conditions. How prescribed fire can affect the microbiome of regionally native *Cornus florida*, which is economically and ecologically valued, is not well understood. The objective of this study was to evaluate shifts in fungal and bacterial communities of *C. florida* in five different niches that occur following a prescribed fire event. Bacterial and fungal communities across five niches from 20 *C. florida* trees were characterized using 16S and ITS2 rRNA gene amplicon analyses. Our results indicate that prescribed burn had variable effects on bacterial and fungal species richness or diversity of different niches as these niches are located at different proximities in respect to the burn treatment. However, these metrics did differ significantly between 2018 and 2019 likely due to the differences in the environmental factors between these years. The relative abundance of ectomycorrhizal species decreased while that of saprotrophic fungi increased in root niche following prescribed burn event. Further studies will be required to determine if this would have any consequences on the stability of mycorrhizal symbioses in *C. florida* trees.

2.2 Introduction

Plant-associated microbial communities have critical roles in water and nutrient uptake, stress tolerance, and plant defense (Cregger et al. 2018; Peiffer et al. 2013; Berendsen, Pieterse, and Bakker 2012). These plant-associated microbial communities also play a fundamental role in driving various ecological processes such as decomposition and carbon cycling (Martiny et al. 2016). Moreover, microbes also have symbiotic relationship with plants – for instance, mycorrhizal fungi interact with plants and can help in initial terrestrial colonization (Humphreys et al. 2010). Additionally, the plant microbiome can greatly extend the ability of the host to adapt under changing environmental conditions (Goh et al. 2013). In comparison to herbaceous plant species, including model organisms such as *Arabidopsis* spp., tree-microbe interactions are extremely complex (treBonfante and Anca 2009).

Abiotic factors such as soil physicochemical properties, particularly soil pH, are major factors affecting soil microbial structure and composition (Fierer and Jackson 2006). The rhizosphere includes the zone of soil that is influenced by exudates released from plants and microorganisms (Rout 2014). The vast microbial diversity within this niche (Egamberdieva et al. 2008; Mendes et al. 2011) provides a microbiome cohort that can serve as the first line of defense against soil-borne plant pathogens (Mendes et al. 2018), or aid in plant responses to stress conditions such as drought and other abiotic situations (Fitzpatrick et al. 2018).
During the last decade, research related to interaction between plant hosts and their associated phyllosphere microbial communities has made great progress (Bodenhausen et al. 2014). The phyllosphere microbiome is diverse and include different bacteria, algae, yeasts, and filamentous fungi taxa, and, less frequently, protozoa and nematodes. Interactions between the phyllosphere (above-ground portion of plant), rhizosphere (below-ground portion of the plant), diversity and

abundance of their associated microbial communities can be influenced by number of environmental and physiological conditions (Rout 2014; Bais et al. 2004). However, their current research findings indicate that bacteria are the most abundant inhabitant of the phyllosphere (Lindow and Brandl 2003).

Host plant genotype has been the recognized as a principal factor affecting both fungal and bacterial phyllosphere communities (Ulrich, Ulrich, and Ewald 2008; Vorholt 2012). Constituents of these communities are associated with the production of growth-promoting nutrients and hormones that aid in plant defenses (Gourion, Rossignol, and Vorholt 2006; Reed et al. 2010). Microbial biodiversity is also an important trait that forms a part of the phenotype of the host organism with important effects on host fitness and evolution (Berendsen, Pieterse, and Bakker 2012; Zilber-Rosenberg and Rosenberg 2008; Benson et al. 2010; Whitham et al. 2003). Because of the importance of the phyllosphere microbiome for plant health, the need to understand the drivers of microbial community assembly has become the cornerstone of microbial ecology research (Kembel et al. 2014; Robinson, Bohannan, and Young 2010).

Prescribed fire describes an intentional application of fire under particular weather conditions to attain certain management objectives such as reducing the hazard of wildfire, control of weeds, insects, and plant pathogenic disease (Wade and Lunsford 1989; Fernandes and Botelho 2003; Kilgore and Curtis 1987). Prescribed fire has been a crucial management tool frequently used in the southeastern USA, as the practice is an integral contributor to influencing the structure and composition of forest systems (Wright, Wright, and Bailey 1982; Heinselman 1973; Brown et al. 2013). In addition to its obvious above-ground effects, fire can alter soil physical characteristics like structure, porosity and water relations, organic matter content, and soil chemistry including mineral nutrient availability (Mao et al. 2002). Soil microorganisms can

also be affected by fire, either as a direct result of heating or by indirect effects of changes to soil physical and chemical properties (Neary et al. 1999). Effects of fire on the biogeochemical characteristics of a forest ecosystem are widely acknowledged, however, how a prescribed fire will shape the microbiome of a specific tree species, like *Cornus florida,* is largely unknown.

Cornus florida L. (flowering dogwood; *Cornaceae*) is a small, shade-tolerant, self-incompatible understory tree species native to the eastern United States (USA) (Sharma et al. 2005; Harrar and Harrar 1962; Sork et al. 2005). This tree species is an important food source for various animals and game species, and is also an economically valuable ornamental plant (Mantooth et al. 2017; McLemore 1990). Fruits of *C. florida* contain one of the highest available levels of calcium and fat among forest plant resources in the eastern USA (Hadziabdic et al. 2010). *Cornus florida* is a staple nursery crop in Tennessee, with more than 70% of trees and cultivars sold commercially having their origins as seedlings and liners started in the state (NASS 2012).

Although two major plant pathogenic diseases have impacted *C. florida* trees (Carr and Banas 2000; McRitchie 1994; Daughtrey et al. 1996), considerable genetic diversity of this host species has been sustained despite high tree mortality rates (Hadziabdic et al. 2012; Hadziabdic et al. 2010). The influence that these tree diseases and the high mortality rates of trees within regional areas have had on host plant-associated fungal and bacterial communities have not been studied. In addition, the role of planned understory management using prescribed fire in shaping microbial community of *C. florida* trees is largely unknown.

Microbiome-mediated defenses can be utilized to reduce pathogen establishment or to interact with the immune response of host plants to reduce pathogen colonization success. The extent to which microbial communities of *C. florida* influence resistance or susceptibility to

pressures like plant disease is largely unknown, especially in post-burn areas of the forest. Knowledge regarding the influence of management activities and ecological perturbations such as prescribed burn and forest fires on the microbiome of *C. florida* also is limited. This report provides a baseline evaluation of the responses of fungal and bacterial communities associated with *C. florida* before and after a prescribed burn management. The main objective of this study was to describe changes in diversity and community composition of fungal and bacterial microbiome across five different niches of *C. florida* in response to a prescribed forest fire. The niches included in this study were soil, roots, bark, stem, and leaves. We hypothesized that alpha diversity of microbial communities would decrease in burned plots after 152 days of the prescribed burn treatment and the extent of reduction in the alpha diversity would vary according to the niche. We also hypothesized that the prescribed burn treatment would have significant impacts on microbial community composition in all five niches. We expected different niches to respond differently to prescribed burn treatment and also expected to have differences in the microbial diversity and community composition due to different environment factors in years 2018 and 2019.

2.3 Materials and Methods

2.3.1 Site Description and Study Design

This study was conducted at the University of Tennessee Highland Rim Forest Unit Research and Education Center in Tullahoma, TN (35.32, -86.15). The experimental site was divided into four plots, each about 4000 m^2 in area that contained mid-aged to mature, established *C. florida* trees. Each plot was randomly assigned to a treatment group, burned or unburned, for a total of two plots per treatment. Within each plot, five *C. florida* trees were randomly selected for

sampling with diameter at breast height (DBH) ranging from 6-10 cm. The mean age of the trees was 38.5 years old and mean height was 8.05 m (Table 2.3).

2.3.2 Habitat and Host Plant Characteristics

Vegetation cover and foliage density were assessed pre- and post-burn. Vegetation cover was assessed within a five m radius around each study tree. In each of the four cardinal directions, the cover of leaf litter, woody debris, standing dead, and live trees was recorded at one m intervals (Table 2.2).

To provide an approximation of tree age, cores were taken from the main trunk at approximately breast height for all *C. florida* trees within plots using an increment borer. Cores were removed from the borer, transported to the lab protected within 15 ml Falcon tubes (Thermo Fisher Scientific™, Waltham, MA), where they were attached with wood glue to six-inch plastic pot tags, and then were air dried beneath weight to prevent curling. Once dried, a flat surface was sanded into the core and Natural #209 wood stain (Minwax Wood Finish Penetrating Stain, Sherwin-Williams Co., Cleveland, OH) was used to provide contrast so that annular rings could be visualized beneath a dissecting microscope on the pale surface of the wood. The age of the trees ranged from 29 to 50 years with 38 years as median age (Table 2.3).

2.3.3 Burn Application

The prescribed burn occurred on March 28, 2019. The fire was applied by UT Forest Resources Research & Education Center staff (Oak Ridge, TN) who were assisted by Tennessee Division of Forestry employees. Application of fire started on east side of the plot and moved towards the west direction and the plots burned for 1 hour. Fire initiated as head fire and started behaving as

back fire after 10 min. Based on infra-red imaging, the maximum temperature recorded at any time during the burn was 680°C.

2.3.4 Pre- and Post-Prescribed Fire Sample Collection

Samples were collected from five different niches: fine roots, soil, bark, stem, and leaves before and after the burn. The pre-burn sample collection was completed on September 20-21, 2018 and the post-burn sample collection was completed on September 6-7, 2019. Tools were cleaned sterilized between niches and trees using 70% non-denatured ethanol to prevent cross-contamination.

For collection of fine roots, surface soil was gently scraped to remove the litter and plant debris from the immediate base of each tree. To ensure that roots from trees of interest and not neighboring trees were sampled, we sampled fine roots from lateral roots that could be identified projecting from the base of each study tree. For bulk soil, soil cores (15 cm deep x 3 cm diameter) were collected in the four cardinal directions approximately 0.3 m from the base of the tree using a sterilized stainless-steel soil probe. Soil cores were pooled per tree and homogenized in the field. Plant debris and larger roots were removed. For microbiome analyses, an approximately 5 g subsample of homogenized soil sample was placed in a small Whirl-Pak® bag (Nasco, Atkinson, WI), transported to the lab in liquid nitrogen, and stored at -80°C until DNA extraction. The remaining bulk soil was stored at 4°C. Soil was air-dried, ground, passed through a 2-mm sieve (No. 10), and sent to Brookside Laboratories (New Bremen, OH) for analysis of pH (1:1), soil organic matter (SOM; loss on ignition 360°C), nitrate (NO_3.N), ammonium (NH_4.N), extractable aluminum (Al), boron (B), calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), sulfur (S), and zinc (Zn).

Bark samples were collected by shaving portions of the cambium at approximately 30 cm from the base of the tree. For sampling of the remaining three niches a pole pruner was used to sample three to four, small to medium sized, branches from each tree. For stem samples, approximately 2-3 branch sections, 8 cm in length by 2 mm in diameter were cut from each sampled branch and pooled per tree for a total of 10-12 bark sections per tree. For leaves, we sampled approximately 3-4 leaves from each branch section for a total of 10-12 leaves per tree. Collected fine roots, bark shavings, branch sections, and leaves were stored in 50 ml Falcon tubes (Thermo Fisher Scientific™, Waltham, MA), transported to the lab in liquid nitrogen, and stored at -80°C until DNA extraction.

2.3.5 DNA Extractions

DNA was extracted from fine roots, bark shavings, branch sections, and leaves following the protocol of the E.Z.N.A. Plant DNA DS Mini Kit (Omega Bio-tek, Norcross, GA). For DNA extraction from soil, we followed the protocol of the PowerLyzer® PowerSoil ® DNA Isolation Kit (Qiagen, Carlsbad, CA). The extracted DNA was stored at -20°C until library preparation for sequencing on the Illumina MiSeq platform.

2.3.6 16S rRNA Gene and ITS Library Preparation and Sequencing

For pre-fire DNA samples, the University of Tennessee Genomics Core (Knoxville, TN, USA) performed library preparation of the 16S (bacteria and archaea) and ITS (fungi) regions. We adopted a two-step PCR approach to barcode templates with the following modifications. Primers for amplifying bacterial amplicons were modified 515F (forward) and 785R (reverse) primers (Walters et al. 2016) with Illumina MiSeq specific adapters for the 16S rRNA V4 region. The ITS2 region in fungal sequences was amplified using ITS3 forward and ITS4 reverse

primers (Martin and Rygiewicz 2005). Amplification reactions for the primary PCR were performed in 25 µL volumes containing 2x KAPA HiFi hot start ready mix, 2.5 µL of genomic DNA and 2 µM of forward and reverse primers each. Thermocycler conditions for the primary PCR were: initial denaturation at 95 °C for 30 s, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 30 s and then final elongation step at 72 °C for 5 min. Primary PCR products were cleaned with 20 µL of AMPure beads and eluted in 50 µL of hydroxymethyl-aminomethane (TRIS) buffer. Secondary PCR had purified DNA tagged with barcoded forward and reverse indexes in the 50 µL reaction having 5 µL of genomic DNA. Thermocycler conditions for secondary PCR were: Denaturation at 95 °C for 3 min, followed by 8 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 30 s and then final elongation step at 72 °C for 5 min. The product was quantified on a NanoDrop 1000 spectrophotometer (NanoDrop Products, Wilmington, DE, USA). After PCRs, the samples were pooled based on the Bioanalyzer (Agilent, Santa Clara, CA, USA) reading. The final loading concentration of the pooled samples was 4 pM. Illumina Miseq sequencing was carried out using a 20% PhiX spike on a V2, 500 cycle flow cell reading 2 x 250 in the UT Genomics Core. The post-fire DNA samples were sent to Psomagen, Inc. (Rockville, MD, USA) and the procedure used for the 16S and ITS library preparation was identical.

2.3.7 16S rRNA Gene Sequence Processing

Sequences were processed into Operational Taxonomic Units (OTUs) using mothur (v.1.43.0) (Schloss et al. 2009). Prior to sequence processing in mothur, forward and reverse primers were first removed using the *cutadapt* program (Martin 2011). Processing of 16S sequences was performed using a modified version of the mothur Illumina MiSeq SOP (Kozich et al. 2013).

Briefly, forward and reverse sequences were merged using the *make.contigs* command in mothur. We removed sequences containing ambiguous bases and homopolymers (>8 bp), using the *screen.seqs* command. Sequences were aligned to SILVA v.138 reference database using the *align.seqs* command (Quast et al. 2012). Aligned sequences were pre-clustered (maxdiff = 2 bp) using the *pre.cluster* command. Pre-clustered sequences were screened for the presence of any chimeras via VSEARCH using *chimera.vsearch* command (Rognes et al. 2016). The sequences were then classified using SILVA reference alignment with the *classify.seqs* command to generate the best species classification. Sequences that were classified as originating from chloroplasts, mitochondria, eukaryota, or unknown were removed using *remove.lineage* command. The sequences were then clustered into OTUs with 97% cut-off level using *cluster.split* command.

2.3.8 ITS Sequence Processing

Sequences were processed into Amplicon Sequence Variants (ASVs) using DADA2 ITS pipeline workflow (https://benjjneb.github.io/dada2/ITS_workflow.html) in R version 3.5.3 (Callahan et al. 2016; R Core Team 2011). Due to the highly variable ITS region, all the possible orientations of forward and reverse primers were removed from both the forward and reverse sequences using *cutadapt* program (Martin 2011). Then, the forward and reverse sequences were filtered and trimmed using *filterAndTrim* command with default parameters. We de-replicated the identical reads using *derepFastq* command. Next, forward and reverse sequences were merged using *mergePairs* command. The ASV table was constructed using *makeSequenceTable* command and chimeras were removed from the merged sequences using *removeBimeraDenovo* with default parameters. The naive Bayesian classifier method was used to assign taxonomy to the sequences using General Fasta file version 8.2 from the UNITE ITS database (Nilsson et al. 2019).

2.3.9 Classification of Fungal Functional Guilds

Fungal ASVs were assigned to fungal functional guilds using the online version of the FUNGuild database (http://funguild.org, accessed 26 November, 2019; (Nguyen et al. 2016)) with a confidence cut-off of "possible". To visualize differences in fungal functional guilds before and after prescribed burn treatment of *C. florida* trees, abundance values for any ASV assigned to multiple functional guilds were divided by the number of functional guilds assigned to an ASV so that each possible function for that ASV were equally weighted. To visualize differences in potential fungal functions, stacked bar plots depicting the relative abundance of each fungal functional guild were created.

2.3.10 Statistical Analyses

We performed all statistical analyses in R program. To visualize differences in soil physicochemical properties before and after the prescribed burn treatment, soil physicochemical properties were scaled and centered and a principal component analysis (PCA) was conducted using the *prcomp* function from the *stats* package.

Prior to alpha and beta-diversity analyses, OTU tables for bacteria and ASV tables for fungi were rarefied to account for differences in sequencing depth. Rarefaction cut-offs were chosen by evaluating rarefaction curves and selecting a minimum sequencing depth that allowed us to maximize coverage and reduce sample loss. Using the rarefied OTU and ASV abundance tables, we calculated the observed species richness and the Shannon diversity index with the *specnumber* and *diversity* functions from the *vegan* package (Oksanen et al. 2018). To test the burn and year differences in bacterial and fungal diversity and richness, we performed repeated measures analysis of variance (ANOVA) using the *lme* function from *nlme* package (Bliese 2006). Singleton OTUs and ASVs were then removed from the respective rarefied tables. To

visualize taxonomic differences for each niche by treatment for each year, we used relative OTU and ASV abundances to construct stacked bar plots of bacterial and fungal phyla, and classes. To analyze bacterial and fungal beta-diversity before and after the burn, we performed a principal coordinate analysis (PCoA) on Bray-Curtis dissimilarity using the *pcoa* function of the *ape* package for each niche (Paradis and Schliep 2019). Dissimilarity indices were calculated from the relative abundance OTU and ASV tables with singletons omitted using the *vegdist* function from the *vegan* package (Oksanen et al. 2018). Additionally, the contribution of burn and year to variation in community composition was tested using PERMANOVA with the *adonis* function in *vegan* with 10,000 permutations (Oksanen et al. 2018).

2.3.11 Indicator Species Analysis

To identify bacterial OTUs and fungal ASVs characteristic of the control and burned plots we performed indicator species analysis for all five niche microbial communities of *C. florida* using the *multipatt* function from the *indicspecies* package with 10,000 permutations (Cáceres and Legendre 2009). We report significant bacterial OTUs and fungal ASVs ($P \leq 0.05$, indicator value ≥ 0.80) for control and burned plots of *C. florida* trees.

2.4 Results

2.4.1 Differences in Soils Physicochemical Properties

Soil physicochemical properties did not significantly differ by prescribed burn treatment (Fig. 2.1; Pseudo-$F_{1,39} = 2.27$, $P = 0.109$, $R^2 = 0.03$, but instead separated out by sampling year along the first principal component (PC). A total of three principal components were retained accounting for 74.7% of the variation in the data. PC1 (50.02% variation explained) correlates with OM, Mg, TEC, Na, Fe, K, P, Al, and Ca. PC2 (14.4% variation explained) correlates with Zn, Cu, Mn, and NH_4.N. Finally, PC3 (10.3% variation explained) correlates with Cu, S, Mn, NH4.N., pH, and Zn.

2.4.2 Bacterial and Fungal Sequence Processing

A total of 17 million 16S rRNA gene and 13 million ITS sequences were assembled from paired-end reads across 200 samples for both 2018 (pre-fire) and 2019 (post-fire) data. Following 16S rRNA gene sequence processing in mothur, a total of 5,388,026 sequences were clustered into 169,999 bacterial OTUs. No sequences identified as archaea were retained following sequence processing. After ITS sequence processing in DADA2, a total of 6,955,451 sequences were retained across 12,456 fungal ASVs. Prior to downstream analysis, bacterial and fungal sequences from samples in each niche were rarefied to allow for within niche comparisons of alpha and beta-diversity (Table 2.3).

2.4.3 Differences in Below-ground (Soil and Roots) Microbiome

Soil bacterial alpha-diversity (richness and Shannon diversity) and community composition did not significantly differ by burn treatment (Fig. 2.2A, B, Fig. 2.3A, Table 2.2). However, alpha-diversity measures and community composition did significantly differ between 2018 and 2019

for all four plots (Fig. 2.2A, B, Fig. 2.3A; Table 2.2). The majority of soil bacterial sequences were classified to Proteobacteria (28%) in 2018 with sequences from Alphaproteobacteria (19%) being the most frequently detected at class level (Fig. 2.4A, B). In 2019, sequences from Acidobacteria (27%) were the most dominant in soil with sequences from the class Acidobacteriae (25%) having the greatest representation (Fig. 2.4A, B). Prescribed burn treatment did not have any significant effect on soil fungal alpha diversity, however, soil fungal community composition significantly different between burned and control plots (Fig. 2.3B, 5A, B; Table 2). Basidiomycota (56%) was the most abundant fungal phyla for soil with most sequences classified to Agaricomycetes (56%) at class level across both years (Fig. 2.6A, B). A total of 3,691 ASVs out of 4,409 ASVs in soil fungal community were assigned to a fungal functional guild. The majority of soil fungal sequences belonging to classifiable ASVs were identified as potential ectomycorrhizal fungi in 2018 (49%) which increased to 61% in 2019 (Fig. 2.7A).

Bacterial alpha diversity for root niche was not significantly different between burned and control plots, however, root bacterial community composition was significantly different (Fig. 2.2C, D, Fig. 2.3C; Table 2). Sequences from Proteobacteria (40%) were the most abundant in roots and those classified to Actinobacteria (32%) were the most dominant at class level across both years (Fig. 2.4C, D). Richness and diversity values for the fungal community in roots did not significantly differ by burn treatment (Fig. 2.5C, D; Table 2). However, root fungal community composition differed significantly by the burn treatment (Fig. 2.3D; Table 2). Ascomycota (48%) was the most dominant fungal phyla for roots with Agaricomycetes (46%) having the most representation at class level across both years (Fig. 2.6C, D). In root fungal communities, a total of 1,197 ASVs out of 1,375 ASVs were assigned to a fungal functional

guild. The majority of root fungal sequences that belonged to classifiable ASVs were identified as potential undefined saprotrophs in 2018 (47%) and 2019 (33%) (Fig. 2.7B). Interestingly, the relative abundance of classifiable OTUs identified as potential wood saprotrophs increased from 5% in 2018 to 36% in 2019 in burned plots, whereas, relative abundance of potential ectomycorrhizal fungi decreased from 44% in 2018 to 15% in 2019 following prescribed burn treatment (Fig. 2.7B).

2.4.4 Differences in Above-ground (Bark, Stem, and Leaves) Microbiome

Prescribed burn did not significantly shift the richness and diversity of bacterial and fungal communities for above-ground niches (i.e. bark, stem, and leaves) (Table 3). Bacterial and fungal community composition did significantly differ by burn treatment for bark and leaves but not for stems (Fig. 2.3E, G, I, Table 3). A majority of sequences were identified as Proteobacteria for bark (30%), stem (63%), and leaves (55%) and most sequences at the class level were classified to Alphaproteobacteria (bark - 25%, stem - 61%, leaves - 54%) for both years (Fig. 2.7). For fungal communities, a majority of the sequences were identified as Ascomycetes (bark - 77%, stem - 84%, leaves - 93%) with Dothideomycetes (bark - 77%, stem - 84%, leaves - 93%) being the most abundant at the class level across both years (Fig. 2.8).

In the bark fungal community, a total of 2,260 ASVs out of 2,767 ASVs were assigned to a fungal functional guild. The majority of bark fungal sequences belonging to classifiable ASVs were identified as potential undefined saprotrophs in 2018 (31%) and potential wood saprotrophs in 2019 (45%) (Fig. 2.7C). In stem fungal communities, a total of 2,948 ASVs out of 3,583 ASVs were assigned to a fungal functional guild. The majority of stem fungal sequences belonging to classifiable ASVs were identified as potential plant pathogens both in 2018 (58%) and 2019 (45%) (Fig. 2.7D). In leaf fungal communities, a total of 2,824 ASVs out of 3,638

ASVs were assigned to a fungal functional guild. The majority of leaf fungal sequences belonging to classifiable ASVs were identified as potential plant pathogens in 2018 (40%) and potential undefined saprotrophs (58%) in 2019 (Fig. 2.7E).

2.4.5 Bacterial and Fungal Indicator Species

A total of 1,285 bacterial indicator OTUs for soil and 96 bacterial indicator OTUs for root communities were detected across control and burned plots before and after prescribed burn treatment. Diplorickettsiaceae was found to be an indicator OTU in soil bacterial communities of burned plots in 2019. In root bacterial communities, Acidothermaceae, which contains thermophilic species, was found as an indicator bacterial OTU post prescribed burn treatment (Berry, Barabote, and Normand 2014).

For above-ground niches, a total of 435 (bark), 98 (stem), and 105 (leaf) bacterial indicator OTUs were detected across control and burned plots before and after prescribed burn treatment. In bark bacterial communities, Oxalobacteraceae, Microbacteriaceae, Beijerinckiazeae, Nocardioidaceae, Micromonosproaceae, and Burkholderiaceae were the indicator OTUs found in *C. florida* plots where prescribed burn treatment was applied, whereas, none of the indicator OTUs were found in stem and leaves bacterial communities for *C. florida* post prescribed burn treatment.

A total of 203 fungal indicator ASVs for soil and 29 fungal indicator ASVs for root communities were detected across control and burned plots of *C. florida* trees before and after the prescribed burn treatment. No fungal indicator ASVs were found in soil and root fungal communities in plots which received prescribed burn treatment.

For above-ground niches, a total of 126 (bark), 130 (stem), and 287 fungal indicator ASVs were detected across control and burned plots of *C. florida* trees before and after the

prescribed burn treatment. In bark fungal communities, Cucurbitariaceae, Rhynchogastermataceae, and Cladosporiaceae were found as indicator fungal ASVs in burned *C. florida* plots. While there was no indicator ASV found for stem fungal communities in post burn plots, Mycosphaerellaceae and Dothideomycetes were detected as fungal ASVs for leaf communities in burned *C. florida* plots.

2.5 Discussion

Fungal and bacterial microbial communities were examined across five different niches associated with 5 different *C. florida* trees that were growing in each of four different plots (two burned, and two unburned). Tree niches were sampled at about the same time of year, and 152 days after imposition of a fire event prescribed for the two burned plots. Our results indicate that prescribed burn had variable effects on bacterial and fungal alpha diversity of different niches likely due to differences in proximity to burn treatments. However, these metrics did differ significantly between 2018 and 2019 likely due to the differences in the environmental factors between the years.

Many studies have focused on the impact of fire on below-ground microbial communities and the importance of these communities for recovery from fire disturbance (Oliver, Callaham Jr, and Jumpponen 2015; Mikita-Barbato, Kelly, and Tate III 2015; Pressler, Moore, and Cotrufo 2019; Dove and Hart 2017). However, the focus on below-ground microbial communities has precluded a comprehensive view of the entire phytobiome response to fire (Kardol and Wardle 2010). Furthermore, much of our understanding of the effects of fire on soil properties comes from wildfires (Oliver, Callaham Jr, and Jumpponen 2015). Wildfires frequently burn hotter, consume more organic matter, and result in greater nutrient volatilization than prescribed burns (Hatten and Zabowski 2010). Therefore, the effects of prescribed burn on microbial communities might be expected to have less dramatic or prolonged effects than those of wildfires (Neary et al. 1999). It is still uncertain whether the impacts of wildfires are comparable to those of prescribed burning (Oliver, Callaham Jr, and Jumpponen 2015). In addition to differences between prescribed and wild fires, microbial responses to fire may be site specific indicating a need for

sampling across a variety of ecosystems and host species (Mikita-Barbato, Kelly, and Tate III 2015).

Soil does not exhibit a single environment, instead, soil encompasses a broad range of diverse microbial habitats (Fierer 2017). This includes the rhizosphere, soil in close proximity to the plant roots; surface layers that are exposed to light, the photic zone; soil found in water flows, and cracks (Fierer 2017). The most important factors that are most likely to influence soil microbiome are soil pH, nitrogen availability, soil organic carbon content, temperature and redox status (Pett-Ridge and Firestone 2005; Oliverio, Bradford, and Fierer 2017; Sul et al. 2013; Cederlund et al. 2014). The immediate effect of fire on soil microbiome is the reduction of their biomass (Certini 2005). Following a fire event, soil pH increases by soil heating because of denaturation of organic acids (Certini 2005). However, significant increases in pH only occur if the fire event reaches high temperature (>450-500 °C) resulting from the complete combustion of present fuel on the ground (Arocena and Opio 2003). For below-ground niches such as soil and roots, fire can directly affect the microbiome by producing high temperatures or indirectly affect them by increasing soil pH (by depositing ash) or by reduction in soil C and N (Grogan, Baar, and Bruns 2000; Grogan, Burns, and Chapin Iii 2000). However, fire disturbances can exert positive, negative, and neutral effects on below-ground microbial communities that are often species specific (Coyle et al. 2017). Previous evidence suggests that fungi are more sensitive to fire than bacteria, probably due to both the lower thermal tolerance of fungi and, for mycorrhizal fungi, the mortality of plant hosts during fire (Pressler, Moore, and Cotrufo 2019; Neary et al. 1999). Our findings of reduction in fungal, but not bacterial alpha diversity following prescribed burns is in line with the findings of these previous studies. Due to the low intensity of the prescribed burn in our study, only the top few centimeters of the soil surface may

have been burned, and because our samples were taken from depths up to 15 cm, deeper soil communities may have been unaffected and the overall response of soil microbial communities to the prescribed burn treatment may have been diluted (Neary et al. 1999; Pressler, Moore, and Cotrufo 2019).

In root fungal communities, ectomycorrhizal species decreased after prescribed burn treatment, but saprophytic species increased. The abundance of saprophytic fungi was higher in post-fire *C. florida* plots, which could be related to findings that spore germination of certain saprophytic fungi is stimulated by heat (Dahlberg 2002). Provided that *C. florida* has shallow root system, saprophytic growth pattern could allow some of these species to survive short periods of live-host absence by persisting on dead host roots or other organic matter (Bonello, Bruns, and Gardes 1998; Martín-Pinto et al. 2006; McLemore 1990). The reduction in ectomycorrhizal species in *C. florida* plots following prescribed burn treatment could be due to the fact that ectomycorrhizal species require more energy from the host plant and have slower growth rates and, therefore, are less competitive after fire events (Torres and Honrubia 1997; Klingeman, Augé, and Flanagan 2002).

Family Acidothermaceae, which was found as an indicator bacterial OTU in root endophytes of burned *Cornus florida* plots, contains thermophilic bacteria which was originally isolated from thermal springs in Yellowstone National Park (WY, USA) (Berry, Barabote, and Normand 2014). *Acidothermus cellulolyticus* is presently the sole species, in the genus *Acidothermus* of the family Acidothermaceae. This bacterial species is capable of degrading cellulose at relatively high growth temperatures (55°C optimum) (Mohagheghi et al. 1986). The *Acidothermus* genome contains genes which encode for thermostable enzymatic cellulose degradation which have been employed in various biotechnological applications. However, the

presence of the family Acidothermaceae in burned *C. florida* plots could affect the stability of the root cell walls which are primarily made of cellulose and hemicellulose (Mohagheghi et al. 1986).

Effects of prescribed burning on the microbiome of *C. florida* have remained largely unknown, despite of the essential roles of microbiome in nutrient cycling as well as in soil and plant health. Intense wildfires tend to have greater impacts and longer lasting effects than low intensity fires, often implemented with prescribed burn management. Thus, it is important to evaluate the effects of prescribed fire as the results from studies on wildfires may not permit extrapolation into prescribed fires. The direct and selective impacts of prescribed fire on microbiome can potentially alter the ecological process that they mediate. Prescribed fire is an important management tool to reduce fuel loads, to remove non-fire adapted species and to sustain fire-adapted taxa in many forested ecosystems of the southeastern USA. Yet, the long-term effects of recurring prescribed fires on microbial communities in these ecosystems remain unclear (Oliver, Callaham Jr, and Jumpponen 2015).

Evaluating the long-term effects of recurring prescribed fires on soil and fungal communities therein is important to properly manage forests to avoid compromising the composition and function of the soil fungal communities that are crucial to ecosystem services through nutrient cycling and facilitation of plant productivity (Wardle et al. 2004). Further work will be needed to determine if the composition of microbial communities will return to the composition of unburned plots or if they will reach some alternative steady state. More detailed functional assays targeting either enzyme activities or genes that code for those enzymes would be helpful to gain further functional inference. Doing genome wide association study can be used

to shed light on the microbial assembly which has been affected by prescribed burn treatment (Tabrett and Horton 2020).

2.6 Appendix: Tables and Figures

Table 2.1 Total exchange capacity (TEC), pH, organic matter (OM), phosphorus (P), calcium (Ca), magnesium (Mg), potassium (K), and ammonium ion concentration (NH_4-N) of *Cornus florida* trees in 2018 and 2019.

Tree Tag	Year	Treatment	TEC	pH	OM	P	Ca	Mg	K	NH4-N
18_CF_71	2018	Control	2.15	4.8	4.27	8	103	29	44	24.3
18_CF_72	2018	Control	7.89	4.5	8.57	9	455	48	53	31.1
18_CF_73	2018	Control	5.38	4.6	9.1	13	274	55	70	42.5
18_CF_74	2018	Control	3.98	4.6	5.28	9	193	43	56	31.8
18_CF_75	2018	Control	3.96	4.6	7.05	9	177	49	61	44.7
18_CF_151	2018	Pre-burned	9.52	4.4	13.55	22	468	74	70	14.7
18_CF_152	2018	Pre-burned	3.61	4.4	6.78	12	127	43	56	7.1
18_CF_153	2018	Pre-burned	8.23	4	11.16	12	249	62	82	8.1
18_CF_154	2018	Pre-burned	6.54	4.1	9	14	243	41	55	15.6
18_CF_156	2018	Pre-burned	4.65	4.2	7.17	9	154	41	60	7.8
18_CF_221	2018	Pre-burned	8.52	4.5	7.01	12	489	48	61	9.2
18_CF_222	2018	Pre-burned	6	4.3	8.84	11	259	44	58	7.7
18_CF_223	2018	Pre-burned	7.46	4	9.08	11	208	68	71	7.9
18_CF_224	2018	Pre-burned	7.45	4.2	9.51	13	301	53	65	8.7
18_CF_225	2018	Pre-burned	6.85	4.2	10.35	11	268	52	59	8.6
18_CF_391	2018	Control	6.87	4.3	7.36	10	279	59	71	8.8
18_CF_392	2018	Control	5.98	4.4	7.01	14	262	46	89	12.9
18_CF_394	2018	Control	6.09	4.3	5.28	6	253	53	53	4.6
18_CF_396	2018	Control	6	4.1	6.81	12	198	51	53	13
18_CF_398	2018	Control	4.02	4.8	6.25	8	244	33	50	14.8
19_CF_71	2019	Control	1.72	4.8	2.86	6	78	22	36	15.5
19_CF_72	2019	Control	2.78	4.7	2.55	5	141	27	54	17.3
19_CF_73	2019	Control	2.34	4.9	2.76	7	122	33	38	17.8
19_CF_74	2019	Control	3.56	4.7	3.28	9	199	32	50	21
19_CF_75	2019	Control	4.26	4.7	3.01	10	256	33	44	22.1
19_CF_151	2019	Post-burned	2.95	4.7	3.1	8	154	31	44	18.8
19_CF_152	2019	Post-burned	2.53	4.8	2.61	7	134	28	45	14.9
19_CF_153	2019	Post-burned	4.43	4.6	2.26	10	274	23	32	13.5

Table 2.1 continued

19_CF_154	2019	Post-burned	3.32	4.6	2.75	8	178	26	43	13.2
19_CF_156	2019	Post-burned	3.94	4.6	1.7	8	238	23	30	13.4
19_CF_221	2019	Post-burned	2.88	4.5	2.59	7	144	22	34	12.6
19_CF_222	2019	Post-burned	2.47	4.6	3.35	6	121	24	33	9.5
19_CF_223	2019	Post-burned	2.16	4.4	2.16	5	78	25	29	11.3
19_CF_224	2019	Post-burned	2.31	4.7	2.41	7	132	18	29	10.2
19_CF_225	2019	Post-burned	2.93	4.2	3.29	6	107	24	33	13.4
19_CF_391	2019	Control	1.83	4.8	2.19	5	79	24	52	15.6
19_CF_392	2019	Control	4.48	4.3	1.96	8	216	21	41	12.7
19_CF_394	2019	Control	1.65	5	1.93	5	92	21	36	21.5
19_CF_396	2019	Control	4.87	4.2	2.86	8	219	25	38	15.7
19_CF_398	2019	Control	1.99	4.8	2.42	5	107	20	36	14.6

Table 2.2 Plant species within 5 m radius of each *Cornus florida* tree in study. *1 – Present, 0 – Absent.

Flowering Dogwood Tree Tag	**White Oak (*Quercus nigra)***	***Sassafras albidum***	***Vitis* spp.**	**Flowering Dogwood (*Cornus florida)***	**Sourwood (*Oxydendrum arboreum)***	***Pinus strobus***	**Persimmon *(Diospyros virginiana)***	**Hickory *(Carya*sp.)**	**Overcup Oak (*Quercus lyrata)***	***Vaccinium* spp.**
71	1	1	1	1	0	0	0	0	0	0
72	1	1	0	1	1	0	0	0	0	0
73	1	0	0	1	0	1	1	0	0	0
74	1	0	0	0	0	0	0	1	0	0
75	1	0	0	1	0	0	1	0	0	0
151	0	0	0	0	0	0	1	0	0	0
152	0	0	0	0	0	1	0	0	0	0
153	0	0	0	0	0	1	0	0	1	0
154	1	0	1	1	0	1	1	0	0	1
156	1	0	0	1	0	0	0	0	0	0
221	1	0	0	1	0	1	0	0	0	0
222	1	0	0	1	1	0	1	0	0	0
223	0	0	0	1	1	1	1	0	0	0
224	0	0	0	1	0	0	1	1	1	0

Table 2.2 continued

225	0	0	0	1	0	1	1	0	1	0
391	1	0	0	1	0	0	0	0	0	0
392	1	0	1	1	0	0	1	0	0	0
394	0	0	1	1	0	1	0	1	0	0
396	1	0	0	1	1	0	0	1	0	0
398	1	0	0	1	0	0	0	0	0	0

Table 2.3 Average age, diameter at breast height (DBH), height, and co-ordinates of *Cornus florida* trees in burned and unburned plots.

Tree Tag	Plot	Burned/Unburned	Average age estimate (years)	DBH (cm)	Height (m)	Latitude	Longitude
71	A	Unburned	39	9	8.8	35.3204087	-86.1523903
72	A	Unburned	38	7	8.9	35.3205077	-86.1533106
73	A	Unburned	50	8.5	9.9	35.3203534	-86.1532791
74	A	Unburned	44	7	7.7	35.3202281	-86.1530394
75	A	Unburned	41	8	11.4	35.3202399	-86.1528422
151	B	Burned	39	8	7.7	35.3206765	-86.1525653
152	B	Burned	35	9	9	35.3207345	-86.1526317
153	B	Burned	37	8	8	35.3205944	-86.1524959
154	B	Burned	42	8	9.4	35.3205827	-86.1527051
156	B	Burned	29	7	6.9	35.320363	-86.1525707
221	C	Burned	32	8	8.3	35.3208499	-86.1523108
222	C	Burned	36	7	6.3	35.3208215	-86.1521985
223	C	Burned	41	7.5	8.4	35.3205367	-86.152399
224	C	Burned	43	10	8.5	35.3205955	-86.1524862
225	C	Burned	40	7	5.4	35.3207684	-86.1526944
391	D	Unburned	34	6	7.2	35.3203096	-86.1526387

Table 2.3 continued

392	D	Unburned	39	8	8.4	35.32038	-86.1523142
394	D	Unburned	34	7	7.1	35.3201761	-86.1520654
396	D	Unburned	NA	6	5.6	35.3202738	-86.1522783

Table 2.4 Rarefaction cutoffs for 16S and ITS sequences across five niches of *Cornus florida* for 2018 and 2019 samples.

Niche	Number of sequences in subsampled libraries	Samples retained (out of 40)
Soils 16S	11,677	40
Soils ITS	14,563	40
Roots 16S	3,614	40
Roots ITS	12,672	39
Bark 16S	10,608	39
Bark ITS	12,184	39
Stem 16S	2,030	38
Stem ITS	17,688	40
Leaves 16S	1,139	40
Leaves ITS	14,362	39

Table 2.5 Two Sample t-test results of alpha diversity (richness and Shannon diversity index) metrics and permutational multivariate ANOVA results with Bray-Curtis distance matrices for both bacterial and fungal communities of belowground niches (soils and roots) of *Cornus florida* before and after prescribed burn treatment (significant P-values bolded).

	Alpha-diversity						**Beta-diversity**			
Niche	Richness			Shannon diversity			Bray-Curtis Dissimilarity			
Organism	Two Sample t-test			Two Sample t-test			PERMANOVA			
Analysis	t	Df	*P*	t	Df	*P*	df	F	R^2	*P*
Soils										
Bacteria										
Treatment	1.28	12.56	0.225	1.68	14.58	0.114	1	2.09	0.03	0.058
Year	7.70	19	**<0.001**	6.29	19	**<0.001**	1	24.52	0.38	**<0.001**
Treatment × Year							1	1.13	0.02	0.269
Plot							2	1.55	0.05	0.107
Year × Plot							2	1.00	0.03	0.381
Fungi										
Treatment	-0.43	17.13	0.673	0.12	16.49	0.905	1	2.01	0.05	**<0.001**
Year	-3.40	19	**0.003**	-4.56	19	**<0.001**	1	3.97	0.09	**<0.001**
Treatment × Year							1	0.63	0.01	0.993
Plot							2	1.75	0.08	**<0.001**
Year × Plot							2	0.54	0.02	1
Roots										
Bacteria										
Treatment	1.08	12.05	0.301	-0.31	12.16	0.762	1	1.78	0.04	**0.017**
Year	0.83	19	0.420	3.53	19	**0.002**	1	2.6	0.06	**<0.001**
Treatment × Year							1	1.19	0.03	0.199
Plot							2	1.35	0.06	0.052
Year × Plot							2	1.13	0.05	0.228
Fungi										

Table 2.5 continued

Treatment	-1.96	14.49	0.069	-0.47	16.85	0.646	1	1.73	0.43	**0.017**
Year	-3.96	18	**0.001**	-6.00	18	**<0.001**	1	1.06	0.03	0.316
Treatment × Year							1	0.95	0.02	0.513
Plot							2	1.87	0.09	**0.001**
Year × Plot							2	0.56	0.03	0.995

Table 2.6 Two Sample t-test results of alpha diversity alpha diversity (richness and Shannon diversity index) metrics and permutational multivariate ANOVA results with Bray-Curtis distance matrices of aboveground niches (bark, stem, and leaves) of *Cornus florida* before and after prescribed burn treatment (significant *P*-values bolded).

	Alpha-diversity						**Beta-diversity**			
Niche	Richness			Shannon diversity			Bray-Curtis Dissimilarity			
Organism	Two Sample t-test			Two Sample t-test			PERMANOVA			
Analysis	t	Df	*P*	t	Df	*P*	Df	F	R^2	*P*
Bark										
Bacteria										
Treatment	1.95	15.02	0.070	0.67	16.35	0.515	1	1.94	0.04	**0.022**
Year	6.9	18	**<0.001**	4.14	18	**<0.001**	1	2.13	0.05	**0.016**
Treatment × Year							1	0.91	0.02	0.514
Plot							2	2.76	0.13	**<0.001**
Year × Plot							2	0.79	0.04	0.841
Fungi										
Treatment	1.18	16.32	0.254	-1.18	11.99	0.259	1	1.38	0.03	**0.04**
Year	-2.32	18	**0.032**	-3.76	18	**0.001**	1	1.95	0.05	**<0.001**
Treatment × Year							1	0.76	0.02	0.92
Plot							2	1.76	0.09	**<0.001**
Year × Plot							2	0.76	0.04	0.979
Stem										
Bacteria										
Treatment	1.95	15.02	0.070	1.91	14.62	0.076	1	1.46	0.03	0.154
Year	5.36	17	**<0.001**	5.16	17	**<0.001**	1	6.89	0.16	**<0.001**
Treatment × Year							1	0.87	0.02	0.477

Table 2.6 continued

Plot							2	1.09	0.05	0.311
Year × Plot							2	0.59	0.03	0.937
Fungi										
Treatment	-1.44	13.66	0.171	-1.48	10.61	0.168	1	1.17	0.02	0.238
Year	-3.02	19	**0.007**	-2.03	19	**0.057**	1	7.09	0.15	**<0.001**
Treatment × Year							1	0.94	0.02	0.520
Plot							2	1.89	0.08	**0.002**
Year × Plot							2	1.03	0.04	0.400
Leaves										
Bacteria										
Treatment	-0.45	12.92	0.664	-0.07	11.28	0.949	1	1.88	0.04	**0.028**
Year	1.19	19	0.248	-1.22	19	0.239	1	6.02	0.13	**<0.001**
Treatment × Year							1	1.03	0.02	0.362
Plot							2	0.86	0.04	0.673
Year × Plot							2	1.04	0.05	0.368
Fungi										
Treatment	1.62	11.99	0.131	1.93	12.78	**0.076**	1	1.76	0.04	**0.045**
Year	-7.64	18	**<0.001**	-10.32	18	**<0.001**	1	10.41	0.21	**<0.001**
Treatment × Year							1	1.26	0.03	0.183
Plot							2	1.53	0.06	0.051
Year × Plot							2	0.94	0.04	0.529

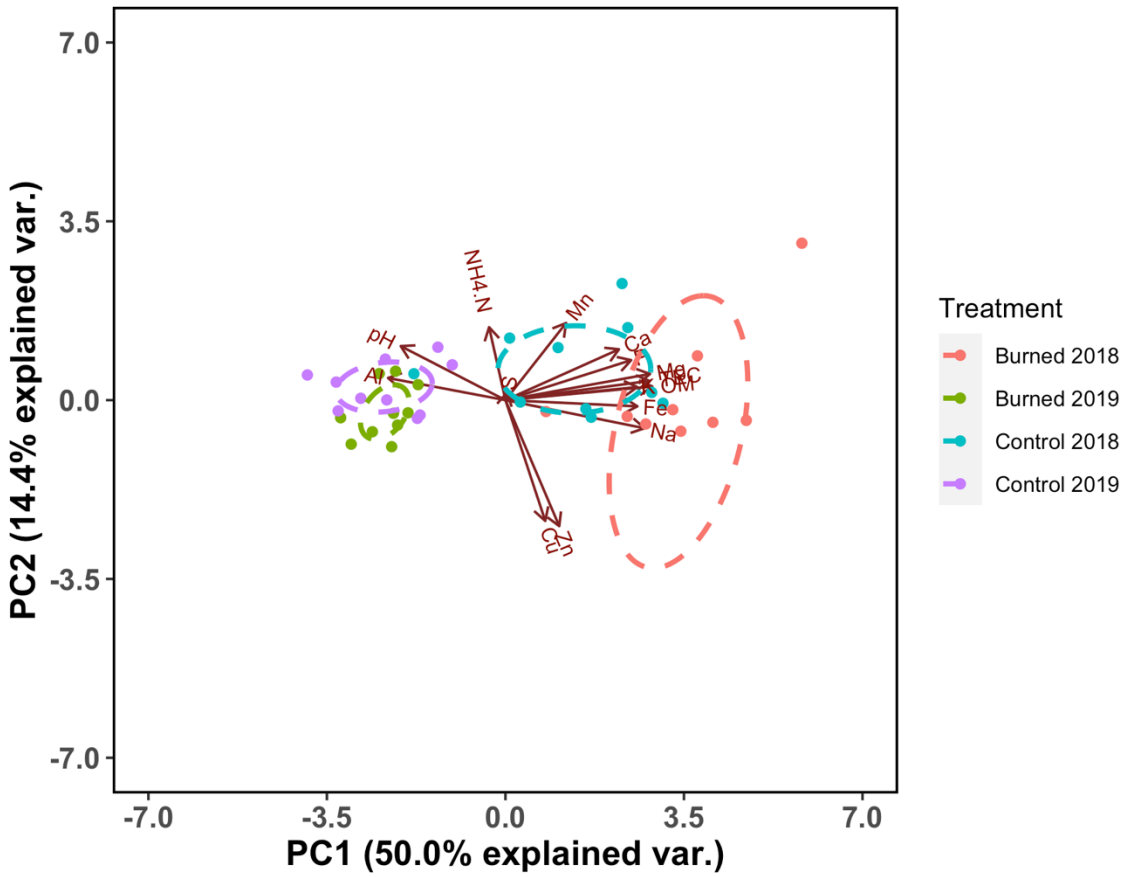

Figure 2.1 Principal component analysis of physicochemical properties of bulk soils collected from 20 *Cornus florida* trees before and after the prescribed burn treatment. PC1 correlates with OM, Mg, TEC, Na, Fe, K, P, Al, and Ca. PC2 correlates with Zn, Cu, Mn, and NH4.N. Soils physicochemical properties did not significantly differ by prescribed burn treatment (Fig. 2.4.1; Pseudo-$F_{1,39} = 2.27$, $P = 0.109$, $R^2 = 0.03$). Points and ellipses are colored by prescribed burn treatment and year, and ellipses represent standard deviation of axis scores from the group centroids. Length of arrows indicate strength of association.

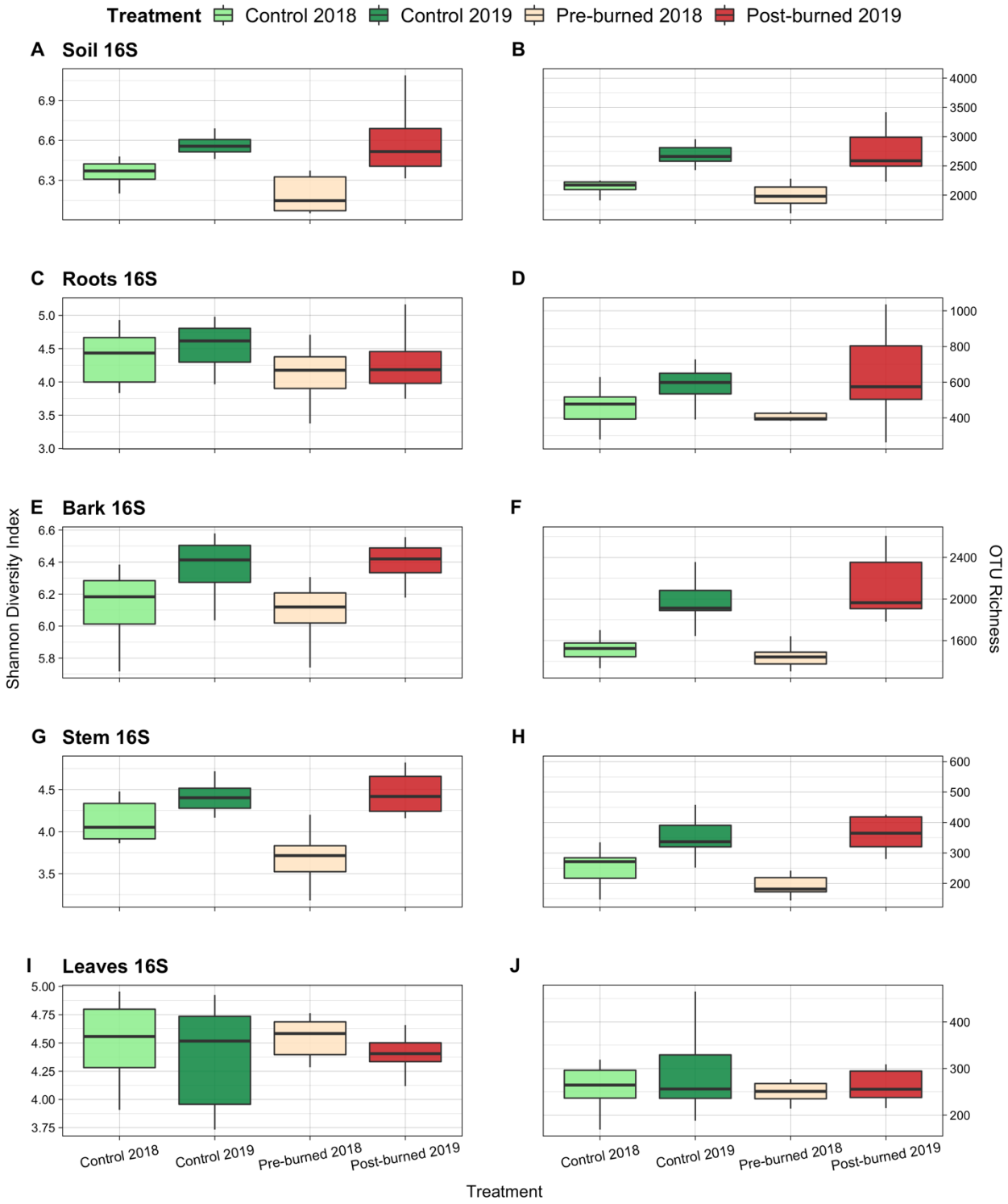

Figure 2.2 Shannon diversity (A, C, E, G, I) and observed OTU richness (B, D, F, H, J) of bacterial communities of belowground niches - soils (A, B) and roots (C, D) and aboveground niches - bark (E, F), stem (G, H), and leaves (I, J) before and after prescribed burn treatment of *Cornus florida* trees. OTU, operational taxonomic unit.

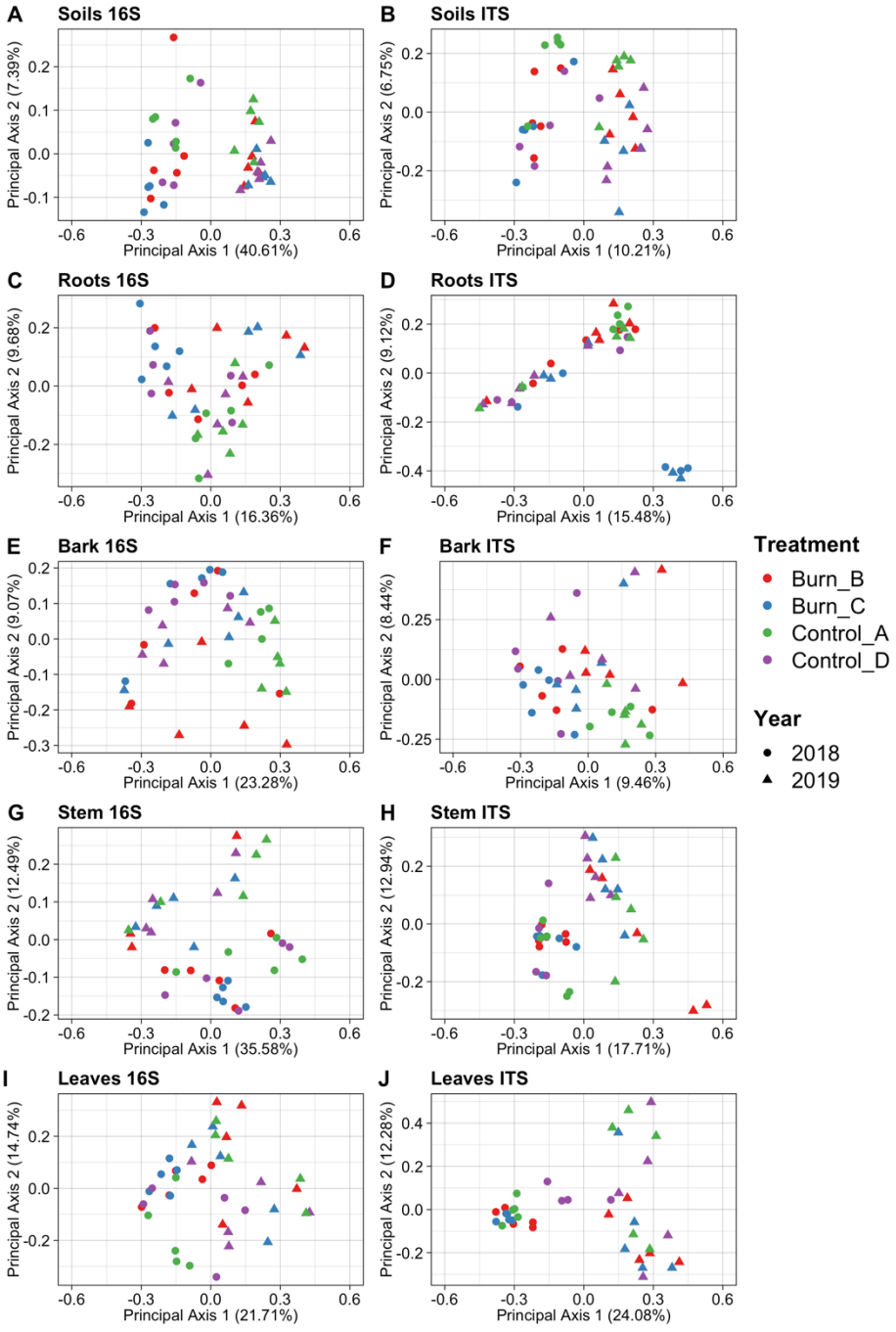

Figure 2.3 Principal coordinates analyses of both bacterial (16S) and fungal (ITS) community composition of soils (A, B), roots (C, D), bark (E, F), stem (G, H), and leaves (I, J) niches before and after prescribed burn treatment of *Cornus florida* plots. Each point represents an individual sample, colored by treatment (control and burn) and shaped by year. See Table 2.2 and 2.3 for statistical test results.

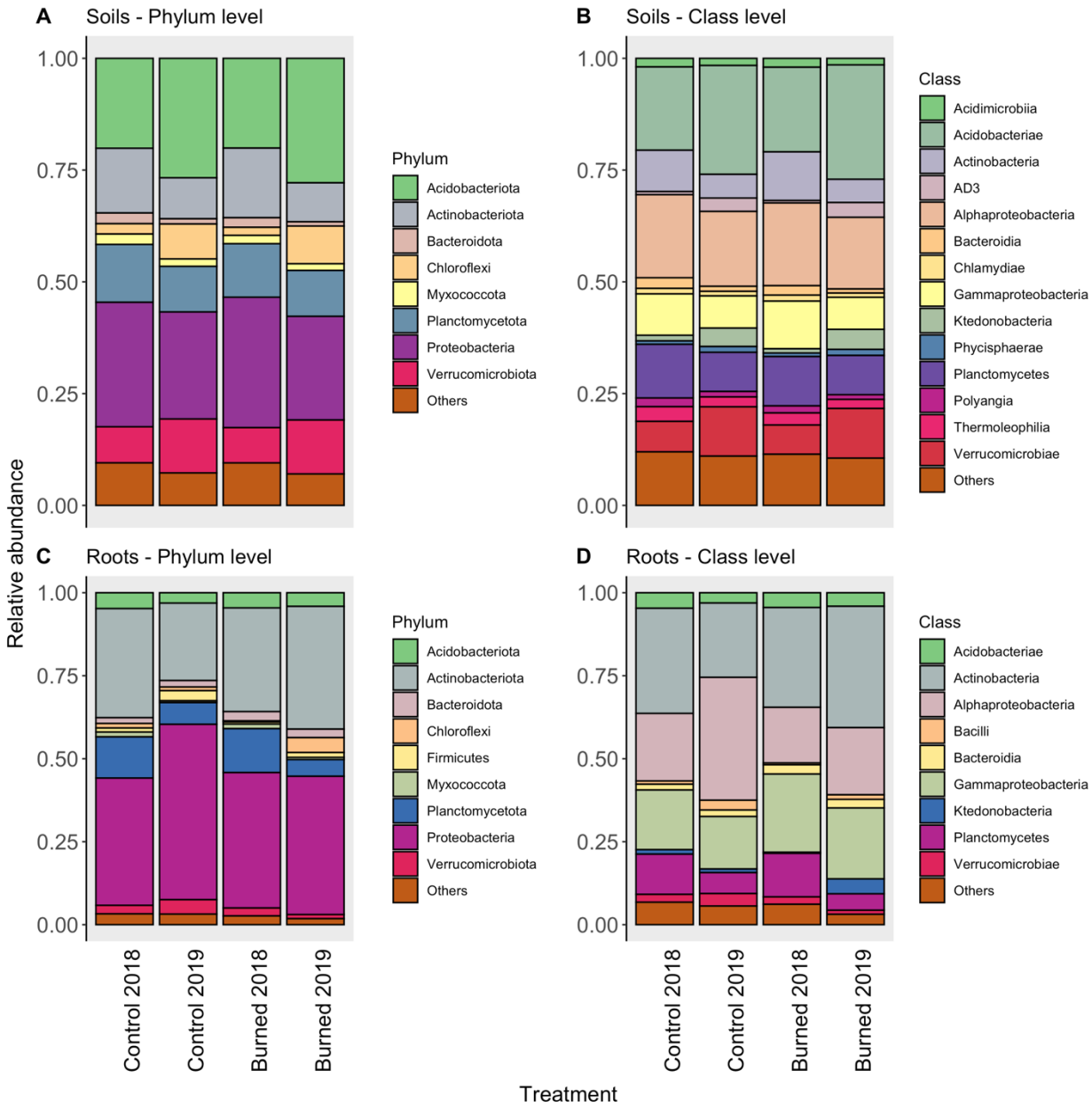

Figure 2.4 Relative abundance of bacterial communities of belowground niches - soils and roots at phylum (A, C) and class (B, D) level. *Others represent taxa that comprised less than 1% of all bacterial sequences.

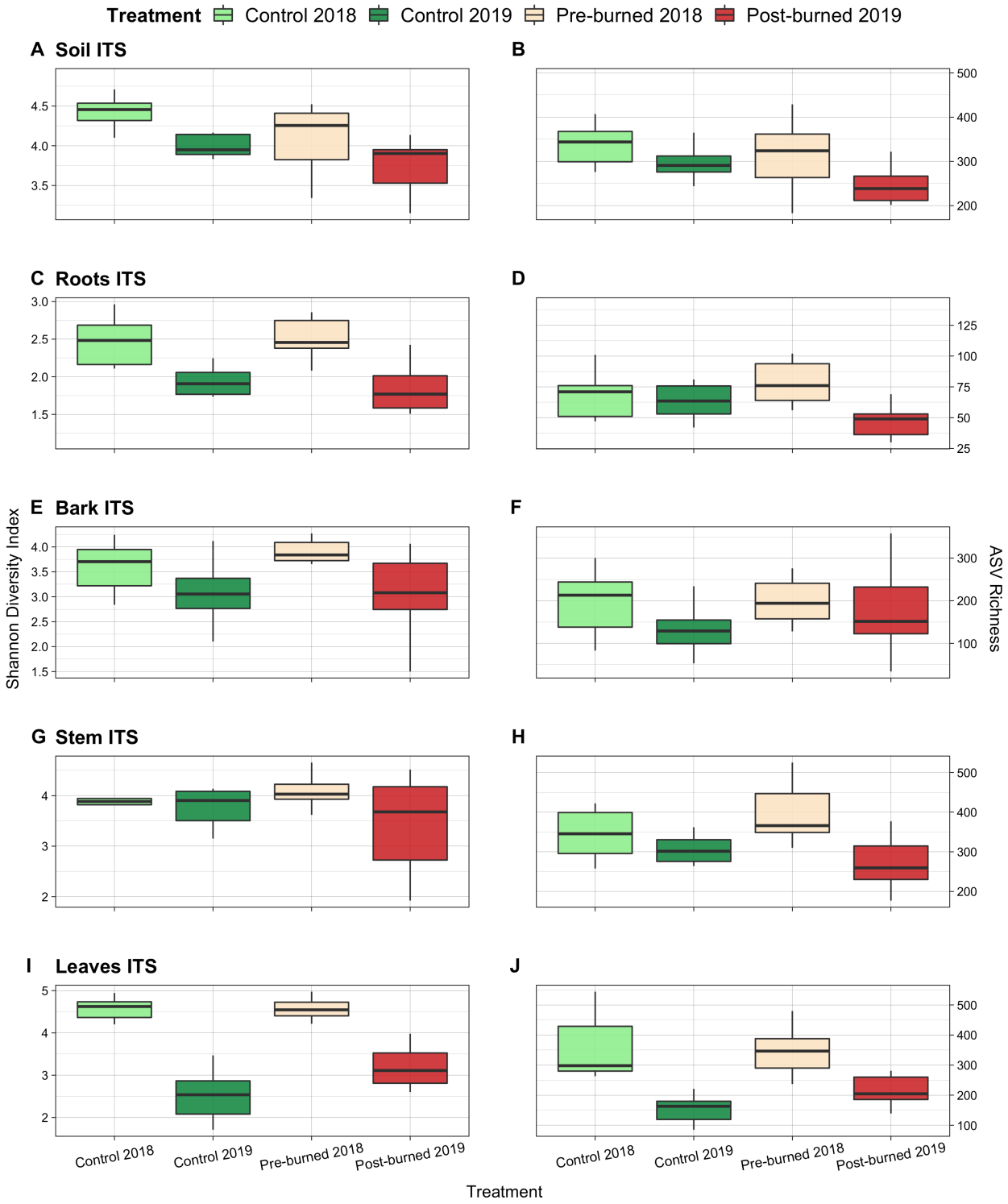

Figure 2.5 Shannon diversity (A, C, E, G, I) and observed ASV richness (B, D, F, H, J) of fungal communities of belowground niches - soils (A, B) and roots (C, D) and aboveground niches - bark (E, F), stem (G, H), and leaves (I, J). ASV, amplicon sequence variant.

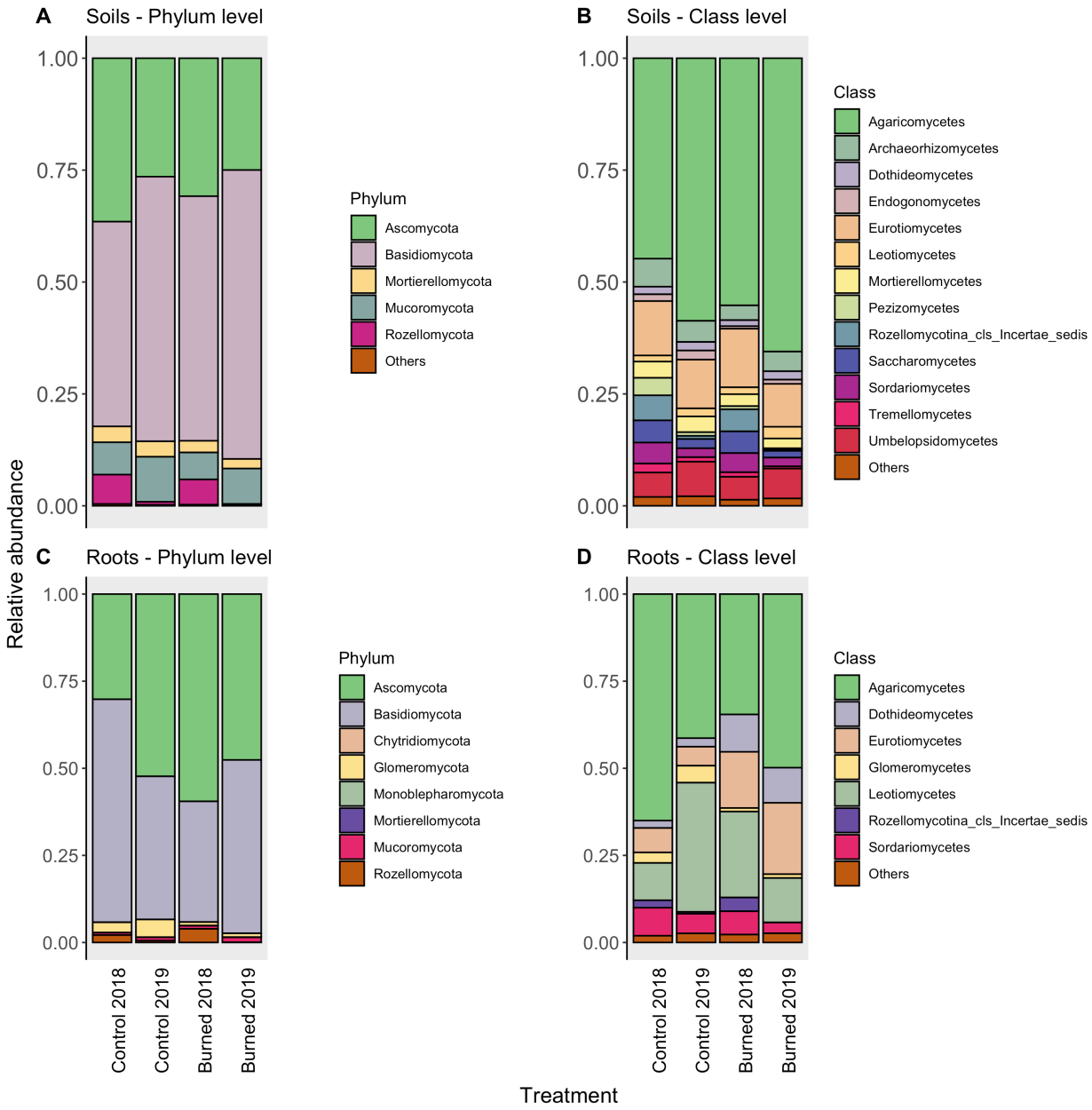

Figure 2.6 Relative abundance of fungal communities of belowground niches - soils and roots at phylum (A, C) and class (B, D) level before and after the prescribed burn treatment of *Cornus florida* trees. *Others represent taxa that comprised less than 1% of all fungal sequences.

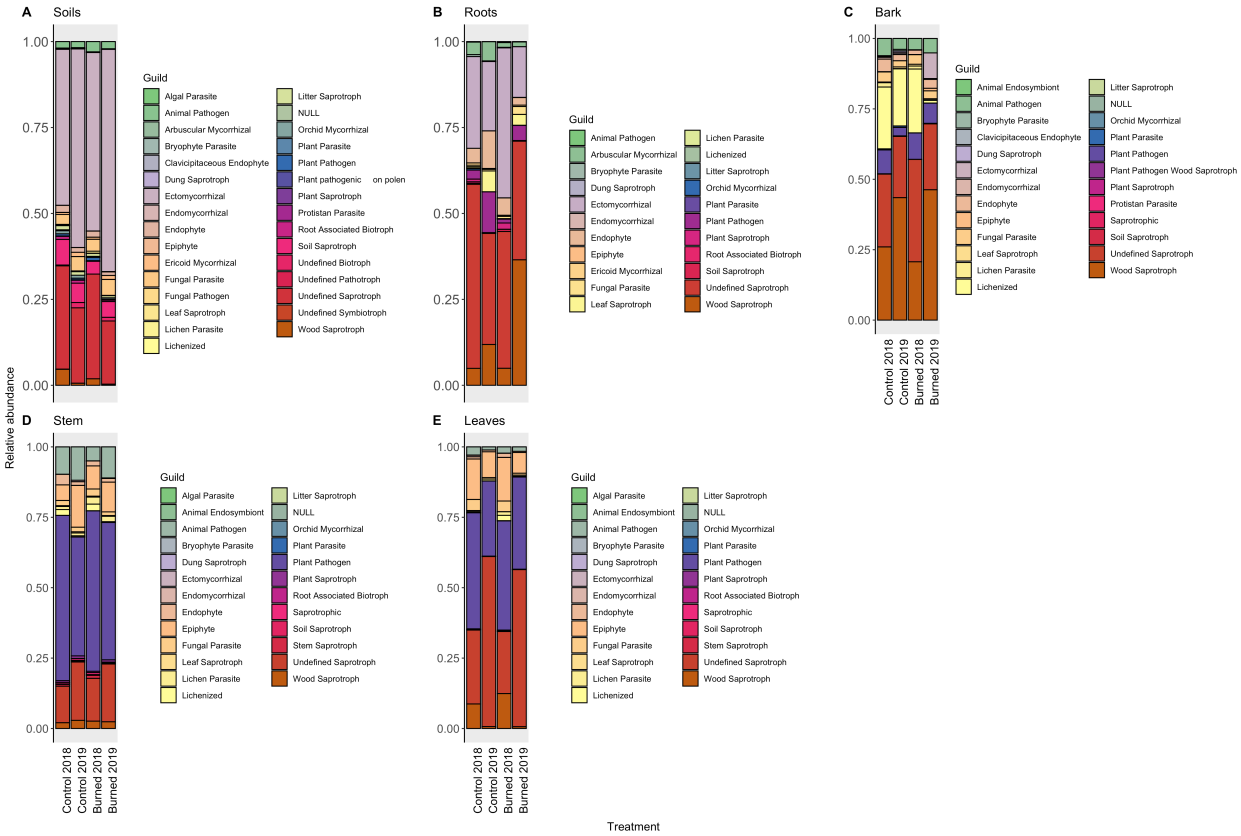

Figure 2.7 Relative abundance of fungal functional guilds of soil (A), roots (B), bark (C), stem (D) and leaves (E) before and after the prescribed burn treatment of *Cornus florida* tree plots.

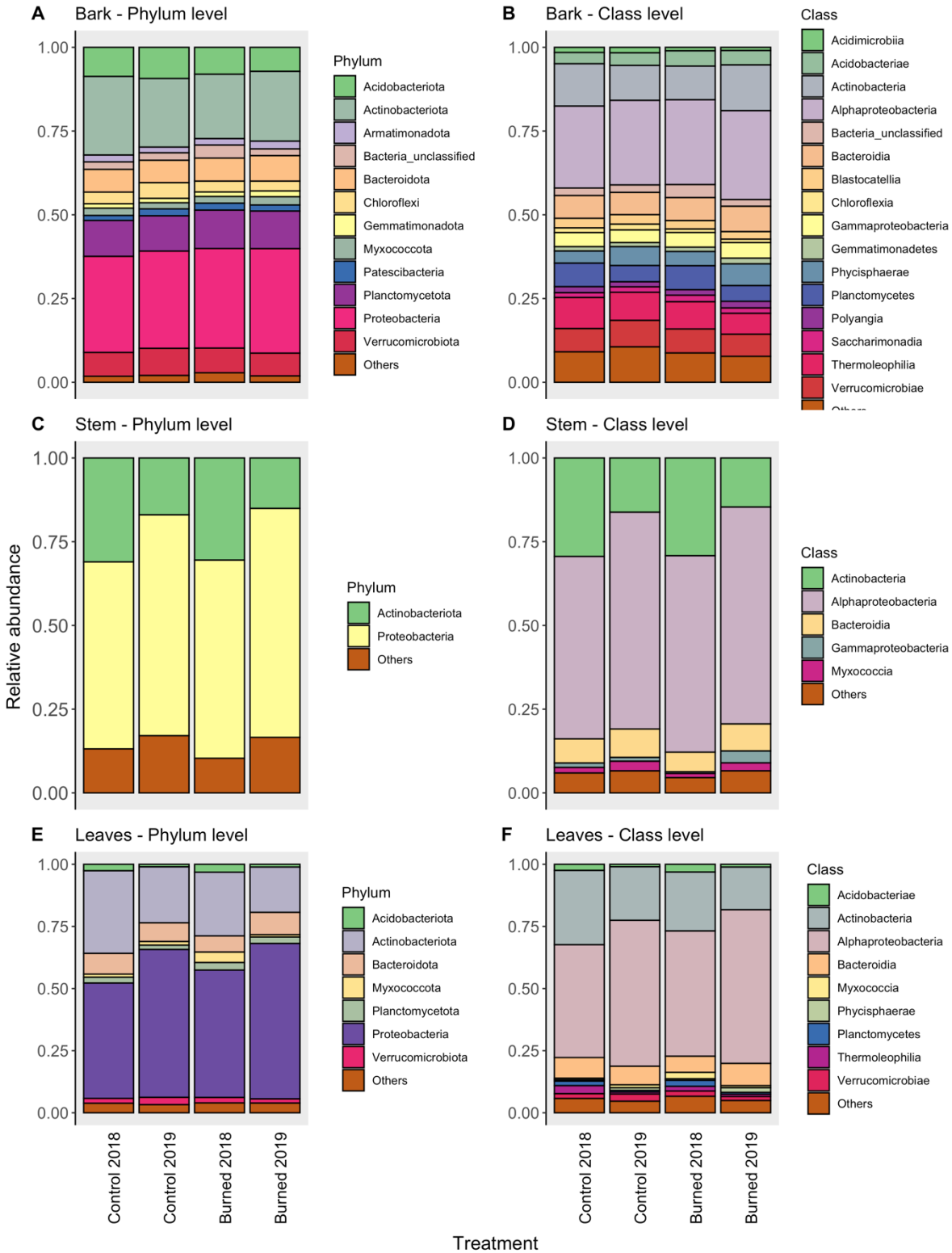

Figure 2.8 Relative abundance of bacterial communities of aboveground niches - bark, stem, and leaves at phylum (A, C, E) and class (B, D, F) level before and after the prescribed burn treatment of *Cornus florida* trees. *Others represent taxa that comprised less than 1% of all bacterial sequences.

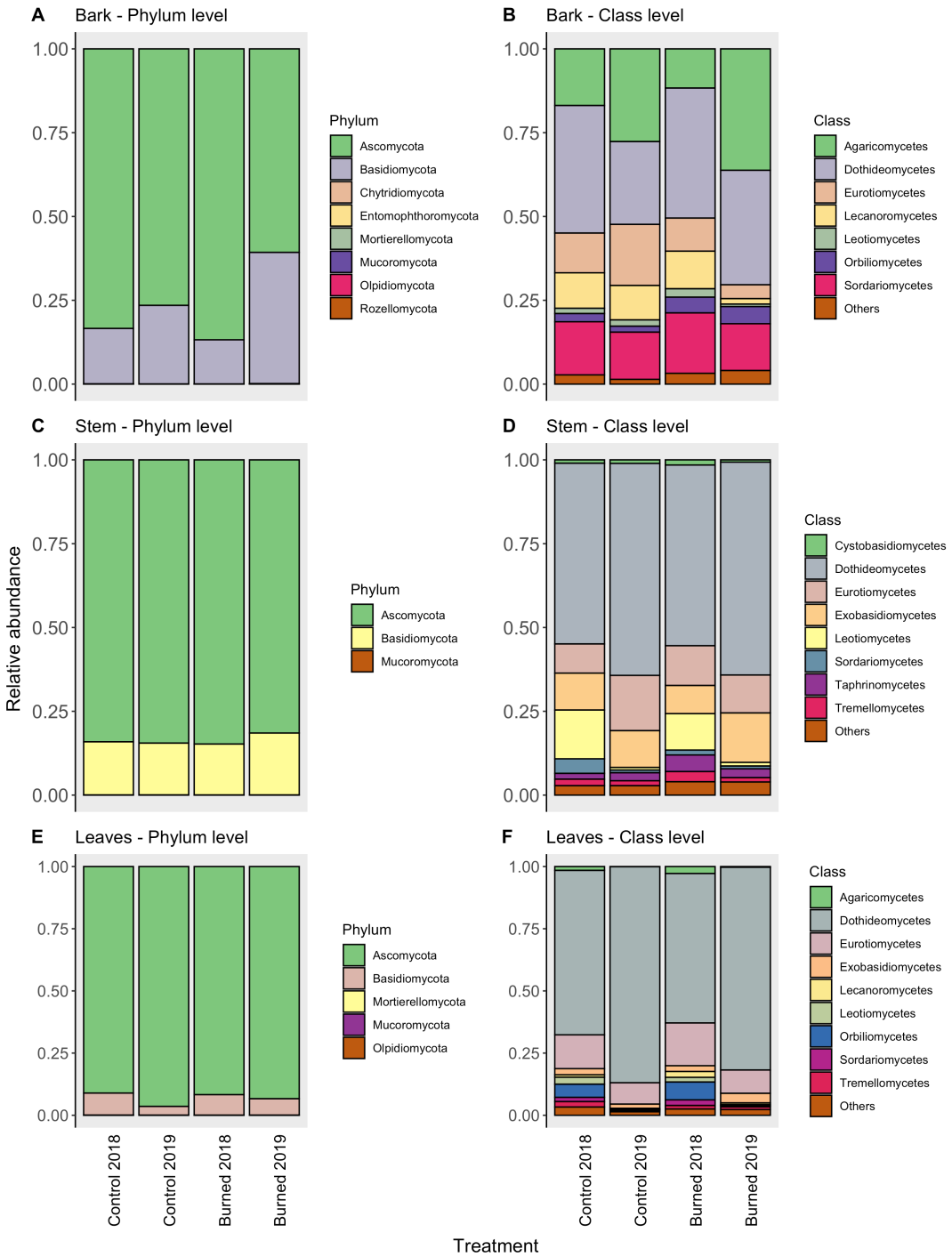

Figure 2.9 Relative abundance of fungal communities of aboveground niches - bark, stem, and leaves at phylum (A, C, E) and class (B, D, F) level before and after the prescribed burn treatment of *Cornus florida* trees. *Others represent taxa that comprised less than 1% of all fungal sequences.

3 Conclusion

This study characterized the microbiome of *C. florida* trees before and after the application of a prescribed burn treatment. Fungal and bacterial microbial communities were examined across five different niches which included soil, fine roots, bark, stem, and leaves associated with five different *C. florida* trees that were growing in each of four different plots (two burned, and two unburned). Tree niches were sampled at about the same time of year and 152 days after imposition of a fire event prescribed for the two burned plots. Our results indicate that prescribed burn had variable effects on bacterial and fungal alpha diversity of different niches likely due to differences in proximity to burn treatment. However, these did differ significantly between 2018 and 2019 likely due to the differences in the environmental factors between the years.

We successfully sampled these niches from *C. florida* trees before and after the prescribed fire. The original plan was to include a fruit niche as a part of study design. Given climatic conditions, prolonged rainy and dry periods, and probable frugivory, our decision to wait to perform the prescribed burn, resulted in loss of this niche as a study possibility. Although *C. florida* fruits ripen from September to late October across its native range, insufficient numbers of fruits were found, and only of a few of the experimental trees, when post-fire sampling was conducted in 2019.

To record the temperature achieved by prescribed burn at different plot locations, temperature indicating liquid paints (Cole-Parmer, Vernon Hills, Illinois) were used. Paints with meltdown temperature indicators at 79, 107, 135, 163, 191, 218, 246, 274, 343, 371, 399, and 427 degrees Celsius were applied parallel to the width of the ceramic white tile (40.64 cm x 10.16 cm) as well as to each other. For each individual tree, four tiles were installed upright at a distance of 5 m from that tree in each of the cardinal directions. However, the evaluation of the temperature achieved during prescribed fire could not be evaluated using this method. Paints did

not melt at those given thermal set points. However, we did install an infrared imaging camera on the site which recorded the temperatures on *C. florida* trees in the burned plots. We used that data to obtain burn temperatures experienced within the plots. Future efforts will further examine this data to extract insight about the behavior of the prescribed burn applied. This issue could have been mitigated if we had added thermocouples in ground soil at a depth of about 5 cm to measure the temperature of the prescribed fire as it was occurring. This would have given us a more comprehensive view of how the prescribed fire behaved with *C. florida* plots and adjacent to study trees.

The alpha diversity of bacterial communities of belowground niches (soil and fine roots of *C. florida* trees) increased in 2019 as compared to 2018 while the alpha diversity of fungal communities decreased. Bacterial and fungal community composition was also significantly different in 2019 as compared to 2018. The relative abundance of bacterial and fungal communities of *C. florida* trees were not significantly changed by prescribed burn treatment. Further analyses such as null model approach would give us greater insight into how the microbial communities in control plots and burned plots varied across time (Zhao et al. 2015). If this experiment could be repeated and additional funds were available for sequence processing, more sampling time points would have been helpful in addressing other experimental questions. For example, additional years of collection would have provided novel information regarding resilience of microbial communities as a result of prescribed fire into extended time points. A microbiome sampling point one month after the prescribed fire, three months after the prescribed fire, and then six months after the prescribed fire would have helped us determine how the microbial communities changed across time. However, given limited funding resources and time needed to complete my M.S. degree, these points were not addressed in this study.

Interestingly, in fungal communities associated with fine roots, ectomycorrhizal species decreased following the prescribed burn treatment, but the abundance of saprophytic fungal species increased. The reduction in ectomycorrhizal species in *C. florida* plots following prescribed burn treatment could be explained by the fact that ectomycorrhizal species require more energy from the host plant, generally have slower growth rates and, therefore, are less competitive after fire events. Furthermore, *Acidothermus* was found as an indicator bacterial species in root niche in burned *C. florida* plots. The presence of this bacterial species can affect the root cell wall stability as these bacterial species contain enzymes that degrade cellulose and hemicellulose (Mohagheghi et al. 1986). The results from this study are intended to be published in the APS journals *Phytobiomes*. Future research will be needed to investigate if this would have any lasting consequences on the stability of microbial symbioses of *C. florida* trees.

The plant microbiome plays an important role in reducing the occurrence of plant disease and increasing nutrient availability under stressful environmental conditions. Evaluating the long-term effects of prescribed fires on plant microbial communities therein is important to avoid compromising the composition and function of these microbial communities (Wardle et al. 2004). Our study demonstrated that the microbial communities of *C. florida* trees exhibited resilience 152 days after prescribed burn treatment. Further work will be needed to determine if the composition of microbial communities of *C. florida* trees will return to the composition of unburned plots, or if they will reach some alternative steady state. More detailed functional assays targeting either enzyme activities or genes that code for those enzymes would be helpful to gain further functional understanding about how the system is affected. Further studies such as metatranscriptomic and genome-wide association studies can be used to evaluate the microbial assemblage that may be influenced by prescribed burn treatment (Tabrett and Horton 2020).

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5 Vita

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