Fungal Community Structural and Functional Responses to Disturbances in a North Temperate Forest

By

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Dedication

To my mother: Melinda Kathryn Fry

For always instilling in me a sense of wonder and curiosity. For all the adventures down dirt roads and imaginations of centuries past. For all your love, Thank you.

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Abstract

Globally soils contain three times as much carbon (C) as the Earth's atmosphere, with an additional 5-10% as much C stored in coarse woody debris as in the atmosphere. Due to the large sizes of these organic C stocks, small shifts in the amounts of soil organic matter, coarse woody debris, and the microbial communities responsible for decomposition could have large effects on global carbon cycle. In this dissertation I combine a series of observational and manipulative experiments to analyze fungal community responses to historical and predicted future disturbances in a forest ecosystem. I explore how fungal communities respond spatially and temporally to clear-cutting and burning, and whether they follow trends in diversity predicted by the intermediate disturbance hypothesis (IDH). In Chapter II, I investigate the short-term effects of clear-cutting and prescribed burning on fungal community composition and function in mineral soils, and how spatial variation in disturbance severity within a 1-ha plot structured these communities. I observed differential effects of clear-cutting or clear-cutting + burning within a 1-ha stand and found that the areas of highest burn disturbance yielded lowest fungal diversity and extracellular enzyme activity (EEA). High burn areas also tended to drive plot level differences in soil physio-chemical properties with increases pH and effective cation exchange capacity (ECEC), while plot level increases in fungal diversity were driven by areas that primarily received the clear-cutting disturbance. My results highlight the importance of spatial variation and scale of sampling when examining both abiotic and biotic responses to disturbances. In Chapter III, I leveraged an existing +100-year cut+burn chronosequence to explore whether soil fungal communities follow decadal patterns in successional trajectories predicted and often observed in plant communities by the IDH. I found that plant community diversity in the chronosequence adhered to patterns predicted by the IDH, however, fungal diversity did not follow similar trajectories. Fungal diversity was lowest in the 61-year old mid-successional plot. The low diversity observed in this plot was primarily attributed competitive exclusion due to a dominance of ectomycorrhizal taxa in the Cortinariaceae that was accompanied by a high abundance of oaks relative to the other experimental plots. When the successional patters were

analyzed with just the remaining chronosequence plots diversity was seen to decrease with successional stage. Conversely, I observed a steady increase in microbial abundance into later successional stages reflecting similar patters observed in primary producer productivity. In Chapter IV, I investigated patters of fungal decomposer communities in coarse woody debris of a pioneering tree species in temperate forests, *Populus grandidentata*, from standing dead trees to the incorporation of woody necromass into soil. I showed that fungal communities are dynamic during and after a decay class continuum, colonizing while trees are standing and continuing to shift throughout coarse woody debris decay. Specifically, I note that nitrogen is an important driver of enzymatic activity in microbial communities and show a correlation with bacterial and fungal species composition and abundance along the continuum of decay. Overall, the work described here offers further support for the importance of disturbances in structuring soil fungal communities. Notably, my work highlights the importance of spatial variability in disturbance severity and long-term legacies on fungal composition, activity, and successional trajectories.

Chapter 1: Introduction

1.1 Ecological Implications of Forest Disturbances in a Changing Global Environment

Throughout history humans have driven changes to ecosystems across the Earth in the pursuit to acquire resources to provide fuel, food, and shelter for ever growing populations. With advances in technology of the 20th century, the rate and magnitude at which humans have modified ecosystems has increased exponentially and has come at the cost of drastic disturbances to abiotic conditions and biotic communities (Bonan, 2008; Dale et al., 2011). The full extent of these disturbances is unknown due to the complexities of interactions and time required for systems to recover. Unravelling the past, present and future effects of disturbances on biological communities and ecosystem processes is essential as we look to moderate human influence moving forward.

Over the next 100 years, both human and natural disturbances are predicted to increase as Earth surface temperatures rise and regional precipitation patterns are altered (IPCC 2013). This is in part due to the altering of biotic communities and biogeochemical cycles leading to increases in atmospheric greenhouse gasses like carbon dioxide, methane, and nitrous oxide. These disruptions are primarily the result of habitat alterations and burning of fossil fuels from human activities. Globally, forests have been shown to provide key ecosystem services by regulating surface energy balances, hydrologic cycles, water quality, and atmospheric composition (Barnes et al., 1998; Bonan, 2008; Fahey et al., 2011). The loss of forests around the Earth has particularly important implications as they have the potential to mediate anthropogenic effects through the sequestration of CO_2 via plant primary production and storage of carbon (C) in biomass and organic deposits in soils. Carbon storage is calculated through measurements of Gross Primary Production (GPP), defined as the total amount of C fixed in organic matter during photosynthesis by primary producers, autotrophic respiration (R_a) of those producers, and heterotrophic respiration (R_h) of heterotrophic organisms (Goulden et al., 2011). Net Ecosystem

Production (NEP) of forests can be calculated as $NEP=GPP- (R_a + R_h)$ to determine whether they are acting as atmospheric C sources or sinks.

Today forests store ~45% of terrestrial C and are responsible for ~50% of terrestrial net primary productivity, thereby partially mitigating previously mentioned anthropogenic CO_2 emissions (Fahey et al., 2011). In the United States (U.S.), forests sequester 13% of the country's annual CO_2 emissions (Vose and Ryan, 2002) with most sequestered C being stored in the form of wood fragments as coarse woody debris (CWD), leaf litter, and partially decomposed organic matter in the soil (Balesdent and Mariotti, 1996; González-Pérez et al., 2004).

Fungal and bacterial decomposers are responsible for breaking down organic C from these various forms and releasing large amounts (~60-80%) back to the atmosphere in the form of CO_2 via respiration (González-Pérez et al., 2004). The importance of the fate of forest organic matter cannot be overstated, as together the pool of C in CWD plus soil organic matter (SOM), is more than double that of the atmosphere. Globally soils store ~1500-3000 Gt of organic C (Sanderman et al., 2018; Scharlemann et al., 2014; Vermeulen et al., 2019) with up to an additional ~36-72 Gt stored in CWD (Magnússon et al., 2016). Each year ~94.3 (±17.9) GT of C is respired from soils which is equal to ~10% of total atmospheric C (Xu and Shang, 2016) and heterotrophic respiration from CWD decomposition can be up to 10%-16% of total respiration in temperate forests (Jomura et al., 2007). Due to the large sizes of these stocks, small shifts in the amount of available SOM, CWD, and the microbial communities responsible for decomposition have undeniably large effects on the global C cycle.

1.2 Effects of Clear-cutting and Burning Disturbances on Biotics and Abiotic Factors of Soil

During the late 19th and early 20th century, widespread logging and subsequent wildfires across the northeastern United States left a legacy of disturbance throughout what was a once mostly contiguous forest (Frelich, 1995; Friedman and Reich, 2005). This has influenced the amount of C these forests have been able to sequester and store in above ground biomass and below ground soil C storage (Gough et al., 2007a). Most C in forests is stored in the form of wood fragments, leaf litter, and partially decomposed organic matter in the soil (Balesdent and Mariotti, 1996; González-Pérez et al., 2004). Rates of C cycling and the ability of forests to store C long-term

have been recently modified. Prior to European settlement, the Great Lakes forests remained intact and upland areas experienced fires or large wind storms on 130-260 year intervals (Whitney, 1987). Aside from the long-term disturbance legacies across the northeastern United States, climate models predict increasingly frequent forest fires, which will have short- and long-term effects on C and nutrient cycles (Stocks et al., 1998).

Soil microbial organisms are critical actors in key ecosystem processes in terrestrial systems around the globe. Temperate forest fungi are particularly important in their roles as saprotrophs (Högberg et al., 2003; Štursová et al., 2012; Talbot et al., 2013) and plant symbionts (Kirk et al., 2004; Smith and Read, 2008). They decompose organic matter, mineralizing nutrients for primary producers and in doing so release enzymes that help to bind soil particles (Sinsabaugh, 2010). Their roles in key processes as well as their sensitivities to perturbations in soil temperature (Frey et al., 2008), moisture (Manzoni et al., 2012), and nutrient availability (Allison et al., 2010, 2008) make them of particular importance to study in light of predicted increases in the frequency and severity of disturbances (Dale et al., 2011; Pechony and Shindell, 2010). Despite the importance of these organisms in temperate ecosystems their ecology remains difficult to study in both time and space (Neary et al., 1999a). Variation in soil properties (Debano, 2000; Gul et al., 2015; Hamman et al., 2007a; Holden et al., 2015; Wan et al., 2001) , plant communities (Chapman et al., 2005; Deka and Mishra, 1983; Hart et al., 2005), micro-climate (Rincón and Pueyo, 2010), and disturbance history (Gough et al., 2007b) within a forested stand make it difficult to generalize trends in community diversity and function. Understanding the variations in the composition and activities of these communities within forests, and how they respond to disturbances is crucial to our understanding of soil ecosystem processes.

Research into the effects of both biotic and abiotic disturbances on soil fungal communities has been recently highlighted as an area of needed focus (Holden and Treseder, 2013). Disturbances can alter soil fungal communities both directly through induced mortality (Dooley and Treseder, 2012; Martín-Pinto et al., 2006; Neary, 2009) and indirectly through alterations to environmental conditions (Gonzalez-Perez et al., 2004; Holden et al., 2015) such as soil temperature (Carney et al., 2007), moisture (Hawkes et al., 2011), nutrient availability, and the quality and quantity of C

inputs (Gonzalez-Perez et al., 2004). Fungi can also be directly affected through mortality of mycorrhizal plant symbionts (Castillo et al., 2018; Courty et al., 2010b). Mycorrhizal fungi form symbioses with most terrestrial primary producers, allowing increased access to nutrients and water (Bucher, 2007; Harrison, 2005; Kirk et al., 2004; Martin and Slater, 2007) while offering protection from soil pathogens (Bonfante and Anca, 2009). These fungi are vulnerable to disturbances to plant communities as they are dependent on their plant counterparts for a source of C.

Alterations to fungal composition, abundances, and function can have direct implications for global cycles via increased or decreased respiration. In temperate forests, soil respiration represents approximately 70% of total ecosystem respiration. Saprotrophic fungi play a particularly important role as regulators of C from terrestrial systems to the atmosphere through decomposition processes (Floudas et al., 2012; Sinsabaugh, 2010). They do so by utilizing suites of hydrolytic and oxidative enzymes (McGuire and Treseder, 2010; Morrissey et al., 2014; Urich et al., 2008) such as cellulases, chitinases, phosphatases and peroxidases. Excretion of these extracellular enzymes is integral in the decomposition of dead plant biomass as they break down bonds in carbohydrates, chitin, xylan, and lignin (Baldrian et al., 2013; López-Mondéjar et al., 2016; Wang et al., 2014). In producing these suites of enzymes fungi respire, releasing C back to the atmosphere as CO2 (Dooley and Treseder, 2012). An estimated 60-80% of organic materials decomposed by saprotrophs in forest soils is returned to the atmosphere (Gonzalez-Perez et al., 2004). Disturbances that directly alter the community or detritus inputs from primary producers may have large implications on extracellular enzyme activity (EEA) in soil fungal communities and, in turn, global C cycles.

1.3 Successional Trends Post Disturbance

Given the long legacy and certain future of anthropogenically driven disturbances, it is our suitable burden to understand the cascading effects on the everchanging forests and the implications for global change. To that end, much work has been done to theorize trends in successional patterns post-disturbance in forested ecosystems. For a century now ecologists have studied the classic debate of Gleason's individualistic concept of community ecology and Clements' deterministic view of climax communities (Clements, 1936; Gleason, 1939). Odum

furthered successional theory including productivity and postulating that annual C storage increases to a maximum in mid-successional stages, thereafter, decreasing as forests mature to late successional stages (Odum, 1969a). This pattern has been rigorously tested and born out to be true across an array of systems spanning the Earth (Bond-Lamberty et al., 2004a; Gough et al., 2007a; Howard et al., 2004; Law et al., 2003). However, recent work has shown that old-growth forests can maintain significant C storage potential through greater resource use efficiency facilitated by increased canopy structural complexity (Gough et al., 2013; Hardiman et al., 2013)

Modern times have further called for theory examining frequency and intensity of disturbances that scatter the globe. Central in importance is the intermediate disturbance hypothesis (IDH), which predicts trends in species diversity as forests progress through successional stages (O'Connell and McCaw, 1997). Despite being often cited, there is no general consensus in support of the IDH. Studies supporting the IDH range from investigations of marine macroalgal and invertebrate communities (Osman, 1977; Sousa, 1979), plant communities (Kershaw & Mallik, 2013), and models incorporating spatial and temporal dynamics (Roxburgh et al., 2004). Meanwhile, abundant evidence, both empirically and theoretically, suggests that the IDH is more often the exception than the rule (Fox, 2013). A recent meta-analysis revealed that most evidence against the IDH occurs in papers focused on aquatic ecology while the majority of terrestrial ecosystem studies supported the IDH (Moi et al., 2020). However important the work that has been done to test these hypotheses, testing of these hypotheses throughout the century in terrestrial ecosystems has focused largely on primary producer communities. In this dissertation I venture to test the applicability of disturbance theory in microbial communities of forested ecosystems.

1.4 Summary of Dissertation Chapters

My dissertation is divided into three chapters. Chapters II-IV focus on historical and future effects of disturbances in north temperate forests on fungal community structure and function as follows. Chapter II focuses on the short-term effects of clear-cutting and subsequent prescribed burning on fungi in mineral soils. Chapter III builds on chapter two by examining the long-term (decadal) trends in succession of fungal communities in mineral soil post disturbance. Finally, in

Chapter IV, I examine fungal succession in the boles of *Populus grandidenta*, a widespread temperate and boreal forest and currently senescing primary successional tree species in north temperate forests, along varying stages of decay.

Chapter II: Short-term effects of clear-cutting and burning on fungal community structure and function in mineral soils

Disturbances to forest ecosystems via clear-cutting and burning can alter soil fungal communities both directly and indirectly. Fungal communities are altered directly through fire induced mortality at the time of disturbance (Dooley and Treseder, 2012; Martín-Pinto et al., 2006; Neary, 2009). The same communities are affected indirectly through alterations of soil environmental conditions (Gonzalez-Perez et al., 2004; Holden et al., 2016), soil physio-chemical properties such as moisture and nutrient availability (Gonzalez-Perez et al., 2004; Hawkes et al., 2011), and mortality of symbiotic plants forming associations with mycorrhizal fungi (Courty et al., 2010a). In this chapter, I explore how fungal communities in mineral soils are affected by an implemented cut +burn experiment in a 130-year old mixed hardwood forest and how variation in disturbance severities within this experimental forest structured communities spatially.

I mapped the variation in disturbance severity within an experimental cut + burn stand to examine location specific effects on fungal communities and abiotic conditions. I sampled mineral soils pre- and post-disturbance to analyze fungal communities' structure via amplicon-based sequencing and function via extracellular enzyme activities. These analyses were combined with analyses of soil physio-chemical properties and mapped to varying severities. I predicted that 1) cutting and burning will lead to a mosaic of varying localized disturbance severities within a 1-ha plot; 2) Fungal diversity and abundances will increase in moderately disturbed areas consisting of cut+low burn severity and decrease in areas of high burn severity; and 3) Extracellular enzyme activities associated with decomposition will track fungal diversity and abundance.

Chapter III: Decadal Trends of Fungal Succession in Mineral Soils Following Clear-Cutting and Burning of a Mixed Hardwood Forest

Forest disturbances have the potential to alter soil physio-chemical properties and successional trajectories of biotic communities (Catford et al., 2012; Nave et al., 2017). Time since disturbance is particularly important to consider when examining successional trajectories and the implications for stand diversity and productivity. The intermediate disturbance hypotheses (IDH) predicts a peak in diversity in mid-successional stages of succession and has often been observed in plant communities (Connell, 1978). The IDH has been often extended to forest productivity which at times follows similar patterns, peaking mid-succession (Kershaw and Mallik). However, productivity has also recently been observed to increase into late successional stages (Gough et al., 2013; Hardiman et al., 2013). In this study I explore whether fungal communities follow the often observed and predicted patterns of mid-successional peaks seen in plant community diversity and productivity.

I leveraged an existing cut + burn experimental chronosequence spanning >100-years of succession in a north mixed hardwood forest. Sampling across the already established chronosequence offered insights into the long-term legacy of deforestation and slash fire across the Northeastern U.S on soil fungal communities. I quantified the long-term effects of fire on microbial diversity and function by measuring 1) mineral soil physio-chemical properties (i.e. carbon and nitrogen stocks, pH, soil moisture, etc.) 2) microbial communities through amplicon-based sequencing of the ITS2 region, and 3) a suite of hydrolytic and oxidative enzymes responsible for SOM decomposition. I predict that 1) peak fungal diversity and enzymatic activity will occur in mid-successional forest stands with the highest productivity, 2) low diversity in early successional stand following disturbance as communities are still recovering to pre-condition levels, and 3) low diversity in late succession stands relative to mid-succession due to competitive exclusions by dominant taxa.

Chapter IV: Fungal Succession of Populus grandidentata (Bigtooth Aspen) During Wood Decomposition

Mortality of early colonizing trees during successional shifts as well as species-specific mortality due to pest and pathogens can directly affect coarse woody debris (CWD) production in forests

(Brais et al., 2006; Gough et al., 2007a). CWD consists of small to large dead branches, snags and downed logs in forests, and can represent up to 20% of total forest C stocks (Boddy, 2001; Forrester et al., 2015). Variations in C fluxes from CWD in forests can be large (Harmon et al., 2011), and recent studies indicate that wood decomposer community richness may explain much of the variation in respiration and decay rates by microbial decomposers (Forrester et al., 2015; Toljander et al., 2006; Valentín et al., 2014). In this chapter, I investigated patterns of fungal decomposer communities in CWD of a pioneering species, *Populus grandidentata*, from standing dead trees to incorporation into soil, and whether those shifts co-vary with substrate chemistry and co-occurring bacterial communities.

I sampled fungal communities from boles of *Populus grandidentata* that were identified as standing dead (SD) or assigned to a decay class (DC) 1-5, with DC1 representing freshly fallen trees and DC5 representing individuals in late stages of decay. I examined fungal community structure through amplicon-based sequencing, function via extracellular enzyme activity assays, abundances and fungal bacterial ratios through quantitative PCR, and physio-chemical properties (C, N, δ_{15}N, pH, and moisture) of CWD along the decompositional gradient. I also compared fungal results with prior analysis of bacterial community structure in the same boles to disentangle the relative importance of biotic vs abiotic drivers structuring fungal communities. I predicted that diversity of fungal community structure would be primarily driven by wood physio-chemistry and increase along the continuum of decay, and that enzyme profiles would shift from predominately lignin associated decomposition to greater rates of cellulose and hemicellulose depolymerization in later stages of decay.

1.5 References

Adamčík, S., Buyck, B., 2011. The Species of Russula Subsection Xerampelinae Described. Cryptogam. Mycol. 32, 63–81. https://doi.org/10.7872/crym.v32.iss1.2012.063

Allison, S.D., McGuire, K.L., Treseder, K.K., 2010. Resistance of microbial and soil properties to warming treatment seven years after boreal fire. Soil Biol. Biochem. 42, 1872–1878. https://doi.org/10.1016/j.soilbio.2010.07.011

Baldrian, P., Kolařík, M., Štursová, M., Kopecký, J., Valášková, V., Větrovský, T., Žifčáková, L., Šnajdr, J., Rídl, J., Vlček, Č., Voříšková, J., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. ISME J. 6, 248–258. https://doi.org/10.1038/ismej.2011.95

Baldrian, P., Šnajdr, J., Merhautová, V., Dobiášová, P., Cajthaml, T., Valášková, V., 2013. Responses of the extracellular enzyme activities in hardwood forest to soil temperature and seasonality and the potential effects of climate change. Soil Biol. Biochem. 56, 60–68. https://doi.org/10.1016/j.soilbio.2012.01.020

Barg, A.K., Edmonds, R.L., 1999. Influence of partial cutting on site microclimate, soil nitrogen dynamics, and microbial biomass in Douglas-fir stands in western Washington. Can. J. For. Res. 29, 705–713. https://doi.org/10.1139/x99-045

Belillas, C.M., Feller, M.C., 1998. Relationships between fire severity and atmospheric and leaching nutrient losses in British Columbia's coastal western hemlock zone forests. Int. J. Wildl. Fire 8, 87–101.

Biswas, A., 2018. Scale–location specific soil spatial variability: A comparison of continuous wavelet transform and Hilbert–Huang transform. Catena 160, 24–31. https://doi.org/10.1016/j.catena.2017.08.019

Boddy, L., Watkinson, S., 1994. Wood decomposition, higher fungi, and their role in nutrient redistribution. Can. J. Bot. 73.

Brais, S., Camire, C., Pare, D., 1995. Impacts of whole-tree harvesting and winter windrowing on soil pH and base status of clayey sites of northwestern Quebec. Can. J. For. Res. 25.

Brais, S., Paré, D., Lierman, C., 2006. Tree bole mineralization rates of four species of the Canadian eastern boreal ... Can. J. For. Res. 36.

Buyck, B., Mitchell, D., Parrent, J., 2006. Russula parvovirescens sp. nov., a common but ignored species in the eastern United States . Mycologia 98, 612–615. https://doi.org/10.1080/15572536.2006.11832664

Byrd, K.B., Parker, V.T., Vogler, D.R., Cullings, K.W., 2000. The influence of clear-cutting on ectomycorrhizal fungus diversity in a lodgepole pine (Pinus centorta) stand, Yellowstone National Park, Wyoming, and Gallatin National Forest, Montana. Can. J. Bot. 78, 149–156. https://doi.org/10.1139/b99-171

Campbell, G.S., Jungbauer, J.D., Bristow, K.L., Hungerford, R.D., 1995. Soil temperature and water content beneath a surface fire. Soil Sci. 6, 363–374.

Catford, J.A., Daehler, C.C., Murphy, H.T., Sheppard, A.W., Hardesty, B.D., Westcott, D.A., Rejmánek, M., Bellingham, P.J., Pergl, J., Horvitz, C.C., Hulme, P.E., 2012. The intermediate disturbance hypothesis and plant invasions: Implications for species richness and management. Perspect. Plant Ecol. Evol. Syst. 14, 231–241. https://doi.org/10.1016/j.ppees.2011.12.002

Certini, G., 2005. Effects of fire on properties of forest soils: A review. Oecologia. https://doi.org/10.1007/s00442-004-1788-8

Chapin, F.S., Matson, P.A., Mooney, H., 2002. Principles of Ecosystem Ecology.

Chapman, S.K., Langley, J. a, Hart, S.C., Koch, G.W., 2005. Plants actively control nitrogen cycling: uncorking the microbail bottleneck. New Phytol. 169, 27–34.
Claridge, A.W., Trappe, J.M., Hansen, K., 2009. Do fungi have a role as soil stabilizers and remediators after forest fire? For. Ecol. Manage. 257, 1063–1069. https://doi.org/10.1016/j.foreco.2008.11.011
Connell, J.H., 1978. Diversity in tropical rain forests and coral reefs. Science (80-.). 199, 1302–1310. https://doi.org/10.1126/science.199.4335.1302
Cortina, J., Vallejo, V.R., 1994. Effects of clearfelling on forest floor accumulation and litter decomposition in a radiata pine plantation. For. Ecol. Manage. 70, 299–310. https://doi.org/10.1016/0378-1127(94)90095-7
Covington, W.W., 1981. Changes in Forest Floor Organic Matter and Nutrient Content Following Clear Cutting in Northern Hardwoods. Ecol. Soc. Am. 62, 41–48.
Czimczik, C.I., Masiello, C.A., 2007. Controls on black carbon storage in soils. Global Biogeochem. Cycles 21. https://doi.org/10.1029/2006GB002798
Debano, L.F., 2000. The role of fire and soil heating on water repellency in wildland environments: A review, in: Journal of Hydrology. pp. 195–206. https://doi.org/10.1016/S0022-1694(00)00194-3
DeBano, L.F., Neary, D.G., Ffolliott, P.F., 1998. Fire's Effects on Ecosystems. John Wiley and Sons, New YOrk.
Deka, H.K., Mishra, R.R., 1983. The effect of slash burning on soil microflora. Plant Soil 73, 167–175. https://doi.org/10.1007/BF02197713
Dooley, S.R., Treseder, K.K., 2012. The effect of fire on microbial biomass: A meta-analysis of field studies. Biogeochemistry 109, 49–61. https://doi.org/10.1007/s10533-011-9633-8
Everett, R.L., Sharrow, S.H., 1985. Response of grass species to tree harvesting in singleleaf pinyon- Utah juniper stands. Res. Pap. - US Dep. Agric. For. Serv. 45. https://doi.org/10.5962/bhl.title.69006
Fahey, T.J., Arthur, M.A., 1994. Studies of Root Decomposition Following Harvest of a Northern. For. Sci. 40, 618–629.
Floudas, D., Binder, M., Riley, R., Barry, K., Blanchette, R., Henrissat, B., Martínez, A.T., Otillar, R., Spatafora, J.W., Yadav, J.S., Coutinho, P.M., Vries, R.P. De, Ferreira, P., Findley, K., Foster, B., 2012. Lignin Decomposition Reconstructed from 31 Fungal Genomes. Science (80-.). 336, 1715–1719.
Fox, J.W., 2013. The intermediate disturbance hypothesis should be abandoned. Trends Ecol. Evol. 28, 86–92. https://doi.org/10.1016/j.tree.2012.08.014
Freeman, S., Horowitz, S., Sharon, A., 2001. Pathogenic and nonpathogenic lifestyles in Colletotrichum acutatum from strawberry and other plants. Phytopathology 91, 986–992. https://doi.org/10.1094/PHYTO.2001.91.10.986
Frey, S.D., 2019. Mycorrhizal Fungi as Mediators of Soil Organic Matter Dynamics. Annu. Rev. Ecol. Evol. Syst. 50, 237–259. https://doi.org/10.1146/annurev-ecolsys-110617-062331
Frey, S.D., Drijber, R., Smith, H., Melillo, J., 2008. Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biol. Biochem. 40, 2904–2907. https://doi.org/10.1016/j.soilbio.2008.07.020
Fukatsu, T., Sato, H., Kuriyama, H., 1997. Isolation, Inoculation to Insect Host, and Molecular Phylogeny of an Entomogenous Fungus Paecilomyces tenuipes. J. Invertebr. Pathol. 70, 203–208. https://doi.org/10.1006/jipa.1997.4696
Fukuzawa, K., Shibata, H., Takagi, K., Nomura, M., Kurima, N., Fukazawa, T., Satoh, F., Sasa,

K., 2006. Effects of clear-cutting on nitrogen leaching and fine root dynamics in a cool-temperate forested watershed in northern Japan. For. Ecol. Manage. 225, 257–261. https://doi.org/10.1016/j.foreco.2006.01.001
Gadgil, R.L., Gadgil, P.D., 1971. Mycorrhiza and litter decomposition. Nature 233, 133. https://doi.org/10.1038/233133a0
Gholz, H.L., Perry, C.S., Cropper, W.P., Hendry, L.C., 1985. Litterfall, decomposition, and nitrogen and phosphorus dynamics in a chronosequence of slash pine (Pinus elliottii) plantations. For. Sci. 31, 463–478. https://doi.org/10.1093/forestscience/31.2.463
Giovannini, G., Lucc, 1983. Effect of fire on hydrophobic and cementing substances of soil aggregates. Soil Sci. 136, 231–236.
Giovannini, G., Lucchesi, S., 1997. Modifications induced in soil physico-chemical parameters by experimental fires at different intensities. Soil Sci. 162, 479–486.
Godbout, C., Fortin, J.A., 1985. Synthesized ectomycorrhizae of aspen: fungal genus level of structural characterization. Can. J. Bot. 63, 252–262. https://doi.org/10.1139/b85-029
González-Pérez, J.A., González-Vila, F.J., Almendros, G., Knicker, H., 2004. The effect of fire on soil organic matter--a review. Environ. Int. 30, 855–870. https://doi.org/10.1016/j.envint.2004.02.003
Gough, C.M., Hardiman, B.S., Nave, L.E., Bohrer, G., Kyle, D., Vogel, C.S., Nadelhoffer, K.J., Curtis, P.S., Gough, C., Hardiman, B.S., Nave, L., Bohrer, G., Maurer, K.D., Vogel, C.S., Nadelhoffer, K.J., Curtis, P.S., 2013. Sustained carbon uptake and storage following moderate disturbance in a Great Lakes forest 23, 1202–1215.
Gough, C.M., Vogel, C.S., Harrold, K.H., George, K., Curtis, P.S., 2007. The legacy of harvest and fire on ecosystem carbon storage in a north temperate forest. Glob. Chang. Biol. https://doi.org/10.1111/j.1365-2486.2007.01406.x
Gough, C.M., Vogel, C.S., Schmid, H.P., Curtis, P.S., 2008. Controls on Annual Forest Carbon Storage: Lessons from the Past and Predictions for the Future. Bioscience. https://doi.org/10.1641/B580708
Gul, S., Whalen, J.K., Thomas, B.W., Sachdeva, V., Deng, H., 2015. Physico-chemical properties and microbial responses in biochar-amended soils: Mechanisms and future directions. Agric. Ecosyst. Environ. https://doi.org/10.1016/j.agee.2015.03.015
Hamman, S.T., Burke, I.C., Stromberger, M.E., 2007a. Relationships between microbial community structure and soil environmental conditions in a recently burned system. Soil Biol. Biochem. https://doi.org/10.1016/j.soilbio.2007.01.018
Hamman, S.T., Burke, I.C., Stromberger, M.E., 2007b. Relationships between microbial community structure and soil environmental conditions in a recently burned system. Soil Biol. Biochem. 39, 1703–1711. https://doi.org/10.1016/j.soilbio.2007.01.018
Hardiman, B.S., Gough, C.M., Halperin, A., Hofmeister, K.L., Nave, L.E., Bohrer, G., Curtis, P.S., 2013. Maintaining high rates of carbon storage in old forests: A mechanism linking canopy structure to forest function. For. Ecol. Manage. 298, 111–119. https://doi.org/10.1016/j.foreco.2013.02.031
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. For. Ecol. Manage. https://doi.org/10.1016/j.foreco.2005.08.012
Hart, S.C., Firestone, M.K., Paul, E.A., 1991. Decomposition and nutrient dynamics of pondersosa pine needles in a Mediterranean-type climate. Can. J. For. Res. 22, 306–314. https://doi.org/10.16309/j.cnki.issn.1007-1776.2003.03.004

Harvey, A.E., Jurgensen, M.F., Larsen, M.J., 1980. Clearcut harvesting and ectomycorrhizae: survival of activity on residual roots and influence on a bordering forest stand in western Montana. Can. J. Bot. 10, 300–303.
Hassett, J.E., Zak, D.R., 2005. Aspen Harvest Intensity Decreases Microbial Biomass, Extracellular Enzyme Activity, and Soil Nitrogen Cycling. Soil Sci. Soc. Am. J. 69, 227. https://doi.org/10.2136/sssaj2005.0227
Hatch, A.B., C.T., H., 1933. Some Hymenomycetes forming mycorrhizae with Pinus strobus. J. Arnold Arbor. 14, 324–334.
Högberg, M.N., Bååth, E., Nordgren, A., Arnebrant, K., Högberg, P., 2003. Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs - A hypothesis based on field observations in boreal forest. New Phytol. 160, 225–238. https://doi.org/10.1046/j.1469-8137.2003.00867.x
Holden, S.R., Treseder, K.K., 2013. A meta-analysis of soil microbial biomass responses to forest disturbances. Front. Microbiol. 4. https://doi.org/10.3389/fmicb.2013.00163
Hu, Z., Xu, C., McDowell, N.G., Johnson, D.J., Wang, M., Luo, Y., Zhou, X., Huang, Z., 2017. Linking microbial community composition to C loss rates during wood decomposition. Soil Biol. Biochem. 104, 108–116. https://doi.org/10.1016/j.soilbio.2016.10.017
Ice, G.G., Neary, D.G., Adams, P.W., 2004. Effects of Wildfire on Processes. J. For. 102, 16–20.
Jensen, M., Michelsen, A., Gashaw, M., 2001. Responses in plant, soil inorganic and microbial nutrient pools to experimental fire, ash and biomass addition in a woodland savanna. Oecologia 128, 85–93. https://doi.org/10.1007/s004420000627
Jiménez Esquilín, A.E., Stromberger, M.E., Massman, W.J., Frank, J.M., Shepperd, W.D., 2007. Microbial community structure and activity in a Colorado Rocky Mountain forest soil scarred by slash pile burning. Soil Biol. Biochem. https://doi.org/10.1016/j.soilbio.2006.12.020
Johnson, C.E., Johnson, A.H., Siccama, T.G., 1991. Whole-Tree Clear-Cutting Effects on Exchangeable Cations and Soil Acidity. Soil Sci. Soc. Am. J. 55, 502. https://doi.org/10.2136/sssaj1991.03615995005500020035x
Jomura, M., Kominami, Y., Tamai, K., Miyama, T., Goto, Y., Dannoura, M., Kanazawa, Y., 2007. The carbon budget of coarse woody debris in a temperate broad-leaved secondary forest in Japan. Tellus, Ser. B Chem. Phys. Meteorol. 59, 211–222. https://doi.org/10.1111/j.1600-0889.2006.00234.x
Kara, O., Bolat, I., 2009. Short-term effects of wildfire on microbial biomass and abundance in black pine plantation soils in Turkey. Ecol. Indic. 9, 1151–1155. https://doi.org/10.1016/j.ecolind.2009.01.002
Kardol, P., Cornips, N.J., van Kempen, M.M.L., Bakx-Schotman, J.M.T., van der Putten, W.H., 2007. Microbe-Mediated Plant-Soil Feedback Causes Historical Contingency Effects in Plant Community Assembly. Ecol. Monogr. 77, 147–162.
Kershaw, H.M., Mallik, A.U., 2013. Predicting Plant Diversity Response to Disturbance: Applicability of the Intermediate Disturbance Hypothesis and Mass Ratio Hypothesis. CRC. Crit. Rev. Plant Sci. 32, 383–395. https://doi.org/10.1080/07352689.2013.791501
Kirk, J.L., Beaudette, L.A., Hart, M., Moutoglis, P., Klironomos, J.N., Lee, H., Trevors, J.T., 2004. Methods of studying soil microbial diversity. J. Microbiol. Methods 58, 169–188. https://doi.org/10.1016/j.mimet.2004.04.006
Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by 14C labeling.

Soil Biol. Biochem. 41, 210–219. https://doi.org/10.1016/j.soilbio.2008.10.016
Kyaschenko, J., Clemmensen, K.E., Hagenbo, A., Karltun, E., Lindahl, B.D., 2017. Shift in fungal communities and associated enzyme activities along an age gradient of managed Pinus sylvestris stands. ISME J. 11, 863–874. https://doi.org/10.1038/ismej.2016.184
Lapin, M., Barnes, B. V, 1995. Society for Conservation Biology Using the Landscape Ecosystem Approach to Assess Species and Ecosystem Diversity Using the Landscape Ecosystem Approach to Assess Species and Ecosystem Diversity. Conserv. Biol. 9, 1148–1158.
Lewandowski, T.E., Forrester, J.A., Mladenoff, D.J., D'Amato, A.W., Palik, B.J., 2016. Response of the soil microbial community and soil nutrient bioavailability to biomass harvesting and reserve tree retention in northern Minnesota aspen-dominated forests. Appl. Soil Ecol. 99, 110–117. https://doi.org/10.1016/j.apsoil.2015.11.001
Likens, G.E., Bormann, F.H., Johnson, N.M., Fisher, D.W., Robert, S., 2010. Effects of Forest Cutting and Herbicide Treatment on Nutrient Budgets in the Hubbard Published by : Ecological Society of America. America (NY). 40, 23–47.
Little, L.R., Maun, M.A., 1996. The Ammophila Problem Revisited : A Role for Mycorrhizal Fungi. Br. Ecol. Soc. 84, 1–7.
López-Mondéjar, R., Zühlke, D., Becher, D., Riedel, K., Baldrian, P., 2016. Cellulose and hemicellulose decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems. Sci. Rep. 6, 1–12. https://doi.org/10.1038/srep25279
Lundgren, B., 1982. Bacteria in a pine forest soil as affected by clear-cutting. Soil Biol. Biochem. 14, 537–542. https://doi.org/10.1016/0038-0717(82)90083-9
Magnússon, R., Tietema, A., Cornelissen, J.H.C., Hefting, M.M., Kalbitz, K., 2016. Tamm Review: Sequestration of carbon from coarse woody debris in forest soils. For. Ecol. Manage. 377, 1–15. https://doi.org/10.1016/j.foreco.2016.06.033
Manzoni, S., Schimel, J.P., Porporato, A., Ecology, S., April, N., Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress : results from a meta-analysis. Ecology 93, 930–938.
Margalef, O., Sardans, J., Fernández-Martínez, M., Molowny-Horas, R., Janssens, I.A., Ciais, P., Goll, D., Richter, A., Obersteiner, M., Asensio, D., Peñuelas, J., 2017. Global patterns of phosphatase activity in natural soils. Sci. Rep. 7, 1–13. https://doi.org/10.1038/s41598-017-01418-8
Martín-Pinto, P., Vaquerizo, H., Peñalver, F., Olaizola, J., Oria-De-Rueda, J.A., 2006. Early effects of a wildfire on the diversity and production of fungal communities in Mediterranean vegetation types dominated by Cistus ladanifer and Pinus pinaster in Spain. For. Ecol. Manage. https://doi.org/10.1016/j.foreco.2006.01.006
McGuire, K.L., Treseder, K.K., 2010. Microbial communities and their relevance for ecosystem models: Decomposition as a case study. Soil Biol. Biochem. 42, 529–535. https://doi.org/10.1016/j.soilbio.2009.11.01
Moi, D.A., García-Ríos, R., Hong, Z., Daquila, B.V., Mormul, R.P., 2020. Intermediate Disturbance Hypothesis in Ecology: A Literature Review. Ann. Zool. Fennici 57, 67. https://doi.org/10.5735/086.057.0108
Morris, M.H., Smith, M.E., Rizzo, D.M., Rejmánek, M., Bledsoe, C.S., 2008. Contrasting ectomycorrhizal fungal communities on the roots of co-occurring oaks (Quercus spp.) in a California woodland. New Phytol. 178, 167–176. https://doi.org/10.1111/j.1469-8137.2007.02348.x

Morrison, E.W., Frey, S.D., Sadowsky, J.J., van Diepen, L.T.A., Thomas, W.K., Pringle, A., 2016. Chronic nitrogen additions fundamentally restructure the soil fungal community in a temperate forest. Fungal Ecol. 23, 48–57. https://doi.org/10.1016/j.funeco.2016.05.011
Morrissey, E.M., Berrier, D.J., Neubauer, S.C., Franklin, R.B., 2014. Using microbial communities and extracellular enzymes to link soil organic matter characteristics to greenhouse gas production in a tidal freshwater wetland. Biogeochemistry 117, 473–490. https://doi.org/10.1007/s10533-013-9894-5
Mueller, G.M., Schmit, J.P., 2007. Fungal biodiversity: What do we know? What can we predict? Biodivers. Conserv. 16, 1–5. https://doi.org/10.1007/s10531-006-9117-7
Nave, L.E., Gough, C.M., Maurer, K.D., Bohrer, G., Hardiman, B.S., Le Moine, J., Munoz, A.B., Nadelhoffer, K.J., Sparks, J.P., Strahm, B.D., Vogel, C.S., Curtis, P.S., 2011. Disturbance and the resilience of coupled carbon and nitrogen cycling in a north temperate forest. J. Geophys. Res. Biogeosciences 116. https://doi.org/10.1029/2011JG001758
Nave, L.E., Gough, C.M., Perry, C.H., Hofmeister, K.L., Le Moine, J.M., Domke, G.M., Swanston, C.W., Nadelhoffer, K.J., 2017. Physiographic factors underlie rates of biomass production during succession in Great Lakes forest landscapes. For. Ecol. Manage. 397, 157–173. https://doi.org/10.1016/j.foreco.2017.04.040
Nave, L.E., Le Moine, J.M., Gough, C.M., Nadelhoffer, K.J., 2019. Multidecadal trajectories of soil chemistry and nutrient availability following cutting vs. Burning disturbances in upper great lakes forests. Can. J. For. Res. 49, 731–742. https://doi.org/10.1139/cjfr-2018-0211
Nave, L.E., Sparks, J.P., Le Moine, J., Hardiman, B.S., Strahm, B.D., Curtis, P.S., 2014. Changes in soil nitrogen cycling in northern temperate forest ecosystem during succession. Biogeochemistry 121, 471–488. https://doi.org/10.1007/s
Neary, D.G., Klopatek, C.C., DeBano, L.F., Ffolliott, P.F., 1999a. Fire effects on belowground sustainability: A review and synthesis, in: Forest Ecology and Management. https://doi.org/10.1016/S0378-1127(99)00032-8
Neary, D.G., Klopatek, C.C., DeBano, L.F., Ffolliott, P.F., 1999b. Fire effects on belowground sustainability: A review and synthesis, in: Forest Ecology and Management. pp. 51–71. https://doi.org/10.1016/S0378-1127(99)00032-8
O'Connell, A.M., McCaw, W.L., 1997. Prescribed burning of thinning slash in regrowth stands of karri (Eucalyptus diversicolor) 2. Nitrogen budgets in pre- and post-burn fuel. Int. J. Wildl. Fire 7, 41–49. https://doi.org/10.1071/WF9970041
Odum, E.P., 1969. The Strategy of Ecosystem Development. Science (80-.). 164, 262–270.
Osman, R.W., 1977. The Establishment and Development of a Marine Epifaunal Community. Ecol. Monogr. 4, 37–63.
Özkan, U., Gökbulak, F., 2017. Effect of vegetation change from forest to herbaceous vegetation cover on soil moisture and temperature regimes and soil water chemistry. Catena 149, 158–166. https://doi.org/10.1016/j.catena.2016.09.017
Palese, A.M., Giovannini, G., Lucchesi, S., Dumontet, S., Perucci, P., 2004. Effect of fire on soil C, N and microbial Biomass. Agronomie 24, 47–53. https://doi.org/10.1051/agro
Pearsall, D.R., 1985. Landscape ecosystems of the University of Michigan Biological Station: ecosystem diversity and ground-cover diversity. University of Michigan, Ann Arbor.
Pellitier, P.T., Zak, D.R., 2018. Ectomycorrhizal fungi and the enzymatic liberation of nitrogen from soil organic matter: why evolutionary history matters. New Phytol. 217, 68–73. https://doi.org/10.1111/nph.14598
Pietikainen, J., Fritze, H., 1995. Clear-cutting and prescribed burning in coniferous forest:

comparision of effects on soil fungal and total microbial biomass, respiration activity and nitrification. Soil Biol. Biochem. 27, 1995.
Ramanathan, V., Carmichael, G., 2008. Global and regional climate changes due to black carbon. Nat. Geosci. https://doi.org/10.1038/ngeo156
Reazin, C., Morris, S., Smith, J.E., Cowan, A.D., Jumpponen, A., 2016. Fires of differing intensities rapidly select distinct soil fungal communities in a Northwest US ponderosa pine forest ecosystem. For. Ecol. Manage. 377, 118–127. https://doi.org/10.1016/j.foreco.2016.07.002
Rincón, A., Pueyo, J.J., 2010. Effect of fire severity and site slope on diversity and structure of the ectomycorrhizal fungal community associated with post-fire regenerated Pinus pinaster Ait. seedlings. For. Ecol. Manage. 260, 361–369. https://doi.org/10.1016/j.foreco.2010.04.028
Roxburgh, S.H., Shea, K., Wilson, J.B., 2004. The intermediate disturbance hypothesis: Patch dynamics and mechanisms of species coexistence. Ecology 85, 359–371. https://doi.org/10.1890/03-0266
Sanderman, J., Hengl, T., Fiske, G.J., 2018. Soil carbon debt of 12,000 years of human land use. Proc. Natl. Acad. Sci. U. S. A. 115, E1700. https://doi.org/10.1073/pnas.1800925115
Scharlemann, J.P.W., Tanner, E.V.J., Hiederer, R., Kapos, V., 2014. Global soil carbon: Understanding and managing the largest terrestrial carbon pool. Carbon Manag. 5, 81–91. https://doi.org/10.4155/cmt.13.77
Schmidt, M.W.I., Noack, A.G., 2000. Black carbon in soils and sediments: Analysis, distribution, implications, and current challenges. Global Biogeochem. Cycles. https://doi.org/10.1029/1999GB001208
Sikes, B.A., Maherali, H., Klironomos, J.N., 2012. Arbuscular mycorrhizal fungal communities change among three stages of primary sand dune succession but do not alter plant growth. Oikos 121, 1791–1800. https://doi.org/10.1111/j.1600-0706.2012.20160.x
Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol. Biochem. 42, 391–404. https://doi.org/10.1016/j.soilbio.2009.10.014
Sinsabaugh, R.L., Linkins, A.E., 1990. Enzymic and chemical analysis of particulate organic matter from a boreal river. Freshw. Biol. 23, 301–309. https://doi.org/10.1111/j.1365-2427.1990.tb00273.x
Smith, S., Read, D., 2008. Mycorrhizal Symbiosis, Third. ed. Elsevier Ltd, New York.
Soil Survey Staff, 1991. Soil Survey of Cheboygan, Michigan.
Sousa, W.P., 1979. Disturbance in Marine Intertidal Boulder Fields : The Nonequilibrium Maintenance of Species. Ecology 60, 1225–1239.
Spatafora, J.W., Blackwell, M., 1993. Molecular systematics of unitunicate perithecial ascomycetes: The clavicipitales-hypocreales connection. Mycologia 85, 919–922. https://doi.org/10.1080/00275514.1993.12026353
Štursová, M., Žifčáková, L., Leigh, M.B., Burgess, R., Baldrian, P., 2012. Cellulose utilization in forest litter and soil: Identification of bacterial and fungal decomposers. FEMS Microbiol. Ecol. 80, 735–746. https://doi.org/10.1111/j.1574-6941.2012.01343.x
Talbot, J.M., Bruns, T.D., Smith, D.P., Branco, S., Glassman, S.I., Erlandson, S., Vilgalys, R., Peay, K.G., 2013. Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. Soil Biol. Biochem. 57, 282–291. https://doi.org/10.1016/j.soilbio.2012.10.004
Taylor, D.L., Hollingsworth, T.N., McFarland, J.W., Lennon, N.J., Nusbaum, C., Ruess, R.W.,

2014. A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning. Ecol. Monogr. 84, 3–20. https://doi.org/10.1890/12-1693.1
Turner, B.L., Condron, L.M., 2013. Pedogenesis, nutrient dynamics, and ecosystem development: The legacy of T.W. Walker and J.K. Syers. Plant Soil 367, 1–10. https://doi.org/10.1007/s11104-013-1750-9
Urich, T., Lanzen, A., Qi, J., Huson, D.H., Schleper, C., Schuster, S.C., 2008. Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. PLoS One 3, e2527. https://doi.org/10.1371/journal.pone.0002527
Vázquez, D.P., Aizen, M.A., 2004. Asymmetric Specialization : A Pervasive Feature of Plant-Pollinator Interactions. Ecology 85, 1251–1257.
Verma, S., Jayakumar, S., 2012. Impact of forest fire on physical, chemical and biological properties of soil: A. Proc. Int. Acad. … 2, 168–176.
Vermeulen, S., Bossio, D., Lehmann, J., Luu, P., Paustian, K., Webb, C., Augé, F., Bacudo, I., Baedeker, T., Havemann, T., Jones, C., King, R., Reddy, M., Sunga, I., Von Unger, M., Warnken, M., 2019. A global agenda for collective action on soil carbon. Nat. Sustain. 2, 2–4. https://doi.org/10.1038/s41893-018-0212-z
Wan, S., Hui, D., Luo, Y., Applications, E., Oct, N., 2001. Fire Effects on Nitrogen Pools and Dynamics in Terrestrial Ecosystems : A Meta-Analysis. Ecol. Appl. 11, 1349–1365.
Wang, W., Li, Y., Wang, H., Zu, Y., 2014. Differences in the activities of eight enzymes from ten soil fungi and their possible influences on the surface structure, functional groups, and element composition of soil colloids. PLoS One 9. https://doi.org/10.1371/journal.pone.0111740
Wicklow, D.T., 1975. Fire as an Environmental Cue Initiating Ascomycete Development in a Tallgrass Prairie. Mycologia 67, 852–862. https://doi.org/10.1080/00275514.1975.12019813
Wicklow, D.T., Hirschfield, B.J., 1979. Competitive Hierarchy in Post-Fire Ascomycetes. Mycologia 71, 47–54. https://doi.org/10.1080/00275514.1979.12020986
Widden, P., Parkinson, D., 1974. The effects of a forest fire on soil microfungi. Soil Biol. Biochem. 7, 125–138.
Xu, M., Shang, H., 2016. Contribution of soil respiration to the global carbon equation. J. Plant Physiol. 203, 16–28. https://doi.org/10.1016/j.jplph.2016.08.007
Zak, D.R., Pellitier, P.T., Argiroff, W.A., Castillo, B., James, T.Y., Nave, L.E., Averill, C., Beidler, K. V., Bhatnagar, J., Blesh, J., Classen, A.T., Craig, M., Fernandez, C.W., Gundersen, P., Johansen, R., Koide, R.T., Lilleskov, E.A., Lindahl, B.D., Nadelhoffer, K.J., Phillips, R.P., Tunlid, A., 2019. Exploring the role of ectomycorrhizal fungi in soil carbon dynamics. New Phytol. 223, 33–39. https://doi.org/10.1111/nph.15679
Zhao, X., Wang, Q., Kakubari, Y., 2009. Stand-scale spatial patterns of soil microbial biomass in natural cold-temperate beech forests along an elevation gradient. Soil Biol. Biochem. 41, 1466–1474. https://doi.org/10.1016/j.soilbio.2009.03.028

Chapter 2: Short-term Effects of Clear-Cutting and Burning on Fungal Community Composition and function in Mineral Soils

2.1 Abstract

Clear-cutting and burning affect fungal community composition and function directly through induced mortality and indirectly through alterations to soil physio-chemical properties. Understanding the variations in the composition and activities of these communities within soils, and how they respond to disturbances is crucial to our understanding of soil ecosystem processes. In this study I explore how fungal communities in mineral soils are affected by an implemented cut+burn disturbance in a 130-year old mixed hardwood forest, and how variation in disturbance severity within the plot structured communities. The results reveal differential effects on soils receiving either clearcutting or clearcutting + burning within a 1-ha stand and highlight the importance of scale when examining disturbance effects. Areas of high disturbance severity showed emphatic decreases in fungal diversity, abundances, and extracellular enzyme activity. The biotic changes were accompanied by an increase in pH, soil moisture, and Ca^{2+} that accompanied a punctuated loss of ECM taxa, a decline in all measures of diversity, and a decline in both fungal and bacterial total abundances. In areas of the plot only where only clear-cutting occurred, I noted an increase in fungal diversity and a shift in dominant ectomycorrhizal taxa from species in the Cortinariaceae to Russulaceae. Additionally, pooling all disturbed soils led to differing results at the plot level for abiotic and biotic properties. High burn areas tended to drive plot level differences in soil physio-chemical properties, while plot-level increases in diversity were driven by areas that primarily received the clear-cutting disturbance. I underscore that the variability in fire severity at the scale of meters is of particular importance due to the large differences in physio-chemical properties and microbial communities. These results offer insight into potentially missed trends in the variable effects of disturbances at a fine scale when measurements are pooled or examined at scales of hectares or larger.

2.2 Introduction

Soil microbial organisms are critical actors in key ecosystem processes in terrestrial systems around the globe. Fungi, in particular, play important roles in global carbon (C) cycles as saprotrophs (Högberg et al., 2003; Štursová et al., 2012; Talbot et al., 2013) and plant symbionts (Kirk et al., 2004; Smith and Read, 2008). Saprotrophic fungi play a particularly important role as regulators of carbon (C) from terrestrial systems to the atmosphere through decomposition processes (Floudas et al., 2012; Sinsabaugh, 2010). They do so by utilizing suites of hydrolytic and oxidative enzymes (McGuire and Treseder, 2010; Morrissey et al., 2014; Urich et al., 2008) such as cellulases, chitinases, phosphatases, and phenol oxidases. Excretion of these extracellular enzymes is integral in the decomposition of dead plant biomass as they break down bonds in carbohydrates, chitin, xylan, and lignin (Baldrian et al., 2012; López-Mondéjar et al., 2016; Wang et al., 2014). In producing these suites of enzymes fungi respire, releasing C back to the atmosphere as CO_2 (Dooley and Treseder, 2012). An estimated 60-80% of organic materials decomposed by saprotrophs in forest soils is returned to the atmosphere (González-Pérez et al., 2004).Disturbances that directly alter fungal communities, disrupt mycorrhizal associations, or detritus inputs from primary producers may have large implications on extracellular enzyme activity (EEA) in soil fungal communities and, in turn, global C cycles (Dooley and Treseder, 2012).

Classic ecosystem theory predicts that soil microbes will increase post disturbance due to increases in soil temperature and C resource availability (Chapin et al., 2002). Increases in temperature have been shown to be linked with increased microbial respiration and EEA (Allison et al., 2010; Ice et al., 2004). Recent studies of fire disturbances challenge this theory and indicate that the type and severity of a disturbance can also lead to an overall reduction of microbial community biomass and activity immediately following perturbations (Dooley and Treseder, 2012; Hamman et al., 2007a). A meta-analysis of fire showed that it often reduced both total microbial abundance (fungi + bacteria) and fungal abundances immediately following a disturbance, but that responses varied significantly among disparate biomes and fire types (Dooley and Treseder, 2012). Microbial biomass as a whole is reduced in burnt temperate forests, however fungal responses were unclear. Similarly, tree harvesting disturbances can decrease fungal biomass by altering soil physio-chemical properties and the quantity and quality

of litter inputs to soils (Hassett and Zak, 2005). Coupled disturbance events of timber harvesting followed by subsequent slash fires increase response complexity and the difficulty in predicting microbial community structure and function.

Despite the importance of these organisms, soil fungal communities remain difficult to study in both time and space (Neary et al., 1999a). Variation in soil properties (Debano, 2000; Gul et al., 2015; Hamman et al., 2007b; Holden and Treseder, 2013; Wan et al., 2001) , plant communities (Chapman et al., 2005; Deka and Mishra, 1983; Hart et al., 2005), micro-climate (Rincón and Pueyo, 2010), and disturbance history (Gough et al., 2007) within a forest stand make it difficult to generalize results. Understanding the variations in the composition and activities of these communities within forests, and how they respond to disturbances is crucial to our understanding of soil ecosystem processes. The previously mentioned meta-analysis by Dooley and Treseder (2012) showed that fire has the propensity to lower fungal and total microbial abundances post fire. However, they noted that community structure was not taken into consideration in most studies. Additionally, more work is needed in temperate systems as results were skewed by a strong positive effect on fungal abundances in a study by (Kara and Bolat, 2009).

Temperate forests of North America have a history of large-scale deforestation and subsequent fires which have shaped recovering ecosystems (Gough et al., 2008, 2007; Hart et al., 2005; Nave et al., 2011). Timber harvesting affects fungal communities indirectly through symbiont mortality and changes to soil microclimates (Hassett and Zak, 2005). The dominant plant taxa in temperate forests typically form associations with one or more mycorrhizal fungal groups. Post harvesting, mycorrhizae on the associated roots senesce due to a dependence on their plant symbiont for a source of C (Hassett and Zak, 2005). Dead roots and mycorrhizae serve as substrates for saprotrophs along with coarse woody debris and leaf litter from harvesting (Covington, 1981). Soil fungi may also be affected as light penetrates to soil surfaces in harvested stands with only herbaceous vegetation as shade, warming the soil. Moisture availability may then be altered via losses through evaporation and conversely may increase due to reduced transpiration. The result of harvesting is a patchy mosaic of microclimates and substrate availability.

Subsequent fires further disturb fungal communities as they alter communities directly through fire induced mortality (DeBano et al., 1998; Dooley and Treseder, 2012; Hart et al., 2005) and indirectly by further altering microclimates and substrate availability. As heat moves through the soil it can alter hydrophobicity (Debano, 2000), nutrient availability (Palese et al., 2004), and soil C quality (González-Pérez et al., 2004). Hydrophobicity can increase post fires resulting from ash that is deposited on soil surfaces during burning but has also been shown to vary depending on fire severity and soil moisture at the time of the fire (Giovannini and Lucc, 1983; Giovannini and Lucchesi, 1997; González-Pérez et al., 2004). Nutrient availability has been demonstrated to be affected in both short timeframes (1-5 years post fire) (Holden and Treseder, 2013) and over longer periods (decadal) where nitrogen availability has been shown to decrease for decades post fires (Nave et al., 2019). Short-term ash deposition may lead to a pulse in nutrient availability post fire followed by losses to leaching and runoff (Jensen et al., 2001). Finally, C availability can be affected through alterations to the chemical structure of SOM. Partial volatilization through pyrolysis can lead to increased recalcitrance as bonds between macromolecules in pre-existing soil C is altered during burning, resulting in pyromorphic humus or black carbon (Czimczik and Masiello, 2007; Kuzyakov et al., 2009; Ramanathan and Carmichael, 2008; Schmidt and Noack, 2000).

Responses of ecosystem functions such as primary production (Hardiman et al., 2013), soil respiration, and decomposition have been well documented (Nave et al., 2011). Odum's (1969) classic intermediate succession theory postulates that productivity of a forest will increase to a maximum in mid successional stages, thereafter, decreasing as forests mature to late successional stages. Recent studies in temperate forests have highlighted the potential for forest productivity to persist into late successional stages (Gough et al., 2013; Hardiman et al., 2013). Similar to and often associated with the theory of intermediate succession proposed by Odum, the intermediate disturbance hypothesis (IDH) predicts plant communities diversity will peak in mid-successional stages, before declining as late successional dominant species take over and early successional species senesce (Connell, 1978). The IDH has also been applied to varying degrees of disturbance severities with moderate levels of disturbance resulting in higher species diversity relative to lesser and more severe disturbances.

Importantly, little work has been done to test these theories on fungal communities during succession and post disturbance. Fungi remain difficult to study and to generalize beyond the stand level due high variation at the fine spatial scale in which they operate. During disturbances natural variation soil fungal community structure is then confounded with spatial variation in disparate severities within a single stand (Hamman et al., 2007a; Neary et al., 1999a). This is particularly true in slash fires that occur in harvested forests where burning is often extremely heterogeneous (Deka and Mishra, 1983; Jiménez Esquilín et al., 2007).

Here I examine the coupled effects of logging and subsequent burning in a mixed deciduous temperate forest in Northern Michigan. This forest is representative of the secondary forests across the Great Lakes and Northeastern United States that regenerated post harvesting in the 18th and 19th centuries. I leveraged a newly implemented prescribed cut + burn at the University of Michigan Biological Station to ask how direct effects of fire-induced mortality and indirect effects through changes in habitat conditions and resource availability affect fungal community structure and function? Additionally, I ask if fungal communities follow predicted patters of diversity and abundance predicted by the intermediate disturbance hypothesis? I hypothesize that 1) Cutting and burning will lead to a mosaic of varying localized disturbance severities within a 1-ha plot; 2) Fungal diversity and abundances will increase in moderately disturbed areas consisting of cut+low burn severity and decrease in areas of high burn severity; and 3) Extracellular enzyme activities associated with decomposition will track fungal diversity and abundance.

2.3 METHODS

Study Site

The study was conducted at the University of Michigan Biological Station (UMBS) "Burn Plots", in northern Michigan, USA (45°35'N 84°43'W). Mean annual temperature is 5.5 ^{o}C with a mean annual precipitation of 817 mm. The study site is located on a high-level sandy outwash plain plane with soils that are primarily well-drained entic Haplorthods of the Rubicon series (Lapin and Barnes, 1995; Pearsall, 1985; Soil Survey Staff, 1991). Soil series profiles, vegetation and landforms of the Burn Plots and surrounding UMBS forests have been described in detail in (Nave et al., 2019, 2017). Briefly, the typical morphology of the soil series at our site consists of

Oi and Oe horizons 1-3 cm thick, a bioturbated A horizon 1-3 cm thick, an E horizon 10-15 cm thick, and Bs and Bc horizons of sand with occasional gravel and cobble (Nave et al., 2014). Approximately half of the fine root biomass is in the upper 20 cm of soil (approximately 5-15 Mg C ha^{-1}), with the remaining root biomass approximately split between the forest floor (O horizons) and depths of 20-40 cm (Nave et al., 2011). The study described here focuses on work in the 2017 burn plot.

This study focuses on a newly-implemented burn plot in the spring and fall of 2017. The experimental plot was 9554 m^2 with the north, west, south and east dimensions of 90 m, 113 m, 85.5 m, and 110.5 m, respectively. The experimental plots reside within a surrounding forest that serves as a reference and was burned in 1911 after clear-cutting by commercial loggers (Figure 2.1). The experimental forests have similar soil profiles and share common plant species but differ from the surrounding reference area in that they have been twice cut and burned, having experienced the same disturbance as the reference forest in 1911 (Gough et al. 2007). Prior to timber harvesting, 30 subplots (approximately 20 m x 20 m) were created to record adult trees (>8 cm DBH (diameter at breast height)), juvenile trees (<8 cm DBH), saplings, ground cover, coarse woody debris and standing dead trees. All trees were stem mapped and DBH measurements were used to determine species specific basal areas. Relative dominance of tree species were calculated by dividing species specific basal areas by stand basal area.

Timber harvesting occurred in May 2017 and the prescribed burn was conducted on October 10, 2017. During timber harvesting the boles of all trees >8 cm DBH were removed and the slash was left in the plot. The controlled burn resulted in a gradient of disturbances that ranged from a cut only disturbance without detectable burning of sampling area to disturbances that combined the cutting disturbance + a range of burn disturbance from superficial to high severity (Table 2.1)

Field Sampling

A sampling grid of 60 m x 90 m was created with six, 90 m transects running north to south containing 10 sampling points 10 m apart. Each transect was 10 m from another with all sampling areas at least 20m from plot borders. At each sampling location (60) a 64 cm^2 soil monolith of the combined O and A horizons was taken pre-disturbance and post cut + burn. Pre-

treatment samples were collected from September 25_{th}-October 5th in 2016. Post cut + burn monoliths were collected from October 16_{th}-25_{th} 2017. Additionally, in 2017 a ten point transect running north to south was sampled on October 25_{th}, 2017 in the adjacent forest to the west of the burn plot serving as the reference stand. The reference stand had similar soils, climate and plant composition and demographic relative to pre-disturbance characteristics. Cumulatively 130 total samples (60 pre-disturbnce, 60 post cut+burn, and 10 reference) were collected during the 2016 and 2017 campaigns. Soil monoliths were immediately stored in a cooler and maintained at 4 $_{o}$C until processed in the lab. All monoliths were processed in the lab within 48 hours.

In the lab, the OA monoliths were sieved with a 2mm mesh screen to remove rocks, coarse and fine roots, and fine earth fractions to isolate mineral soil. After sieving, monolith material was subsampled for RNA/DNA analysis, extracellular enzymatic activity quantitation, C:N and gravimetric water content (GWC). A 5-gram subsample was flash frozen in liquid nitrogen and stored at -80 °C for molecular analyses. Soils for C:N and gravimetric water content were lyophilized until constant mass with no residual ice apparent (ca. 48 h; Labconco Freezone One; Labconco, Kansas City, MO). The remaining soils were stored in a 20 fridge until subsamples for GWC were dried.

Fungal Enzymes

A 1-gram dry soil equivalent of homogenized field moist soil was used to create an aqueous extract in 125 ml of 50 mM sodium acetate buffer solution at pH 5. Samples were homogenized first in 60 ml solution for 30 seconds with a Fisher Scientific Tissuemizer® in a 250 ml Nalgene bottle. Mixed solutions were then placed on a shaker table for 10 minutes. After the first shaking cycle an additional 65 ml of buffer solution was added and samples were mixed again for an additional 30 seconds. Bottles were then shaken for another 10 minutes. The aqueous solutions were then divided into 3 x 40ml aliquots in sterile 50ml polypropylene centrifuge tubes and frozen at -20 °C until analysis. Six enzymes (Table A 3) associated with EEA were analyzed using p-nitrophenyl-linked substrate microplate assays similar to (Sinsabaugh and Linkins, 1990). Phenol oxidase (PHENOL_OX)and peroxidase activities were measured colorimetrically (460 nm) with L-3,4-dihydroxyphenylalanine (L-DOPA, 6.5mM) using sodium bicarbonate

buffer. Potential peroxidase activity was determined by the difference between soil with L-DOPA + 0.3% H_2O_2 and L-DOPA.

Cellulase activity was analyzed by measuring beta-glucosidase (BG) (EC 3.2.1.21). Hydrolosis of starches was analyzed measuring alpha-glucosidase (AG) (EC 3.2.1.2) activity. Chitinase activity was determined by measuring N-acetylglutamate (NAG) (EC 2.3.1.1) reduction of a glucosaminide substrate. Hydrolysis of polyphosphates was determined by measuring alkaline phosphatase (ALK) (EC 3.1.3.1) activity. Artificial substrates and incubation times used were: 4-nitrophenyl a-D-glucopyranoside (4 hours), 4-nitropheynyl B-D-gluco (4 hours), 4-nitrophenyl B-D-cellobioside (4 hours), 4-nitrophenyl N-acetyl-B-D-glucosaminide (4 hours), 4-nitrophenyl phosphate (4 hours) and activity was determined by measuring the amount of liberated p-nitrophenolate upon addition of 3M KOH stopping solution. All substrates and reagents were obtained from Sigma-Aldrich Co. Ltd. (Saint Louis, MO, USA), and measurements were made using a Biotek ELX800 plate reader (Biotek, Winooski, VT, USA). Three technical replicates were prepared for each assay, blank and standard curve and means were used to determine per sample values. Enzyme activities were calculated in international "units" (U; 1 U= 1000 mU) defined as the amount of enzyme that forms 1 μmol of product per minute.

Chemical analysis

Chemical analyses were conducted to determine changes in substrate quality and nutrient content in various aged stands. Percent C, N and $\delta^{15}N$ were analyzed on homogenized and ball-milled subsamples of mineral soil at the UMBS (Pellston, MI, USA) using a SPEX Certiprep 8000D Mixer/Mill (Metuchen, New Jersey, USA) to pulverize soils and a Thermo Scientific EA Isolink elemental analyzer coupled with a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer for analysis (Bremen, GE). Soil pH was collected using a 0.2 g of composite, ball-ground material using a model 8000 pH meter (VWR Scientific, Radnor, PA, USA). Samples were added to 15 ml of deionized water, incubated for 30 minutes at room temperature and equilibrated with a probe for 1 minute prior to taking each reading.

Organic matter was determined by loss on ignition at 375 ^{o}C. Nutrients in cations and anions assays were extracted in pH 4.8 ammonium acetate. A subset of paired pre- and post-disturbance

samples (30 pre-disturbance, 30 post-disturbance) were analyzed for effective cation exchange capacity (ECEC), calculated by the sum of the milliequivalent levels of Ca, K, Mg, Na, and acidity. Soluble nutrients (extractable NO_{3-}, NH_{4+}, PO_{4-}) were determined by automated colorimetry at the University of Michigan Biological Station Chemistry Lab, while all other nutrients were determined via ICP-MS (Perkin Elmer ELAN DRCe) at the Maine Soil Testing Service Lab.

RNA and DNA extraction, RT-PCR & PCR amplification and sequencing of ITS2

RNA and DNA analysis of fungal communities was conducted on homogenized soil samples from soil cores. Genomic RNA and DNA were co-extracted from 2 g frozen soil using a MoBio RNA Powersoil Total RNA Isolation Kit (MoBio) combined with an RNA Powersoil DNA Elution Kit (MoBio) following manufacturer's instructions. Extraction quantity was determined using a Qubit Fluorometer (Invitrogen, San Jose, CA, USA) and frozen (-80 °C).

Prior to amplification, a subset of pre- and post-disturbance (25 pre- and 25 post-) RNA extractions were treated with Dnase 1 following manufactures instructions. After cleaning all RNA products were diluted and standardized to 4.5 ng/ul for template in reverse transcription. RNA was reverse transcribed using a reverse primer, ITS4-Fun (GCAWAWCAAWAAGCGGAGGACCCTGACTGACT) (Taylor et al., 2014). with a High Capacity cDNA Reverse Transcription Kit from (Applied Biosystems) under the following conditions: 2.0ul 10x RT buffer, 0.8ul 25X dNTP, 2.0ul primer, 1.0ul MultiScribe Reverse Transcriptase, 1.0ul RNase inhibitor, 7.7ul H2O and 5.5ul RNA template. PCR cycle parameters were as followed: 10 min at 25 $_{o}$C, 120 min at 37 $_{o}$C, 5 min at 85 $_{o}$C followed by a final rest temperature at 4 $_{o}$C.

The fungal ITS2 region was amplified from genomic fungal DNA and reverse transcribed cDNA using forward primer 5.8S-FUN (5'-AGWGATCCRTTGYYRAAAGTTCCCTGACTGACT) and reverse primer ITS4-FUN (GCAWAWCAAWAAGCGGAGGACCCTGACTGACT) (Taylor et al., 2014). Each soil sample had paired forward and reverse barcodes appended to the primer ends for multiplexing. PCR reactions were carried out using Phusion High-Fidelity DNA Polymerase (New England BioLabs, Ipswich, MA) under the following conditions: 5.9ul PCR

grade H2O, 4ul 5X Phusion HF Buffer, 0.4ul 10mM dNTP mix, 0.75ul forward primer (10mM), 0.75ul reverse primer (10 mM), 8ul DNA template, 0.2ul Phusion Taq. PCR cycle parameters were as follows: 3 min of initial denaturation at 94 $_{o}$C, 28 cycles of 30 sec denaturation at 94C, 45 sec annealing at 55 $_{o}$C, and 90 sec at 72 $_{o}$C with a final extension at 72 $_{o}$C for 10 min. PCR products were submitted to the Microbial Systems Molecular Biology Laboratory at the University of Michigan where they were normalized, purified and sequences were generated on a MiSeq500-V2 platform yielding a mean of ~25K sequences per sample (Illumina, San Diego, CA, Unites States).

Sequence Analysis

Sequences were demultiplexed by the Microbial Systems Molecular Biology Laboratory at University of Michigan. Fungal sequences were processed using QIIME 2 package version 1.8.2. Prior to downstream analysis both forward and reverse reads were analyzed for QC scores in Qiime2-view. All reverse reads generated from primer ITS4-FUN (Taylor et al., 2014) were excluded due to poor quality and only forward reads were used in downstream analyses. Additionally, 10 pairs of pre- and post-disturbance and 4 reference samples (24 total samples) were removed from all downstream DNA community and environmental analyses due to low quantity and/or quality sequencing data. Similarly, 11 pairs of pre- and post-disturbance RNA samples were excluded due to low quality sequences. The remaining sequences were trimmed for poor quality end reads using denoise-single by trimming the first 12 bp and truncating at 245 bp. Sequences were clustered into OTUs at 97% similarity and filtered for chimeras using vsearch cluster-features-de-novo pipeline. OTUs were then classified using a classifier created from the UNITE database using classify-sklearn command. A phylogenetic tree was generated with QIIME2 phylogeny fasttree. The dataset was then filtered for sequences that appeared less than 5 times and fewer than 2 samples with filter-features and rarefied at a sampling depth of 10,721.

Statistics

Due to the exclusion of the 24 previously mentioned DNA samples, the resulting DNA dataset included 50 pre-disturbance, 50 post-disturbance, and 6 reference samples used for statistical analyses of fungal community structure, fungal abundances, EEA, and environmental datasets. The resulting dataset included 19 cut only samples, 8 superficial burn severity, 9 low burn, 6

medium burn, and 8 high burn samples. Analyses pertaining to fungal RNA utilized the dataset that included 14 pre-disturbance and 14 post-disturbance samples including: 6 no burn, 2 superficial burn, 4 low burn, 2 medium burn and 1 high burn sample. Analyses on ECEC, cations, anions, and base saturation were run using 30 pre- and 30 post-disturbance samples. Additionally, ECEC, cations, anions and base saturation were excluded from downstream stepwise regression analyses due to the lack of data for >50% of pre-, post-, and reference samples.

Alpha and beta diversity indices were generated in QIIME2 with alpha-rarefaction and core-metrics-phylogenetic, respectively. Differential abundance tests were run with composition ANCOM in QIIME2 to identify taxonomic groups driving community dissimilarities. A biplot was generated with a Bray-Curtis distance matrix with QIIME2 pcoa-biplot function. Biom files were exported and used with R software to generate regressions and ANOVAS for environmental variable analysis. Finally, a dbRDA using a Bray-Curtis dissimilarity matrix was run with a step-wise regression function in R to determine variables correlated with community dissimilarities. A Mantel test was generated to test for significance in community dissimilarity prior to the redundancy analysis. I accept as significant statistical tests with $\alpha \leq 0.05$ and ascribe marginal significance for $0.05 < \alpha < 0.10$.

2.4 RESULTS

Pre-harvest Plant Community

The forest species composition is comprised of a mixed hardwood canopy and understory species. Canopy trees include but are not limited to *Populus grandidentata* (bigtooth aspen), *Betula papyrifera* (paper birch), *Quercus rubra* (northern red oak), *Acer rubrum* (red maple), and *Pinus strobus* (eastern white pine). Ground flora consist mainly of *Pteridium aqualinum*, *Gaultheria procumbens*, *Maianthemum canadense*, and *Vaccinium angustifolium*. *Q. rubra*, *P. resinosa* and *P. grandidentata* dominated the canopy basal area and relative abundances (Table A 1). Juvenile (<8 cm DBH) tree communities were overwhelmingly dominated by *P. strobus* while seedling communities were dominated by *A. rubrum*, *Q. rubra* and *P. strobus* (Table A 2).

Post Cut + Burn Heterogeneity

All sampling areas were subject to the effects of harvesting such that trees >8cm DBH were removed from the entire plot. The timing of the harvest resulted in an immediate increase in randomly distributed coarse woody debris to the forest floor, but little additional leaf litter due to the harvesting being conducted prior to leaf out. Five months later, the controlled burn of the plot resulted in a gradient of disturbances that ranged from a cut only disturbance without detectable burning to disturbances that combined the cutting disturbance + a range of burn intensities ranging from superficial to high severity (Table 2.1). The prescribed burn resulted in a patchy mosaic of disturbance that resulted in ~60% of the sampling areas having been cut + burned with the remaining areas only receiving the cutting disturbance (Figure 2.2). A rain event 3 days prior to the burn led to a primarily low intensity fire with patches of slash and O horizons remaining completely unburned and intact. Only 13% of the monoliths were in areas of high intensity burning that almost completely volatilized the slash on soil surface and reduced %C in mineral soils. ANOVA and Tukey post-hoc analyses revealed that %C in high burn severity areas decreased relative to other disturbances ($p>0.01$). However, no differences in %C were detected between any other disturbance severities indicating that only high severity areas reached temperatures high enough to combust SOM in mineral soils.

Fungal community composition

After quality filtering and rarefaction, a total of 1,176,010 sequences were used in community analyses. The quality filtered raw reads of fungi from soil communities were assigned to a total of 1862 operational taxonomic units (OTUs). Of these, 81%, 75%, 70%, 67% and 61% could be taxonomically assigned to phylum, order, family, genus and species, respectively. Out of the 1862 OTUS, 19 accounted individually for 1% or more of total sequences across all samples, with 4 of those OTUs accounting for more than 3% (Table 2.2). The mean number of fungal OTUs across all samples was 175 (± 65 s.d.). Within reference, pre-disturbance and post-disturbance mean OTUs were 144 (± 31 s.d), 154 (± 37 s.d) and 198 (±79 s.d), respectively.

An analysis of diversity showed no differences in overall species evenness or richness (Shannon) between pre- and post-disturbance communities, however, there were significant differences in total OTUs (paired t-test, $p<0.01$) and phylogenetic diversity (paired t-test, $p<0.01$) (Figure A 1).

An ANOVA of diversity indices of communities from the reference, pre-disturbance, and post-disturbance sites shows a significant reduction in all measures of community diversity in samples that received the cut + high intensity burn (Figure A 2). Conversely, relative to the pre-disturbance sites, all sites that received a less severe disturbance (cut only through cut+med burn) tended to increase in diversity. Samples from areas experiencing cutting only significantly increased in all measures of diversity. There were also significant increases in Shannon values, observed OTUs and phylogenetic diversity in sites that were both cut only and cut+superficially burned.

A Mantel test using a Bray-Curtis distance matrix of the fungal communities revealed significant overall community differences. A PCoA analysis of the fungal communities based on the Bray-Curtis distance matrix shows pre-disturbance communities generally clustering independently from disturbed communities with approximately 32% of the total variation explained by community differences at the family level (Figure 2.3). No differences in communities experiencing low to medium intensity disturbances (cut only through cut + med burn) could be detected in the ordination. In contrast, cut + high burn sites clustered independently of other disturbed communities (with the exception of 2 low burn and 1 medium burn intensity communities), and independently of all pre-disturbance and reference communities.

Differences between pre- and post-disturbance fungal communities are driven by the stark loss of ectomycorrhizal taxa from Cortinariaceae post-disturbance and an overall increase in the abundance of ascomycetous fungi in the Sordariaceae and Pyronemataceae and the Mucoromycete family Umbelopsidaceae (Figure A 3). Taxa in the Russulaceae were persistent in disturbed sites that received cutting + (no to moderate burning), however we found reduced relative abundance in high burn communities (Figure A 3). High burn severity sites were dominated by fungi that could not be classified at the genus level, *Anthracobia*, and *Umbelopsis* with less than 5% of the diversity accounted for by other genera (Figure A 4). More specifically, there were detectable increases in ascomycetes such as *Umbelopsis dimorpha*, Mortierella humilis, Anthacobia melaloma and species from the Sordariaceae and Pyronemataceae that were detected in medium to high intensity burn sites (Figure A 5).

There were 19 OTUs that were only found in cut + high burn severity sampling areas (Table A 4). Of the 19 OTUs found in high severity burn areas >50% were ascomyceteous fungi. A total of 143 and 553 OTUs were unique to pre-disturbance and post-disturbance communities, respectively. Of those unique OTUs, there was an increase in taxa that could not be classified past the Kingdom level from 14 pre-disturbance to 140 post-disturbance. Fungi found only in pre-burn samples that were not found post-disturbance primarily belonged to members of the Agaricomycetes.

Fungal community RNA

Subsamples of coextracted fungal RNA were also analyzed for community composition. As with the DNA sequences, pre-disturbance communities tended to cluster apart from disturbed communities (Figure A 6). An ANCOM analysis indicates there are taxa strongly associated with community clustering. Similar to DNA results, at the family-level, pre-disturbance communities are defined by the high representation from the ectomycorrhizal families Cortinariaceae and Russulaceae. RNA communities of post-disturbance soils showed varying results between the severities. Cut only through cut + medium burn areas were noted to have elevated relative abundances of unknown fungal taxa and cut only and cut + superficial burn areas saw an increase in arbuscular mycorrhizae (AM) in the Glomeraceae relative to pre-disturbance communities (Figure A 7). The sole high severity disturbances area that yielded RNA sequences was overwhelmingly represented (>50%) by saprotrophs in the Umbelopsidaceae.

Fungal and Bacterial qPCR

Gene copy abundances for fungi and bacteria (ITS or 16S rRNA, respectively) were used as proxies to examine potential changes in population size pre- and post-disturbance. At the plot level, neither fungal nor bacterial gene abundances post-disturbance were different from pre-disturbed conditions (Figure A 8). As a result, there was no change in fungal:bacterial ratios. However, an ANOVA examining varying disturbance severities revealed differences in both fungal ($p < 0.01$) and bacterial ($p < 0.01$) abundances (Figure A 9). Fungal abundances were elevated in low severity disturbances (cut only through cut+medium burn) and peaked in the cut + low burn sites. Bacteria also showed elevated abundances in low severity disturbances and were highest in sites that experienced only the cutting treatment. Both fungal and bacterial

abundances were detectably lower in the high-burn severity sites relative to all other disturbances and the pre-disturbed conditions. No differences were detected in fungal:bacterial ratios as both reflected similar trends.

Soil Physical and Chemical Properties

Percent moisture, pH, and cation exchange capacity (CEC) all increased in post-disturbance relative to pre-disturbance samples, but no differences were detected in bulk density (Figure 2.4). An ANOVA for these variables separately on the post-disturbance communities revealed the only detectable difference in soil chemistry to be an elevated soil pH in high burn communities (Figure A 10), indicating ECEC, moisture and bulk density were plot level effects post-disturbance. Coinciding with elevated pH and ECEC in post disturbance plots, there were significant differences in available cation and anions. As a whole, base cations (Ca^{2+}, K^{+}, Mg^{2+} and Na^{+}), also increased significantly post burn ($p < 0.01$). In addition to elevated base cations there were significant increases in P and Mn (Figure 2.5). Conversely, there were detectible decreases in Cu ($p < 0.1$) and S ($p < 0.1$). The increases in cation and anion availability were largely driven by elevated availability in high burn localities (Figure A 11). An ANOVA and post hoc analyses revealed base saturation (ECEC-acidity) was significantly reduced in the cut only sites and significantly elevated in high burn soils ($p<0.01$) (Figure A 12).

Available phosphate (PO_{4-}) and ammonium ($NH_{4=}$) were both significantly greater in the post-disturbance samples relative to the pre-disturbance (Figure 2.6 B &C). No detectable difference was found in available nitrate (NO_{3-}). Within the disturbed localities differences in $NH_{4=}$ and PO_{4-} were driven by increases in low severity + high severity burns and medium + high severity burns, respectively (Figure A 13).

Extracellular Enzyme Activity (EAA)

Enzyme analyses revealed differences in the activities of 3 of 6 enzymes assayed, with a reduction in two hydrolytic enzymes (N-acetylglutamate and alkaline phosphatase) and one oxidative enzyme (polyphenol oxidase) (Figure 2.7). Pre-disturbance N-acetylglutamate (NAG) activity (~37 µmol/min) was approximately 3x greater than post-disturbance activity (~13 µmol/min). Similarly, Alkaline phosphatase (ALK) activity pre-disturbance (~80 µmol/min) was

greater than double post-disturbance activity (~32 µmol/min). Pre-disturbance phenol oxidase (PHENOL_OX) activity was ~17500 µmol/min relative to ~12500 µmol/min post-disturbance. An ANOVA within disturbance severities did not detect differences in enzymes (Figure A 14). However, if all low to medium severity disturbances were pooled and compared to high severity areas a t-test revealed lower EEA in NAG, AG, ALK and PHENOL_OX (Figure A 15). EEA was not correlated with moisture availability in soil in pre- or post-disturbance samples.

Fungal Community and Environmental Interactions

A redundancy analysis using a Bray-Curtis distance matrix combined with a forward stepwise regression model indicated several significant drivers of community structure. The predictors of community structure were primarily driven by pH, alkaline phosphatase activity, PO_4 availability, and percent moisture. Additional drivers in the model included % N, %C, fine earth dry weight, and both peroxidase and phenol oxidase activities. The cumulative sum of the variation explained by the model is 47% with 18% and 29% explained by the x and y axes, respectively (Figure 2.8). While there was overlap of pre-burn, reference, and low severity disturbance communities in the ordination space, there were 3 distinct clusters of communities: pre-disturbance, cut only through cut + medium burn, and high burn severity. Pre-disturbance communities are primarily associated with higher soil %C and elevated extracellular enzymatic activity in poly-phenol oxidase and alkaline phosphatase. High burn severity samples were separated along axis 1 and were driven by elevated pH, phosphate availability and percent moisture. It's also of note that variance within a given disturbance intensity tended to increase until reaching the high severity disturbance (Figure 2.8).

2.5 DISCUSSION

Disturbance Heterogeneity

As predicted, the cut + burn treatment resulted in a mosaic of disturbance severities allowing me to examine the heterogeneous effects of both cutting and cut+burning within the disturbed plot. With the mixed hardwood tree species and canopy removed, the remaining plant community consisted of seedling and sampling communities that were dominated by *Pinus strobus* and *Acer rubrum* (Table A 2). Timing of the spring harvest resulted in slash that primarily consisted of coarse woody debris (CWD) with little leaf litter as cutting occurred before leaf out of deciduous

trees. The remaining slash consisted of needles from *Pinus strobus* and *Pinus resinosa.* With slash consisting of primarily CWD and slowly decomposing pine needles, residue additions from decomposed slash substrates between harvesting and burning were likely small contributors to any abiotic changes in mineral soils as little if any slash was highly decomposed at the time of burning. Pine needles being relatively high in lignin and low in P and N are slower to decompose than deciduous tree leaf litter (Gholz et al., 1985). Mass loss of pine litter can have an average rate of decay as low as 15% per year (Cortina and Vallejo, 1994; Gholz et al., 1985; Hart et al., 1991). Coarse woody debris on forest floors has an even slower decay rates and longer residence times due to high lignin concentrations and nutrient poor substrates (Boddy and Watkinson, 1994; Hu et al., 2017).

While the fire was generally low in intensity and severity, this patchy distribution of burning within a stand (See Results) was typical of previously observed results in both wildfires and prescribed burns (Hamman et al., 2007b; Neary et al., 1999a). The majority of studies, however, examine effects exclusively on a stand level (Holden and Treseder, 2013; Kara and Bolat, 2009; Martín-Pinto et al., 2006) whereas I investigated spatial effects within a single disturbance event to disentangle the effects of cutting and burning on soil fungal communities and soil physical and chemical properties.

Soil Physical Properties

Cutting and burning in the experimental plot had no on bulk density and soil moisture. Due to the intensive harvesting using heavy machinery there was a possibility of soil compaction and partial disturbance to A and O horizons. However, the O and A horizons of the soil monoliths post-disturbance were primarily intact with the exception of high burn samples that were volatilized. Machine harvesting has been shown to affect soil horizons through compaction and removal or redistribution of organic matter (Johnson et al., 1991). Soil compaction associated with these highly intensive harvesting methods can lead to increases in bulk density. Bulk density may also increase post fire due to compaction and SOM volatilization during burning, as soil aggregates collapse without OM acting as a binding agent (Verma and Jayakumar, 2012). As ash is deposited in high intensity burns it can percolate into voids in mineral soils further leading to increased bulk densities (Certini, 2005). Notwithstanding these previous findings, bulk density

did not differ between pre- and post-disturbance soils (Figure 2.4) or between the varying disturbance severities (Figure A 9).

There was a detected increase in soil moisture at the plot level (Figure 2.4), but no differences between the disparate disturbance severities (Figure A 9). Harvesting of trees can reduce transpiration as root systems are no longer providing above ground biomass with water necessary for photosynthesis (Everett and Sharrow, 1985). Concurrently, post-harvest the removal of the overstory can lead to an increase in evaporation as more sunlight reaches the soil surface raising soil temperatures and rates of evaporation. Although the entire plot was clear-cut, most of the plot area had a persisting herbaceous layer shading soil surfaces potentially negating effects of increased sunlight. To this end, increases in soil moisture have been previously detected in forest systems transitioning from tree to herbaceous vegetation cover (Özkan and Gökbulak, 2017).

Fires have commonly been linked to decreases in soil moisture with the mechanism being ascribed to increases in soil hydrophobicity. Increased water repellency is a result of the combined effects of deposition and translocation of hydrophobic substances created by organic matter volatilization during burning (Debano, 2000). This is particularly true in arid systems. However, soils that are wet at the time of burning combined with a low intensity fire can lead to no change or to soils becoming less hydrophobic. Our soil sampling campaigns occurred in autumn with a heavy rain event ~1 week prior to sampling. Additionally, temperate forest soils in fall tend to have moist soils due to increased precipitation, cooler temperatures, shorter photoperiods, and decreased transpiration as deciduous plants senesce (Baldrian et al., 2013).

Chemical Properties

The detected increases in soil pH, ECEC (Figure 3AB) and base cations (Figure 2.4ABCH) seen post disturbance are consistent with the effects of responses of previously been reported burn disturbances (González-Pérez et al., 2004; Nave et al., 2019). Clear-cutting has been linked to decreased soil pH and base saturation attributed to decreases in forest floor litter mass and mixing of mineral soils with forest floor materials during harvesting (Brais et al., 2006; Johnson et al., 1991). However, ash deposition has been shown to directly lead to increases in these properties immediately post fire as soluble nutrients are deposited on soil surfaces (Holden and

Treseder, 2013). The elevated pH I detected occurred even in cut only sites indicating ash deposition was ubiquitous throughout the harvested stand. I expected these results to be driven by high intensity burn areas where ash deposits would be higher than other sites. While no differences in ECEC were found between disturbance severities, differences were detected in pH, Ca^{2+}, and total base saturation.

Biomass residues deposited from harvesting and soluble ash deposition from fires have resulted in both elevated and decreased base cation availability and overall base saturation (Nave et al., 2019). The significant reduction in base saturation in cut only sites relative to pre-disturbance samples was similar to those previously reported from timber harvests in temperate forests (Brais et al., 1995; Johnson et al., 1991). In contrast, base saturation in high burn intensity areas was elevated relative to both pre-disturbance sites and all soils with a lower severity disturbance. Elevated base saturation post-disturbance was primarily driven by Ca^{2+} which was found in quantities an order of magnitude higher than any other cation (Figure A 6). While all base cations were found to be elevated post disturbance, post-hoc analyses showed only Ca^{2+} and P^{3-} differed between varying disturbance severities.

Soil Nutrients

The detected increases in NH_4^+ and PO_4^3 are consistent with ash deposition and partial combustion of debris seen in other low intensity fires (Wan et al., 2001). Increases in NH_4^+ post-fire has often been attributed to organic matter pyrolysis, increased N mineralization, and leaching of N from the forest floor into mineral horizons. The amount of nitrogen post fire is typically strongly correlated with the amount of organic matter combusted and is typically higher under high intensity fires (Belillas and Feller, 1998; O'Connell and McCaw, 1997; Wan et al., 2001). Soil NO_3^- was not significantly elevated relative to pre-disturbance sites due to the short retention time of NO_3 in these soils consisting mostly of Rubicon sands (Nave et al., 2014, 2011). A rain event occurred between the burn implementation and post-disturbance sampling campaign, likely exacerbating the leaching of NO_3 from soils. I attribute elevated phosphate availability primarily to ash deposition as P mineralization through phosphatase activities in post disturbance soils were significantly lower than pre-disturbance soils (Figure 2.7).

Fungal DNA

As predicted, I detected both plot level (pre- vs post-) and within plot differences in fungal community composition. Differences between pre- and post-disturbance fungal communities were driven by mortality of ectomycorrhizal associated fungi in the basidiomycetes, and an influx in an array of saprotrophic ascomycetes and mucoromycetes post-disturbance. Pre-disturbance plant communities were dominated the adult *Quercus rubra*, *Populus grandidentata* and *Pinus resinosa* that associate with EM fungi (Godbout and Fortin, 1985; Morris et al., 2008). This shift was characterized by the loss of Cortinariaceae that were dominant in pre-disturbed soils (Figure A 3). Declines in taxa in the Cortinariaceae were detected in both burned and unburned soils indicating losses of these taxa were due to the harvesting as opposed to the fire. Without the ability to persist in absence of a plant symbiont these EM and associated roots senesce in soils, providing a nutrient rich substrate for saprobic fungi.

It is important to note that mortality of EM taxa due to harvesting was not phylogenetically or spatially ubiquitous. Species in the Russulaceae persisted in low to moderately disturbed soils (Figure B 3). While Cortinariaceae taxa have strong symbiotic relationships with tree species removed during harvesting, EM in the Russulaceae have been shown to form associations with *Pinus strobus* (Adamčík and Buyck, 2011; Buyck et al., 2006; Hatch and C.T., 1933) which dominated seedling and understory plant communities. With approximately 40% of the plot going unburnt and the majority of the burn disturbance being relatively low severity, I noted little seedling and no juvenile mortality or *Pinus strobus* post fire. In addition to plausible host persistence in the plot, Russulaceae include nitrophilic EM species whose relative abundances can increase in soils that are enriched with inorganic N products (Morrison et al., 2016). An ANCOM analysis (Figure 2.3) and stepwise regression of soil physio-chemical properties (Figure 2.8) revealed mirroring trends in describing community dissimilarities. Russulaceae species that persisted and elevated %N tended to be indicators of communities that experienced cutting and no to low severity burning.

Importantly, within the varying disturbance severities in the post cut + burn plot, I did detect evidence of support for the intermediate disturbance hypothesis. A suite of multiple diversity indices revealed significant differences in fungal communities with a unimodal peak in low to

moderate disturbances. Areas of high severity disturbance were markedly lower in diversity relative to all other soils including the reference, pre-disturbance, and all areas of low to moderate disturbance (cut only through cut + moderate severity burn) (Figure A 2). Concurrently, fungal diversity in the low to moderately disturbed areas showed consistent increases in diversity relative to pre-disturbance, reference, and high severity areas within the plot (Figure A 2).

Given that no difference could be detected in percent C between low to medium severity disturbances and pre-disturbance soils, it is unlikely that high enough temperatures for fungal mortality in mineral soils were reached. Fire induced mortality in fungi has been shown to result from temperatures less than 100 °C (DeBano et al., 1998), but it is likely that temperatures in the mineral soils never exceed this level at our site. Charring of organic matter in soils and on soil surfaces begins to occur above 200 °C (Gonzalez-Perez et al., 2004). While medium burn severity sites did show some signs of surface charring, only a fraction of heat generated at soil surfaces is radiated to mineral soils. Dry soils can act as good insulators causing temperatures to rise slowly, requiring prolonged burning (DeBano et al., 1998), whereas in moist soils evaporation of water can prevent a moist organic layer from exceeding temperatures above water boiling point (Campbell et al., 1995). Soils during the time of the burn implementation were noted as being moist from a rain event earlier in the week. The resulting fire induced mortality of fungi in mineral soil was likely minimal due to relatively low temperatures in subsurface soils in superficial to moderately burned areas.

Under high severity fire conditions there was punctuated dominance by Ascomycetes and Zygomycetous fungi and loss of EM. Ascomycetes have previously been shown to have developed high tolerances to organic byproducts of burning (Widden and Parkinson, 1974) and inorganic physio-chemical changes to soil C (Wicklow, 1975; Wicklow and Hirschfield, 1979). The high severity burn areas did contrast starkly from less severe disturbances with elevated pH (Figure A 9), base saturation (Figure A10) and PO4 (Figure A 12). The high burn area communities were consistent with previous studies where mycorrhizal basidiomycetes were replaced by fungi in the Pyronemataceae, Sordariaceae (Reazin et al., 2016; Wicklow and Hirschfield, 1979). Organisms in all these families have been previously shown to increase post

fires in a number of ecosystems. Anthracobia, a genus of fungi in the Pyronemataceae, was found in particularly high abundance in these soils. Anthracobia have been shown to form extensive mycelial mats in heavily burned microsites (Claridge et al., 2009) and are hypothesized to play a role in soil aggregation post fire in sites with erodible soils.

The observed increase in saprobic ascomycetes throughout the plot post-disturbance has been similarly reported after clear-cutting (Byrd et al., 2000) and fire disturbances (Widden and Parkinson, 1974). The influx of ascomycetous saprotrophs throughout the plot post-disturbance, coupled with a partial reduction in EM dominance in low to moderately disturbed areas, likely resulted in the observed increases in diversity. These areas stand in contrast to the high severity areas where fire induced mortality was a driver for diversity loss of all EM taxa. Root and hyphal biomass were most likely volatilized along with SOM in high burn areas, whereas they potentially served as a substrate for saprotrophs potentially priming decomposition throughout the rest of the plot.

In addition to increases in substrate availability from root and hyphal mortality, another driver for saprotroph increases in clear-cut areas has been attributed to a release in competition as EM decline post harvesting (Kyaschenko et al., 2017). The Gadgil effect posits that the presence of EM in a system can inhibit saprotroph growth and activity through competition for nutrients, water and space (Gadgil and Gadgil, 1971). This shift in fungal functional groups from EM to saprotroph dominated may be important for C cycling dynamics. Unlike saprotrophs, EM have not been shown to decompose organic matter for the liberation of C via extracellular enzymes (Frey, 2019; Zak et al., 2019). While some ectomycorrhizal fungi have the genetic capacity required to produce extracellular enzymes secreted during decomposition (Floudas et al., 2012; Talbot et al., 2013), they have yet to be shown to be active in soils free from association with their plant symbionts (Pellitier and Zak, 2018).

While the result of decreasing EM abundance was consistent with earlier findings, results showing an increase in taxa belonging to the Umbelopsidaceae family stand in contrast to previously published results. A recent study examining the effects of differing fire intensities in ponderosa pine ecosystems found that taxa in the Umbelopsidaceae were seen to show ten-fold

decreases in relative abundance compared to pre-burn communities (Reazin et al., 2016). The most common OTU was *Umbelopsis dimorpha* which was found in particularly high abundances post-disturbance. They also detected stark declines in taxa in the Mortierella genus in high soils experiencing high intensity burns. While I did not detect a strong representation in our pre-disturbance soils, the high intensity burn areas had noticeably lower relative abundances of Mortierella species relative to the reference stand and areas of lower severity disturbance.

Fungal RNA

RNA analyses of fungal communities confirmed results from DNA describing pre-disturbance dominated by mycorrhizal taxa in the Cortinariaceae and Russulaceae. However, unlike DNA results, in soils that received the lowest severity disturbances (cut only and cut + superficial burn) there was an increase in AM taxa in the Glomeraceae relative to pre-disturbance conditions. This response may be partially attributed to *Acer rubrum* seedlings and saplings in the ground flora that have been shown to form associations with AM fungi (Sikes et al., 2012). Sapling associations with AM fungi during succession post disturbances, potentially providing plants with protection from soil pathogens that may otherwise inhibit plant growth (Kardol et al., 2007; Little and Maun, 1996), while also helping saplings acquire soil P.

Harvest of *Acer rubrum* boles accounted for 6% of the overstory (Table A 1,) while the relative abundances of juvenile and seedling *A. rubrum* stems were 17.4% and 53.6%, respectively (Table A 2). With *Quercus rubra*, *Populus grandidenta,* and *Pinus resinosa* as the dominant species harvested and the coinciding decrease in their EM symbionts abundances in soils, roots of *A. rubrum* and their AM symbionts may have experienced releases in competition as soil resources became available. AM fungi in the Glomeromycota can be more phylogenetically diverse in later successional stages but it is yet unclear whether that is a result of dispersal capabilities or asymmetries in plant mutualist specialization (Sikes et al., 2012; Vázquez and Aizen, 2004).

The high severity RNA sample had similar fungal community characteristics of those described by DNA analyses with lower phylogenetic diversity, a complete absence of EM or AM fungi and a dominance by Mucoromycete fungi in the Umbelopsidaceae. Interestingly, the high severity

sample did not show strong representation from taxa in the Pyrenometaceae that were noted to be prevalent by DNA. While pyrenomycetous fungi can include plant pathogens (Freeman et al., 2001), insect pathogens (Fukatsu et al., 1997) and fungal parasites (Spatafora and Blackwell, 1993), the high abundance of fungi in the Anthracobia genus reported in the DNA results would suggest that main Pyrenomycetes in our system were saprotrophs (Claridge et al., 2009). Despite this, they appear to be less active post-disturbance relative to saprobes in the Umbelopsidaceae. It is however important to note that it's difficult to generalize these results as only a single sample in a high severity area provided RNA data.

Fungal and Bacterial Abundances

Neither fungi nor bacteria showed a response in abundance, as measured by gene copies (Figure A 7). Clearcutting harvests have repeatedly indicated a significant negative response from total soil microbes as well as fungal and bacterial abundances separately (Holden and Treseder, 2013; Pietikainen and Fritze, 1995). Declines in total microbial biomass have primarily been attributed to soil compaction and a loss of inputs due to vegetation removal (Barg and Edmonds, 1999). Fungi in particular have repeatedly shown to decline as root systems lose the ability to support mycorrhizae (Harvey et al., 1980; Pietikainen and Fritze, 1995). I did find a decline in relative abundances of EM taxa (Figure A 3 & A 4), but was unable to measure relative abundances of EM and saprobic fungi using qPCR because of the polyphyletic nature of EM. It is possible that increases in both fungal and bacterial saprobic abundances offset EM decline as they decomposed and assimilated the readily available root and EM hyphal biomass post-harvest (Lundgren, 1982). The findings that bacterial abundances tended to increase in cut only and fungi increased in cut +low severity burns (Figure A 8) is in line with these previous studies (Lewandowski et al., 2016). The fungal results observed in low severity disturbances may be a product of contrasting but intertwined forces of decreased EM abundances and priming of the saprobic communities as a result of harvesting.

In contrast with lower severity disturbances, I detected lower abundances of both fungi and bacteria at high severity areas (Figure A 8). The relatively short time (1 week) between burning and post-burn sampling was likely indicative of direct effects of fire induced mortality. A complete combustion of the O horizon and charring begin to occur at 200 C° (González-Pérez et

al., 2004) exposing the mineral soils of the A horizon to temperatures >100 C that has been shown to cause both fungal and bacterial mortality (DeBano et al., 1998; Neary et al., 1999b). While bacteria as a whole have been reported to be more resilient to fire compared to fungi (Dooley and Treseder, 2012) and tend to increase in abundance as pH increases, I noted similar responses of bacterial abundance to high severity burning (Figure A 8). The detected decline in abundances in high burn areas concurrent with increased fungal diversity in the cut + low burn offers further support for the IDH, as patterns of biomass are often reported to accompany trends in diversity.

Extracellular Enzyme Activity

I detected varying effects of disturbances on the suite of enzymes measured. No differences were detected in the enzymatic activity of cellulase BG and AG associated with starch hydrolysis despite altered shifts in fungal community composition. Previously published rates of root decomposition in a clear-cut northern hardwood forest suggest that fine roots in the mineral soil died slowly and were not effectively leached of K or N in the first year, while woody roots in mineral soils took years to decay (Fahey and Arthur, 1994). Indeed, for much of the substrate N would first need to be recruited into the roots in order for their decomposition to commence. The effects I did detect in EEA were declines in EEA of chitinases (NAG), xylanases (ALK) and laccases (PHEN_OX) post cut + burn (Figure 2.7). EEA of ALK and PHEN_OX was particularly descriptive of pre-disturbance soils (Figure 2.8) for which I reported lower nutrient availability.

I predicted that plot level reductions in EEA would be driven by high severity burn areas due to reduced diversity and fungal abundances relative to less severely disturbed areas within the plot. As previously discussed, fungal and bacterial abundances were detectably lower in high severity soils relative to all other severities (Figure A 8). Despite this, an ANOVA on disparate disturbance severities did not reveal any significant differences in NAG, ALK or PHEN_OX that would suggest EEA was lower in high intensity burns (Figure A 14). However, if all areas of low to moderate disturbance were pooled and compared with high severity areas I did detect a difference with lower EEA of NAG ($p<0.01$), ALK ($p<0.01$), PHEN_OX ($p<0.01$) and AG ($P=0.03$) in high severity sites (Figure A 15).

Activity of ALK is a good indicator of P mineralization in forest soils. Pre-disturbed soils were low in available PO_4 relative to disturbed soils. This limitation likely stimulated the high ALK activity in these soils as mining from organic P forms would be necessary for continued primary production (Margalef et al., 2017; Turner and Condron, 2013). Once the trees were harvested their demand for P for photosynthates would be removed from the system as would a primary source of C allocated to soil that would serve as fuel for further EEA. While there has been previously documented proliferation of root production and turnover by juveniles, saplings, and herbaceous ground cover after canopy trees are harvested (Fukuzawa et al., 2006; Likens et al., 2010), this is likely not enough to replace the resource of C and demand for P lost from tree harvesting. In contrast, elevated nutrients in disturbed soils, including PO4, would likely result in a decrease in the need for liberation of P from organic pools. The detected elevated inorganic N concentrations would have had a similar effect on N mineralization from organic pools by EEA in NAG and PHENOL_OX.

Conclusions

Results reveal the differential effects on soils receiving either clearcutting or clearcutting + burning treatments within a 1-ha stand and highlight the importance of scale when examining disturbance effects across landscapes. Previous studies of forests at the University of Michigan Biological Station have inferred the importance of understanding underlying variation at the landscape scale when examining effects of disturbance. Most have examined long-term trajectories and few studies to date, however, have examined the effects of disturbances at a fine spatial scale (i.e. within 1 ha). Variation in disturbance intensities at the scale of tens of meters is often lost when examining the effects of disturbances at large scales.

Although I did detect some plot level differences, I underscore that the variability in fire severity at a scale of meters is of particular importance due to the large differences in physio-chemical properties and microbial communities in soils experiencing high temperature disturbances relative to low intensity burning. This is highlighted by complete volatilization of the organic horizons and by increases in pH, soil moisture and Ca^{2+}. These physio-chemical changes were accompanied by a major restructuring of the fungal communities in mineral soil with a

punctuated loss of ECM taxa, a decline in all measures of diversity and a decline in both fungal and bacterial total abundances.

Those trends were markedly different than soils in areas that did not experience high enough temperatures to volatilize the organic horizon or mineral soil carbon. In those areas (cut only through cut + medium burn) where clearcutting was the primary driver led to drastically different fungal communities where I observed an increase in diversity indices. Instead of the complete loss of ECM I noted a shift in the dominant ECM taxa from Cortinariaceae to Russulaceae that was likely driven by plant community shifts from *Quercus rubra* and *Pinus resinosa* dominated forest to a sampling community that is overwhelmingly dominated by *Acer rubrum*, an AM associated plant, and *Pinus strobus*. Together the trends in diversity offer support for fungal communities adhering to patterns predicted by the IDH often observed in plant communities.

These results stand in light of the fact that only 13% of 2017 burn plot area had a high intensity burn. Interestingly, pooling all disturbed soils led to differential results at the plot level for abiotic and biotic measurements. High burn samples tended to drive plot level differences in soil physio-chemical properties. In contrast I noted that the plot-level increases in diversity were driven by areas that primarily received the clear-cutting disturbance. Those contrasted with the punctuated drop community diversity and abundance in the high burn localities. These results offer insight into potentially missed trends in the variable effects of disturbances at a fine scale when measurements are pooled or examined at scales of hectares or larger. Finally, I identify the disproportionate weight of highly disturbed soils when examining soil physio-chemical properties. In total, the work in this chapter underlines the importance of variation in disturbance severity for structuring soil fungal communities and highlights the differential effects of clear-cutting and clear-cutting + burning on fungal diversity, abundance, and enzymatic activity.

2.6 References

Adamčík, S., Buyck, B., 2011. The Species of Russula Subsection Xerampelinae Described. Cryptogam. Mycol. 32, 63–81. https://doi.org/10.7872/crym.v32.iss1.2012.063

Allison, S.D., McGuire, K.L., Treseder, K.K., 2010. Resistance of microbial and soil properties to warming treatment seven years after boreal fire. Soil Biol. Biochem. 42, 1872–1878. https://doi.org/10.1016/j.soilbio.2010.07.011

Baldrian, P., Kolařík, M., Štursová, M., Kopecký, J., Valášková, V., Větrovský, T., Žifčáková, L., Šnajdr, J., Rídl, J., Vlček, Č., Voříšková, J., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. ISME J. 6, 248–258. https://doi.org/10.1038/ismej.2011.95

Baldrian, P., Šnajdr, J., Merhautová, V., Dobiášová, P., Cajthaml, T., Valášková, V., 2013. Responses of the extracellular enzyme activities in hardwood forest to soil temperature and seasonality and the potential effects of climate change. Soil Biol. Biochem. 56, 60–68. https://doi.org/10.1016/j.soilbio.2012.01.020

Barg, A.K., Edmonds, R.L., 1999. Influence of partial cutting on site microclimate, soil nitrogen dynamics, and microbial biomass in Douglas-fir stands in western Washington. Can. J. For. Res. 29, 705–713. https://doi.org/10.1139/x99-045

Belillas, C.M., Feller, M.C., 1998. Relationships between fire severity and atmospheric and leaching nutrient losses in British Columbia's coastal western hemlock zone forests. Int. J. Wildl. Fire 8, 87–101.

Biswas, A., 2018. Scale–location specific soil spatial variability: A comparison of continuous wavelet transform and Hilbert–Huang transform. Catena 160, 24–31. https://doi.org/10.1016/j.catena.2017.08.019

Boddy, L., Watkinson, S., 1994. Wood decomposition, higher fungi, and their role in nutrient redistribution. Can. J. Bot. 73.

Brais, S., Camire, C., Pare, D., 1995. Impacts of whole-tree harvesting and winter windrowing on soil pH and base status of clayey sites of northwestern Quebec. Can. J. For. Res. 25.

Brais, S., Paré, D., Lierman, C., 2006. Tree bole mineralization rates of four species of the Canadian eastern boreal ... Can. J. For. Res. 36.

Buyck, B., Mitchell, D., Parrent, J., 2006. Russula parvovirescens sp. nov., a common but ignored species in the eastern United States . Mycologia 98, 612–615. https://doi.org/10.1080/15572536.2006.11832664

Byrd, K.B., Parker, V.T., Vogler, D.R., Cullings, K.W., 2000. The influence of clear-cutting on ectomycorrhizal fungus diversity in a lodgepole pine (Pinus centorta) stand, Yellowstone National Park, Wyoming, and Gallatin National Forest, Montana. Can. J. Bot. 78, 149–156. https://doi.org/10.1139/b99-171

Campbell, G.S., Jungbauer, J.D., Bristow, K.L., Hungerford, R.D., 1995. Soil temperature and water content beneath a surface fire. Soil Sci. 6, 363–374.

Catford, J.A., Daehler, C.C., Murphy, H.T., Sheppard, A.W., Hardesty, B.D., Westcott, D.A., Rejmánek, M., Bellingham, P.J., Pergl, J., Horvitz, C.C., Hulme, P.E., 2012. The intermediate disturbance hypothesis and plant invasions: Implications for species richness and management. Perspect. Plant Ecol. Evol. Syst. 14, 231–241. https://doi.org/10.1016/j.ppees.2011.12.002

Certini, G., 2005. Effects of fire on properties of forest soils: A review. Oecologia. https://doi.org/10.1007/s00442-004-1788-8

Chapin, F.S., Matson, P.A., Mooney, H., 2002. Principles of Ecosystem Ecology.
Chapman, S.K., Langley, J. a, Hart, S.C., Koch, G.W., 2005. Plants actively control nitrogen cycling: uncorking the microbail bottleneck. New Phytol. 169, 27–34.
Claridge, A.W., Trappe, J.M., Hansen, K., 2009. Do fungi have a role as soil stabilizers and remediators after forest fire? For. Ecol. Manage. 257, 1063–1069. https://doi.org/10.1016/j.foreco.2008.11.011
Connell, J.H., 1978. Diversity in tropical rain forests and coral reefs. Science (80-.). 199, 1302–1310. https://doi.org/10.1126/science.199.4335.1302
Cortina, J., Vallejo, V.R., 1994. Effects of clearfelling on forest floor accumulation and litter decomposition in a radiata pine plantation. For. Ecol. Manage. 70, 299–310. https://doi.org/10.1016/0378-1127(94)90095-7
Covington, W.W., 1981. Changes in Forest Floor Organic Matter and Nutrient Content Following Clear Cutting in Northern Hardwoods. Ecol. Soc. Am. 62, 41–48.
Czimczik, C.I., Masiello, C.A., 2007. Controls on black carbon storage in soils. Global Biogeochem. Cycles 21. https://doi.org/10.1029/2006GB002798
Debano, L.F., 2000. The role of fire and soil heating on water repellency in wildland environments: A review, in: Journal of Hydrology. pp. 195–206. https://doi.org/10.1016/S0022-1694(00)00194-3
DeBano, L.F., Neary, D.G., Ffolliott, P.F., 1998. Fire's Effects on Ecosystems. John Wiley and Sons, New YOrk.
Deka, H.K., Mishra, R.R., 1983. The effect of slash burning on soil microflora. Plant Soil 73, 167–175. https://doi.org/10.1007/BF02197713
Dooley, S.R., Treseder, K.K., 2012. The effect of fire on microbial biomass: A meta-analysis of field studies. Biogeochemistry 109, 49–61. https://doi.org/10.1007/s10533-011-9633-8
Everett, R.L., Sharrow, S.H., 1985. Response of grass species to tree harvesting in singleleaf pinyon- Utah juniper stands. Res. Pap. - US Dep. Agric. For. Serv. 45. https://doi.org/10.5962/bhl.title.69006
Fahey, T.J., Arthur, M.A., 1994. Studies of Root Decomposition Following Harvest of a Northern. For. Sci. 40, 618–629.
Floudas, D., Binder, M., Riley, R., Barry, K., Blanchette, R., Henrissat, B., Martínez, A.T., Otillar, R., Spatafora, J.W., Yadav, J.S., Coutinho, P.M., Vries, R.P. De, Ferreira, P., Findley, K., Foster, B., 2012. Lignin Decomposition Reconstructed from 31 Fungal Genomes. Science (80-.). 336, 1715–1719.
Freeman, S., Horowitz, S., Sharon, A., 2001. Pathogenic and nonpathogenic lifestyles in Colletotrichum acutatum from strawberry and other plants. Phytopathology 91, 986–992. https://doi.org/10.1094/PHYTO.2001.91.10.986
Frey, S.D., 2019. Mycorrhizal Fungi as Mediators of Soil Organic Matter Dynamics. Annu. Rev. Ecol. Evol. Syst. 50, 237–259. https://doi.org/10.1146/annurev-ecolsys-110617-062331
Frey, S.D., Drijber, R., Smith, H., Melillo, J., 2008. Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biol. Biochem. 40, 2904–2907. https://doi.org/10.1016/j.soilbio.2008.07.020
Fukatsu, T., Sato, H., Kuriyama, H., 1997. Isolation, Inoculation to Insect Host, and Molecular Phylogeny of an Entomogenous Fungus Paecilomyces tenuipes. J. Invertebr. Pathol. 70, 203–208. https://doi.org/10.1006/jipa.1997.4696
Fukuzawa, K., Shibata, H., Takagi, K., Nomura, M., Kurima, N., Fukazawa, T., Satoh, F., Sasa, K., 2006. Effects of clear-cutting on nitrogen leaching and fine root dynamics in a cool-

temperate forested watershed in northern Japan. For. Ecol. Manage. 225, 257–261. https://doi.org/10.1016/j.foreco.2006.01.001
Gadgil, R.L., Gadgil, P.D., 1971. Mycorrhiza and litter decomposition. Nature 233, 133. https://doi.org/10.1038/233133a0
Gholz, H.L., Perry, C.S., Cropper, W.P., Hendry, L.C., 1985. Litterfall, decomposition, and nitrogen and phosphorus dynamics in a chronosequence of slash pine (Pinus elliottii) plantations. For. Sci. 31, 463–478. https://doi.org/10.1093/forestscience/31.2.463
Giovannini, G., Lucc, 1983. Effect of fire on hydrophobic and cementing substances of soil aggregates. Soil Sci. 136, 231–236.
Giovannini, G., Lucchesi, S., 1997. Modifications induced in soil physico-chemical parameters by experimental fires at different intensities. Soil Sci. 162, 479–486.
Godbout, C., Fortin, J.A., 1985. Synthesized ectomycorrhizae of aspen: fungal genus level of structural characterization. Can. J. Bot. 63, 252–262. https://doi.org/10.1139/b85-029
González-Pérez, J.A., González-Vila, F.J., Almendros, G., Knicker, H., 2004. The effect of fire on soil organic matter--a review. Environ. Int. 30, 855–870. https://doi.org/10.1016/j.envint.2004.02.003
Gough, C.M., Hardiman, B.S., Nave, L.E., Bohrer, G., Kyle, D., Vogel, C.S., Nadelhoffer, K.J., Curtis, P.S., Gough, C., Hardiman, B.S., Nave, L., Bohrer, G., Maurer, K.D., Vogel, C.S., Nadelhoffer, K.J., Curtis, P.S., 2013. Sustained carbon uptake and storage following moderate disturbance in a Great Lakes forest 23, 1202–1215.
Gough, C.M., Vogel, C.S., Harrold, K.H., George, K., Curtis, P.S., 2007. The legacy of harvest and fire on ecosystem carbon storage in a north temperate forest. Glob. Chang. Biol. https://doi.org/10.1111/j.1365-2486.2007.01406.x
Gough, C.M., Vogel, C.S., Schmid, H.P., Curtis, P.S., 2008. Controls on Annual Forest Carbon Storage: Lessons from the Past and Predictions for the Future. Bioscience. https://doi.org/10.1641/B580708
Gul, S., Whalen, J.K., Thomas, B.W., Sachdeva, V., Deng, H., 2015. Physico-chemical properties and microbial responses in biochar-amended soils: Mechanisms and future directions. Agric. Ecosyst. Environ. https://doi.org/10.1016/j.agee.2015.03.015
Hamman, S.T., Burke, I.C., Stromberger, M.E., 2007a. Relationships between microbial community structure and soil environmental conditions in a recently burned system. Soil Biol. Biochem. https://doi.org/10.1016/j.soilbio.2007.01.018
Hamman, S.T., Burke, I.C., Stromberger, M.E., 2007b. Relationships between microbial community structure and soil environmental conditions in a recently burned system. Soil Biol. Biochem. 39, 1703–1711. https://doi.org/10.1016/j.soilbio.2007.01.018
Hardiman, B.S., Gough, C.M., Halperin, A., Hofmeister, K.L., Nave, L.E., Bohrer, G., Curtis, P.S., 2013. Maintaining high rates of carbon storage in old forests: A mechanism linking canopy structure to forest function. For. Ecol. Manage. 298, 111–119. https://doi.org/10.1016/j.foreco.2013.02.031
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. For. Ecol. Manage. https://doi.org/10.1016/j.foreco.2005.08.012
Hart, S.C., Firestone, M.K., Paul, E.A., 1991. Decomposition and nutrient dynamics of pondersosa pine needles in a Mediterranean-type climate. Can. J. For. Res. 22, 306–314. https://doi.org/10.16309/j.cnki.issn.1007-1776.2003.03.004
Harvey, A.E., Jurgensen, M.F., Larsen, M.J., 1980. Clearcut harvesting and ectomycorrhizae:

survival of activity on residual roots and influence on a bordering forest stand in western Montana. Can. J. Bot. 10, 300–303.
Hassett, J.E., Zak, D.R., 2005. Aspen Harvest Intensity Decreases Microbial Biomass, Extracellular Enzyme Activity, and Soil Nitrogen Cycling. Soil Sci. Soc. Am. J. 69, 227. https://doi.org/10.2136/sssaj2005.0227
Hatch, A.B., C.T., H., 1933. Some Hymenomycetes forming mycorrhizae with Pinus strobus. J. Arnold Arbor. 14, 324–334.
Högberg, M.N., Bååth, E., Nordgren, A., Arnebrant, K., Högberg, P., 2003. Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs - A hypothesis based on field observations in boreal forest. New Phytol. 160, 225–238. https://doi.org/10.1046/j.1469-8137.2003.00867.x
Holden, S.R., Treseder, K.K., 2013. A meta-analysis of soil microbial biomass responses to forest disturbances. Front. Microbiol. 4. https://doi.org/10.3389/fmicb.2013.00163
Hu, Z., Xu, C., McDowell, N.G., Johnson, D.J., Wang, M., Luo, Y., Zhou, X., Huang, Z., 2017. Linking microbial community composition to C loss rates during wood decomposition. Soil Biol. Biochem. 104, 108–116. https://doi.org/10.1016/j.soilbio.2016.10.017
Ice, G.G., Neary, D.G., Adams, P.W., 2004. Effects of Wildfire on Processes. J. For. 102, 16–20.
Jensen, M., Michelsen, A., Gashaw, M., 2001. Responses in plant, soil inorganic and microbial nutrient pools to experimental fire, ash and biomass addition in a woodland savanna. Oecologia 128, 85–93. https://doi.org/10.1007/s004420000627
Jiménez Esquilín, A.E., Stromberger, M.E., Massman, W.J., Frank, J.M., Shepperd, W.D., 2007. Microbial community structure and activity in a Colorado Rocky Mountain forest soil scarred by slash pile burning. Soil Biol. Biochem. https://doi.org/10.1016/j.soilbio.2006.12.020
Johnson, C.E., Johnson, A.H., Siccama, T.G., 1991. Whole-Tree Clear-Cutting Effects on Exchangeable Cations and Soil Acidity. Soil Sci. Soc. Am. J. 55, 502. https://doi.org/10.2136/sssaj1991.03615995005500020035x
Jomura, M., Kominami, Y., Tamai, K., Miyama, T., Goto, Y., Dannoura, M., Kanazawa, Y., 2007. The carbon budget of coarse woody debris in a temperate broad-leaved secondary forest in Japan. Tellus, Ser. B Chem. Phys. Meteorol. 59, 211–222. https://doi.org/10.1111/j.1600-0889.2006.00234.x
Kara, O., Bolat, I., 2009. Short-term effects of wildfire on microbial biomass and abundance in black pine plantation soils in Turkey. Ecol. Indic. 9, 1151–1155. https://doi.org/10.1016/j.ecolind.2009.01.002
Kardol, P., Cornips, N.J., van Kempen, M.M.L., Bakx-Schotman, J.M.T., van der Putten, W.H., 2007. Microbe-Mediated Plant-Soil Feedback Causes Historical Contingency Effects in Plant Community Assembly. Ecol. Monogr. 77, 147–162.
Kirk, J.L., Beaudette, L.A., Hart, M., Moutoglis, P., Klironomos, J.N., Lee, H., Trevors, J.T., 2004. Methods of studying soil microbial diversity. J. Microbiol. Methods 58, 169–188. https://doi.org/10.1016/j.mimet.2004.04.006
Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by 14C labeling. Soil Biol. Biochem. 41, 210–219. https://doi.org/10.1016/j.soilbio.2008.10.016
Kyaschenko, J., Clemmensen, K.E., Hagenbo, A., Karltun, E., Lindahl, B.D., 2017. Shift in fungal communities and associated enzyme activities along an age gradient of managed Pinus sylvestris stands. ISME J. 11, 863–874. https://doi.org/10.1038/ismej.2016.184

Lapin, M., Barnes, B. V, 1995. Society for Conservation Biology Using the Landscape Ecosystem Approach to Assess Species and Ecosystem Diversity Using the Landscape Ecosystem Approach to Assess Species and Ecosystem Diversity. Conserv. Biol. 9, 1148–1158.

Lewandowski, T.E., Forrester, J.A., Mladenoff, D.J., D'Amato, A.W., Palik, B.J., 2016. Response of the soil microbial community and soil nutrient bioavailability to biomass harvesting and reserve tree retention in northern Minnesota aspen-dominated forests. Appl. Soil Ecol. 99, 110–117. https://doi.org/10.1016/j.apsoil.2015.11.001

Likens, G.E., Bormann, F.H., Johnson, N.M., Fisher, D.W., Robert, S., 2010. Effects of Forest Cutting and Herbicide Treatment on Nutrient Budgets in the Hubbard Published by : Ecological Society of America. America (NY). 40, 23–47.

Little, L.R., Maun, M.A., 1996. The Ammophila Problem Revisited : A Role for Mycorrhizal Fungi. Br. Ecol. Soc. 84, 1–7.

López-Mondéjar, R., Zühlke, D., Becher, D., Riedel, K., Baldrian, P., 2016. Cellulose and hemicellulose decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems. Sci. Rep. 6, 1–12. https://doi.org/10.1038/srep25279

Lundgren, B., 1982. Bacteria in a pine forest soil as affected by clear-cutting. Soil Biol. Biochem. 14, 537–542. https://doi.org/10.1016/0038-0717(82)90083-9

Magnússon, R., Tietema, A., Cornelissen, J.H.C., Hefting, M.M., Kalbitz, K., 2016. Tamm Review: Sequestration of carbon from coarse woody debris in forest soils. For. Ecol. Manage. 377, 1–15. https://doi.org/10.1016/j.foreco.2016.06.033

Manzoni, S., Schimel, J.P., Porporato, A., Ecology, S., April, N., Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress : results from a meta-analysis. Ecology 93, 930–938.

Margalef, O., Sardans, J., Fernández-Martínez, M., Molowny-Horas, R., Janssens, I.A., Ciais, P., Goll, D., Richter, A., Obersteiner, M., Asensio, D., Peñuelas, J., 2017. Global patterns of phosphatase activity in natural soils. Sci. Rep. 7, 1–13. https://doi.org/10.1038/s41598-017-01418-8

Martín-Pinto, P., Vaquerizo, H., Peñalver, F., Olaizola, J., Oria-De-Rueda, J.A., 2006. Early effects of a wildfire on the diversity and production of fungal communities in Mediterranean vegetation types dominated by Cistus ladanifer and Pinus pinaster in Spain. For. Ecol. Manage. https://doi.org/10.1016/j.foreco.2006.01.006

McGuire, K.L., Treseder, K.K., 2010. Microbial communities and their relevance for ecosystem models: Decomposition as a case study. Soil Biol. Biochem. 42, 529–535. https://doi.org/10.1016/j.soilbio.2009.11.016

Morris, M.H., Smith, M.E., Rizzo, D.M., Rejmánek, M., Bledsoe, C.S., 2008. Contrasting ectomycorrhizal fungal communities on the roots of co-occurring oaks (Quercus spp.) in a California woodland. New Phytol. 178, 167–176. https://doi.org/10.1111/j.1469-8137.2007.02348.x

Morrison, E.W., Frey, S.D., Sadowsky, J.J., van Diepen, L.T.A., Thomas, W.K., Pringle, A., 2016. Chronic nitrogen additions fundamentally restructure the soil fungal community in a temperate forest. Fungal Ecol. 23, 48–57. https://doi.org/10.1016/j.funeco.2016.05.011

Morrissey, E.M., Berrier, D.J., Neubauer, S.C., Franklin, R.B., 2014. Using microbial communities and extracellular enzymes to link soil organic matter characteristics to greenhouse gas production in a tidal freshwater wetland. Biogeochemistry 117, 473–490. https://doi.org/10.1007/s10533-013-9894-5

Mueller, G.M., Schmit, J.P., 2007. Fungal biodiversity: What do we know? What can we predict? Biodivers. Conserv. 16, 1–5. https://doi.org/10.1007/s10531-006-9117-7

Nave, L.E., Gough, C.M., Maurer, K.D., Bohrer, G., Hardiman, B.S., Le Moine, J., Munoz, A.B., Nadelhoffer, K.J., Sparks, J.P., Strahm, B.D., Vogel, C.S., Curtis, P.S., 2011. Disturbance and the resilience of coupled carbon and nitrogen cycling in a north temperate forest. J. Geophys. Res. Biogeosciences 116. https://doi.org/10.1029/2011JG001758

Nave, L.E., Gough, C.M., Perry, C.H., Hofmeister, K.L., Le Moine, J.M., Domke, G.M., Swanston, C.W., Nadelhoffer, K.J., 2017. Physiographic factors underlie rates of biomass production during succession in Great Lakes forest landscapes. For. Ecol. Manage. 397, 157–173. https://doi.org/10.1016/j.foreco.2017.04.040

Nave, L.E., Le Moine, J.M., Gough, C.M., Nadelhoffer, K.J., 2019. Multidecadal trajectories of soil chemistry and nutrient availability following cutting vs. Burning disturbances in upper great lakes forests. Can. J. For. Res. 49, 731–742. https://doi.org/10.1139/cjfr-2018-0211

Nave, L.E., Sparks, J.P., Le Moine, J., Hardiman, B.S., Strahm, B.D., Curtis, P.S., 2014. Changes in soil nitrogen cycling in northern temperate forest ecosystem during succession. Biogeochemistry 121, 471–488. https://doi.org/10.1007/s

Neary, D.G., Klopatek, C.C., DeBano, L.F., Ffolliott, P.F., 1999a. Fire effects on belowground sustainability: A review and synthesis, in: Forest Ecology and Management. https://doi.org/10.1016/S0378-1127(99)00032-8

Neary, D.G., Klopatek, C.C., DeBano, L.F., Ffolliott, P.F., 1999b. Fire effects on belowground sustainability: A review and synthesis, in: Forest Ecology and Management. pp. 51–71. https://doi.org/10.1016/S0378-1127(99)00032-8

O'Connell, A.M., McCaw, W.L., 1997. Prescribed burning of thinning slash in regrowth stands of karri (Eucalyptus diversicolor) 2. Nitrogen budgets in pre- and post-burn fuel. Int. J. Wildl. Fire 7, 41–49. https://doi.org/10.1071/WF9970041

Odum, E.P., 1969. The Strategy of Ecosystem Development. Science (80-.). 164, 262–270.

Özkan, U., Gökbulak, F., 2017. Effect of vegetation change from forest to herbaceous vegetation cover on soil moisture and temperature regimes and soil water chemistry. Catena 149, 158–166. https://doi.org/10.1016/j.catena.2016.09.017

Palese, A.M., Giovannini, G., Lucchesi, S., Dumontet, S., Perucci, P., 2004. Effect of fire on soil C, N and microbial Biomass. Agronomie 24, 47–53. https://doi.org/10.1051/agro

Pearsall, D.R., 1985. Landscape ecosystems of the University of Michigan Biological Station: ecosystem diversity and ground-cover diversity. University of Michigan, Ann Arbor.

Pellitier, P.T., Zak, D.R., 2018. Ectomycorrhizal fungi and the enzymatic liberation of nitrogen from soil organic matter: why evolutionary history matters. New Phytol. 217, 68–73. https://doi.org/10.1111/nph.14598

Pietikainen, J., Fritze, H., 1995. Clear-cutting and prescribed burning in coniferous forest: comparision of effects on soil fungal and total microbial biomass, respiration activity and nitrification. Soil Biol. Biochem. 27, 1995.

Ramanathan, V., Carmichael, G., 2008. Global and regional climate changes due to black carbon. Nat. Geosci. https://doi.org/10.1038/ngeo156

Reazin, C., Morris, S., Smith, J.E., Cowan, A.D., Jumpponen, A., 2016. Fires of differing intensities rapidly select distinct soil fungal communities in a Northwest US ponderosa pine forest ecosystem. For. Ecol. Manage. 377, 118–127. https://doi.org/10.1016/j.foreco.2016.07.002

Rincón, A., Pueyo, J.J., 2010. Effect of fire severity and site slope on diversity and structure of

the ectomycorrhizal fungal community associated with post-fire regenerated Pinus pinaster Ait. seedlings. For. Ecol. Manage. 260, 361–369. https://doi.org/10.1016/j.foreco.2010.04.028
Sanderman, J., Hengl, T., Fiske, G.J., 2018. Soil carbon debt of 12,000 years of human land use. Proc. Natl. Acad. Sci. U. S. A. 115, E1700. https://doi.org/10.1073/pnas.1800925115
Scharlemann, J.P.W., Tanner, E.V.J., Hiederer, R., Kapos, V., 2014. Global soil carbon: Understanding and managing the largest terrestrial carbon pool. Carbon Manag. 5, 81–91. https://doi.org/10.4155/cmt.13.77
Schmidt, M.W.I., Noack, A.G., 2000. Black carbon in soils and sediments: Analysis, distribution, implications, and current challenges. Global Biogeochem. Cycles. https://doi.org/10.1029/1999GB001208
Sikes, B.A., Maherali, H., Klironomos, J.N., 2012. Arbuscular mycorrhizal fungal communities change among three stages of primary sand dune succession but do not alter plant growth. Oikos 121, 1791–1800. https://doi.org/10.1111/j.1600-0706.2012.20160.x
Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol. Biochem. 42, 391–404. https://doi.org/10.1016/j.soilbio.2009.10.014
Sinsabaugh, R.L., Linkins, A.E., 1990. Enzymic and chemical analysis of particulate organic matter from a boreal river. Freshw. Biol. 23, 301–309. https://doi.org/10.1111/j.1365-2427.1990.tb00273.x
Smith, S., Read, D., 2008. Mycorrhizal Symbiosis, Third. ed. Elsevier Ltd, New York.
Soil Survey Staff, 1991. Soil Survey of Cheboygan, Michigan.
Spatafora, J.W., Blackwell, M., 1993. Molecular systematics of unitunicate perithecial ascomycetes: The clavicipitales-hypocreales connection. Mycologia 85, 919–922. https://doi.org/10.1080/00275514.1993.12026353
Štursová, M., Žifčáková, L., Leigh, M.B., Burgess, R., Baldrian, P., 2012. Cellulose utilization in forest litter and soil: Identification of bacterial and fungal decomposers. FEMS Microbiol. Ecol. 80, 735–746. https://doi.org/10.1111/j.1574-6941.2012.01343.x
Talbot, J.M., Bruns, T.D., Smith, D.P., Branco, S., Glassman, S.I., Erlandson, S., Vilgalys, R., Peay, K.G., 2013. Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. Soil Biol. Biochem. 57, 282–291. https://doi.org/10.1016/j.soilbio.2012.10.004
Taylor, D.L., Hollingsworth, T.N., McFarland, J.W., Lennon, N.J., Nusbaum, C., Ruess, R.W., 2014. A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning. Ecol. Monogr. 84, 3–20. https://doi.org/10.1890/12-1693.1
Turner, B.L., Condron, L.M., 2013. Pedogenesis, nutrient dynamics, and ecosystem development: The legacy of T.W. Walker and J.K. Syers. Plant Soil 367, 1–10. https://doi.org/10.1007/s11104-013-1750-9
Urich, T., Lanzen, A., Qi, J., Huson, D.H., Schleper, C., Schuster, S.C., 2008. Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. PLoS One 3, e2527. https://doi.org/10.1371/journal.pone.0002527
Vázquez, D.P., Aizen, M.A., 2004. Asymmetric Specialization : A Pervasive Feature of Plant-Pollinator Interactions. Ecology 85, 1251–1257.
Verma, S., Jayakumar, S., 2012. Impact of forest fire on physical, chemical and biological properties of soil: A. Proc. Int. Acad. … 2, 168–176.
Vermeulen, S., Bossio, D., Lehmann, J., Luu, P., Paustian, K., Webb, C., Augé, F., Bacudo, I., Baedeker, T., Havemann, T., Jones, C., King, R., Reddy, M., Sunga, I., Von Unger, M.,

Warnken, M., 2019. A global agenda for collective action on soil carbon. Nat. Sustain. 2, 2–4. https://doi.org/10.1038/s41893-018-0212-z
Wan, S., Hui, D., Luo, Y., Applications, E., Oct, N., 2001. Fire Effects on Nitrogen Pools and Dynamics in Terrestrial Ecosystems : A Meta-Analysis. Ecol. Appl. 11, 1349–1365.
Wang, W., Li, Y., Wang, H., Zu, Y., 2014. Differences in the activities of eight enzymes from ten soil fungi and their possible influences on the surface structure, functional groups, and element composition of soil colloids. PLoS One 9. https://doi.org/10.1371/journal.pone.0111740
Wicklow, D.T., 1975. Fire as an Environmental Cue Initiating Ascomycete Development in a Tallgrass Prairie. Mycologia 67, 852–862. https://doi.org/10.1080/00275514.1975.12019813
Wicklow, D.T., Hirschfield, B.J., 1979. Competitive Hierarchy in Post-Fire Ascomycetes. Mycologia 71, 47–54. https://doi.org/10.1080/00275514.1979.12020986
Widden, P., Parkinson, D., 1974. The effects of a forest fire on soil microfungi. Soil Biol. Biochem. 7, 125–138.
Xu, M., Shang, H., 2016. Contribution of soil respiration to the global carbon equation. J. Plant Physiol. 203, 16–28. https://doi.org/10.1016/j.jplph.2016.08.007
Zak, D.R., Pellitier, P.T., Argiroff, W.A., Castillo, B., James, T.Y., Nave, L.E., Averill, C., Beidler, K. V., Bhatnagar, J., Blesh, J., Classen, A.T., Craig, M., Fernandez, C.W., Gundersen, P., Johansen, R., Koide, R.T., Lilleskov, E.A., Lindahl, B.D., Nadelhoffer, K.J., Phillips, R.P., Tunlid, A., 2019. Exploring the role of ectomycorrhizal fungi in soil carbon dynamics. New Phytol. 223, 33–39. https://doi.org/10.1111/nph.15679
Zhao, X., Wang, Q., Kakubari, Y., 2009. Stand-scale spatial patterns of soil microbial biomass in natural cold-temperate beech forests along an elevation gradient. Soil Biol. Biochem. 41, 1466–1474. https://doi.org/10.1016/j.soilbio.2009.03.028

2.7 TABLES & FIGURES

Table 2.1. Disturbance severity classification guide defining varying burn descriptions. All disturbances were found within the 2017 burn plot and include a full cut and bole removal of all stems >8 cm diameter at breast height. Harvesting of boles occurred in May 2017 and the prescribed burn on October 10th 2017

Disturbance severity Classification

Disturbance	Burn description	Photo
Cut + no burn	No physical signs of burning within 0.5 m radius	
Cut + superficial burn	Burning along the edge of the 0.5 m radius	
Cut + low burn	Light burning. Patchy blackening of leaf litter and small twigs	
Cut + medium burn	Approximately 50% of the area within a 0.5 m radius of sampling location charred. Leaf litter and small twigs combusted. Charring of larger wood	
Cut + high burn	Full combustion of leaf litter and coarse woody debris around sampling point	

Table 2.2. Operational Taxonomic Units (OTUs) that individually accounted for >1% relative abundance of total sequences. Species accounting for >3% of total relative abundance are indicated with an asterisk (*).

Family	Genus	Species	Relative Abundance
Umbelopsidaceae	Umbelopsis	Umbelopsis dimorpha	4.8*
Russulaceae	Lactifluus	Lactifluus allardii	4.3*
Russulaceae	unidentified	unidentified	4.0*
Gloniaceae	Cenococcum	Cenococcum geophilum	3.1*
Cantharellaceae	Craterellus	Craterellus tubaeformis	2.4
Russulaceae	Russula	Russula cyanoxantha	2.2
Sordariaceae	unidentified	unidentified	2.1
Hydnangiaceae	Laccaria	unidentified	2.1
Cortinariaceae	Cortinarius	unidentified	2.0
Pyronemataceae	Anthracobia	Anthracobia melaloma	1.9
Omphalotaceae	Rhodocollybia	Rhodocollybia butyracea	1.9
Russulaceae	Russula	Russula subsulphurea	1.8
Hydnodontaceae	Trechispora	Trechispora invisitata	1.7
Hymenogastraceae	Hebeloma	Hebeloma radicosum	1.5
Amanitaceae	Amanita	Amanita brunnescens	1.5
Mortierellaceae	Mortierella	Mortierella humilis	1.3
Herpotrichiellaceae	unidentified	unidentified	1.1
unidentified	unidentified	unidentified	1.1
Russulaceae	Russula	Russula cremoricolor	1.0

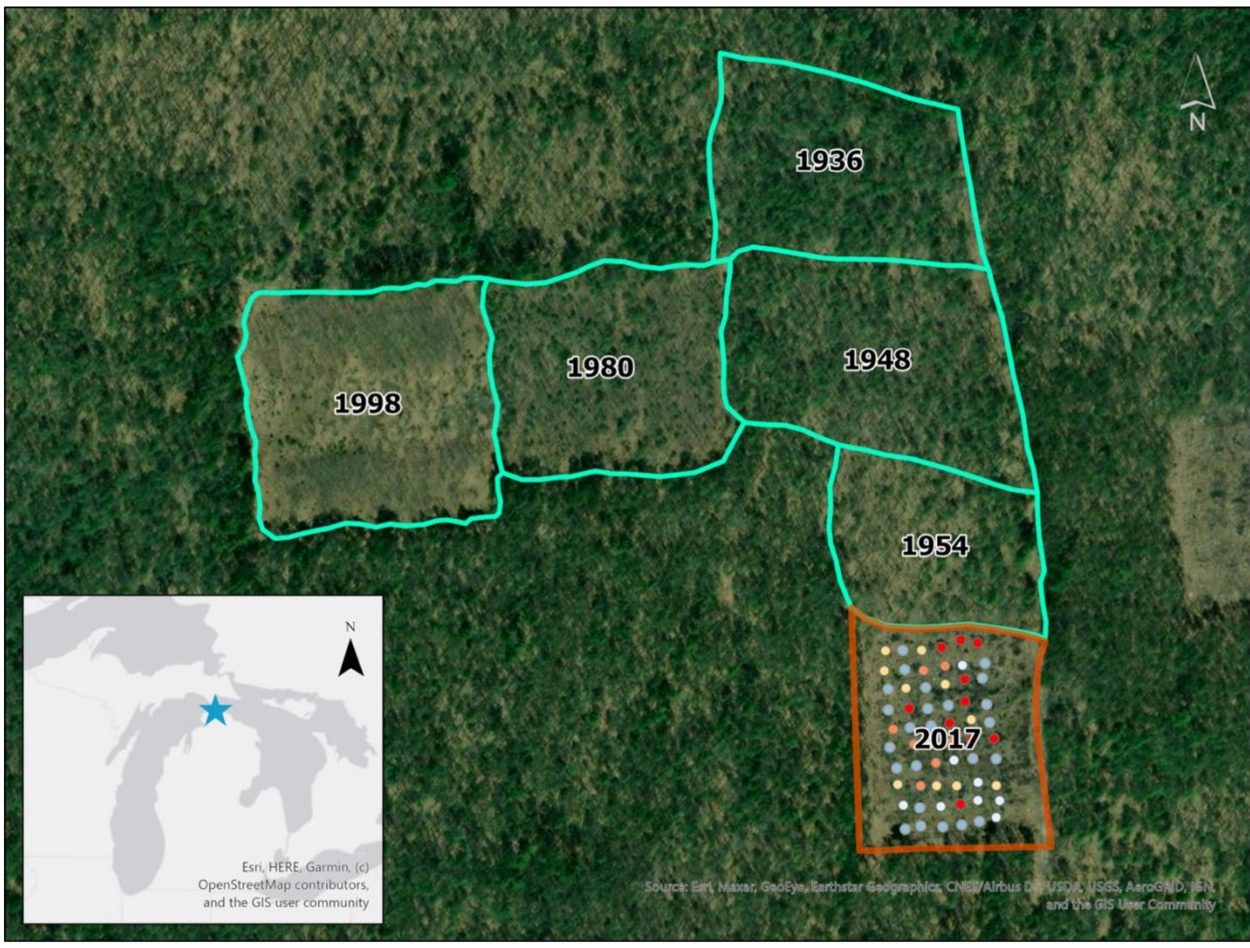

Figure 2.1. Location of 2017 cut + burn plot location in relation to the existing burn plots and Michigan. The 2017 experimental plot is outlined with a red border and existing plots with green borders

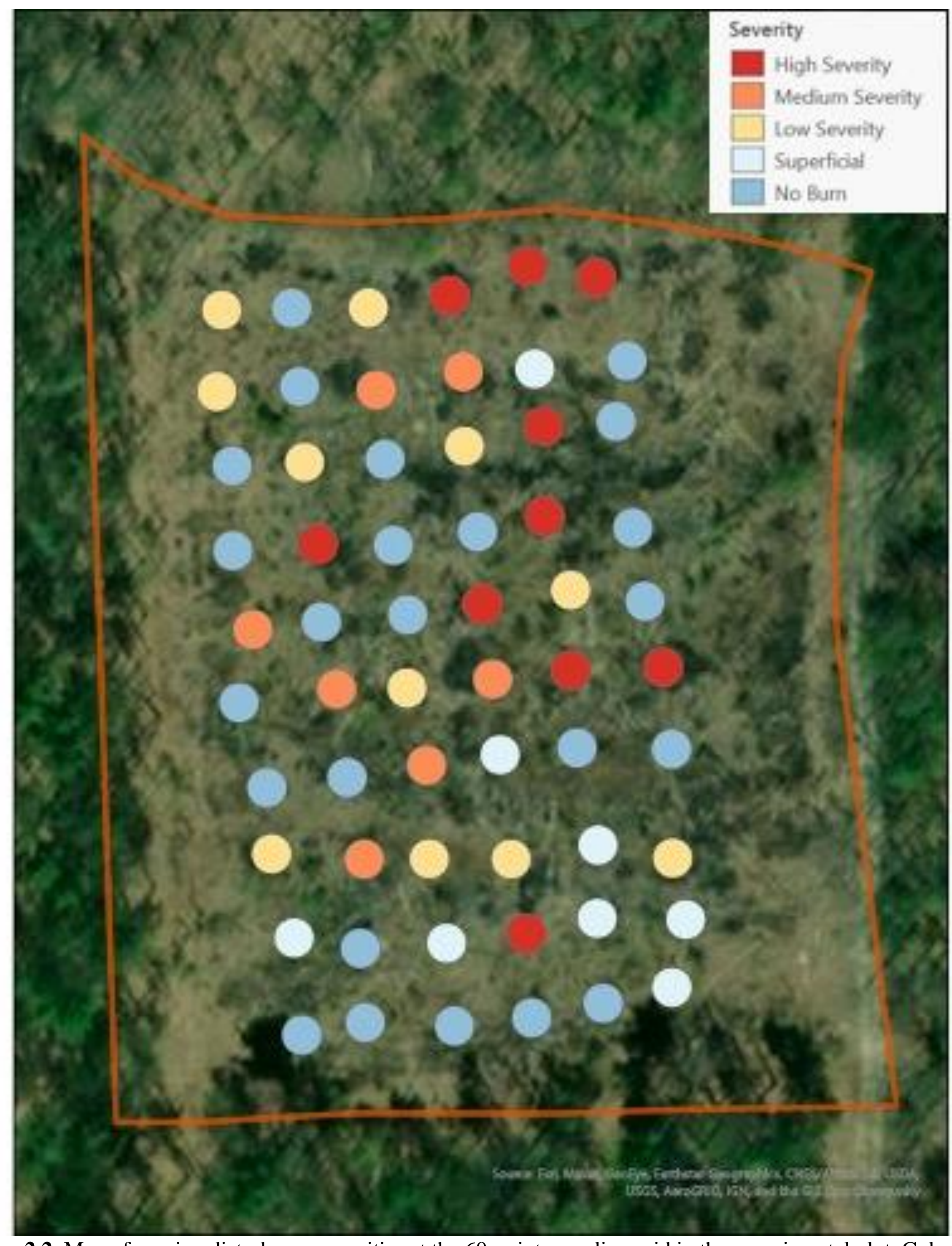

Figure 2.2. Map of varying disturbance severities at the 60-point sampling grid in the experimental plot. Colors represent varying severities of disturbance: High burn severity (red), medium burn severity (orange), low burn severity (yellow), superficial burn severity (pale blue), and no burn (dark blue).

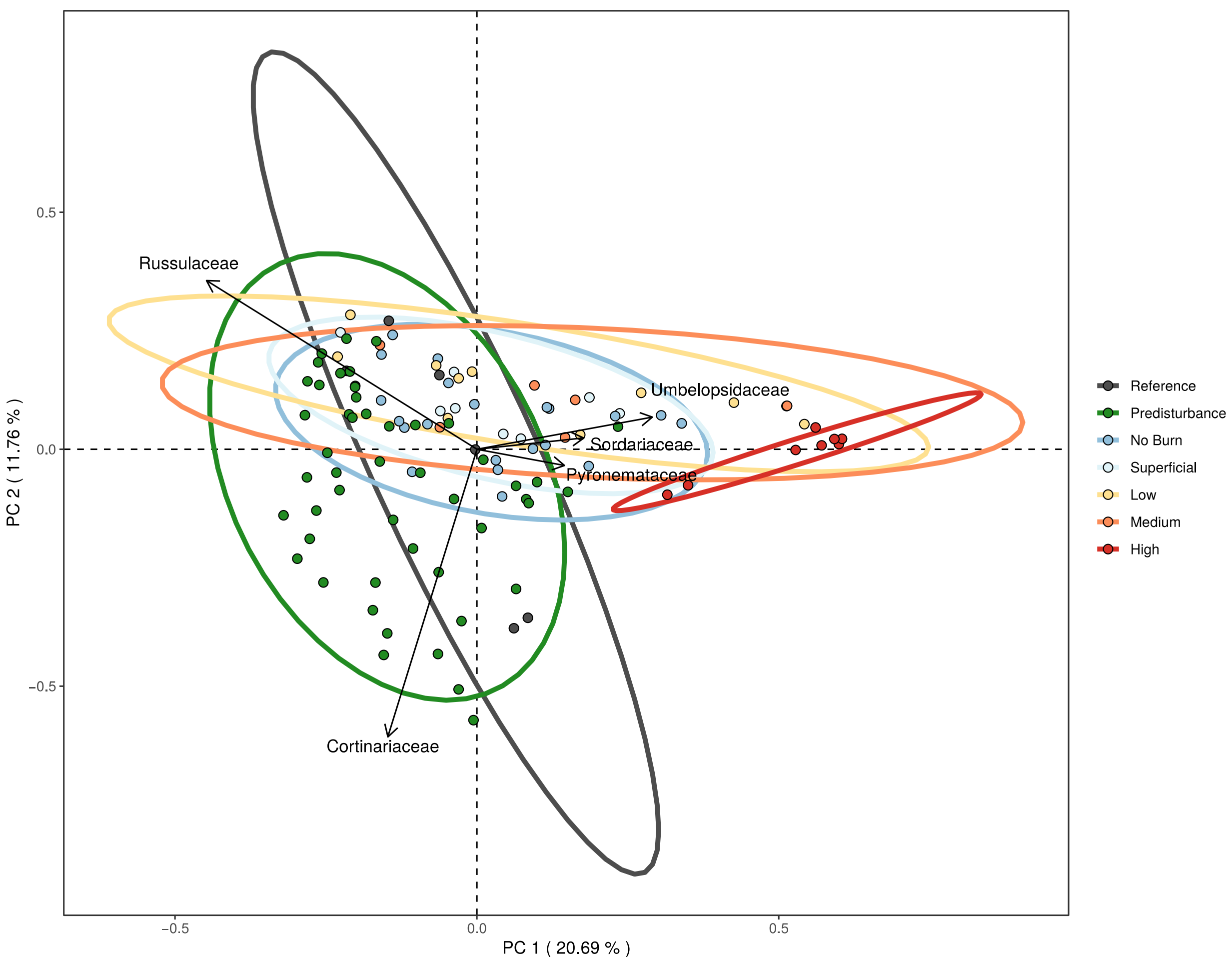

Figure 2.3. Principal coordinates analysis (PCoA) of fungal communities in soils from reference (grey), pre-disturbance (green), cut + no burn (dark blue), superficial burn (light blue), low burn (yellow), medium burn (orange) and high burn (red) Bray-Curtis abundances using ThetaYC calculator of dissimilarity. A Mantel test indicated significant community differences based on disturbance severities ($p<0.05$). Ellipses signify a 95% confidence interval of distributions for the varying disturbances. The PCoA explains 32.45% of the total variation in fungal community dissimilarity at the family level. The top 5 families responsible for community dissimilarity are represented by vectors.

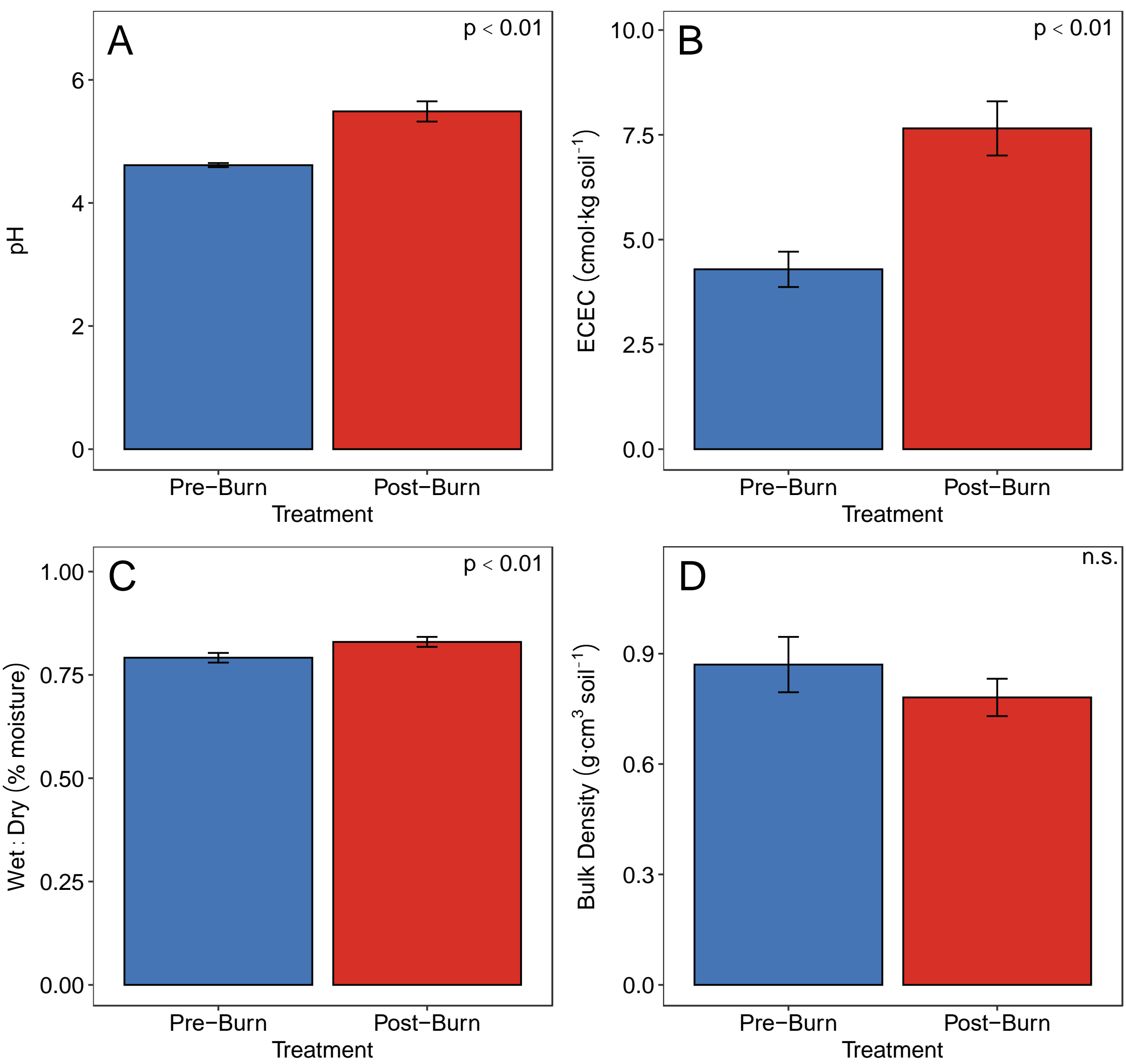

Figure 2.4. Pre- vs Post-disturbance means of pH, ECEC, percent moisture, and bulk density. (A) pH, (B) ECEC, (C) wet:dry ratios and (D) bulk density responses due to cut and burn treatment. Mean pH, ECEC and wet:dry measurements were all significantly higher post disturbance ($p < 0.01$). Bulk density of mineral soils decreased post disturbance ($p = 0.02$). Bars indicate mean values for pre-burn (blue) and post-burn (red). Whiskers represent std. error.

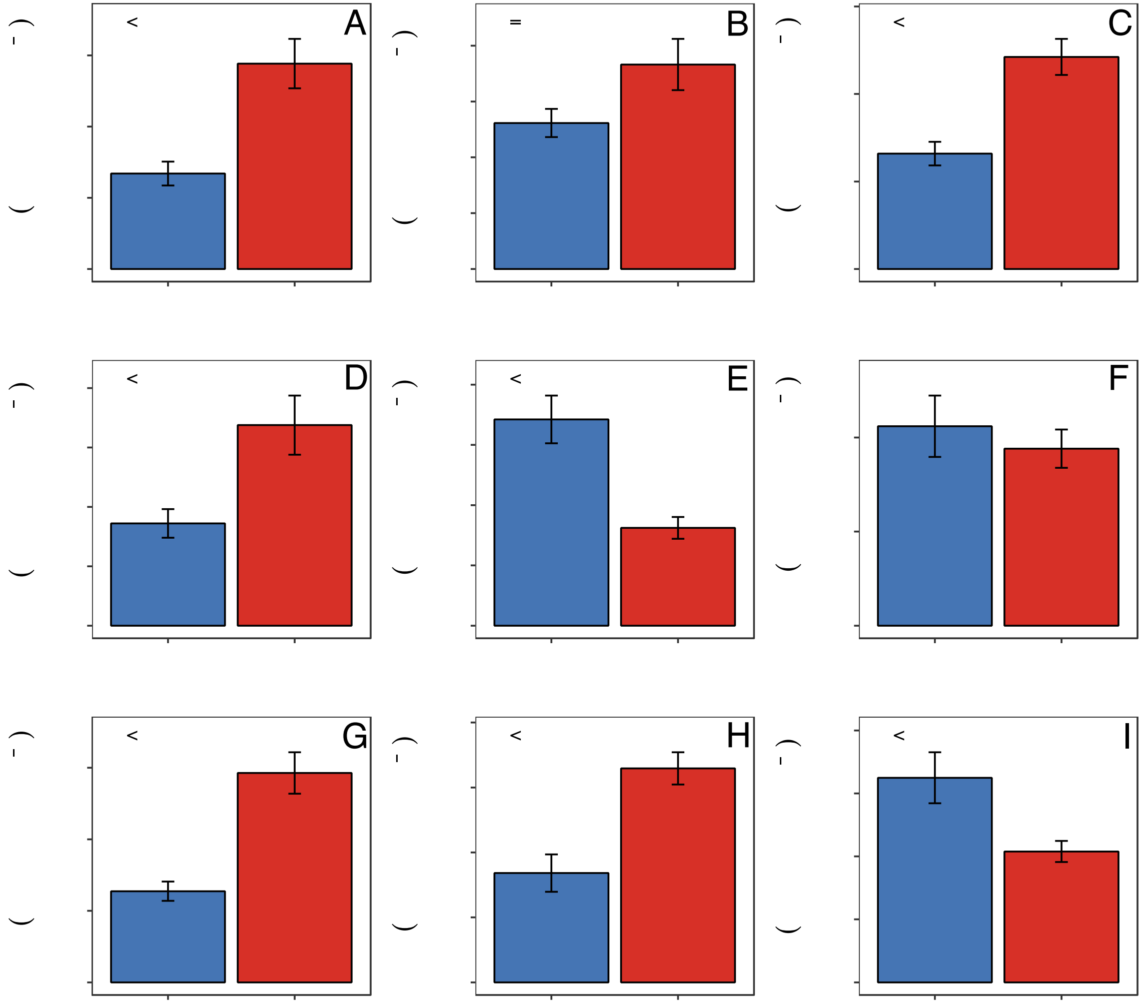

Figure 2.5. Summary of mean cation and anion responses to experimental disturbance on a subsample of pre (blue) and post (red) soils (n=30). Ca, K, Mg, P, Mn and Na (panes A,B,C,D,G and H, respectively) all showed significant ($p < 0.05$) increases in mean abundances post disturbance relative to pre-disturbance conditions. Conversely, Cu and S (Panes E & I, respectively) analyses revealed a decrease in availability post-disturbance ($p < 0.05$). No difference was found in available Fe pre and post-disturbance. Whiskers indicate standard errors.

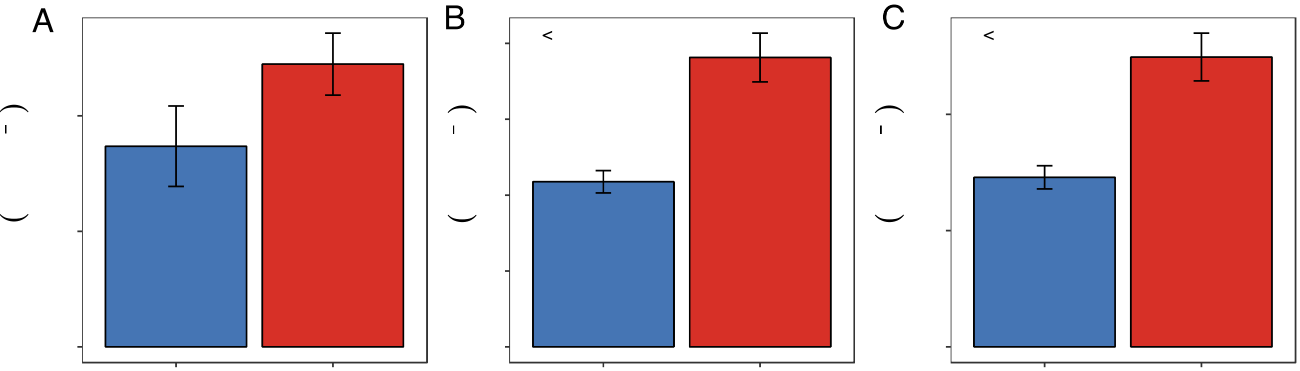

Figure 2.6. Mean available mineralized nutrients A) nitrate, B) ammonium and C) phosphate, respectively. pre- and post-disturbance in mineral soils determined by colorimetric analysis. Ammonium and phosphate were significantly higher post disturbance relative to pre-disturbance conditions ($p < 0.01$). Whiskers represent standard errors.

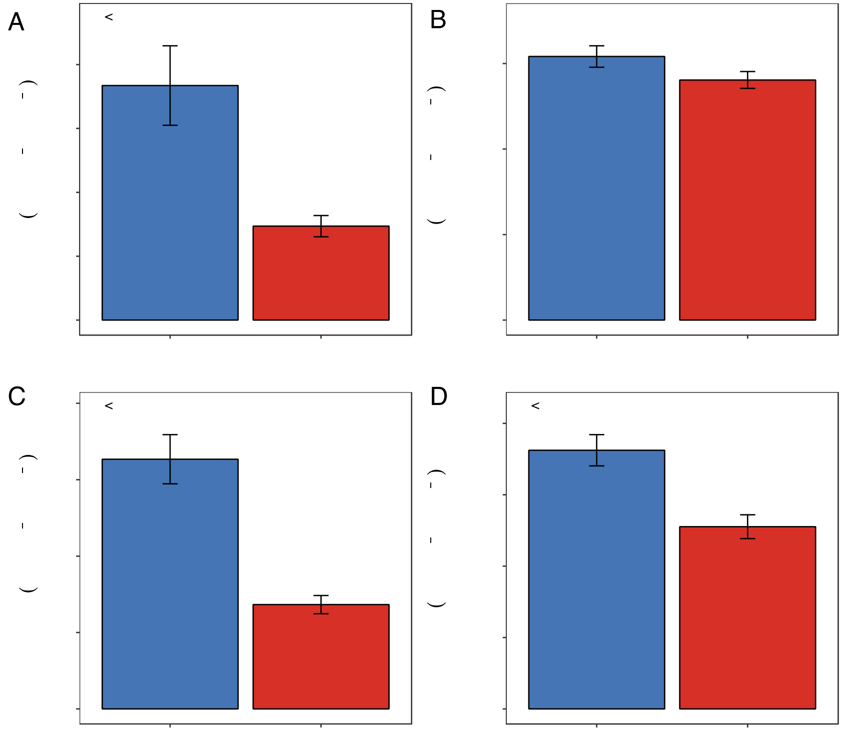

Figure 2.7. Summary of enzymatic activity (ummol/min/g soil) responses of (A) Nag, (B) alpha glucosidase, (C) alkaline phosphatase and (D) phenol oxidase in mineral soil to the experimental cut + burn. Nag, Alk-phos and phenol oxidase (A,C,D) all show significant decreases in enzyme activity a week post burn relative to pre-burn conditions ($p < 0.01$). Alpha glucosidase illustrated a trend toward lower activity post burn but was not a significant decrease ($0.1 < p < 0.05$)

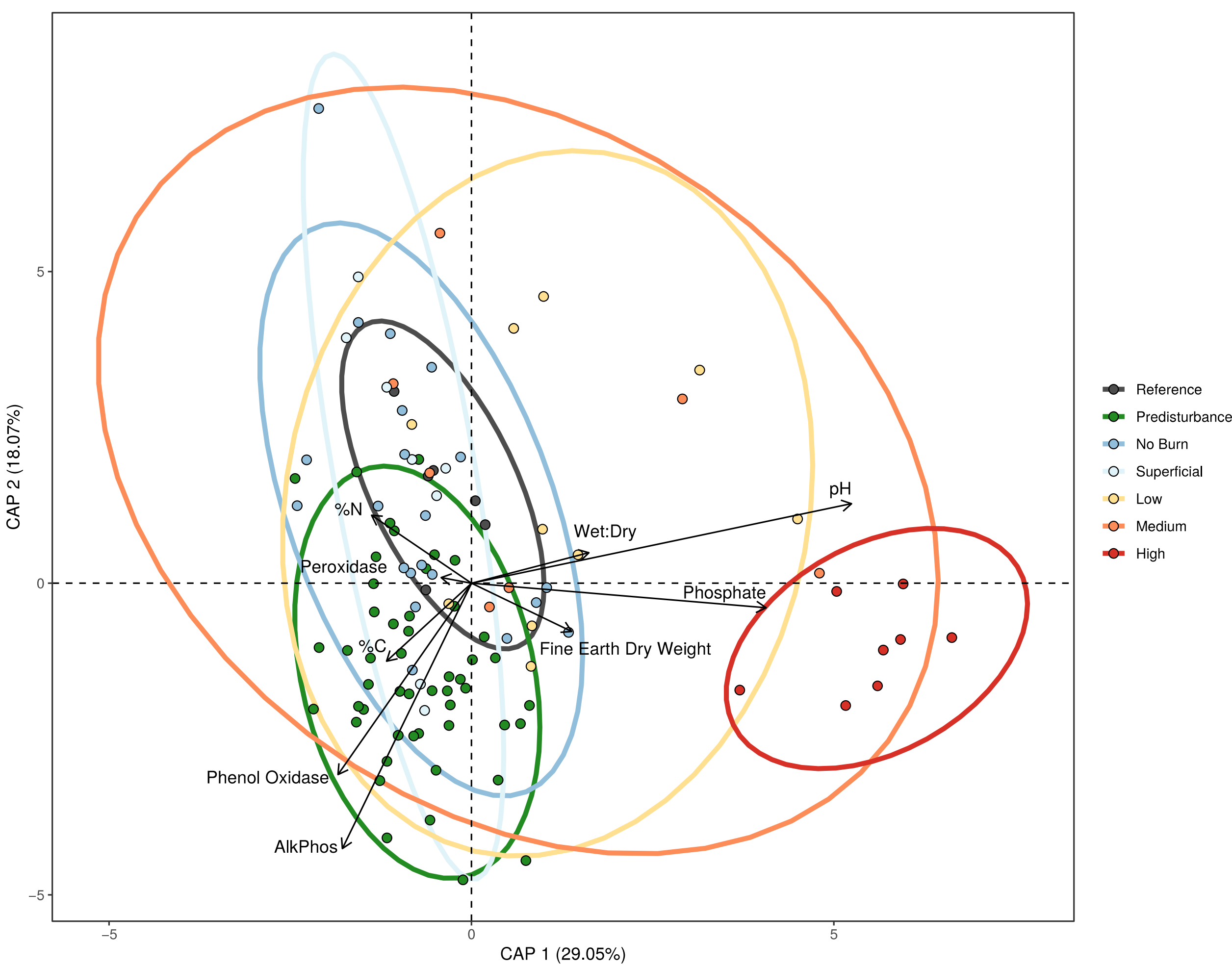

Figure 2.8. A db-RDA plot of the fungal communities of pre- and post-disturbance soil cores. Colors represent the level of disturbance: reference (grey), pre-disturbance (green), cut + no burn (dark blue), cut + superficial burn (light blue), cut + low burn (yellow), cut + medium burn (orange) and cut + high burn (red) fungal communities. Vectors represent environmental conditions that are significant ($p < 0.05$) predicters of fungal community differences in a stepwise regression analysis. A cumulative total of 47.12% of the variation was explained by the x and y axes.

Chapter 3: Decadal Trends of Fungal Succession in Mineral Soils following Clear-Cutting and Burning of a Mixed Hardwood Forest

3.1 Abstract

Time since disturbance is particularly important to consider when examining successional trajectories and the implications for stand diversity and productivity. The intermediate disturbance hypotheses (IDH) predicts a peak in diversity in mid-successional stages of succession and has often been observed in plant communities. Forest productivity often follows similar patterns and has been observed to peak in mid-successional stages. In this study, I explore whether fungal communities follow the same patterns seen in plant community diversity and productivity. I leverage an established cut + burn chronosequence to examine long-term trends in soil fungal community structure and function in a post-disturbance aspen dominated forest. While plant community diversity in the chronosequence adhered to patterns predicted by the IDH, similar trends were not detected in fungal diversity over time. The lowest fungal diversity was detected in the 61-year-old mid-successional plot and was attributed to a dominance of ectomycorrhizal taxa. Additionally, there was an observed an increase in microbial biomass early to late stages, reflecting previously reported increasing trends in stand productivity into late successional stages. These results offer insight into the lasting legacy of disturbance that was shown to have a persistent effect on soil chemistry in elevated pH and suggest a decline in fungal diversity and increase in abundance through 100 years of succession.

3.2 Introduction

Historically, anthropogenic and natural disturbances have altered the landscapes across North America with a particularly intensive legacy of deforestation and wildfire in the northeastern United States. What was once primarily old growth forests was clear-cut around the turn of the 20th century (Frelich, 1995; Friedman and Reich, 2005). In the years following timber harvesting wildfires spread through much of the northeast due to high amounts of burnable fuel in the form

of slash. Prior to European settlement and deforestation, the Great Lakes forests and upland areas experienced fires on 130-260 year intervals (Whitney, 1987). Much work has been done to analyze differences in net primary production (NPP) of pre-disturbed forests from those stands that replace them (Clein et al., 2002; Goetz et al., 2012; Gough et al., 2016, 2007; Goulden et al., 2011).

The variation in disturbance severity and frequency, landscape, pre-disturbance community composition, and time since disturbance have led to a mosaic of forests that have differed in their successional trajectories. Time since disturbance is particularly important to consider when interested in examining successional trajectories and the implications for stand diversity and productivity. They are particularly important when considering structural functional diversity. Classic successional trends predicted by Odum (1969) postulate that annual C storage increases to a maximum in mid-successional stages and then decreases as forests progress to late successional stages. While this trend has been repeatedly reported to be true in a number of systems following disturbances (Bond-Lamberty et al., 2004a; Gough et al., 2007; Howard et al., 2004; Law et al., 2003), recent work has shown that old-growth forests can maintain significant C storage potential through greater resource use efficiency (Gough et al., 2013; Hardiman et al., 2013). Additionally, the intermediate disturbance hypothesis (IDH) predicts that species diversity will follow a unimodal 'humpback' relationship as a forest progresses through successional stages (Connell, 1978). The implications of these historical disturbances and subsequent restructuring of forest biota over time loom large for global change as they vary in their ability to sequester and store carbon (C).

An abundance of studies have since been conducted asking questions about how these forested have regenerated over the past century. These include studies of the re-establishment of primary producer communities (Scheiner and Teeri, 1981), primary production rates (Gough et al., 2013, 2007; Hardiman et al., 2013), nitrogen cycling (Fisk et al., 2002), arthropod communities (Fisk et al., 2010), soil properties (Nave et al., 2019; Roberts and Gilliam, 1995), and microbial properties (Lundgren, 1982). Studies on plant successional trends widely report the development of aspen (*Populus grandidentata*) dominated stands over much of the Northeastern United States (Gough et al., 2008; Roberts and Gilliam, 1995; Scheiner and Teeri, 1981). However, few studies

have examined soil fungal community responses, and those that have are limited to observational studies comparing a modern forest with historical records or undisturbed forest sites as reference. Studies that do examine successional trends in varying aged plots are often limited to opportunistic chronosequences created by natural disturbances or unique glaciation history that can introduce uncontrolled landscape or climatic variation (Bond-Lamberty et al., 2004b, 2004a; Pare and Bergeron, 1995; Richardson et al., 2004; Serbin et al., 2013).

An experimental chronosequence at the University of Michigan Biological Station comprised of a set of contiguous 1-ha stands that were clear-cut and burned has offered researchers a unique opportunity to study forest succession in a controlled manner. This design has allowed researchers to examine the effects of disturbance on successional patterns and processes by minimizing variations due to landscape and climatic dissimilarity (Gough et al., 2007). These studies have elucidated much about the regeneration of aspen dominated forests during varying stages of succession, addressing concepts community assembly, net ecosystem production (NEP, or carbon storage rate), soil physio-chemical properties, and pedology (Gough et al., 2007; Nave et al., 2019; Scheiner and Teeri, 1981). Importantly, they show that clear-cutting and burning had long-term detrimental effects on C storage, in effect lowering the successional trajectory of NEP (Gough et al., 2007). This reduction in NEP was primarily attributed to a lower site quality, eluding to reduced nutrient availability post-disturbance. Importantly, while total soil respiration increased over the first ~50 years of succession, soil heterotrophic respiration showed a 28% decrease from pre-disturbance conditions over the same time period (Gough et al., 2007).

The findings underscoring reduced site quality and heterotrophic respiration highlight the need for investigation into the restructuring and subsequent successional trends of soil fungal communities' post-disturbance. To date no studies have been conducted on long term trajectories of fungal communities in this chronosequence. Research has shown that fungal community composition and function can be significantly altered both clear-cutting (Everett and Sharrow, 1985; Harvey et al., 1980; Lewandowski et al., 2016; Muñoz Delgado et al., 2019) and burning disturbances (Dooley and Treseder, 2012; Holden and Treseder, 2013; Reazin et al., 2016; Wicklow, 1975; Wicklow and Hirschfield, 1979; Zak and Wicklow, 1980). This includes alterations to various functional groups including mycorrhizal and saprotrophic fungi.

Previous work has shown that both ecto- and arbuscular mycorrhizal associations can facilitate the presence, health, and competitive ability of associated primary producers in forest systems (Janos, 2017; Kormanik et al., 1982). Similarly, studies within aspen dominated temperate forests have shown that altered plant communities, whether through natural succession or disturbance, have the propensity to alter mycorrhizal communities (Castillo et al., 2018; Nave et al., 2013). Importantly, mycorrhizal community shifts during succession have been linked to patterns of carbon sequestration in forest soils (Bödeker et al., 2014; Clemmensen et al., 2015; Hobbie, 2006). Saprotrophs are an equally important functional group in forest soils as the primary conduits to biogeochemical cycling of C back to the atmosphere. Saprotrophs excrete extracellular enzymes to degrade coarse woody debris (Boddy and Watkinson, 1994; Hiscox et al., 2015), leaf litter (Romaní et al., 2006; Štursová et al., 2012), and SOM in forest soils (Cairney and Meharg, 2002; Sinsabaugh, 2010), in doing so they return C to the atmosphere via respiration (Fisk et al., 2010).

A meta-analysis on fire effects to fungal communities and chronosequence studies in Alaskan boreal forest ecosystems both suggest that more than 10 years may be required for post-fire communities to recover in high intensity burn sites (Dooley and Treseder, 2012; Holden and Treseder, 2013). Moreover, results from a study of low intensity fires in coniferous forests in the southern U.S. reflect similar results, suggesting that soil fungal community returns to pre-disturbance states can require greater than a decade (Oliver et al., 2015). Few studies examine fungal succession on longer time scales, and none utilize a chronosequence consisting of contiguous plots.

In this chapter I examine the legacy of logging and slash burning during the early 20th century on soil fungal succession. Specifically, I ask if fungal communities follow predicted patterns of the intermediate disturbance hypothesis often seen in primary producers. To address this question, I leverage the established forest cut+burn chronosequence to examine the long-term trends in soil fungal community structure and function in a post-disturbance aspen dominated forest. I hypothesize that fungal community diversity will follow predicted trends of the intermediate disturbance hypothesis. I predict that 1) peak fungal diversity will occur in mid-successional

forest stands with the highest productivity, 2) low diversity in early successional stand following disturbance as communities are still recovering to pre-condition levels, and 3) low diversity in late succession stands relative to mid-succession due to competitive exclusions of dominant taxa.

3.3 Methods

Study Site

The study was conducted at the University of Michigan Biological Station (UMBS) "Burn Plots", in northern Michigan, USA (45°35'N 84°43'W). Mean annual temperature is 5.5 ^{o}C with a mean annual precipitation of 817 mm. The study site was previously described in chapter 2and soil series profiles, vegetation and landforms of the burn plots and surrounding UMBS forests have been described in detail in Nave et. al (2017;2019). The typical morphology of the soil series at our site consists of Oi and Oe horizons 1-3 cm thick, a bioturbated A horizon 1-3 cm, an E horizon 10-15 cm thick, and Bs and Bc horizons of sand with occasional gravel and cobble (Nave et al, 2014f). Across the soils, approximately half of the fine root biomass is in the upper 20 cm of soil (approximately 5-15 Mg C ha^{-1}), with the remaining biomass split between the forest floor (O horizon) and depths of 20-40 cm (Nave et al., 2011).

Prior to disturbance at the end of the 19th and beginning of the 20th century forests in the area were dominated by *Pinus Strobus* (eastern white pine), *Pinus resinosa* (red pine) and *Tsuga canadensis* (eastern hemlock) (Gates, 1926). Harvesting and subsequent fires led to monotypic aspen-dominated stands early in succession. Over time these even aged canopy of primarily *Populus grandidentata* gave way to a mixed canopy with *Quercus rubra* (northern red oak), *Acer rubrum* (red maple), *Betula papyrifera* (white birch), *Pinus strobus* (eastern white pine) and to a lesser extent *Pinus resinosa* (red pine) and *Fagus grandifolia* (American beech) establishing and competing for codominance in the overstory. The understory vegetation shifts from a primarily *Pteridium aquilinum* and *vaccinium angustifolium* dominated composition to one a more diverse community with the addition of mixed hardwood saplings.

The experiment was constructed in the UMBS "Burn Plots" which were installed over the last century to mimic the disturbance created from clear-cutting and subsequent fires around the turn of the 20th century. The burn plots consist of 6 adjacent, 1-ha stands that have been clear-cut and

burned in 1936, 1948, 1954, 1980, 1998, and 2017 (the 2017 stand was implemented after sampling for this study and was therefore not included in this study). The experimental plots reside within a surrounding forest that serves as a reference and was burned in 1911 after clear-cutting from the logging industry. This study focuses on work in the 5 oldest plots as well as the surrounding reference forest. The experimental forests have similar soil profiles and share common plant species but differ from the surrounding reference stand in that they have been twice cut and burned, having experienced the same disturbance as the reference forest in 1911 (Gough et al. 2007).

Field Sampling

During July of 2015, a field sampling campaign was conducted across the burn sequence spanning the 1911 reference forest and 5 ~1ha experimental burn plots. A total of 15 soil samples were collected in each stand with 5 cores collected from 3 locations in each burn plot (Figure 3.1). In the field soil cores of the O and A horizons were taken using a 5.2cm diameter soil core from each of the 15 sampling locations within the varying age stands for a total of 90 soil cores. At the time of sampling 5 soil depth measurements were taken per core to determine average depth of the O+A horizons. Once samples were collected in the field they were immediately put on ice until further processing.

Prior to sieving of mineral soil the Oe and Oi horizons were separated from the A horizons. Once separated the litter comprising Oi horizons were separated by species, acorns and sticks and both the Oe and Oi horizons were dried at 60 C to determine total dry weights. All additional assays were conducted solely on sieved A horizon soils. The A horizons were sieved with a 2mm mesh screen to remove rocks, coarse and fine root, and fine earth fractions to isolate mineral soil. During sieving all roots greater than 1cm in length were extracted by hand from soil cores and coarse and fine roots were pooled. Roots were oven dried at 60 C for 24 hours to determine total root biomass. Immediately post sieving and root removal mineral soils were subsampled for RNA/DNA, enzymes, C:N and gravimetric water content. A 5-gram subsample for molecular analyses was flash frozen in liquid nitrogen and placed on dry ice until being stored at -80 C. Subsamples for C:N and gravimetric water content were lyophilized until constant mass with no residual ice apparent (ca. 48h; Labconco Freezone One; Labconco, Kansas City, MO).

Gravimetric water content was determined by the difference of wet soil weights and post lyophilization dry weights.

Extracellular Enzyme Activity (EEA)

As in chapter 2, a 1 gram dry soil equivalent of homogenized field moist soil was used to create an aqueous extract in 125ml 50mM sodium acetate buffer solution at pH 5. Samples were homogenized first in 60ml solution for 30 seconds with a Fisher Scientific Tissuemizer in a 250 ml Nalgene bottle. Mixed solutions were then placed on a shaker table for 10 minutes. After the first shake an additional 65ml of buffer solution was added and samples were mixed again for an additional 30 seconds. Bottles were then shaken for another 10 minutes. The aqueous solutions were then aliquoted into 3 40ml aliquots in sterile 50 ml polypropylene centrifuge tubes and frozen at -20 C until analysis. Six enzymes associated with EEA were analyzed using p-nitrophenyl-linked substrate microplate assays similar to (Sinsabaugh and Linkins, 1990) (Table B 1). Hydrolytic enzymes, phenol oxidase and peroxidase activities were measured colorimetrically (460 nm) with L-3,4-dihydroxyphenylalanine (L-DOPA, 6.5mM) using sodium bicarbonate buffer. Potential peroxidase activity was determined by the difference between soil with L-DOPA + 0.3% H202 and L-DOPA.

Cellulase activity was analyzed by measuring beta-glucosidase (BG) (EC 3.2.1.21). Hydrolysis of starches were analyzed measuring alpha-glucosidase (AG) (EC 3.2.1.2) activity. Chitinase activity was determined by measuring N-acetylglutamate (NAG) (EC 2.3.1.1) reduction of a glucosaminide substrate. Hydrolysis of polyphosphates was determined by measuring alkaline phosphatase (ALK) (EC 3.1.3.1) activity. Artificial substrates and incubation times used were: 4-nitrophenyl a-D-glucopyranoside (4 hours), 4-nitropheynyl B-D-gluco (4 hours), 4-nitrophenyl B-D-cellobioside (4 hours), 4-nitrophenyl N-acetyl-B-D-glucosaminide (4 hours), 4-nitrophenyl phosphate (4 hours) and activity was determined by measuring the amount of liberated p-nitrophenolate upon addition of 3M KOH stopping solution. All substrates and reagents were obtained from Sigma-Aldrich Co. Ltd. (Saint Louis, MO, USA), and measurements were made using a Biotek ELX800 plate reader (Biotek, Winooski, VT, USA). Three technical replicates were prepared for each assay, blank and standard curve and means were used to determine per

sample values. Enzyme activities were calculated in international "units" (U; 1 U= 1000 mU) defined as the amount of enzyme that forms 1 μmol of product per minute.

Chemical Analyses

Chemical analyses were conducted to determine changes in substrate quality and nutrient content in various aged stands. Percent C, N and $\delta_{15}N$ were analyzed on homogenized and ball-milled subsamples of mineral soil at the UMBS (Pellston, MI, USA) using a SPEX Certiprep 8000D Mixer/Mill (Metuchen, New Jersey, USA) to pulverize soils and a Costech ECS 4010 elemental analyzer coupled with a Thermo Scientific Delta Plus XP isotope ratio mass spectrometer for analysis (San Jose, CA, USA). Soil pH was collected using a 0.2 g of composite, ball-ground material using a model 8000 pH meter (VWR Scientific, Radnor, PA, USA). Substrates were added to 15 ml of deionized water, incubated for 30 minutes at room temperature and equilibrated with a probe for 1 minute prior to taking each reading.

Microbial Biomass Measurements

Mineral soil microbial biomass was determined via chloroform ($CHCL_3$) fumigation and potassium sulfate (K_2SO_4) extractions similar to Joergensen and Brookes (1990). Briefly, two moist 4-gram (dry equivalent) subsamples from each of the sieved mineral soil samples were weighed into 15ml polypropylene centrifuge vials. One of the subsamples from each core was placed in a desiccator with a sterilized 125 ml Erlenmeyer flask containing ~20 boiling chips and 75 ml of ethanol free $CHCl_3$ for 48 hours to lyse soil microbial biomass. Following fumigation both fumigated and non-fumigated soils were extracted with 0.1 M K_2SO_4 (5:1 solution: soil ratio) by shaking at a rate of 180 strokes per minute for 1-hour. Extractions were then poured through a No. 42 filter paper. All results were expressed on an oven-dry soil basis (105°C, 24h).

Microbial C from the extracts was measured via persulphate oxidation procedure (Wu et al., 1990). Total organic C (TOC) was determined with a Seal AA3 auto-analyzer by mixing 15 ml soil extract with 15 ml of sodium hexametaphosphate and using 2% potassium persulphate as an oxidant. TOC was calculated by subtracting TOC extracted from non-fumigated soils from TOC extracted from fumigated soils. Total N was measured via Kjeldahl digestion that was modified to include NO_3 (Brookes et al., 1985; Pruden et al., 1985) and was the summation of Ninhydrin-

reactive N + NH_{4+} NO_{3-}. Breifly, 15 ml of the K2SO4 extract was digested for 2 hours with a reducing agent comprised of 75 g of KCr(SO4) in 200 ml of H2SO4 with 450 mg of zinc powder (Joergensen and Brookes, 1990). NH_{4+}-N and NO_{3-}-N were then extracted in 2 M KCl solution and determined using a Seal AA3 automated colorimeter following standard methods 4500-NH_{4+} and 4500-NO_{3-}, respectively. Ninhydrin-reactive N was the sum of α-amino N and ammonium-N. Therefore, microbial α-amino N was determined by the difference of (ninhydrin-N minus NH_{4+} in fumigated soils) and (ninhydrin-N minus NH_{4+} in non-fumigated soils). Total microbial N was determined from α-amino N plus the difference (NH_{4+} +NO_{3-}) from fumigated soil and (NH_{4+} +NO_{3-}) extracted from non-fumigated soils.

DNA extraction, PCR amplification and sequencing of ITS2

DNA analysis of fungal communities was conducted on homogenized soil samples from soil cores. Genomic DNA was extracted from 2 g frozen soil using a MoBio RNA Powersoil Total RNA Isolation Kit (MoBio) combined with an RNA Powersoil DNA Elution Kit (MoBio) following manufactures instructions. Extraction quantity was determined using a Qubit Fluorometer (Invitrogen, San Jose, CA, USA) and frozen (-80 °C) until PCR amplification was performed. The fungal ITS2 region was amplified from genomic fungal DNA using forward primer 5.8S-FUN (5'-AGWGATCCRTTGYYRAAAGTTCCCTGACTGACT) and reverse primer ITS4-FUN (GCAWAWCAAWAAGCGGAGGACCCTGACTGACT). Each soil sample had paired forward and reverse barcodes appended to the primer ends for miltiplexign. PCR reactions were carried out using Phusion High-Fidelity DNA Polymerase (New England BioLabs, Ipswich, MA) under the following conditions: 5.9ul PCR grade H2O, 4ul 5X Phusion HF Buffer, 0.4ul 10mM dNTP mix, 0.75ul forward primer (10mM), 0.75ul reverse primer (10mM), 8ul DNA template, 0.2ul Phusion Taq. PCR cycle parameters were as follows: 3 min of initial denaturation at 94C, 28 cycles of 30 sec denaturation at 94C, 45 sec annealing at 55°C, and 90 sec at 72C with a final extension at 72C for 10 min. PCR products were submitted to the Microbial Systems Molecular Biology Laboratory at the University of Michigan and sequenced similarly to chapter 2 sequences.

Sequence Analysis

Sequences were demultiplexed by the Microbial Systems Molecular Biology Laboratory at University of Michigan after sequences were generated. Fungal sequences were processed using QIIME2 package version qiime2-2019.4. Prior to downstream analysis both forward and reverse reads were analyzed for QC scores in Qiime2-view. All reverse reads were excluded due to poor and only forward reads were used in downstream analyses. Sequences were trimmed for poor quality end reads using denoise-single trimming the first 12 bp and truncating at 245 bp. Sequences were clustered into OTUs at 97% similarity and filtered for chimeras using vsearch cluster-features-de-novo pipeline. OTUs were then classified using a classifier created from the UNITE database using classify-sklearn command. A phylogenetic tree was generated with QIIME phylogeny fasttree. The dataset was then filtered for sequences that appeared less than 5 times and fewer than 2 samples with filter-features and rarefied to 6845, representing the minimum sequences total for an individual sample.

Statistics

Alpha and beta diversity indices were generated in QIIME with alpha-rarefaction and core-metrics-phylogenetic, respectively. Differential abundance tests were run with composition ANCOM in QIIME to identify taxonomic groups driving community dissimilarities. A biplot was generated with a Bray-Curtis distance matrix with QIIME pcoa-biplot function. Biom files were exported and used with R software to generate regressions and ANOVAS for environmental variable analysis. Finally, a dbRDA using a bray-curtis dissimilarity matrix was run with a step-wise regression function in R to determine variables correlated with community dissimilarities. A mantel test was generated to test for significance in community dissimilarity prior to the redundancy analysis. I accept as significant statistical tests with $\alpha \leq$ and ascribe marginal significance for $0.05 < \alpha < 0.10$.

3.4 RESULTS

Organic horizon

The separated and dried Oi and Oe horizon weights averaged 6.01 g (±2.5 s.d.) and 9.47 g (± 3.7 s.d.), respectively. An aNOVA revealed no differences between the stands for either horizons. The relative abundances of sorted Oi litter did reveal a significant positive correlation between

stand age and both evenness ($p<0.01$, $r_2=0.09$) and richness ($p<0.01$, $r_2=0.0.31$) (Figure 3.2). I did note however that when acorns and sticks were removed from the analyses the relative abundances of leaf litter only showed a significant trend when both richness and evenness were considered (Figure B 1). Differences in diversity of leaf litter are primarily driven by the 17 and 35-year-old stands being predominately *Populus grandidentata* and *Populus tremuloides*, while *Quercus rubra* and *Pinus strobus* increase in relative abundance as stands increase in age (Figure B 2).

Soil Physio-chemical Properties and nutrients

A suite of linear regressions indicates significant patterns in A horizon soil chemistry along a successional gradient. Soil pH was elevated in the youngest stand (~4.7) post-slash and burn disturbance and was shown to decrease ($p=0.03$) as stand age progressed to >4.0 in the 106-year-old stand (Figure 3.3). Conversely, soil %C and soil %N both significantly increased ($p=0.03$ and $p=0.021$, respectively) along a successional trajectory as stands recovered (Figure 3.4). Despite the overall increase in overall % C in mineral soils, no increase was detected in A horizon soil carbon stocks (ranging from 781 (±356) to1320 (±697) g C/m_2) as stands increased in time since disturbance. Additionally, I detected no differences in soil bulk density or percent moisture across varying ages stands.

Total available inorganic N (NH_{4+} + NO_{3-}) was positively correlated with stand age ($p<0.01$). This was driven by available N in the form of NH_4 ($p<001$, $r_2=0.08$), as NO3 was not shown to differ as stan age progressed (Figure 3.5). This trend was detected despite the 1954 plot having lower available NH_4, relative to all other stands, and results did not differ for either NH4 or NO3 when analyzed without 1954 (Figure B 3).

Root and Microbial Biomass

Microbial biomass derived from chloroform fumigations indicated microbial C ranged from ~400-800 µg C/g soil, while microbial N ranged from ~40 – 100 µg N/g soil. Differences in microbial biomass along a successional gradient were detected, as both microbial C and microbial N were positively correlated ($p<0.01$, $R_2=0.804$; $p<0.01$, $R_2=0.914$, respectively) with increasing stand age (Figure 3.6). Additionally, both microbial C and N were positively

correlated $p<0.01$, $R_2=0.46$; $p<0.01$, $R_2=0.46$, respectively) with soil %C and %N as stand age increases (Figure 3.7). However, when microbial biomass was examined per gram soil C these trends did not hold true (Figure B 4). No discernible trends in root mass density (g/m2) could be detected along the successional gradient. Average root mass density was highest in the 104-year old stand at 144 g/m2, which was ~60 g/m2 higher than any other plot average, but there was extremely high variance both within and between plots. Additionally, no relationship could be detected between root mass density and microbial biomass.

Fungal community structure

A total of 622,320 sequences were used in analyses after quality filtering and rarefaction. The quality filtered raw reads of fungi from soil communities were assigned to 1271 operational taxonomic units (OTUs). A total of 86%, 79%, 75%, 71% and 65% were classified at the phylum, order, family, genus and species levels, respectively. Of the 1271 OTUS, 23 individually accounted for more than 1% of total sequences, with 4 of those OTUs (*Cenococcum geophilum, Cortinarius alpinus, Meliniomyces bicolor,* and *Lactifluus allardii)* accounting for more than 3% of all sequences (Table 3.1). Approximately 60% of these taxa are represented by fungi known to form ectomycorrhizal (13 OTUs) or ericoid mycorrhizal (1 OTU) associations. The mean number of fungal OTUs across all samples was ~195 (±39 s.d.) and ranged from 102 to 275 observed OTUs.

Basiodiomyceteous fungi were dominant taxa throughout the chronosequence, accounting for >50% of OTUs in all stands (Figure B 5). Fungi in the Ascomycota were also strongly represented across the successional stages with >25% representation in all stand ages. Aside from these two phyla only Mortierellomycota and the Mucoromycota had >1% relative abundance. There were 18 families (Figure B 6) and 17 genera (Figure B 7) that accounted for >1% of relative abundance at each respective taxonomic level. Of particular note, taxa in the Cortinariaceae family, and in turn the genus Cortinarius, were the most strongly represented at all successional stages.

I detected no discernable trends in any measure of diversity among the varying age stands. However, the 61-year-old plot indices were outside the 95% confidence intervals of the other 5

plots and contrasted starkly to the other plots in showing much lower diversity indices than both younger and older stands (Figure 3.8). The 3 locations with the lowest diversity indices were all from the 61-year old plot and communities were dominated by several mycorrhizal species (*Cortinarius caperatus*, *Lactifluus allardii*, and an unidentified *Cortinarius* sp.). When diversity was analyzed excluding the 61-year old plot there was a detectable negative correlation with stand age in richness ($p=0.04$, $R^2=0.88$), total observed OTUs ($p<0.01$, $R^2=0.156$), and phylogenetic diversity ($p=0.03$, $R^2=0.103$) (Figure B 8). Contributing to the low diversity in the 104-year old plots was a high representation of 8 out of 13 of the most prevalent ectomycorrhizal taxa, with over 20% of the sequences from these taxa having been found in the 104-year old stand.

A PCoA generated with a bray-curtis distance matrix collapsed at the family level show a large amount of variation within and between differing aged stands. Despite the large variation in community structure a Mantel test indicated significant differences in community structure in differing aged stands ($p=0.025$). There is a general separation between the youngest stand (17) and the oldest stand (104 years) along the y axes (Figure 3.9). Cumulatively, the x and y axes explain 44.8% of the variation in communities. Later successional stage communities are primarily driven by ectomycorrhizal taxa in the Cortinariceae and to a lesser extent the Russulaceae. In contrast, younger communities are more strongly represented by members of the Amanitaceae and Clavariaceae.

Extracellular Enzyme Activity

Fungal EEA of both hydrolytic and oxidative enzymes were relatively minimal and varied widely within plots (Table B 2) resulting in no discernable trends in EEA of NAG, BG, Alk, PHEN-OX, or PER. An ANOVA examining alpha glucosidase activity and Tukey post-hoc analysis detected the a significant difference in activity with elevated levels of alpha glucosidase activity in the 104-year-old plot relative to younger stands ($p<0.01$). Additionally, no correlation was found between any EEA and soil moisture or %C.

Fungal community and environmental interactions

A mixed effect redundancy model using a Bray-Curtis distance matrix of fungal communities revealed significant environmental predictors of community structure ($p<0.05$). The forward step regression resulted in pH, NH_{4+}, alpha glucosidase activity, percent moisture, and roots per gram as significant predictors of community dissimilarity between different aged stands. When plotted as vectors over the fungal community PCoA two primary trends were identified: 1) Low pH is the strongest predictor of early community composition dissimilarities as seen by the pH vector separating out the 17-year-old stand along the x axis. 2) an increase in total root biomass and AG EEA are the strongest predictors of fungal communities in the oldest stand (figure 3.10).

3.5 Discussion

Successional Trends in Diversity

The primary objective of this chapter was to examine whether or not fungal communities followed predicted trends of the IDH (1978) as are often detected in plant communities (Catford et al., 2012; Kershaw and Mallik, 2013). I first looked to see if plant community succession reflected the IDH and did in fact note through Oi horizon leaf litter that the plant communities in the stand did follow predicted patterns of diversity in the experimental plots, peaking in the 61-year mid-successional stand (Figure 3.2). The higher diversity in the reference stand is likely due to experimental plots having been twice cut and burned in the last century. These results mirror successional trends in annual C storage (Mg C ha_{-1} yr_{-1}) previously reported in the chronosequence and reference stands (Gough et al., 2007). These trends in plant diversity and annual carbon storage suggest that primary succession in the chronosequence is progressing in accordance with classic ecosystem theory predicting highest productivity and diversity in mid-successional stages (Connell, 1978; Odum, 1969).

Despite support for successional trends reflecting the IDH predictions in plant succession, I could not detect trends in fungal diversity that would support the IDH. To the contrary, the 61-year old stand was exceptionally low in richness, evenness, total observed OTUs, and phylogenetic diversity (Figure 3.8). These results were driven by high abundances of several EM species (Figure B 6), with Cortinariaceae being a particularly important family responsible for community dissimilarity (Figure 9). It was noted during sample collection and processing that a

high abundance of the soil cores collected from the 61-year old stand were located in the footprint of, or in close proximity to adult oaks that commonly form associations with EM in the Cortinariaceae (Dickie et al., 2004; Ortega et al., 2009; Trappe, 1962; Zotti et al., 2014).

Due to the noted irregularity in soil core sampling in the 61-year plot was also examined trends in fungal diversity when the plot was removed. I did not detect evidence of fungal diversity adhering to the IDH, and instead found a negative correlation with fungal diversity and stand age, regardless of stands having received 1 or 2 cut+burn disturbances over the past century (Figure B 8). Moreover, I found an inverse relationship between plant and fungal diversity in the youngest and oldest stands, with high fungal diversity and low plant diversity in the earliest successional stage, and low fungal diversity and high plant diversity in the oldest successional stand (Figure 3.2; Figure B 8).

Microbial Biomass and EEA

Detected trends in microbial biomass differed significantly from those of fungal diversity (Fig B 8). Whereas fungal diversity decreased during succession, both microbial C (g C/g soil$_{-1}$) and microbial N (g N/g soil$_{-1}$) illustrated a strong positive correlation with stand age (Figure 3.6). This increase in measures of microbial pools coincides with increases in EM relative abundances and their symbionts, oaks and pines, over time since disturbance. Low microbial abundances post-harvest and burning likely result from a combination of fire induced mortality (Dooley and Treseder, 2012; Holden and Treseder, 2013), indirect mortality through the death of mycorrhizal associated plants, and alterations to environmental conditions (Gonzalez-Perez et al., 2004), and reductions or alterations in resources availability (Czimczik and Masiello, 2007; Kuzyakov et al., 2009).

As in Chapter 2, in high burn severity areas of the 2017 cut+burn plot and a number of previous studies, fungi are susceptible to mortality when subjected to high temperatures leading to immediate reductions in fungal biomass and diversity after fires (Dooley and Treseder, 2012; Holden and Treseder, 2013). Although mycorrhizal fungi are susceptible to fire induced mortality, they can also experience indirect mortality through the loss of their plant symbiont. Unlike saprotrophs that acquire C from SOM and dead plant tissues during decomposition,

mycorrhizal fungi are dependent on plant host associates for carbohydrates to drive their metabolism (Högberg et al., 2003; Zak et al., 2019). The resulting effect on mycorrhizal fungi is often post-harvest mortality as their carbohydrates supply is cut off. I posit that the death of pre-disturbance saprotrophs and mycorrhizal fungi is responsible for both the reduced biomass and increased diversity. Fungal mortality provides opportunities for additional fungi to colonize and compete for resources in early successional stages (Frey, 2019). This reduction in fungal biomass post fire and, in turn, reduction in competition for available resources can result in higher diversity as fungi disperse into the disturbed sites.

The increase in fungal biomass and decrease in diversity as stands recover from disturbance through time may be due to competitive exclusion as superior saprotroph competitors and mycorrhizal fungi re-establish. Indeed, I found that as EM taxa became more prevalent through succession (Figure 6 B; Figure 7 B), fungal diversity decreased (Figure 8 B). Previous chronosequence studies have noted a decrease in diversity in late successional stages as Cortinariaceae species became abundant (Holden et al., 2013). As plant communities recolonize, they provide resources through leaf litter, root exudates, and carbohydrates to re-establishing fungal mycorrhizal symbionts. It is widely suggested that mycorrhizal fungi directly compete with saprotrophs for resources as they seek to liberate nutrients from SOM to either provide their host with N or assimilate into biomass (Averill et al., 2014; Gadgil and Gadgil, 1971; Sterkenburg et al., 2018). The steady supply of organic materials provided from hosts offers them a competitive advantage from saprotrophs that must acquire C through decomposition (Zak et al., 2019).

It is also interesting to note that when microbial biomass is examined on a per gram soil carbon basis it follows a unimodal distribution with highest abundances in mid-successional stands (Fig 4 B). This trend mirrors that of annual C storage (Mg C ha-1 yr-1) found in a previous study of the chronosequence plots (Gough et al., 2007). The peak in microbial abundances in the 61-year stand coincides with the low diversity and high EM abundances also found. This trend of high productivity, could be resulting in a high contribution of C to EM fungi that are competitively excluding other fungi, resulting in the low diversity detected.

Despite these distinct differences, whether expressed on a per gram soil or soil C basis, no trends in EEA could be detected across the experimental plots (Table B 2). The only detectable difference across the chronosequence was higher alpha glucosidase activity in the reference stand. This result may be indicative of the long-term effect on fungal community functional ability and diversity that persists for decades post-disturbance. While I did detect a difference in EEA in the previous chapter, with reduced activity post-disturbance, the trend was not detectable after ~2 decades of succession. I do note that all enzymes activities reported here were significantly lower than those in both the reference and experimental plot during the 2017 field campaign. While I did conduct the field collection here during the height of summer, previous work examining seasonal variation in EEA of burned and unburned soil showed no detectable differences based on seasonality (Boerner et al., 2005).

Disturbance Effects on soil C, N, and pH

Soil physio-chemical properties are often used as indicators of fire severity in disturbed systems. The detection of elevated soil pH in the youngest stand relative to the reference stand is consistent with results from the recently burned plot in the previous chapter as well as those reported in other studies (Gonzalez-Perez et al., 2004). Our detected negative correlation with stand age here is also consistent with the effects of responses typical over successional trajectories as forested stands recover (Gonzalez-Perez et al., 2004; Nave et al., 2019). These effects commonly resulting from burning stand in contrast with studies showing declines in stands experiencing clear-cutting only (Brais et al., 1995; Johnson et al., 1991). The response due to clear-cutting is typically attributed to mixing of litter with mineral soils during intensive harvesting. However, as in the short-term effects documented at our site in Chapter 2, the deposition of ash during organic matter combustion by burning can outweigh any effects of soil mixing even during intensive machined harvesting. I see here that even 79 years post disturbance pH remains elevated, highlighting the long-term consequences of disturbances in forest soils.

Soil %C and %N were both increased with stand age in this study. Effects on soil C and N can vary depending on both harvest and burn intensities. Intensive harvesting practices can displace organic horizons, mixing them with mineral soils in effect increases detectable soil %C and %N (Johnson, 1992). Conversely, harvesting can lead to a net loss of C and N in mineral soils after

whole-tree harvesting (Johnson et al., 1991; Johnson and Curtis, 2001). Confounding these effects, under high burn conditions organic matter in soils can be volatilized through pyrolysis with enough heat transfer to mineral soils. The effects of this pyrolysis have the potential to lead to either an increase or decrease in soil C and N. On one had soil organic matter can be lost under conditions of high severity burning and has been highlighted across an array of studies (Carney et al., 2007; Gonzalez-Perez et al., 2004; Holden et al., 2015). This was indicative of high severity burn locations in the recently implemented burn plot. However, soil C and N may also increase due to the percolation of partially combusted organic matter (charcoal) and hydrophobic organic matter into mineral soil (Johnson, 1992). The trend in lower %C and %N here is consistent with the effects of both whole tree harvesting and volatilization of mineral SOM during high severity burning. As stands recover and leaf litter is deposited, decomposed, and incorporated into mineral soils during succession both soil C and N will increase.

Conclusions

This study did not support the hypothesis that soil fungal communities follow successional trends predicted by the intermediate disturbance hypothesis. I detected the highest fungal diversity in early successional communities with a precipitous drop throughout ~100 years of succession. The drastic reduction in diversity as detected on short-term timescales post disturbance was lost at the decadal scale. No particular group of fungi, whether functional or taxonomic, was a significant indicator of early successional stages (Figure 3.9). This fact combined with a heightened level of diversity may suggest a continued struggle for dominance in mineral soils by colonizing taxa post-disturbance.

Contrary to the high diversity expected in mid-successional stands I detected the least diverse community in the stand with the highest plant diversity. I attribute this trend to a dominance by EM taxa that I predicted to see in later successional stages. This highlights the potential for stochasticity in community assembly as I detected a higher than expected contribution of oaks to the plant community. I propose that the association with Cortinariacaea taxa likely led to heightened competition with saprotrophs and other EM taxa, driving down competition in this mid-successional stand.

These results offer insight into the lasting legacy of disturbance that was shown to have a persistent effect on soil chemistry and fungal structure and abundance through 100 years of succession. This research highlights the importance of decadal studies on disturbance effects due to the century long legacy of effects on fungal communities and soil properties.

3.6 References

Averill, C., Turner, B.L., Finzi, A.C., 2014. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. Nature 505, 543–545. https://doi.org/10.1038/nature12901

Barg, A.K., Edmonds, R.L., 1999. Influence of partial cutting on site microclimate, soil nitrogen dynamics, and microbial biomass in Douglas-fir stands in western Washington. Can. J. For. Res. 29, 705–713. https://doi.org/10.1139/x99-045

Boddy, L., Watkinson, S., 1994. Wood decomposition, higher fungi, and their role in nutrient redistribution. Can. J. Bot. 73.

Bödeker, I.T.M., Clemmensen, K.E., de Boer, W., Martin, F., Olson, Å., Lindahl, B.D., 2014. Ectomycorrhizal *Cortinarius* species participate in enzymatic oxidation of humus in northern forest ecosystems. New Phytol. 203, 245–256. https://doi.org/10.1111/nph.12791

Boerner, R.E.J., Brinkman, J.A., Smith, A., 2005. Seasonal variations in enzyme activity and organic carbon in soil of a burned and unburned hardwood forest. Soil Biol. Biochem. 37, 1419–1426. https://doi.org/10.1016/j.soilbio.2004.12.012

Bond-Lamberty, B., Wang, C., Gower, S.T., 2004a. Net primary production and net ecosystem production of a boreal black spruce wildfire chronosequence. Glob. Chang. Biol. 10, 473–487. https://doi.org/10.1111/j.1529-8817.2003.0742.x

Bond-Lamberty, B., Wang, C., Gower, S.T., 2004b. Contribution of root respiration to soil surface CO2 flux in a boreal black spruce chronosequence. Tree Physiol. 24, 1387–1395. https://doi.org/10.1093/treephys/24.12.1387

Brais, S., Camire, C., Pare, D., 1995. Impacts of whole-tree harvesting and winter windrowing on soil pH and base status of clayey sites of northwestern Quebec. Can. J. For. Res. 25.

Cairney, J.W., Meharg, A.A., 2002. Interactions between ectomycorrhizal fungi and soil saprotrophs: implications for decomposition of organic matter in soils and degradation of organic pollutants in the rhizosphere. Can. J. Bot. 80, 803–809. https://doi.org/10.1139/b02-072

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., Mcdonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. correspondence QIIME allows analysis of high- throughput community sequencing data Intensity normalization improves color calling in SOLiD sequencing. Nat. Publ. Gr. 7, 335–336. https://doi.org/10.1038/nmeth0510-335

Carney, K.M., Hungate, B.A., Drake, B.G., Megonigal, J.P., 2007. Altered soil microbial community at elevated CO2 leads to loss of soil carbon. Proc. Natl. Acad. Sci. 104, 4990–4995. https://doi.org/10.1073/pnas.0610045104

Castillo, B.T., Nave, L.E., Le Moine, J.M., James, T.Y., Nadelhoffer, K.J., 2018. Impacts of experimentally accelerated forest succession on belowground plant and fungal communities. Soil Biol. Biochem. 125. https://doi.org/10.1016/j.soilbio.2018.06.022

Catford, J.A., Daehler, C.C., Murphy, H.T., Sheppard, A.W., Hardesty, B.D., Westcott, D.A., Rejmánek, M., Bellingham, P.J., Pergl, J., Horvitz, C.C., Hulme, P.E., 2012. The intermediate disturbance hypothesis and plant invasions: Implications for species richness and management. Perspect. Plant Ecol. Evol. Syst. 14, 231–241. https://doi.org/10.1016/j.ppees.2011.12.002

Clein, J.S., McGuire, A.D., Zhang, X., Kicklighter, D.W., Melillo, J.M., Wofsy, S.C., Jarvis, P.G., Massheder, J.M., 2002. Historical and projected carbon balance of mature black spruce ecosystems across north america: The role of carbon-nitrogen interactions. Plant Soil 242, 15–32. https://doi.org/10.1023/A:1019673420225

Clemmensen, K.E., Finlay, R.D., Dahlberg, A., Stenlid, J., Wardle, D.A., Lindahl, B.D., 2015. Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. New Phytol. 205, 1525–1536. https://doi.org/10.1111/nph.13208

Connell, J.H., 1978. Diversity in tropical rain forests and coral reefs. Science (80-.). 199, 1302–1310. https://doi.org/10.1126/science.199.4335.1302

Czimczik, C.I., Masiello, C.A., 2007. Controls on black carbon storage in soils. Global Biogeochem. Cycles 21. https://doi.org/10.1029/2006GB002798

Dickie, I.A., Guza, R.C., Krazewski, S.E., Reich, P.B., 2004. Shared ectomycorrhizal fungi between a herbaceous perennial (Helianthemum bicknellii) and oak (Quercus) seedlings. New Phytol. 164, 375–382. https://doi.org/10.1111/j.1469-8137.2004.01177.x

Dooley, S.R., Treseder, K.K., 2012. The effect of fire on microbial biomass: A meta-analysis of field studies. Biogeochemistry 109, 49–61. https://doi.org/10.1007/s10533-011-9633-8

Everett, R.L., Sharrow, S.H., 1985. Response of grass species to tree harvesting in singleleaf pinyon- Utah juniper stands. Res. Pap. - US Dep. Agric. For. Serv. 45. https://doi.org/10.5962/bhl.title.69006

Fisk, M.C., Fahey, T.J., Groffman, P.M., 2010. Carbon resources, soil organisms, and nitrogen availability: Landscape patterns in a northern hardwood forest. For. Ecol. Manage. 260, 1175–1183. https://doi.org/10.1016/j.foreco.2010.07.009

Fisk, M.C., Zak, D.R., Crow, T.R., 2002. Nitrogen storage and cycling in old- and second-growth northern hardwood forests. Ecology 83, 73–87. https://doi.org/10.1890/0012-9658(2002)083[0073:NSACIO]2.0.CO;2

Frelich, L.E., 1995. Old Forest in the Lake States Today and before European Settlement. Nat. Areas J. 15, 157–167.

Frey, S.D., 2019. Mycorrhizal Fungi as Mediators of Soil Organic Matter Dynamics. Annu. Rev. Ecol. Evol. Syst. 50, 237–259. https://doi.org/10.1146/annurev-ecolsys-110617-062331

Friedman, S.K., Reich, P.B., 2005. Regional legacies of logging: Departure from presettlement forest conditions in northern Minnesota. Ecol. Appl. 15, 726–744. https://doi.org/10.1890/04-0748

Gadgil, R.L., Gadgil, P.D., 1971. Mycorrhiza and litter decomposition. Nature 233, 133. https://doi.org/10.1038/233133a0

Gates, F., 1926. Plant Successions About Douglas Lake , Cheboygan County , Michigan. Bot. Gaz. 82, 170–182.

Goetz, S.J., Bond-Lamberty, B., Law, B.E., Hicke, J.A., Huang, C., Houghton, R.A., McNulty, S., O'Halloran, T., Harmon, M., Meddens, A.J.H., Pfeifer, E.M., Mildrexler, D., Kasischke, E.S., 2012. Observations and assessment of forest carbon dynamics following disturbance in North America. J. Geophys. Res. Biogeosciences 117, 1–17. https://doi.org/10.1029/2011JG001733

Gonzalez-Perez, J., Gonzalez-Vila, F., Almendros, G., Knicker, H., 2004. The effect of fire on soil organic matter-- a review. Environ. Int. 30, 855–870.

Gough, C.M., Curtis, P.S., Hardiman, B.S., Scheuermann, C.M., Bond-Lamberty, B., 2016. Disturbance, complexity, and succession of net ecosystem production in North America's

temperate deciduous forests. Ecosphere 7, 1–15. https://doi.org/10.1002/ecs2.1375
Gough, C.M., Hardiman, B.S., Nave, L.E., Bohrer, G., Kyle, D., Vogel, C.S., Nadelhoffer, K.J., Curtis, P.S., Gough, C., Hardiman, B.S., Nave, L., Bohrer, G., Maurer, K.D., Vogel, C.S., Nadelhoffer, K.J., Curtis, P.S., 2013. Sustained carbon uptake and storage following moderate disturbance in a Great Lakes forest 23, 1202–1215.
Gough, C.M., Vogel, C.S., Harrold, K.H., George, K., Curtis, P.S., 2007. The legacy of harvest and fire on ecosystem carbon storage in a north temperate forest. Glob. Chang. Biol. https://doi.org/10.1111/j.1365-2486.2007.01406.x
Gough, C.M., Vogel, C.S., Schmid, H.P., Curtis, P.S., 2008. Controls on Annual Forest Carbon Storage: Lessons from the Past and Predictions for the Future. Bioscience. https://doi.org/10.1641/B580708
Goulden, M.L., Mcmillan, A.M.S., Winston, G.C., Rocha, A. V., Manies, K.L., Harden, J.W., Bond-Lamberty, B.P., 2011. Patterns of NPP, GPP, respiration, and NEP during boreal forest succession. Glob. Chang. Biol. 17, 855–871. https://doi.org/10.1111/j.1365-2486.2010.02274.x
Hamman, S.T., Burke, I.C., Stromberger, M.E., 2007. Relationships between microbial community structure and soil environmental conditions in a recently burned system. Soil Biol. Biochem. https://doi.org/10.1016/j.soilbio.2007.01.018
Hardiman, B.S., Gough, C.M., Halperin, A., Hofmeister, K.L., Nave, L.E., Bohrer, G., Curtis, P.S., 2013. Maintaining high rates of carbon storage in old forests: A mechanism linking canopy structure to forest function. For. Ecol. Manage. 298, 111–119. https://doi.org/10.1016/j.foreco.2013.02.031
Harvey, A.E., Jurgensen, M.F., Larsen, M.J., 1980. Clearcut harvesting and ectomycorrhizae: survival of activity on residual roots and influence on a bordering forest stand in western Montana. Can. J. Bot. 10, 300–303.
Hiscox, J., Savoury, M., Müller, C.T., Lindahl, B.D., Rogers, H.J., Boddy, L., 2015. Priority effects during fungal community establishment in beech wood. ISME J. 9, 2246–2260. https://doi.org/10.1038/ismej.2015.38
Hobbie, E.A., 2006. Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. Ecology 87, 563–569. https://doi.org/10.1890/05-0755
Högberg, M.N., Bååth, E., Nordgren, A., Arnebrant, K., Högberg, P., 2003. Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs - A hypothesis based on field observations in boreal forest. New Phytol. 160, 225–238. https://doi.org/10.1046/j.1469-8137.2003.00867.x
Holden, S.R., Berhe, A.A., Treseder, K.K., 2015. Decreases in soil moisture and organic matter quality suppress microbial decomposition following a boreal forest fire. Soil Biol. Biochem. 87, 1–9. https://doi.org/10.1016/j.soilbio.2015.04.005
Holden, S.R., Gutierrez, A., Treseder, K.K., 2013. Changes in Soil Fungal Communities, Extracellular Enzyme Activities, and Litter Decomposition Across a Fire Chronosequence in Alaskan Boreal Forests. Ecosystems 16, 34–46. https://doi.org/10.1007/s10021-012-9594-3
Holden, S.R., Treseder, K.K., 2013. A meta-analysis of soil microbial biomass responses to forest disturbances. Front. Microbiol. 4. https://doi.org/10.3389/fmicb.2013.00163
Howard, E.A., Gower, S.T., Foley, J.A., Kucharik, C.J., 2004. Howard et al. (2004)_Effects of logging on carbon.pdf. Glob. Chang. Biol. 10, 1267–1284. https://doi.org/10.1111/j.1365-2486.2004.00804.x

Janos, D., 1980. Mycorrhizae Influence Tropical Succession. Biotropica 12, 56–64.
Joergensen, R.G., Brookes, P.C., 1990. Ninhydrin-reactive nitrogen measurements of microbial biomass in 0.5 m K2SO4 soil extracts. Soil Biol. Biochem. 22, 1023–1027. https://doi.org/10.1016/0038-0717(90)90027-W
Johnson, C.E., Johnson, A.H., Siccama, T.G., 1991. Whole-Tree Clear-Cutting Effects on Exchangeable Cations and Soil Acidity. Soil Sci. Soc. Am. J. 55, 502. https://doi.org/10.2136/sssaj1991.03615995005500020035x
Johnson, D.W., 1992. Natural sinks of CO2, Ecological Modelling. https://doi.org/10.1016/0304-3800(95)90065-9
Johnson, D.W., Curtis, P.S., 2001. Effects of forest management on soil C and N storage: Meta analysis. For. Ecol. Manage. 140, 227–238. https://doi.org/10.1016/S0378-1127(00)00282-6
Kershaw, H.M., Mallik, A.U., 2013. Predicting Plant Diversity Response to Disturbance: Applicability of the Intermediate Disturbance Hypothesis and Mass Ratio Hypothesis. CRC. Crit. Rev. Plant Sci. 32, 383–395. https://doi.org/10.1080/07352689.2013.791501
Koljalg, U., Nilsson, R.H., Taylor, A.F.S., Bates, S.T., Thomas, D., Bengtsson-palme, J., Callaghan, T.M., Douglas, B., Griffith, G.W., Ucking, R.L., Suija, A.V.E., Taylor, D.L.E.E., Teresa, M., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277.
Kormanik, P.P., Schultz, R.C., Bryan, W.C., 1982. The Influence of Vesicular-Arbuscular Mycorrhizae on the Growth and Development of Eight Hardwood Tree Species Recommended Citation The Influence of Vesicular-Arbuscular Mycorrhizae on the Growth and Development of Eight Hardwood Tree Species. For. Publ. 15.
Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by 14C labeling. Soil Biol. Biochem. 41, 210–219. https://doi.org/10.1016/j.soilbio.2008.10.016
Law, B.E., Sun, O.J., Campbell, J., Van Tuyl, S., Thornton, P.E., 2003. Changes in carbon storage and fluxes in a chronosequence of ponderosa pine. Glob. Chang. Biol. 9, 510–524. https://doi.org/10.1046/j.1365-2486.2003.00624.x
Lewandowski, T.E., Forrester, J.A., Mladenoff, D.J., D'Amato, A.W., Palik, B.J., 2016. Response of the soil microbial community and soil nutrient bioavailability to biomass harvesting and reserve tree retention in northern Minnesota aspen-dominated forests. Appl. Soil Ecol. 99, 110–117. https://doi.org/10.1016/j.apsoil.2015.11.001
Lundgren, B., 1982. Bacteria in a pine forest soil as affected by clear-cutting. Soil Biol. Biochem. 14, 537–542. https://doi.org/10.1016/0038-0717(82)90083-9
Muñoz Delgado, B.L., Kenefic, L.S., Weiskittel, A.R., Fernandez, I.J., Benjamin, J.G., Dibble, A.C., 2019. Northern mixedwood composition and productivity 50 years after whole-tree and stem-only harvesting with and without post-harvest prescribed burning. For. Ecol. Manage. 441, 155–166. https://doi.org/10.1016/j.foreco.2019.03.032
Nave, L.E., Gough, C.M., Maurer, K.D., Bohrer, G., Hardiman, B.S., Le Moine, J., Munoz, A.B., Nadelhoffer, K.J., Sparks, J.P., Strahm, B.D., Vogel, C.S., Curtis, P.S., 2011. Disturbance and the resilience of coupled carbon and nitrogen cycling in a north temperate forest. J. Geophys. Res. Biogeosciences 116. https://doi.org/10.1029/2011JG001758
Nave, L.E., Gough, C.M., Perry, C.H., Hofmeister, K.L., Le Moine, J.M., Domke, G.M., Swanston, C.W., Nadelhoffer, K.J., 2017. Physiographic factors underlie rates of biomass production during succession in Great Lakes forest landscapes. For. Ecol. Manage. 397, 157–173. https://doi.org/10.1016/j.foreco.2017.04.040

Nave, L.E., Le Moine, J.M., Gough, C.M., Nadelhoffer, K.J., 2019. Multidecadal trajectories of soil chemistry and nutrient availability following cutting vs. Burning disturbances in upper great lakes forests. Can. J. For. Res. 49, 731–742. https://doi.org/10.1139/cjfr-2018-0211

Nave, L.E., Nadelhoffer, K.J., Le Moine, J.M., van Diepen, L.T.A., Cooch, J.K., Van Dyke, N.J., 2013. Nitrogen Uptake by Trees and Mycorrhizal Fungi in a Successional Northern Temperate Forest: Insights from Multiple Isotopic Methods. Ecosystems 16, 590–603. https://doi.org/10.1007/s10021-012-9632-1

Nave, L.E., Sparks, J.P., Le Moine, J., Hardiman, B.S., Strahm, B.D., Curtis, P.S., 2014. Changes in soil nitrogen cycling in northern temperate forest ecosystem during succession. Biogeochemistry 121, 471–488. https://doi.org/10.1007/s

Odum, E.P., 1969. The Strategy of Ecosystem Development. Science (80-.). 164, 262–270.

Oliver, A.K., Callaham, M.A., Jumpponen, A., 2015. Soil fungal communities respond compositionally to recurring frequent prescribed burning in a managed southeastern US forest ecosystem. For. Ecol. Manage. 345, 1–9. https://doi.org/10.1016/j.foreco.2015.02.020

Ortega, A., Suárez-Santiago, V., Vila, J., 2009. Two new species of Cortinarius collected under Quercus rotundifoha in the Mediterranean area of southern Spain. Fungal Divers. 36, 89.

Pare, D., Bergeron, Y., 1995. Above-Ground Biomass Accumulation along a 230-Year Chronosequence in the Southern Portion of the Canadian Boreal Forest. J. Ecol. 83, 1001–1007.

Pietikainen, J., Fritze, H., 1995. Clear-cutting and prescribed burning in coniferous forest: comparision of effects on soil fungal and total microbial biomass, respiration activity and nitrification. Soil Biol. Biochem. 27, 1995.

Reazin, C., Morris, S., Smith, J.E., Cowan, A.D., Jumpponen, A., 2016. Fires of differing intensities rapidly select distinct soil fungal communities in a Northwest US ponderosa pine forest ecosystem. For. Ecol. Manage. 377, 118–127. https://doi.org/10.1016/j.foreco.2016.07.002

Richardson, S.J., Peltzer, D.A., Allen, R.B., McGlone, M.S., Parfitt, R.L., 2004. Rapid development of phosphorus limitation in temperate rainforest along the Franz Josef soil chronosequence. Oecologia 139, 267–276. https://doi.org/10.1007/s00442-004-1501-y

Roberts, M.R., Gilliam, F.S., 1995. Disturbance effects on herbaceous layer vegetation and soil nutrients in Populus forests of northern lower Michigan. J. Veg. Sci. 6, 903–912. https://doi.org/10.2307/3236405

Romaní, A.M., Fischer, H., Mille-Lindblom, C., Tranvik, L.J., 2006. Interactions of bacteria and fungi on decomposing litter: Differential extracellular enzyme activities. Ecology 87, 2559–2569. https://doi.org/10.1890/0012-9658(2006)87[2559:IOBAFO]2.0.CO;2

Scheiner, S.M., Teeri, J.A., 1981. Succession in the dominant tree species of the Douglas Lake region . MIchigan Bot. 20.

Serbin, S.P., Ahl, D.E., Gower, S.T., 2013. Spatial and temporal validation of the MODIS LAI and FPAR products across a boreal forest wildfire chronosequence. Remote Sens. Environ. 133, 71–84. https://doi.org/10.1016/j.rse.2013.01.022

Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol. Biochem. 42, 391–404. https://doi.org/10.1016/j.soilbio.2009.10.014

Sinsabaugh, R.L., Linkins, A.E., 1990. Enzymic and chemical analysis of particulate organic matter from a boreal river. Freshw. Biol. 23, 301–309. https://doi.org/10.1111/j.1365-2427.1990.tb00273.x

Sterkenburg, E., Clemmensen, K.E., Ekblad, A., Finlay, R.D., Lindahl, B.D., 2018. Contrasting

effects of ectomycorrhizal fungi on early and late stage decomposition in a boreal forest. ISME J. 12, 2187–2197. https://doi.org/10.1038/s41396-018-0181-2
Štursová, M., Žifčáková, L., Leigh, M.B., Burgess, R., Baldrian, P., 2012. Cellulose utilization in forest litter and soil: Identification of bacterial and fungal decomposers. FEMS Microbiol. Ecol. 80, 735–746. https://doi.org/10.1111/j.1574-6941.2012.01343.x
Taylor, D.L., Hollingsworth, T.N., McFarland, J.W., Lennon, N.J., Nusbaum, C., Ruess, R.W., 2014. A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning. Ecol. Monogr. 84, 3–20. https://doi.org/10.1890/12-1693.1
Trappe, J.M., 1962. Fungus associates of ectotrophic mycorrhizae. Bot. Rev. 28, 538–606. https://doi.org/10.1007/BF02868758
Whitney, G.G., 1987. an Ecological History of the Great-Lakes Forest of Michigan. J. Ecol. 75, 667–684. https://doi.org/10.2307/2260198
Wicklow, D.T., 1975. Fire as an Environmental Cue Initiating Ascomycete Development in a Tallgrass Prairie. Mycologia 67, 852–862. https://doi.org/10.1080/00275514.1975.12019813
Wicklow, D.T., Hirschfield, B.J., 1979. Competitive Hierarchy in Post-Fire Ascomycetes. Mycologia 71, 47–54. https://doi.org/10.1080/00275514.1979.12020986
Zak, D.R., Pellitier, P.T., Argiroff, W.A., Castillo, B., James, T.Y., Nave, L.E., Averill, C., Beidler, K. V., Bhatnagar, J., Blesh, J., Classen, A.T., Craig, M., Fernandez, C.W., Gundersen, P., Johansen, R., Koide, R.T., Lilleskov, E.A., Lindahl, B.D., Nadelhoffer, K.J., Phillips, R.P., Tunlid, A., 2019. Exploring the role of ectomycorrhizal fungi in soil carbon dynamics. New Phytol. 223, 33–39. https://doi.org/10.1111/nph.15679
Zak, J.C., Wicklow, D.T., 1980. Structure and composition of a post-fire ascomycete community: role of abiotic and biotic factors. Can. J. Bot. 58, 1915–1922. https://doi.org/10.1139/b80-222
Zotti, M., Ambrosio, E., Di Piazza, S., Bidaud, A., Boccardo, F., Pavarino, M., Mariotti, M.G., Vizzini, A., 2014. Ecology and diversity of Cortinarius species (Agaricales, Basidiomycota) associated with Quercus ilex L. in the Mediterranean area of Liguria (North-western Italy). Plant Biosyst. https://doi.org/10.1080/11263504.2013.877538

3.7 Tables & Figures

Table 3.1. Operational Taxonomic Units (OTUs) that individually accounted for >1% relative abundance of total sequences. Species accounting for >3% of total relative abundance are indicated with an asterisk (*). Ecology was assigned to OTUs classified (>97%) at the genus level.

Ecology	Family	Genus	Species	% relative abundance	Total sequence #
Ectomycorrhizal	Gloniaceae	Cenococcum	*Cenococcum geophilum*	5.7*	35496
Ectomycorrhizal	Cortinariaceae	Cortinarius	*Cortinarius alpinus*	5.4*	33752
Ericoid mycorrhizal	Helotiaceae	Meliniomyces	*Meliniomyces bicolor*	3.3*	20523
Ectomycorrhizal	Russulaceae	Lactifluus	*Lactifluus allardii*	3.1*	19456
Ectomycorrhizal	Amanitaceae	Amanita	*Amanita brunnescens*	2.9	18217
Ectomycorrhizal	Cortinariaceae	Cortinarius	*Cortinarius caperatus*	2.8	17664
Unidentified	Russulaceae	unidentified	*unidentified*	2.7	17104
Ectomycorrhizal	Hydnangiaceae	Laccaria	*unidentified*	2.3	14509
Ectomycorrhizal	Atheliaceae	Piloderma	*Piloderma bicolor*	2.0	12364
Ectomycorrhizal	Cortinariaceae	Cortinarius	*unidentified*	1.6	9770
Unidentified	unidentified	unidentified	*unidentified*	1.4	8716
Endophytic	Umbelopsidaceae	Umbelopsis	*Umbelopsis dimorpha*	1.3	8379
unidentified	unidentified	unidentified	*unidentified*	1.3	8055
Ectomycorrhizal	Russulaceae	Russula	*unidentified*	1.3	7883
Ectomycorrhizal	Cortinariaceae	Cortinarius	*Cortinarius cruentiphyllus*	1.2	7590
unidentified	Russulaceae	unidentified	*unidentified*	1.2	7487
Saprobic	Omphalotaceae	Rhodocollybia	*Rhodocollybia butyracea*	1.2	7273
Ectomycorrhizal	Cortinariaceae	Cortinarius	*Cortinarius leiocastaneus*	1.1	6763
Saprobic	Clavariaceae	Clavulinopsis	*Clavulinopsis laeticolor*	1.1	6749
unidentified	Herpotrichiellaceae	unidentified	*unidentified*	1.1	6580
Ectomycorrhizal	Cortinariaceae	Cortinarius	*Cortinarius alboviolaceus*	1.1	6537
Ectomycorrhizal	Cantharellaceae	Craterellus	*Craterellus tubaeformis*	1.0	6378

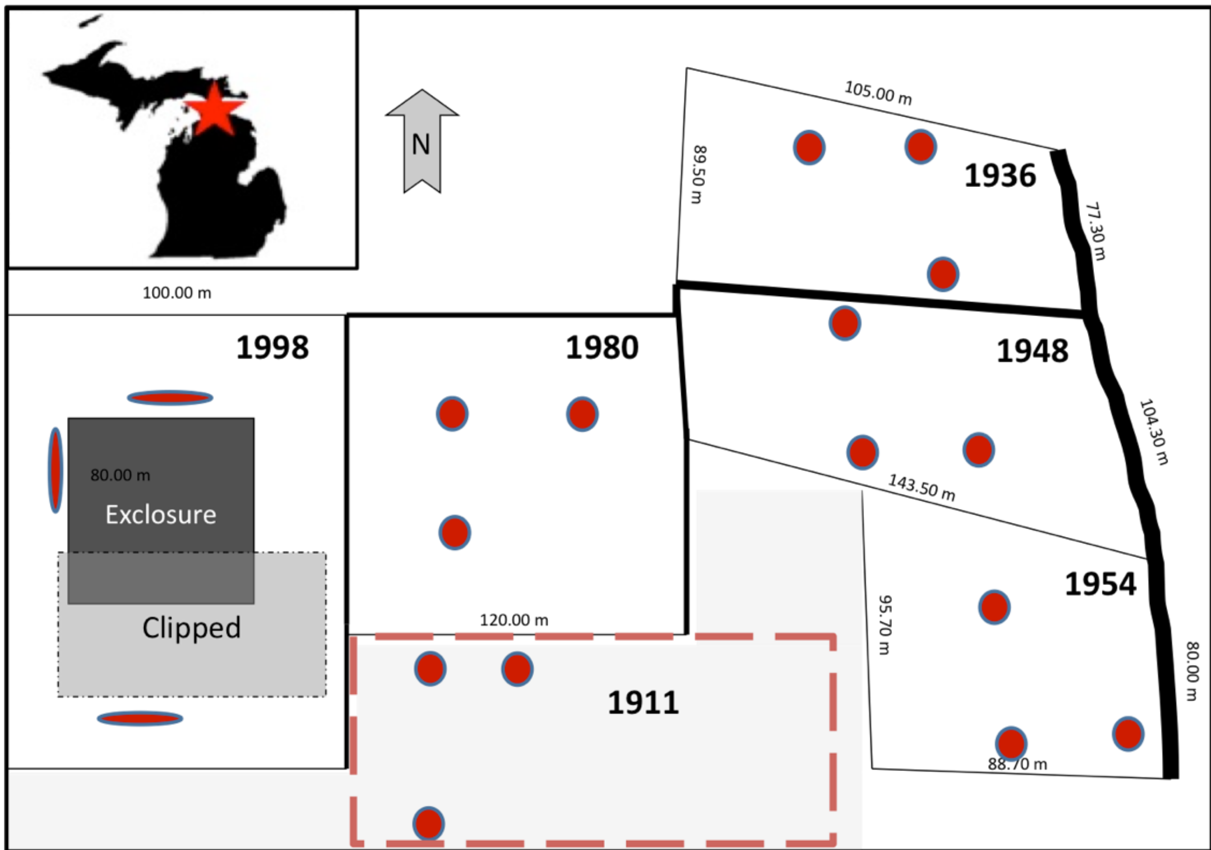

Figure 3.1. Diagram of the UMBS burn plots. A map of Michigan is inset with a red star representing the location of UMBS. Red circles represent sites within each plot where 5 replicate soil samples were extracted (n=15 per plot). The red hashed lines represent artificial boundaries that define the boundaries of the 1911 (104 year) reference stand

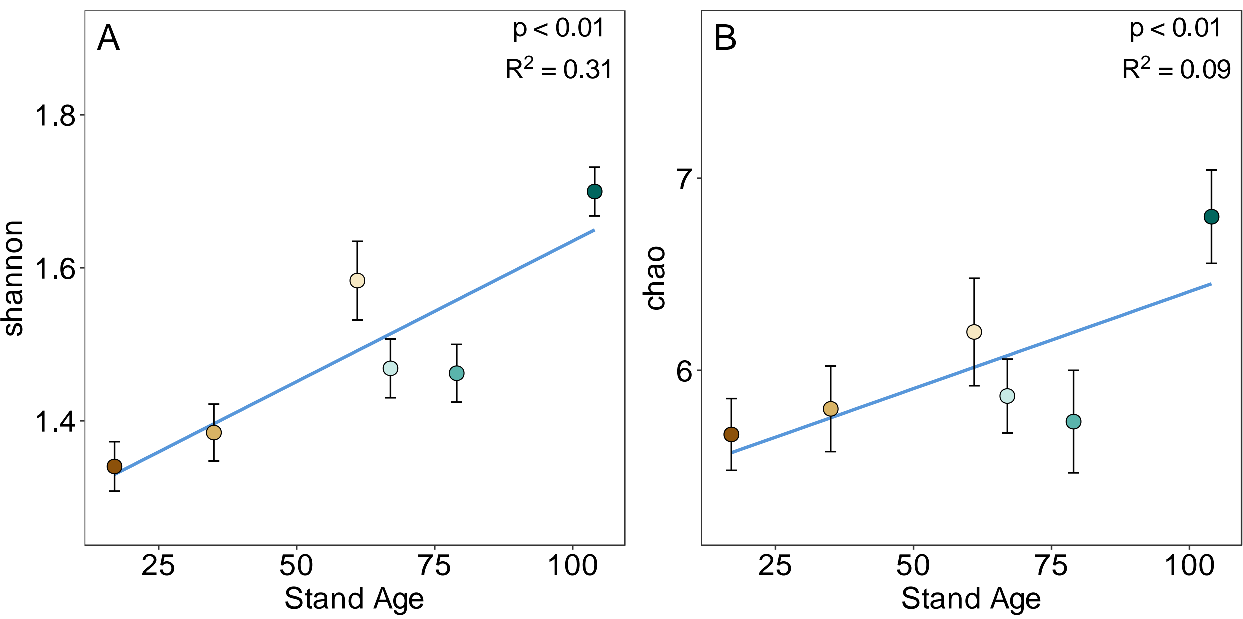

Figure 3.2. Diversity indices of sorted Oi horizons including leaf litter, acorns, and sticks across varying age chronosequence stands from 17-year-old stands to 104-year old stands. Stand ages: 17 year (red), 35 year (orange), 61 year (yellow), 67 year (light blue), 79 (blue), 104 year (dark blue). The shaded band is the 95% confidence interval of the linear model.

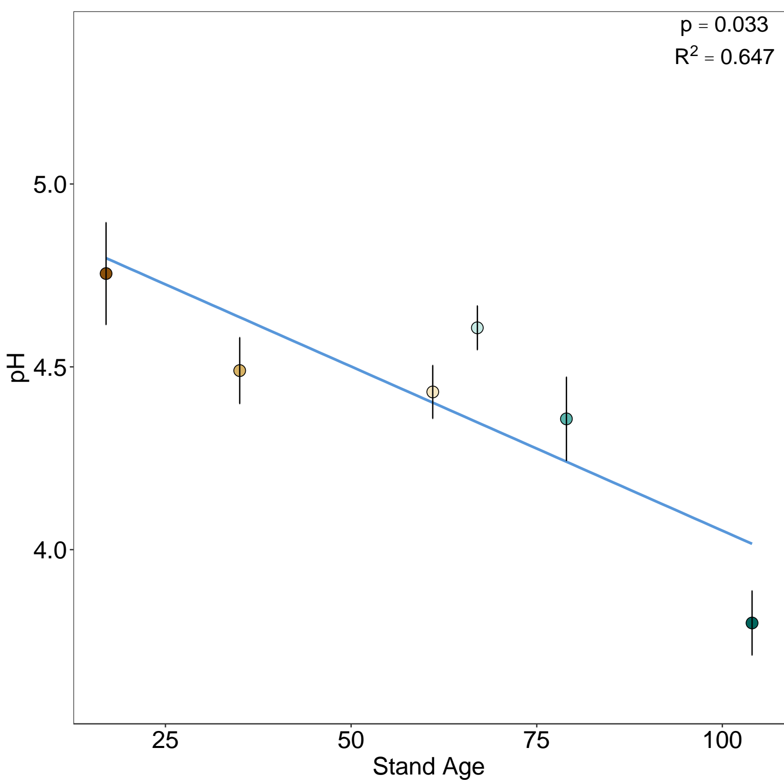

Figure 3.3. Mineral soil pH across the burn chronosequence significantly decreases as stand age increases. Means are represented by circles with vertical hashed lines indicating ±SE. Stand ages: 17 year (red), 35 year (orange), 61 year (yellow), 67 year (light blue), 79 (blue), 104 year (dark blue). The shaded band is the 95% confidence interval of the linear model.

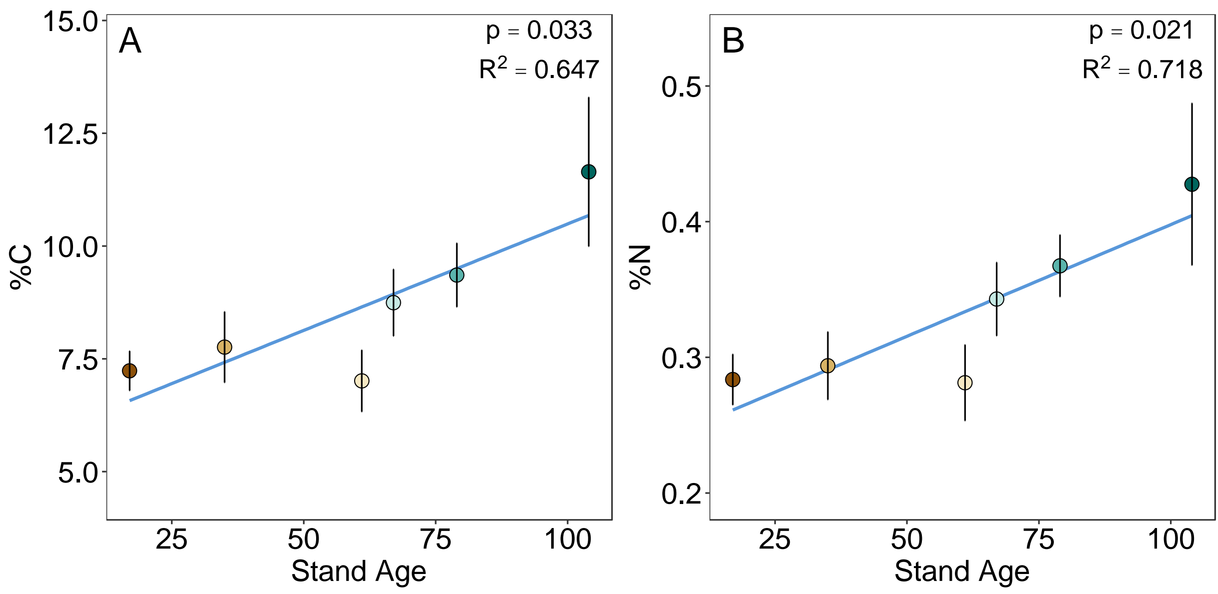

Figure 3.4. Regression between %C and %N and stand age. A) percent C increase with stand age ($p=0.033$, $r^2=0.58$) B) %N increases with stand age ($p=0.021$, $r^2=0.719$). Means are represented by closed circles and vertical hashed lines indicate ±1 S.E. Shaded bands indicate 95% confidence intervals.

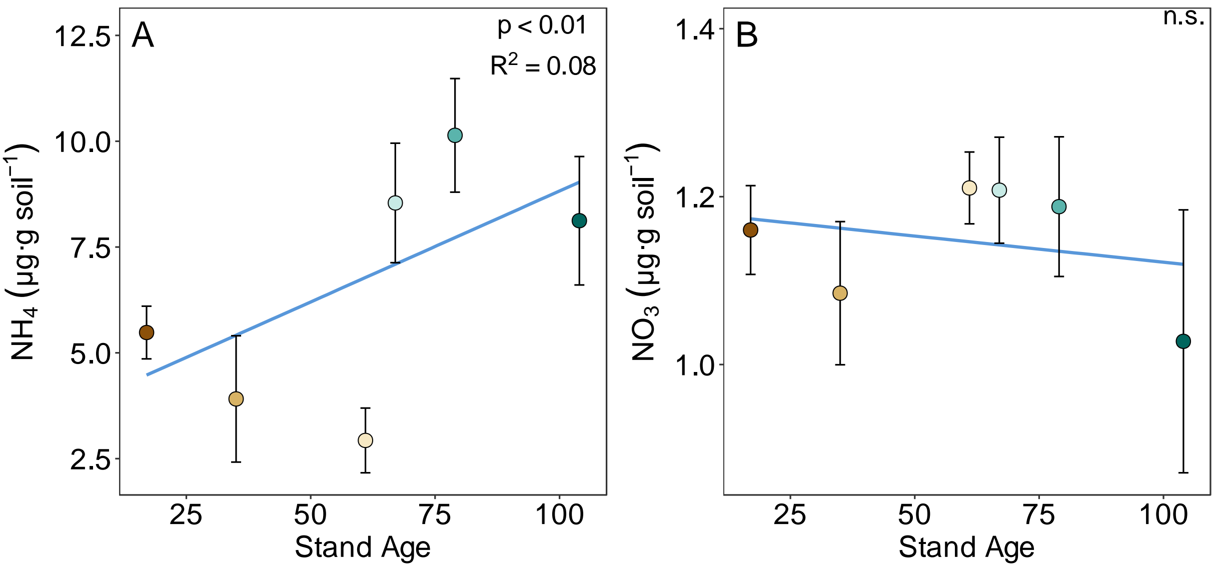

Figure 3.5. Available mineralized N in A horizon soils. A) Available NH_4 increases with stand age ($p<0.01$, $r^2=0.08$) B) No differences were detected in available NO_3 across stands. Means are represented by closed circles and vertical hashed lines indicate ±1 S.E. Shaded bands indicate 95% confidence intervals.

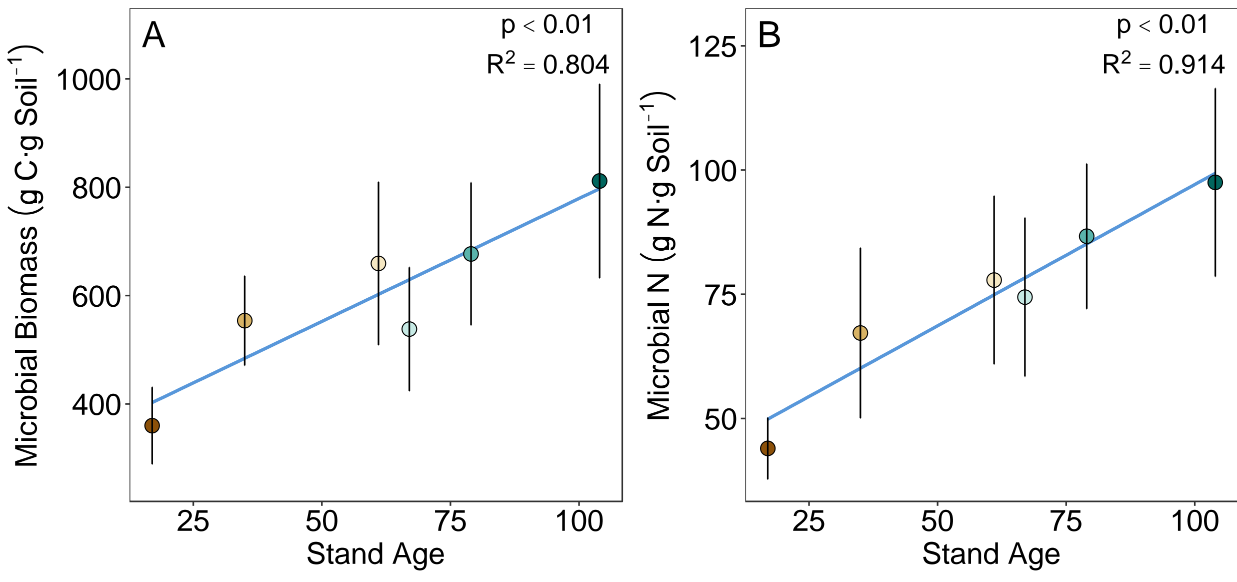

Figure 3.6. Relationship between Microbial biomass C and N concentrations per gram soil along the chronosequence. A) Microbial C per gram of soil increased as stand age increased (p=0.01, r^2=0.804). B) Microbial N per gram soil increases with stand age (p=0.01, r^2=0.914). Means are represented by closed circles and vertical hashed lines indicate ±1 S.E. Shaded bands indicate 95% confidence intervals.

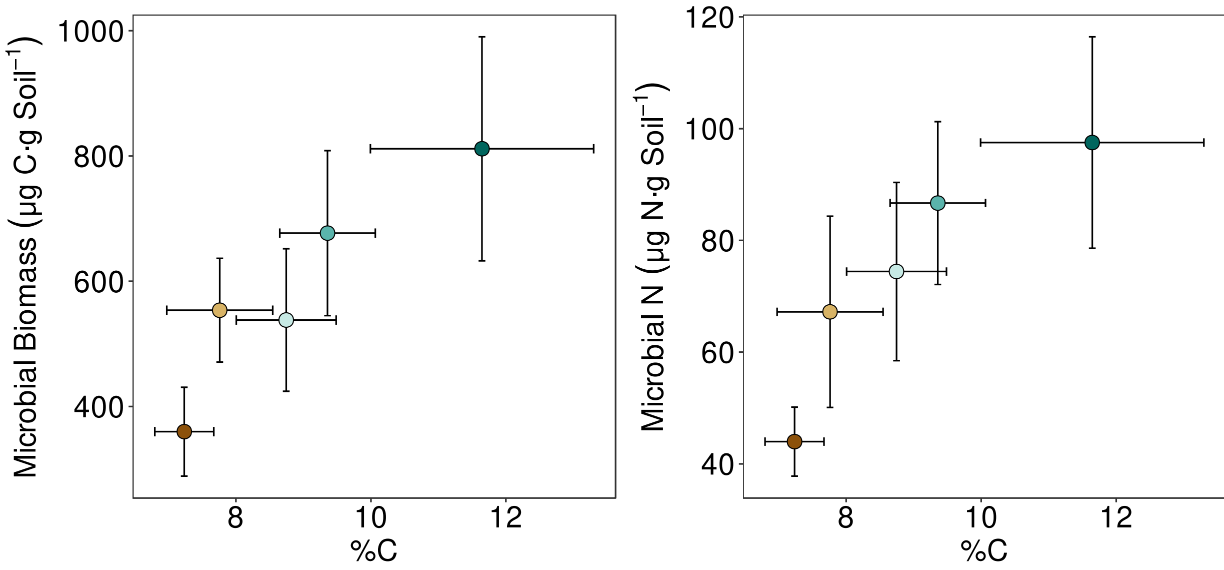

Figure 3.7. Relationship between Microbial biomass C (µg C/g soil) and N (µg N/g soil) concentrations per gram soil and percent soil C and N. A) Microbial C per gram of soil was positively correlated with mineral soil %C (p<0.01, r^2=0.46). B) Microbial N per gram soil was positively correlated with mineral soil %N (p<0.01, r^2=0.46). Means are represented by closed circles and vertical hashed lines indicate ±1 S.E. Shaded bands indicate 95% confidence intervals.

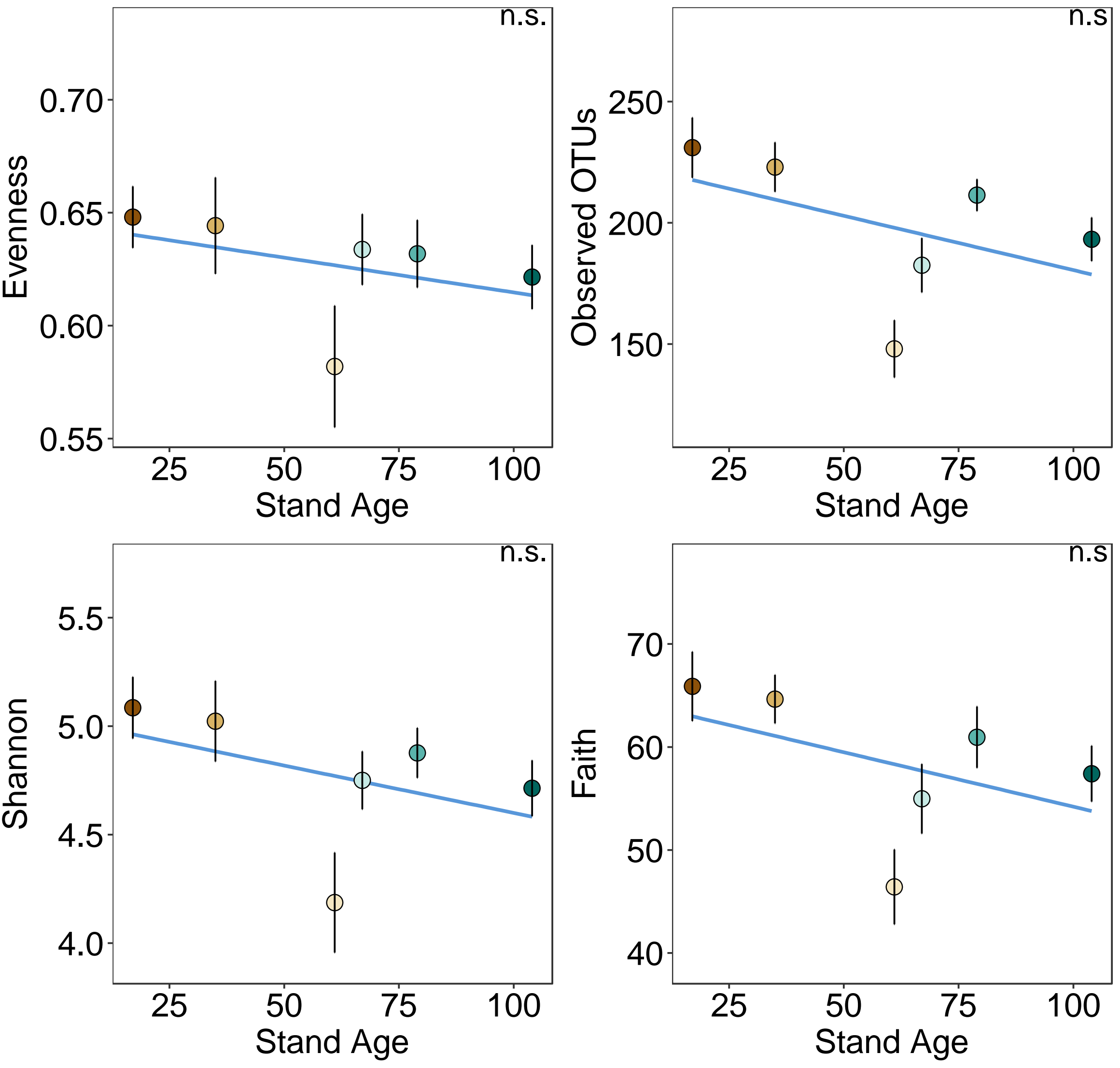

Figure 3.8. Diversity indices across varying age chronosequence stands from 17-year-old stands to 104 year old stands. Stand ages: 17 year (red), 35 year (orange), 61 year (yellow), 67 year (light blue), 79 (blue), 104 year (dark blue). The shaded band is the 95% confidence interval of the linear model.

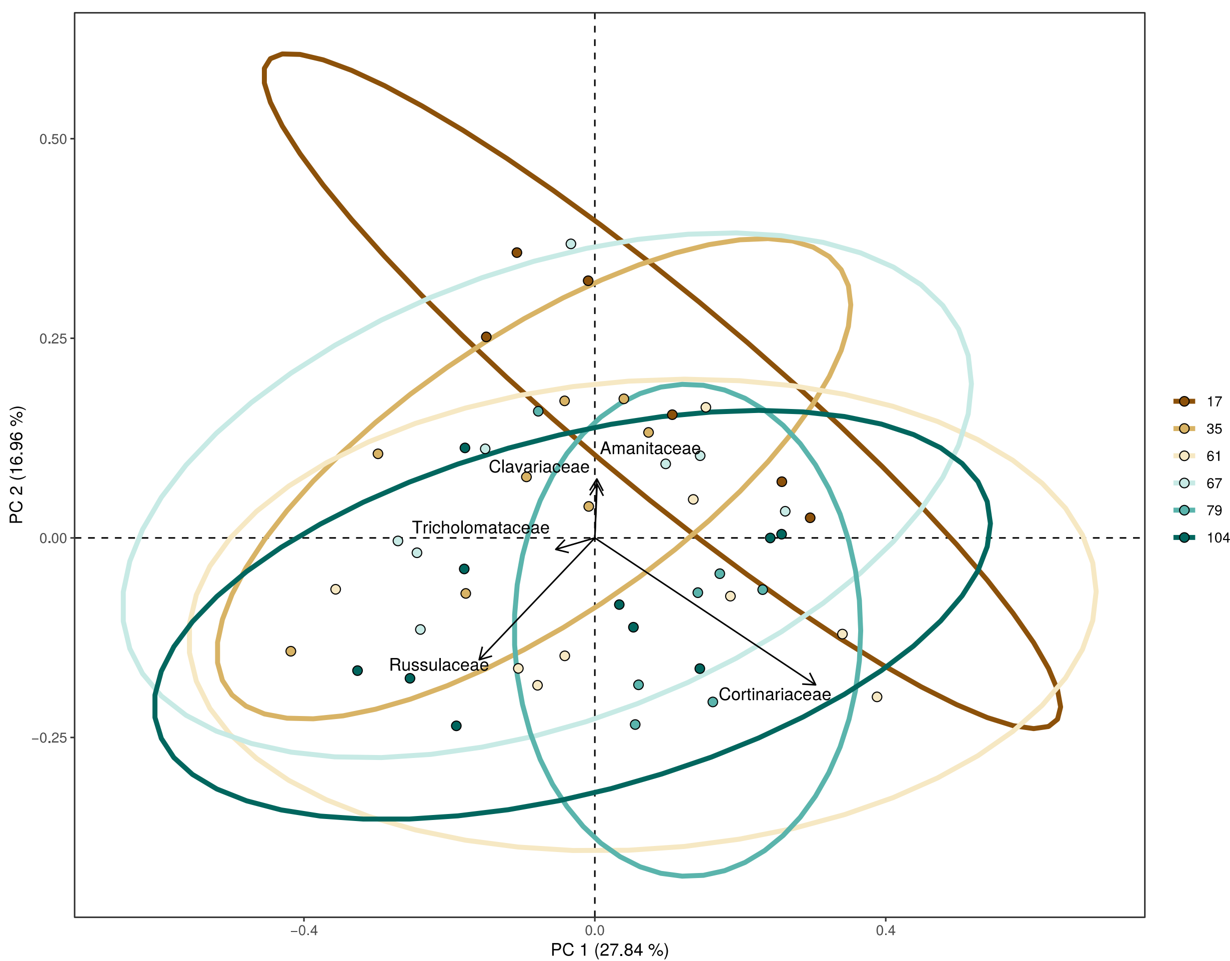

Figure 3.9. Principal coordinates analysis (PCoA) of fungal communities' bray-curtis abundances using ThetaYC calculator of dissimilarity in varying aged stands: 17 year (red), 35 year (orange), 61 year (yellow), 67 year (light blue), 79 (blue), 104 year (dark blue). A Mantel test indicated significant community differences based on disturbance severities ($p<0.05$). Ellipses signify a 95% confidence interval of distributions for the varying disturbances. The PCoA explains ~45% of the total variation in fungal community dissimilarity at the family level. The top 5 families responsible for community dissimilarity are represented by vectors.

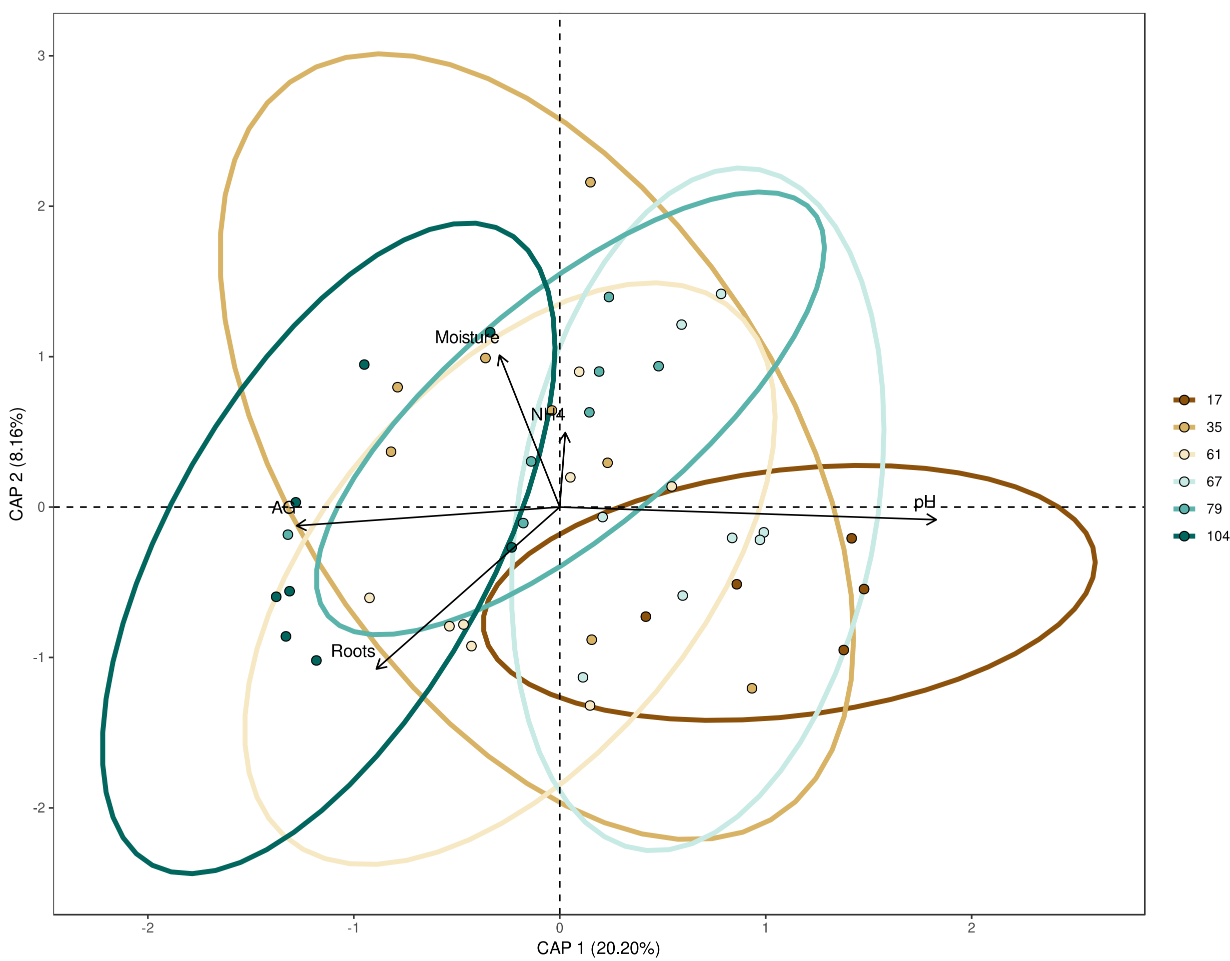

Figure 3.10. A dbRDA (distance base redundancy analysis) plot of the fungal communities along a 100+ year successional gradient of cut+burned disturbed stands and significant predictors as vectors.. Stand ages: 17 year (red), 35 year (orange), 61 year (yellow), 67 year (light blue), 79 (blue), 104 year (dark blue). Soil pH, alpha glucosidase (AG), roots g/m2 and moisture were identified as significant predictive variables of community dissimilarities from a forward-step regression. Ellipses signify a 95% confidence interval of distributions for the varying disturbances.

Chapter 4: Fungal Community Succession of *Populus grandidentata* (Bigtooth Aspen) During Wood Decomposition

4.1 Abstract

Studies indicate that the richness of wood-decay fungal communities explains much of the variation in the decay and respiration rates of coarse woody debris (CWD). I investigated how patterns of fungal decomposer communities in CWD differ across stages of decay, from standing dead trees to the incorporation of woody necromass into soil, and whether shifts in fungal community composition co-vary with substrate chemistry and co-occurring bacterial communities. While no differences were found in diversity or abundance, I show that fungal communities are dynamic in composition during and after the decay class continuum, colonizing while trees are standing and continuing to shift throughout CWD decay. Fungal communities and substrate chemistry of a decay class were most similar to neighboring stages of decay, suggesting gradual transitions as wood becomes decomposed and percent nitrogen increases. Additionally, I note that nitrogen is an important driver of enzymatic activity in microbial communities and show a correlation with bacterial and fungal species composition and abundance along the continuum of decay.

4.2 Introduction

Coarse woody debris (CWD), consisting of small to large branches, snags (dead trees that have not fallen to the forest floor) and downed logs, and in some forests composes ~ 20% of total forest carbon stocks (Boddy, 2001; Forrester et al., 2015). Natural mortality of early colonizing trees during successional shifts and species-specific pest/pathogenic disturbances may directly affect CWD production in forests (Brais et al., 2006; Gough et al., 2007). As forest succession progresses and as the severity and frequency of disturbances increase, the effects of decomposing standing dead wood and fallen CWD on ecosystem carbon (C) fluxes via respiration of CO2 may

also increase (Forrester et al., 2015; Schmid et al., 2016). Variations in C fluxes from CWD in forests can be large (Harmon et al., 2011) and wood decomposer community richness explains much of the variation in respiration and decay rates by microbial decomposers (e.g. Forrester et al., 2015; Toljander et al., 2006; Valentín et al., 2014).

Fungi function as the primary decomposers of CWD (van der Wal et al., 2013). Wood decay fungi are predominantly responsible for the breakdown of cellulose, hemicellulose and lignin, and subsequent respiration of 60-80% of the constituent C back to the atmosphere as CO_2 (Gonzalez-Perez et al., 2004). Fungi are particularly important in early stages of decay, when wood is low in moisture and high in quality. In contrast, bacterial communities can thrive in the later stages of wood decomposition, when moisture content is higher and access to carbohydrates and partially-degrade wood polymers is greater (Hu et al., 2017). Identifying the mechanisms that underlie the community structuring of microorganisms in CWD is important to the understanding of decomposer roles in ecosystem function and, in particular, C emissions from ecosystems (Kubartová et al., 2012).

Fungal succession within woody plant tissues begins prior to tree mortality and continues through incorporation of CWD into soil organic matter (SOM). Fungi can colonize and inhabit wood in bark, sap wood, and heart wood, acting as pathogens prior to tree death (Boddy, 2001; Harmon et al., 1986). These early colonizers may be important determinants of successional pathways in decomposer communities through priority effects and intraspecific competition (Fukami et al., 2010). As trees fall to the forest floor, additional decomposers arrive as spores from wind or via mycelial growth through soil networks (Holmer and Stenlid, 1996; Jönsson et al., 2008). In fallen logs, nutrients can then be translocated by fungal mycelial networks both into the wood and throughout the forest floor (Boddy, 2001; Boddy and Watkinson, 1994; Fricker et al., 2008). The establishment and growth of decomposers is influenced by factors such as wood chemistry, moisture content, and temperature, all of all of which determine community structure as shifts in wood chemistry and successional trajectories unfold in tandem (Boddy, 1983; Brischke and Rapp, 2008; Jonsson et al., 2005; Jönsson et al., 2008).

Wood consists mainly of cellulose and hemicellulose embedded in a lignin matrix, collectively termed lignocellulose. Breakdown of CWD relies on organisms capable of decomposing these structurally complex high-polymeric materials. Lignin content is a key initial determinant of breakdown rates and pathways because it can act as a physical barrier preventing enzymatic decomposition of the labile C contained in wood polysaccharides (Kirk and Cowling, 1984). Disintegration of this lignin-barrier primarily relies on extracellular enzymes that are produced by specific groups of fungi, mostly Basidiomycetes and a few Ascomycetes (Floudas et al., 2012). White rot fungi break down lignin and cellulose through production of laccases, lignin peroxidases, and other enzymes (Bonnarme and Jeffries, 1990; Hatakka, 1994). They are a particularly important assemblage, being the primary organisms that break down lignin. Brown rot fungi can also facilitate degradation of the lignocellulose complex non enzymatically through the production of hydroxyl radicals produced by Fenton chemistry (Arantes et al., 2012). The rates of lignin decay can have particularly important implications for long-term C storage in detritus and soils (Eisenlord et al., 2013).

Studying the relationship between decomposition and microbial communities in CWD can be difficult for a myriad of reasons. Due to its slow rate of decomposition, the temporal effects of CWD decay are particularly difficult to study (Harmon et al., 1986). Moreover, many studies of fungal community diversity in CWD use sporocarp assays or culturing to measure diversity. Metabarcoding enables more complete studies of fungal communities in logs during various stages of decomposition (Kubartová et al., 2012; Yuan et al., 2017). Knowing the identities of decomposing organisms, their diversity and abundance, and the enzyme suites they use to decompose CWD throughout the successional process is critical to improving understanding of how CWD is integrated into SOM.

The woody plant genus *Populus* can serve as an important model to study fungal succession given its widespread distribution throughout the Northern hemisphere and its role as a primary successional forest species (Marks, 1975; Reichle, 1981). The fungal disease ecology of living *Populus* spp. brought on by various conks and root rot fungi has been studied extensively (Brandt et al., 2003; Chapela, 1989; Wall and Kuntz, 1964), and several researchers have

examined the degradative effects of certain fungi in culture (Choi et al., 2006; Holmer et al., 1994; Reid, 1985). A recent study examining bacterial succession associated with decomposition in *Populus grandidentata* found community diversity increased as decomposition progressed peaking in the most highly decomposed logs (Kuramae et al., 2019). However, to date no study has examined fungal community succession and enzymatic productivity along a decompositional gradient of dead *Populus* logs.

I investigated fungal decomposer communities in the CWD of *Populus grandidentata* (bigtooth aspen) across successional stages of decomposition, from standing dead trees to soil. To understand the potential drivers of these successional patterns, I considered concurrent changes in moisture content, wood chemistry, and extracellular enzyme activity. I hypothesized that fungal community diversity would increase along the continuum of decay, with early dominance of heart- and white rot fungi, and that the enzyme profiles would shift from predominantly lignin breakdown toward greater rates of cellulose and hemicellulose depolymerization. I anticipated an increase in bacterial abundance as CWD decomposition progressed, and an overall increase in bacteria:fungi ratios. By comparing these results with our prior analysis of bacterial community structure (Kuramae et al., 2019), I was able to disentangle the relative importance of biotic versus abiotic drivers in structuring microbial community succession in wood decomposition.

4.3. METHODS

Study Site

The study was conducted at the University of Michigan Biological Station (UMBS), in northern Michigan, USA (45$_0$35’N 84$_0$43’W). Mean annual temperature is 5.5 $_o$C and mean annual precipitation is 817 mm. The forest is a mixed hardwood with canopy and understory species composition primarily composed of *Populus grandidentata* (bigtooth aspen), *Betula papyrifera* (paper birch), *Quercus rubra* (northern red oak), *Acer rubrum* (red maple), and *Pinus strobus* (eastern white pine). Maturing *Populus* are rapidly senescing and consequently are the primary constituent of CWD in this regionally representative ecosystem that emerged a century ago following widespread clear-cutting and fires (Gough et al. 2010). Ground flora consists of *Pteridium aquilinum, Gaultheria procumbens, Maianthemum canadense*, and *Vaccinium*

angustifolium. The study site is on a high-level sandy outwash plain and an adjacent gently sloping moraine (Lapin and Barnes, 1995; Pearsall, 1985). Soils are excessively well-drained Entic Haplorthods of the Rubicon series (Soil Survey Staff, 1991). The typical morphology of this series consists of Oi and Oe horizons 1-3 cm thick, a bioturbated A horizon 1-3 cm, an E horizon 10-15 cm thick, and Bs and BC horizons of sand with occasional gravel and cobble (Nave et al., 2014). Across all of these soils, approximately half of the fine root biomass is in the upper 20 cm of soil and the forest floor C mass is approximately 5-15 Mg C ha^{-1}.

Field Sampling

Sampling was conducted in a plot (60 m radius) encircling the US-UMB Ameriflux tower (Curtis et al., 2002). A total of 24 *P. grandidentata* trees were identified as either standing dead (SD) or assigned a decay class (DC) 1-5 (USDA Forest Service, 2018), with DC 1 representing freshly fallen trees with minimal visual decay, and DC 5 representing individuals in late stages of decay (Table C 1). Due to difficulty in identification of highly decayed logs, DC 5 logs were selected from a set of known *Populus grandidentata* logs that were part of a prior long-term CWD experiment. All downed logs included in the analyses were at least 3 m in length and were in direct contact with the forest floor.

A total of 9 sampling points along each log were identified (Figure 4.1). To locate these 9 points, 3 transects were established running perpendicular to the log, with the transects located ~0.5 m from each other and from either end of the log. Along each transect, wood samples were taken from three points located at the top of the log (360°) and points as close to 120 and 240 degrees as possible. Wood samples were collected using a flash sterilized 19-mm drill bit, and logs were drilled with bark intact. A similar protocol was followed for sampling standing dead trees with the center transect for sampling at diameter at breast height DBH (1.3 m), and additional transects 0.5 m above and below this point. Additionally, 3 soil samples were collected 30 m from plot center base at azimuths 120, 240, and 360. Oe and A horizons were collected at each soil sampling location, with depths ranging from 3-5 cm. All samples were kept in a cooler on ice in the field and during transport back to the lab.

Sample Processing

In the lab, 1 g of wood was removed from each drill point sample and refrigerated (4ºC) until enzyme assays could be completed (<1 week). The remainder of each drill point sample was lyophilized, and pre- and post-lyophilization mass difference used to determine relative moisture content. Once lyophilized, 1 gram from each of the 9 points drilled were compiled into a single 9 g sample for each of the 24 logs. The composite sample for each log was ball-ground using a SPEX Certiprep 8000D Mixer/Mill (Metuchen, NJ, USA). Samples were then stored at -80$_0$C until analyzed. Soils were sieved through a 2.0-mm screen to remove pebbles and coarse particulate organic matter. A 10-g subsample from each sieved sample was taken for enzyme assays and refrigerated (4ºC) prior to lyophilization of the remaining soil samples.

DNA Extraction and Amplicon Sequencing

Genomic DNA was extracted from frozen soil and composite wood samples using a PowerMax Soil DNA Isolation Kit (MoBio, Carlsbad, CA, USA) following the manufacturer’s instructions. Extraction quantity was determined using a Qubit fluorometer (Invitrogen, San Jose, CA, USA) and frozen (-80ºC) until PCR amplification was performed. Amplification of the ITS2 region was conducted using the forward primer ITS2_KYO1 (5’- CTHGGTCATTTAGAGGAASTAA) and reverse primer ITS4_KYO3 (5’-CTBTTVCCKCTTCACTCG) using HotStarTaq Plus Master Mix (Qiagen, Hilden Germany), following methods described by Toju et al. (2012). PCR products were assessed on a 2% agarose gel and stored (-80ºC) until shipment to MR DNA (Shallowater, TX, USA) for library construction and sequencing. Illumina libraries were constructed using the TruSeq DNA library preparation protocol (Illumina, San Diego, California, USA), purified using Ampure XP beads (Beckman Coulter, etc), and sequencing was performed on an Illumina MiSeq to generate paired-end reads (~300bp).

Quantitative PCR

Quantitative PCR (qPCR) was used to determine the relative abundance of bacteria and fungi in each genomic DNA sample. Bacterial assays targeted the *16S rRNA* gene using the 799F and 1193R primer pair (Kuramae et al., 2019). Reaction mixtures (15 µl) included 0.1 µM of each primer, 1.8 ng of template DNA, and SsoAdvanced™ Universal SYBR® Green Supermix

(BioRad, Hercules, CA, USA). Thermal cycling conditions were: 95^{0}C for 4 min followed by 40 cycles of 30 sec at 95^{0}C, 30 sec at 53^{0}C, and 60 sec at 72^{0}C, and then melt curve analysis to verify product size. Fungal qPCR reactions (15 µl) were performed as above but included 6 ng of template DNA and 0.75 µM each of the ITS2 KYO1 and ITS4 KYO3 primers. Reaction conditions were: 95^{0}C for 6 min followed by 35 cycles of 15 sec at 95^{0}C, 30 sec at 53.5^{0}C, and 45 sec at 72^{0}C. Three technical replicates were prepared for each sample for each assay, and data were analyzed with BioRad CFX Manager (Version 3.1). Standard curves were prepared using genomic DNA from *Escherichia coli* (ATCC strain 11775, obtained from the American Type Culture Collection (Manassas, VA, USA)) and *Saccharomyces cerevisiae* (ATCC strain S288c) for bacteria and fungi, respectively. All reaction efficiencies were >99.5% and all r^2 were >0.990. Results were standardized to the original dry mass of substrate (wood or soil) and are reported as gene copies g^{-1}.

Chemical Analyses

Chemical analyses were conducted to determine changes in substrate quality and nutrient content along the wood decomposition gradient and in soil. Percent C, N and $\delta^{15}N$ were analyzed on composite wood and soil samples at the University of Michigan Biological Station (Pellston, MI, USA) using a Costech ECS 4010 elemental analyzer (Valencia, CA, USA) coupled with a Thermo Scientific Delta Plus XP isotope ratio mass spectrometer (San Jose, CA, USA). Wood and soil pH was collected using a 0.2 g of composite, ball-ground material using a model 8000 pH meter (VWR Scientific, Radnor, PA, USA). Substrates were added to 15 mL of deionized water, incubated for 30 minutes at room temperature and equilibrated with a probe for 1 minute prior to taking each reading.

Extracellular Enzyme Activity (EEA)

For each log, a composite sample for enzyme analysis was created by combining 1 g of fresh wood from each of the 9 drill point samples. An aqueous extract was then prepared by combining 0.5 (± 0.05) g of homogenized material with 7.5 ml of sterile deionized water in a sterile 15-ml centrifuge tube. Tubes were placed horizontally on a shaker table at 160 rpm for 2 hours and then moved to the refrigerator to incubate overnight. The next morning, I measured

EEA associated with six enzymes (Table C 2) using microplate assays similar to Morrissey et al. (2014) and Neubauer et al. (2014). Hydrolytic enzymes were measured fluorometrically (360 nm excitation/460 nm emission) using 4-methylumbelliferone (MUB) or 7-amino-4-methylcoumarin (AMC) labeled substrates dissolved in ethylene glycol monomethyl ether (EGME) and brought up to desired concentration with MES (0.1 M, pH 6.1) or Trisma (50 mM, pH 7.8) buffer. Phenol oxidase and peroxidase activities were measured colorimetrically (460 nm, using an empirically determined micromolar extinction coefficient of 7.9 per µmol) with L-3,4-dihydroxyphenylalanine (L-DOPA, 6.5 mM) using sodium bicarbonate buffer (50 mM, pH 6.1). Potential peroxidase activity was determined by the difference between soil with L-DOPA + 0.3% H2O2 and L-DOPA. Artificial substrates and incubation times used were: 4-MUB-β-D-glycopyranoside (6 hours), 4-MUB-β-D-cellobioside (2 hours), 4-MUB-β-D-xylopyranoside (6 hours), L-Leucine-7-AMC (2 hours), 3,4-dihydroxy-L-phenylalanine (4 hours), and 3,4-dihydroxy-L-phenylalanine + H_2O_2 (4 hours). All substrates and reagents were obtained from Sigma-Aldrich Co. Ltd. (Saint Louis, MO, USA), and measurements were made using a Synergy 2 plate reader (Biotek, Winooski, VT, USA). Three technical replicates were prepared for each assay, blank, and quench curve, and means were used to determine per sample values. Enzymatic activities were calculated in international "units" (U; 1 U = 1000 mU = 16.67×10^{-9} kat) defined as the amount of enzyme that forms 1 µmol of product per minute under the assay conditions. Data were standardized to the original dry mass of substrate (wood or soil) and are reported as: mU g^{-1}.

Sequence Analyses

Fungal DNA sequences were processed for quality control using Mothur v.1.39.0 software (Schloss et al., 2009). Contigs were created with *make.contigs*, *check orient = true* since forward and reverse fastq files contained both forward and reverse reads. Forward and reverse raw fasta sequences were trimmed of primers and adapters and demultiplexed. All short sequences (<200 bp) with ambiguous bases were removed from downstream analyses. Clustering was performed with a cutoff of 97% similarity using *opticlust* to determine Operational Taxonomic Units (OTUs). Taxonomy was classified using the UNITE fungal database (Koljalg et al., 2013). Sequences with low total representation (<5 sequences across all samples) were removed from

the dataset. All samples were rarified to 69,143 sequences prior to community analyses, which represents the lowest sequence depth. Raw data were deposited in GenBank Short Read Archive under accession (numbers pending).

Alpha diversity within treatments was measured using Chao1, a non-parametric Shannon and inverse Simpson diversity indices in Mothur v1.39.0. Following rarefication, analyses of variance (ANOVA) were run in R to determine if alpha diversity indices changed significantly through decay. Beta-diversity was visualized with a principal coordinate analysis (PCoA) using Bray-Curtis distance matrices (Borcard et al., 2008). A between-class analysis (BCA) and Monte-Carlo test was applied to both the fungal community and substrate chemistry datasets to analyze variability explained by decay class. A suite of co-inertia analyses using the RV.rtest function of the *ade4* package (Dray and Dufour, 2007) were applied to examine co-variance between fungal community data, substrate chemistry, and a previously published bacterial data set from the same system (Kuramae et al., 2019). Analysis of molecular variance (AMOVA) was also used to test for significant population structure based on decay class (Schloss, 2008). Lastly, a dissimilarity percentage analysis (SIMPER) was conducted using the vegan package in R to test for contributions to community dissimilarity of taxa at the species and order taxonomic levels.

Additional Statistical Analysis

ANOVA was used to determine whether there were significant differences in wood chemistry across the decay classes and between CWD and soil, and Tukey's HSD test was used for *post-hoc* pairwise comparisons. Kruskal-Wallis tests followed by Dunn's *post hoc* comparisons were used for analogous comparisons of qPCR and enzyme data. To visualize overall patterns in enzyme activity, a PCoA was performed using the Bray-Curtis coefficient. Lastly, Pearson correlation analysis was used to identify significant relationships between environmental data and alpha diversity metrics, qPCR gene abundances, and enzyme activity rates. These analyses were all performed using the PAST statistical package version 3.20 (Hammer et al., 2001).

4.4 RESULTS

Fungal Community Structure

The quality filtered raw reads of fungi from wood and soil communities were assigned to a total of 1,848 fungal OTUs spanning most major fungal phyla and subphyla (Ascomycota, Basidiomycota, Mucoromycota, Chytridiomycota) (Figure C 1). Of these, 86.3%, 58.6%, 48.5% and 37.5% could be taxonomically assigned to phylum, order, family, and genus, respectively. Out of the 1,848 total OTUs, 26 accounted for 1% or more of total sequences across all samples, with only 6 accounting for more than 3%. The mean number of fungal OTUs per log was 588 (~ 106 s.d.). The number of fungal OTUs ranged from 809 in a DC 5 log to 371 in a DC1 log (Table C 3). There were a total of 6 and 59 OTUs that were only detected in SD and S, respectively. These SD-only and S-only OTUs were very rarely detected, with the most abundant of them representing 0.0035% of the total pooled communities.

Distinct community differences were detected between decay classes at both the species and order level. The 20 most abundant fungal OTUs and their tentative genera/species identification showed a predominance of wood decaying Agaricomycetes and yeasts (Table C 4). *Rigidoporus corticola* was the most abundant OTU overall, with several yeasts including an unclassified saccharomycetous yeast, *Scheffersomyces shehatae*, and *Rhodoturula lignophila* as the next most abundant species. These were predominantly represented in standing dead and early decay stage (DC1-DC2) samples. The Saccharomycetales was the most abundantly represented order followed by the Polyporales and Auriculariales. The Saccharomycetales, Hymenochaetales and Sporidiobolales were dominant orders in early decay stages (SD-DC2) while the Agaricales, Trichosporonales and mycorrhizal taxa in the Thelephorales and Russulales established in later decay stages (DC3-DC5) (Figure 4.2). The Polyporales were strongly represented in early decay stages and remained persistent members of successional communities until a precipitous drop off in member association in late decay stages (DC4-DC5). Despite these community differences an ANOVA on the alpha diversity indices showed no statistical difference between any decay class or between wood and soil (Figure C 3).

A PCoA analysis of the fungal communities shows a general clustering within decay class treatments, with late stages (DC4-5) separating from earlier stages. There was a general trend of decay class overlapping predominantly with neighboring classes, with the first two axes explaining 10% and 9% of the clustering, respectively (Figure C 4). A between class analysis (BCA) with the sole explanatory variable being decay stage explained 30% of total variability in fungal community composition along a gradient of decay (Figure 4.3) and showed similar clustering and overlap as observed in the PCoA. In both analyses, soil samples were distinctly separated from the CWD samples. I found significant structural differences of fungal communities between decay stages using an ANOVA ($p<0.01$). A Tukey *post-hoc* analysis revealed that all CWD samples were significantly different from the soil community (Table 4.1, all $p<0.028$). Within CWD samples, fungal communities in the early decay stage (SD) were distinctly different from communities in the later decay stages (DC3-DC5). Though pairwise comparisons were only significant for SD, it is worth noting that DC 1 showed a strong trend in differentiation in community structure from DC4 and DC5 ($p=0.053$ and $p=0.071$, respectively; Table 4.1). There were no significant differences between any of the remaining decay stages.

A SIMPER analysis of community dissimilarity revealed that, on average, ~75% of dissimilarity between soil and all decay class communities can be explained by differences in abundances of the top 10 taxonomic orders. However, between soil and DC5, the stage closest to incorporation into soil, only ~50% of dissimilarity could be explained by the top 10 contributors to community difference. Early stages of decay (i.e., SD, DC1) had a high diversity of Saccharomycetales and Hymenochaetales, differentiating them from later decay stages and soil. Taxa in these orders were responsible for ~23% of community dissimilarity between SD samples and logs from later decay stages (DC3-DC5). Saccharomycetales, being highly represented in DC1, accounted for ~25% of community dissimilarity between DC1 and both DC4 and DC5.

At the OTU level, most of the community dissimilarity between classes could be attributed to the most abundant OTUs of each decay class. The SIMPER analysis identified two OTUs assigned to *Russula* sp. and *Cortinarius* sp. that were found predominantly in soil and responsible for ~25% of community dissimilarity between soils and all stages of CWD. The top 10 species

across all soil vs wood comparisons accounted for ~ 57% of community dissimilarity, highlighting the marked differences between these communities. On average, the top 10 OTUs responsible for community dissimilarity between SD and DC3-5 accounted for 48% of differences. Six of these OTUs among the top 10 were found predominantly in SD (*Trichaptum biforme*, 2 unclassified Saccharomycetales, *Bjerkandera* sp., a *Pichia* sp. and an unclassified Hypocreaceae) and accounted for ~29% of community dissimilarity between SD and DC3-5. Similarly, in comparisons between DC1 and late decay stages DC4-5, 6 OTUs (an unclassified Saccharomycete, *Scheffersomyces shehatae*, *Rhodotorula lignophila*, *Pichia*, *Phanerochaete sordida*, and an unclassified fungus) were found predominantly in DC1 and accounted for ~34% of community dissimilarity.

Wood Chemical Composition and Fungal Succession

Chemical profiles for early decay stages (SD, DC1-2) were similar in makeup and were characterized by low %N, low moisture, high C:N ratios, and a low δ15N (Figure 4.4). As logs progressed through mid-decay (DC3) and toward later decay stages, I observed an increase in δ15N, %N, and moisture while C:N ratios begin to decline. An ANOVA and Tukey post-hoc pairwise test reveal significantly higher %N, δ15N and moisture and lower C:N in late stages of decay (DC4-5) relative to all earlier stages of decay. No differences in pH were detected between decay classes or wood and soil (mean across all samples: 4.99, standard error: 0.11). A BCA examining the effects of decay stage on wood chemical composition indicated that 65% of the variation is explained by decay class and showed clustering of DC4-DC5 separate from earlier decay classes (Figure 4.3B). Soil was distinctly different in chemical profiling than any decay class. Overall, diversity measures were positively correlated with increasing levels of %N (Figure 4.5).

Enzyme Activity

The enzyme assays revealed differences in the activity between decay stages, with the strongest treatment effects evident for enzymes that breakdown more complex organic polymers (Figure 4.6). A Kruskal-Wallis test revealed no differences in EEA associated with cellulose degradation (β-1,4-glucosidase and 1,4-β-cellobiosidase) across decay classes and soil, though there is a trend

toward higher activity in later decay stages, especially for 1,4-β-cellobiosidase in DC3-5. Enzymes used in the degradation of hemicellulose (β-D-xylosidase) and for N-acquisition from polypeptides (leucyl aminopeptidase) increased in activity in late stages relative to early decay stages (SD-DC2) and peaked in DC4-5 (Figure 4.6). For EEA associated with lignin breakdown, I saw two different patterns. Phenol oxidase (laccase) activity peaked in early stages of decay (SD-DC2) whereas peroxidase activity increased in the later stages (DC3-5). Overall, EEA was lowest in soils for all enzymes except for peroxidase. This is likely due to the low C content of soil (2-3%) relative to C content of wood (~45-50%). A PCoA using Bray Curtis metric showed three distinct clusters of enzyme profiles separating early decay stages (SD-DC2), late decay stages (DC3-DC4) and soil, with the first two axes accounting for ~65% of variation (Figure 4.6). Correlation analyses revealed a significant positive relationship between %N and the activity of all enzymes except phenol oxidase (Table C 5). In contrast, we found no significant correlations between any of the enzyme data and pH (all $|r|<0.39$ and all $p>0.06$) and only a few relationships with C:N (all $|r|<0.38$ and all $p>0.05$ except peroxidase ($r=-0.45$, $p=0.03$)) and $\delta_{15}N$ (all $|r|< 0.36$ and all $p>0.09$ except phenol oxidase ($r=-0.47$, $p=0.01$)). Correlations with moisture were not evaluated since data were normalized per g dry weight of material.

Fungal and Bacterial Interactions

Gene copy abundance (either ITS or *16S rRNA*) was used as a proxy to examine how fungal and bacterial population size changed with decay stage. Fungal gene abundance was consistently higher in CWD compared to soil, by 3-fold on average, but did not differ significantly across treatments (Figure 4.8A). This is in stark contrast to bacterial gene copies where Kruskal-Wallis followed by Dunn's *post hoc* tests revealed bacteria significantly increased in abundance with decomposition stage ($p=0.002$, Figure 4.8B). As a result, coincident with the increase in bacterial abundance, a gradual increase in the bacteria:fungi ratio was observed along the decomposition gradient. Bacteria:fungi ratios in wood were significantly lower than those of soil, a pattern that was driven in large part by the relatively lower abundance of fungi in soil samples. Within the CWD series, I found lower ratios in the early decay classes (SD, DC1-3) compared to the later ones (DC3-5). Correlation analysis revealed a significant positive relationship between fungal abundance and %N (Figure 4.9A), but no significant correlation with other chemical properties

(all $|r|<0.33$ and all $p>0.21$). For bacteria, a strong positive correlation with %N was also evident (Figure 4.9B).

To further assess fungal and bacterial community co-variation, I compared the fungal community composition data presented here with prior published bacterial data across this wood decomposition sequence (Kuramae et al., 2019). The suite of co-inertia analyses revealed that fungal and bacterial communities have a stronger co-variance with each other (0.66) than either does with substrate chemistry (0.51 and 0.52, respectively), suggesting a strong influence of fungal-bacterial interactions along the successional gradient of wood decay.

4.5 DISCUSSION

Our study provides an extensive taxonomic view of the complexity of, shifts in, and drivers of fungal community composition and associated chemical substrate changes along a successional continuum of *Populus grandidenta* wood decomposition, from standing dead trees to soil. While no differences in evenness, richness or fungal abundances were found along the woody decay gradient, I did detect changes in fungal community structure, enzyme activity and substrate chemistry. Notably, I found fungal and bacterial community succession (Kuramae et al, 2019) were closely coupled. Abiotic factors such as moisture content, %N, and δ15N tended to increase while C:N decreased in later decay stages, an indication of more porous, highly decomposed wood (Brischke and Rapp, 2008; Forrester et al., 2015). Shifts in these abiotic factors have been shown to correspond with shifts in growth rate and community composition of wood decay fungi (Blanchette, 1991). Importantly, in this study these associated changes in chemistry were accompanied by changes in fungal communities at various taxonomic levels.

At the phylum level, I found the greatest diversity was accounted for by the Basidiomycetes and Ascomycetes, with approximately 90% of OTUs at all stages of decay belonging to these two phyla. Early decay stages, particularly SD and DC1, had large abundances of ascomycetous fungi. Members of these early ascomycete-dominated communities belonged predominately to the Saccharomycetales, a group of saprotrophic yeasts (Suh et al., 2006). Similarly, much of the basidiomycete community in early decay stages consisted of non-filamentous yeasts, which were

also strongly represented in standing dead trees, illustrating early colonization and establishment by yeasts prior to felling.

Although both ascomycetous and basidiomycetous yeasts are commonly associated with the early stages of wood decay and can be isolated easily from bark (Hutchinson and Hiratsuka, 2019; Sampaio and Gonçalves, 2008), they are often considered non-wood decay fungi because they target simple sugars in the bark and callous tissue prior to colonization by filamentous basidiomycetous fungi that break down more recalcitrant woody substrates (Bhadra et al., 2008; Kurtzman et al., 2011). Colonization and establishment in early stages of decay is likely a combination of arrival via dispersal by wind and precipitation (Boddy, 2001), but their persistence through the decay process suggests a role in the decomposition process. These yeasts are restricted to localized decomposition in the portion of the log in which they become established and may assimilate nutrients made available from the decomposition of hyphae during early and mid stages of decay (Johnson et al., 2014).

Yeasts were accompanied in early stage dominance by sapwood- and heart rot species in the Hymenochaetales (e.g., the sapwood-rotter *Trichaptum biforme*) as well as white-rot taxa in the Polyporales. Our standing dead samples had a strong representation of asco- and basidomycetous yeasts as well as filamentous basidiomycetes from the Polyporales and Hymenochaetales. Both orders contain fungi important in colonization and decomposition of CWD prior to the dead fall of standing boles. Hymenochaetales contains species that saptrophically decay dead wood following tree mortality as well as species that cause heart rot in living trees (Larsson and Fischer, 2018). Invasion and early decomposition of SD boles by wood rotters in the Polyporales and Hymenochaetales may facilitate colonization by Saccharomycetes. Although the yeasts and wood decay fungi may be spatially separated, yeasts can establish in tracheid cell walls that have been previously decayed by basidiomycetous hyphae (Blanchette & Shaw, 1978). One mechanism for this facilitation is the use of phenol oxidases by white-rots to degrade lignin compounds that, in turn, free up more readily degradable compounds like cellulose and xylan. Our study confirms high phenol oxidase activity early in the decay process, particularly in standing dead wood and logs from DC1 and 2.

As *Populus* logs transition into mid-successional decay stages, I detected a shift in saprotrophic communities, marked by a precipitous decline in representation of yeasts in the Saccharomycetales and Sporidiobolales, while species in the Polyporales maintain abundance in DC3. This decline in ascomycetous yeasts may be due, at least in part, to increased competition with basidiomycetes that out compete them for substrates through mycelial growth and expiration of simple sugars readily available in early decay (Fricker et al., 2008). Trending with the Saccharomycetales, there is decline in the Hymenochaetales order compared to DC1 and SD logs, emphasized by the disappearance of *Trichaptum biforme*, a degrader of lignin and one of the most abundant taxa in early decay stages. *Trichaptum biforme* may be outcompeted in later successional stages of decay as its primary substrate, sap wood, declines rapidly with increasing decay.

In late decay stages (DC4-DC5), taxa that were previously dominant begin to be replaced by the functionally diverse Agaricales that are often associated with wood decay, along with several ectomycorrhizal taxa (Russulales and Thelephorales) and basidiomycetous yeasts in the Trichosporonales. Members of the Trichosporonales have been shown to be abundant in both litter and soil where they likely degrade hemicelluloses and produce antifungal cellobiose lipids that can suppress the growth of other yeast species (Mašínová et al., 2017; Middelhoven et al., 2001; Yurkov et al., 2012). An increase in ectomycorrhizal fungi (ECM) in late decay stages is likely driven through foraging of this guild for the N-rich substrate made available by the decomposer community (Averill, 2016; Zak et al., 2019). It is of great interest to understand the role ECM fungi play in the wood decay process, given that they lack most enzymes for wood decomposition (Pellitier and Zak, 2018).

A shift in wood chemistry accompanies community changes in later stages of decay with sharp increases in %N and moisture during decay stages 4 and 5, a product of fungal and bacterial processing of wood in prior successional stages (Brischke and Rapp, 2008). The progressive enrichment of CWD nitrogen that I observed is consistent with previous findings, a result of mycelial networks importing N from surrounding soils to aid in further degradation of C

compounds remaining in logs (Fricker et al., 2008). The elevated %N in wood was also positively correlated with an increase in the activity of all enzymes except phenol oxidase (Table C 5). This drop-off in phenol oxidase activity is concurrent with that of polypore abundances, underpinning the C recalcitrance bottleneck that white-rot fungi overcome, with the end result of making the labile C and N components of lignocellulose accessible to other decomposers.

The observed changes in fungal taxa with decay class are consistent with the extracellular enzyme assays I performed, as white-rot taxa representation and phenol oxidase activity both declined in later stages of decay. Interestingly, as lignin is broken down in early decay stages, the activities of all of the hydrolytic enzymes I measured increased with decay class, such as those targeting the more readily degradable hemicellulose molecules ($p=0.01$). This could correspond to changes in the fungal community composition and shifts in taxa associated with polysaccharide degradation. Alternatively, because the ability to decompose cellulose and hemicellulose is also common in bacteria, this shift could reflect increased bacterial contributions to the extracellular enzyme pool in dead wood. Supporting this, I found a strong positive correlation between total bacterial abundance (*16S* gene copies via qPCR) and EEA associated with breakdown of cellulose (β-1,4-glucosidase: $r=0.68$, $p<0.001$; 1,4- β-cellobiosidase: $r=0.78$, $p<0.001$), hemicellulose (β-D-xylosidase: $r=0.57$, $p=0.004$), and polypeptides (Leucyl aminopeptidase: $r=0.77$, $p<0.001$) across the wood decay sequence (i.e., excluding soil samples). Moreover, the strong correlation of bacterial and fungal communities that I observed suggest strongly that the two groups work in concert during the decay process. This likely involves inter-domain consortia of heterotrophic organisms that utilize each other's secreted enzymes (Johnston et al., 2016). The relative contribution of bacteria versus fungi to hydrolytic enzyme production in deadwood is poorly understood and will require additional study using proteomic and transcriptomic approaches to resolve.

I observed stark differences between wood (SD-DC5) and soil fungal communities and how they relate to bacterial changes previously reported (Kuramae et al., 2019). These differences in fungal communities are not surprising given the large contrasts in soil and wood chemical composition, with soil substrates containing less than half the standard moisture levels and an

order of magnitude less %C. Soils in our study also had a much higher $\delta^{15}N$ values indicating internal cycling of N in soil substrates, as the enzymes of fungal decomposers discriminate against the heavier ^{15}N isotope. Interestingly, $\delta^{15}N$ values increased from DC1 through DC5, becoming more similar to soil as decay proceeds. This suggests a translocation of isotopically heavier soil N from soil to CWD throughout the decay sequence. Soil was also starkly different than CWD in bacteria:fungi ratios, as soils had both much lower abundance of fungi and much greater abundance of bacteria than any of the wood samples. These ratios are likely driven by differences in both the quantity (<5% in soil compared to >47% in wood) and quality of C substrates. Though not measured in this study, the latter may be particularly important since bacteria and fungi may not mineralize the same forms of C substrate (Bossuyt et al., 2001; Lauber et al., 2008; Six et al., 2006).

In conclusion, our research fills a critical knowledge gap, demonstrating that fungal community structure changes substantially and in a functionally predictable way over the course of wood decomposition despite no changes in overall diversity. I show that fungal communities are dynamic before, during, and after the decay class continuum, colonizing while trees are still standing and continuing to shift throughout incorporation into SOM. I documented a shift in community composition from early decay stages (SD to DC1-2), which are dominated by non-filamentous yeasts, heart-rot fungi and white-rot fungi, to late-successional stage communities with strong representations of ectomycorrhizal taxa and yeasts typically found in leaf litter and soil. Additionally, I note that N is a particularly important driver of enzymatic activity in fungal and bacterial communities and highlight the importance of bacteria:fungi interactions along a continuum of decay. Finally, I emphasize the need for further studies examining both the relative contributions of bacteria vs fungi in hydrolytic enzyme activity and available C compounds in late stages of decay to further disentangle fungal and bacterial contributions to decay.

4.6 References

Averill, C., 2016. Slowed decomposition in ectomycorrhizal ecosystems is independent of plant chemistry. Soil Biol. Biochem. 102, 52–54. https://doi.org/10.1016/j.soilbio.2016.08.003

Bhadra, B., Rao, R.S., Singh, P.K., Sarkar, P.K., Shivaji, S., 2008. Yeasts and yeast-like fungi associated with tree bark: Diversity and identification of yeasts producing extracellular endoxylanases. Curr. Microbiol. 56, 489–494. https://doi.org/10.1007/s00284-008-9108-x

Blanchette, R.A., 1991. Delignification by wood-decay fungi. Annu. Rev. Phytopathol. 29, 381–398.

Boddy, L., 2001. Oikos Editorial Office Fungal Community Ecology and Wood Decomposition Processes in Angiosperms : From Standing Tree to Complete Decay of Coarse Woody Debris processes decomposition ecology and wood Fungal community coarse tree to in angiosperms : from st. Ecol. Bull. 43–56. https://doi.org/10.2307/20113263

Boddy, L., 1983. Microclimate and moisture dynamics of wood decomposing in terrestrial ecosystems. Soil Biol. Biochem. 15, 149–157. https://doi.org/10.1016/0038-0717(83)90096-2

Boddy, L., Watkinson, S., 1994. Wood decomposition, higher fungi, and their role in nutrient redistribution. Can. J. Bot. 73.

Bonnarme, P., Jeffries, T.W., 1990. Mn (II) Regulation of Lignin Peroxidases and Manganese-Dependent Peroxidases from Lignin-Degrading White Rot Fungi Mn (II) Regulation of Lignin Peroxidases and Manganese-Dependent Peroxidases from Lignin-Degrading White Rot Fungi. Appl. Environ. Microbiol. 56, 210–217.

Borcard, D., Gillet, F., Legendre, P., 2008. Numerical Ecology With R !, Applied Spatial Data Analysis with R. Springer. https://doi.org/10.1007/978-0-387-78171-6

Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R., Paustian, K., 2001. Influence of microbial populations and residue quality on aggregate stability. Appl. Soil Ecol. 16, 195–208. https://doi.org/10.1016/S0929-1393(00)00116-5

Brais, S., Paré, D., Lierman, C., 2006. Tree bole mineralization rates of four species of the Canadian eastern boreal ... Can. J. For. Res. 36.

Brandt, J.P., Cerezke, H.F., Mallett, K.I., Volney, W.J.A., Weber, J.D., 2003. Factors affecting trembling aspen (Populus tremuloides Michx.) health in the boreal forest of Alberta, Saskatchewan, and Manitoba, Canada. For. Ecol. Manage. 178, 287–300. https://doi.org/10.1016/S0378-1127(02)00479-6

Brischke, C., Rapp, A.O., 2008. Dose-response relationships between wood moisture content, wood temperature and fungal decay determined for 23 European field test sites. Wood Sci. Technol. 42, 507–518. https://doi.org/10.1007/s00226-008-0191-8

Chapela, I.H., 1989. Fungi in healthy stems and branches of American beech and aspen: a comparative study. New Phytol. 113, 65–75. https://doi.org/10.1111/j.1469-8137.1989.tb02396.x

Choi, J.W., Choi, D.H., Ahn, S.H., Lee, S.S., Kim, M.K., Meier, D., Faix, O., Scott, G.M., 2006. Characterization of trembling aspen wood (Populus tremuloides L.) degraded with the white rot fungus Ceriporiopsis subvermispora and MWLs isolated thereof. Holz als Roh - und Werkst. 64, 415–422. https://doi.org/10.1007/s00107-006-0133-9

Curtis, P.S., Hanson, P.J., Bolstad, P., Barford, C., Randolph, J.C., Schmidt, H.P., Wilson, K.B., D., 2002. Biometric and eddy covariance based estimates of annual carbon storage in five eastern North American deciduous forests. Agric. For. Meteorol. 113, 3–19.

Dray, S., Dufour, A.., 2007. Ade4: Analysis of Ecological Data. Explor. Euclidean Methods Environ. Sci. 22.

Eisenlord, S.D., Freedman, Z., Zak, D.R., 2013. Microbial Mechanisms Mediating Increased Soil C Storage under Elevated Atmospheric N deposition. Appl. Environ. Microbiol. 79, 1191–1199. https://doi.org/10.1128/AEM.03156-12

Floudas, D., Binder, M., Riley, R., Barry, K., Blanchette, R., Henrissat, B., Martínez, A.T., Otillar, R., Spatafora, J.W., Yadav, J.S., Coutinho, P.M., Vries, R.P. De, Ferreira, P., Findley, K., Foster, B., 2012. Lignin Decomposition Reconstructed from 31 Fungal Genomes. Science (80-.). 336, 1715–1719.

Forrester, J.A., Mladenoff, D.J., D'Amato, A.W., Fraver, S., Lindner, D.L., Brazee, N.J., Clayton, M.K., Gower, S.T., 2015. Temporal trends and sources of variation in carbon flux from coarse woody debris in experimental forest canopy openings. Oecologia 179, 889–900. https://doi.org/10.1007/s00442-015-3393-4

Fricker, M.D., Bebber, D., Boddy, L., 2008. Mycelial Networks : Structure and Dynamics. Ecol. Saprotrophic Basidiomycetes 3–18. https://doi.org/10.1016/S0275-0287(08)80003-3

Fukami, T., Dickie, I.A., Paula Wilkie, J., Paulus, B.C., Park, D., Roberts, A., Buchanan, P.K., Allen, R.B., 2010. Assembly history dictates ecosystem functioning: Evidence from wood decomposer communities. Ecol. Lett. 13, 675–684. https://doi.org/10.1111/j.1461-0248.2010.01465.x

Gonzalez-Perez, J., Gonzalez-Vila, F., Almendros, G., Knicker, H., 2004. The effect of fire on soil organic matter-- a review. Environ. Int. 30, 855–870.

Gough, C.M., Vogel, C.S., Kazanski, C., Nagel, L., Flower, C.E., Curtis, P.S., 2007. Coarse woody debris and the carbon balance of a north temperate forest. For. Ecol. Manage. 244, 60–67. https://doi.org/10.1016/j.foreco.2007.03.039

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. Paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4, 9.

Harmon, M.E., Bond-Lamberty, B., Tang, J., Vargas, R., 2011. Heterotrophic respiration in disturbed forests: A review with examples from North America. J. Geophys. Res. 116, G00K04. https://doi.org/10.1029/2010JG001495

Harmon, M.E., Franklin, J.F., Swanson, F.J., Sollins, P., Gregory, S. V., Lattin, J.D., Anderson, N.H., Cline, S.P., Aumen, N.G., Sedell, J.R., Lienkaemper, G.W., Cromack, K., Cummins, K.W., 1986. Ecology of Coarse Woody Debris in Temperate Ecosystems. Adv. Ecol. Res. https://doi.org/10.1016/S0065-2504(03)34002-4

Hatakka, A., 1994. Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. FEMS Microbiol. Rev. 13, 125–135. https://doi.org/10.1111/j.1574-6976.1994.tb00039.x

Holmer, L., Nitare, L., Stenlid, J., 1994. Population structure and decay pattern of Phellinus tremulae in Populus tremula as determined by somatic incompatibility. Can. J. Bot. 72, 1391–1396. https://doi.org/10.1139/b94-171

Holmer, L., Stenlid, J., 1996. Diffuse competition for heterogeneous substrate in soil among six species of wood-decomposing basidiomycetes. Oecologia 106, 531–538. https://doi.org/10.1007/BF00329712

Hu, Z., Xu, C., McDowell, N.G., Johnson, D.J., Wang, M., Luo, Y., Zhou, X., Huang, Z., 2017. Linking microbial community composition to C loss rates during wood decomposition. Soil Biol. Biochem. 104, 108–116. https://doi.org/10.1016/j.soilbio.2016.10.017

Hutchinson, L.J., Hiratsuka, Y., 2019. Some Wood-Inhabiting Yeasts of Trembling Aspen (Populus tremuloides) from Alberta and Northeastern British Columbia Author (s): Leonard J . Hutchison and Yasuyuki Hiratsuka Stable URL : https://www.jstor.org/stable/3760569 Some wood-inhabiting yeasts. Mycologia 86, 386–391.

Johnson, C.E., Siccama, T.G., Denny, E.G., Koppers, M.M., Vogt, D.J., 2014. In situ decomposition of northern hardwood tree boles: decay rates and nutrient dynamics in wood and bark. Can. J. For. Res. 44, 1515–1524. https://doi.org/10.1139/cjfr-2014-0221

Johnston, S.R., Boddy, L., Weightman, A.J., 2016. Bacteria in decomposing wood and their interactions with wood-decay fungi. FEMS Microbiol. Ecol. 92, 1–12. https://doi.org/10.1093/femsec/fiw179

Jonsson, B.G., Kruys, N., Ranius, T., 2005. Ecology of species living on dead wood - Lessons for dead wood management. Silva Fenn. 39, 289–309.

Jönsson, M.T., Edman, M., Jonsson, B.G., 2008. Colonization and extinction patterns of wood-decaying fungi in a boreal old-growth Picea abies forest. J. Ecol. 96, 1065–1075. https://doi.org/10.1111/j.1365-2745.2008.01411.x

Kirk, T.K., Cowling, E.B., 1984. Biological Decomposition of Solid Wood. American Chemical Society. https://doi.org/10.1021/ba-1984-0207.ch012

Koljalg, U., Nilsson, R.H., Taylor, A.F.S., Bates, S.T., Thomas, D., Bengtsson-palme, J., Callaghan, T.M., Douglas, B., Griffith, G.W., Ucking, R.L., Suija, A.V.E., Taylor, D.L.E.E., Teresa, M., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277.

Kubartová, A., Ottosson, E., Dahlberg, A., Stenlid, J., 2012. Patterns of fungal communities among and within decaying logs, revealed by 454 sequencing. Mol. Ecol. https://doi.org/10.1111/j.1365-294X.2012.05723.x

Kuramae, E.E., Leite, M.F., Suleiman, A.K., Gough, C.M., Castillo, B., Faller, L., Franklin, R., Syring, J., 2019. Wood Decay Characteristics and Interspecific Interactions Control Bacterial Community Succession in Populus grandidentata (Bigtooth Aspen). Front. Microbiol.

Kurtzman, C.P., Fell, J.W., Boekhout, T., 2011. The Yeasts: A Taxonomic Study. Volume 1., 5th ed. Elsevier Ltd.

Lapin, M., Barnes, B. V, 1995. Society for Conservation Biology Using the Landscape Ecosystem Approach to Assess Species and Ecosystem Diversity Using the Landscape Ecosystem Approach to Assess Species and Ecosystem Diversity. Conserv. Biol. 9, 1148–1158.

Larsson, K., Fischer, M., 2018. Hymenochaetales : A Molecular Phylogeny for the Hymenochaetoid Clade Author (s): Karl-Henrik Larsson , Erast Parmasto , Michael Fischer , Ewald Langer , Karen K . Nakasone and Scott A . Redhead Source : Mycologia , Vol . 98 , No . 6 , A Phylogeny for Ki 98, 926–936.

Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol. Biochem. 40, 2407–2415. https://doi.org/10.1016/j.soilbio.2008.05.021

López-Mondéjar, R., Zühlke, D., Becher, D., Riedel, K., Baldrian, P., 2016. Cellulose and hemicellulose decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems. Sci. Rep. 6, 1–12. https://doi.org/10.1038/srep25279

Marks, P.L., 1975. On the Relation between Extension Growth and Successional Status of Deciduous Trees of the Northeastern United States. Bull. Torrey Bot. Club 102, 172–177.
Mašínová, T., Bahnmann, B.D., Větrovský, T., Tomšovský, M., Merunková, K., Baldrian, P., 2017. Drivers of yeast community composition in the litter and soil of a temperate forest. FEMS Microbiol. Ecol. 93, 1–10. https://doi.org/10.1093/femsec/fiw223
Middelhoven, W.J., Scorzetti, G., Fell, J.W., 2001. Trichosporon porosum comb. nov., an anamorphic basidiomycetous yeast inhabiting soil, related to the loubieri/laibachii group of species that assimilate hemicelluloses and phenolic compounds. FEMS Yeast Res. 1, 15–22. https://doi.org/10.1016/S1567-1356(00)00002-7
Nave, L.E., Sparks, J.P., Le Moine, J., Hardiman, B.S., Strahm, B.D., Curtis, P.S., 2014. Changes in soil nitrogen cycling in northern temperate forest ecosystem during succession. Biogeochemistry 121, 471–488. https://doi.org/10.1007/s
Pearsall, D.R., 1985. Landscape ecosystems of the University of Michigan Biological Station: ecosystem diversity and ground-cover diversity. University of Michigan, Ann Arbor.
Pellitier, P.T., Zak, D.R., 2018. Ectomycorrhizal fungi and the enzymatic liberation of nitrogen from soil organic matter: why evolutionary history matters. New Phytol. 217, 68–73. https://doi.org/10.1111/nph.14598
Reichle, D.E., 1981. Forest Succession: Conepts and Application, 1st ed. Springer Berlin Heidelberg, New York.
Reid, I.D., 1985. Biological delignification of aspen wood by solid-state fermentation with the white-rot fungus Merulius tremellosus. Appl. Environ. Microbiol. 50, 133–139.
Romaní, A.M., Fischer, H., Mille-Lindblom, C., Tranvik, L.J., 2006. Interactions of bacteria and fungi on decomposing litter: Differential extracellular enzyme activities. Ecology 87, 2559–2569. https://doi.org/10.1890/0012-9658(2006)87[2559:IOBAFO]2.0.CO;2
Sampaio, J.P., Gonçalves, P., 2008. Natural populations of Saccharomyces kudriavzevii in Portugal are associated with Oak bark and are sympatric with S. cerevisiae and S. paradoxus. Appl. Environ. Microbiol. 74, 2144–2152. https://doi.org/10.1128/AEM.02396-07
Schloss, P.D., 2008. Evaluating different approaches that test whether microbial communities have the same structure. ISME J. 2, 265–275. https://doi.org/10.1038/ismej.2008.5
Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09
Schmid, A. V., Vogel, C.S., Liebman, E., Curtis, P.S., Gough, C.M., 2016. Coarse woody debris and the carbon balance of a moderately disturbed forest. For. Ecol. Manage. 361, 38–45. https://doi.org/10.1016/j.foreco.2015.11.001
Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci. Soc. Am. J. 70, 555–569. https://doi.org/10.2136/sssaj2004.0347
Soil Survey Staff, 1991. Soil Survey of Cheboygan, Michigan.
Suh, S.-O., Blackwell, M., Kurtzman, C.P., Lachance, M.-A., 2006. Phylogenetics of Saccharomycetales, the ascomycete yeasts. Mycologia 98, 1006–1017. https://doi.org/10.3852/mycologia.98.6.1006

Toju, H., Tanabe, A.S., Yamamoto, S., Sato, H., 2012a. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PLoS One 7. https://doi.org/10.1371/journal.pone.0040863

Toju, H., Tanabe, A.S., Yamamoto, S., Sato, H., 2012b. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PLoS One. https://doi.org/10.1371/journal.pone.0040863

Toljander, Y.K., Lindahl, B.D., Holmer, L., Högberg, N.O.S., 2006. Environmental fluctuations facilitate species co-existence and increase decomposition in communities of wood decay fungi. Oecologia 148, 625–631. https://doi.org/10.1007/s00442-006-0406-3

USDA Forest Service, ., 2018. Forest Inventory and Analysis National Core Field Guide. Volume I: Field Data Collection Procedures.

Valentín, L., Rajala, T., Peltoniemi, M., Heinonsalo, J., Pennanen, T., Mäkipää, R., 2014. Loss of diversity in wood-inhabiting fungal communities affects decomposition activity in Norway spruce wood. Front. Microbiol. 5, 1–11. https://doi.org/10.3389/fmicb.2014.00230

van der Wal, A., Geydan, T.D., Kuyper, T.W., de Boer, W., 2013. A thready affair: linking fungal diversity and community dynamics to terrestrial decomposition processes. FEMS Microbiol. Rev. 37, 477–494. https://doi.org/10.1111/1574-6976.12001

Wall, R.E., Kuntz, J.E., 1964. Water-soluble substances i n dead branches o f aspen (populus tremuloides michx.) and t h e i r effects on fomes igniarius1 r. e. wall. Can. J. Bot. 42, 969–997.

Yuan, J., Zheng, X., Cheng, F., Zhu, X., Hou, L., Li, J., Zhang, S., 2017. Fungal community structure of fallen pine and oak wood at different stages of decomposition in the Qinling Mountains, China. Sci. Rep. 7, 1–11. https://doi.org/10.1038/s41598-017-14425-6

Yurkov, A.M., Kemler, M., Begerow, D., 2012. Assessment of yeast diversity in soils under different management regimes. Fungal Ecol. 5, 24–35. https://doi.org/10.1016/j.funeco.2011.07.004

Zak, D.R., Pellitier, P.T., Argiroff, W.A., Castillo, B., James, T.Y., Nave, L.E., Averill, C., Beidler, K. V., Bhatnagar, J., Blesh, J., Classen, A.T., Craig, M., Fernandez, C.W., Gundersen, P., Johansen, R., Koide, R.T., Lilleskov, E.A., Lindahl, B.D., Nadelhoffer, K.J., Phillips, R.P., Tunlid, A., 2019. Exploring the role of ectomycorrhizal fungi in soil carbon dynamics. New Phytol. 223, 33–39. https://doi.org/10.1111/nph.15679

4.7 Tables & Figures

Table 4.1. Analysis of Molecular Variance (AMOVA) of fungal community composition and structure between soil and wood decay samples (overall $p<0.01$). Significant ($p<0.05$) post-hoc pairwise comparisons are identified with * and marginally significant trends ($0.05<p<0.10$) are marked as **.

Pair	**p-value**
Soil X SD	*0.028
Soil X DC1	*0.021
Soil X DC2	*0.022
Soil X DC3	*0.023
Soil X DC4	*0.027
Soil X DC5	*0.026
SD X DC1	0.753
SD X DC2	0.115
SD X DC3	*0.030
SD X DC4	*0.042
SD X DC5	0.026
DC1 X DC2	0.583
DC1 X DC3	0.321
DC1 X DC4	**0.059
DC1 X DC5	**0.082
DC2 X DC3	0.773
DC2 X DC4	0.613
DC2 X DC5	0.622
DC3 X DC4	0.748
DC3 X DC5	0.221
DC4 X DC5	0.884

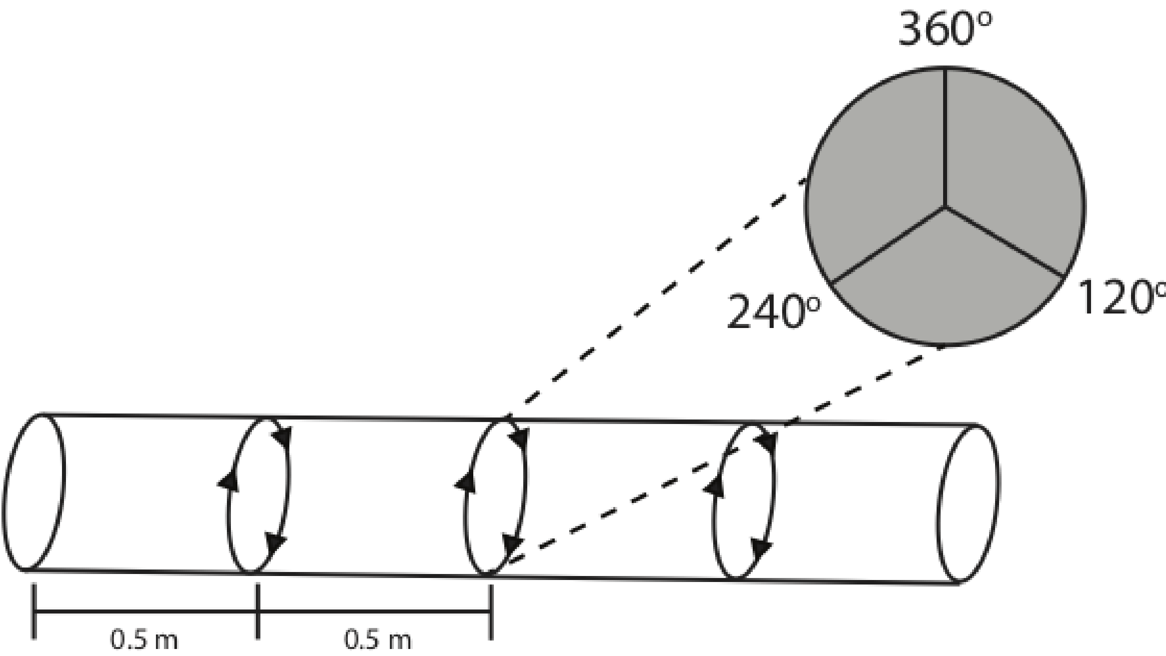

Figure 4.1: Logs were sampled in boles of *Populus grandidentata* at 9 points. First, 3 transects running perpendicular to the length of the log were found, ~0.5 m from each other or from the end of the log. Then 3 drilling points were identified along each transect (at 360 (top of the log), 120, and 240 degrees) from which to collect samples. The 9 samples were composited for community and chemistry analyses.

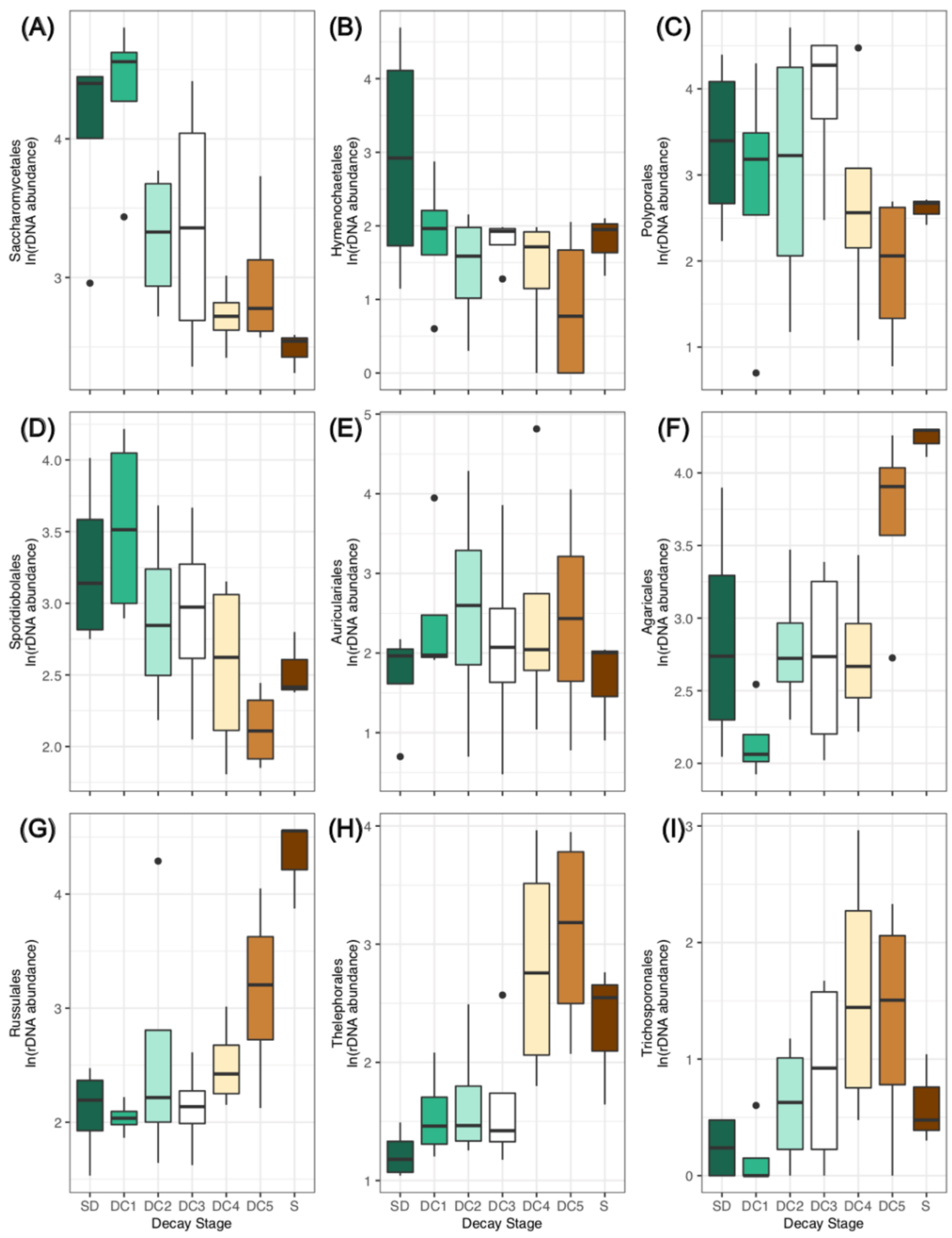

Figure 4.2: Fungal orders with groups that peak across varying decay stages and soil. Panes ABCD are orders with known wood decay fungi or yeasts that are most abundant in early decay stages and decline during successional decay stages. Auriculariales (Pane E) showed consistent representation across decay classes and soil. Panes FGHI represent orders of fungi that peaked in late decay stages. Whiskers represent maximum and minimum values (non-outlier range) and black dots indicate outliers. (SD=standing dead, DC1=decay class 1, DC2=decay class 2, DC3=decay class 3, DC4=decay class 4, DC5=decay class 5). N=27 (4 per decay stage of log, 3 soil).

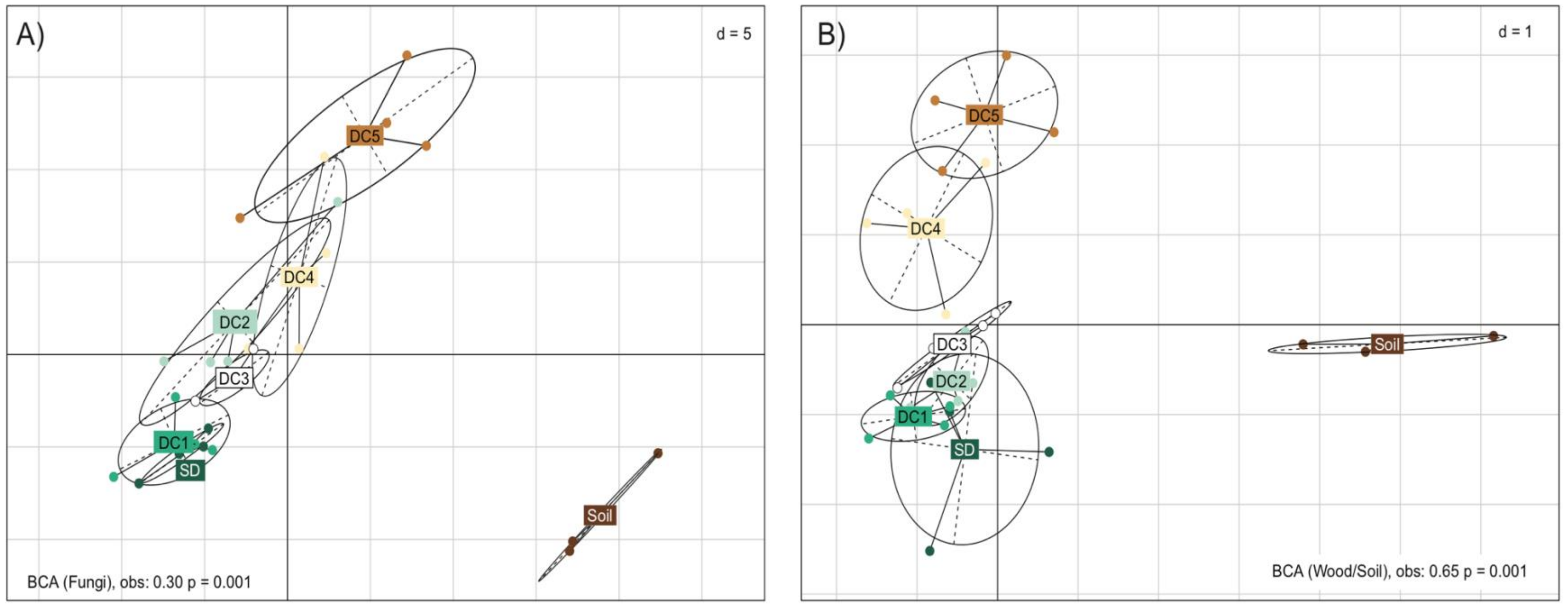

Figure 4.3: Between Class Analyses (BCA) of (A) fungal community composition and (B) substrate chemistry (%N, δ15N, %C, C:N, % moisture, and pH) due solely to decay stage. These differences in decay stage explained 30% of total variability in in fungal community and 65% of total variability in substrate chemistry. (SD=standing dead, DC1=decay class 1, DC2=decay class 2, DC3=decay class 3, DC4=decay class 4, DC5=decay class 5). N=27 (4 per decay stage of log, 3 soil).

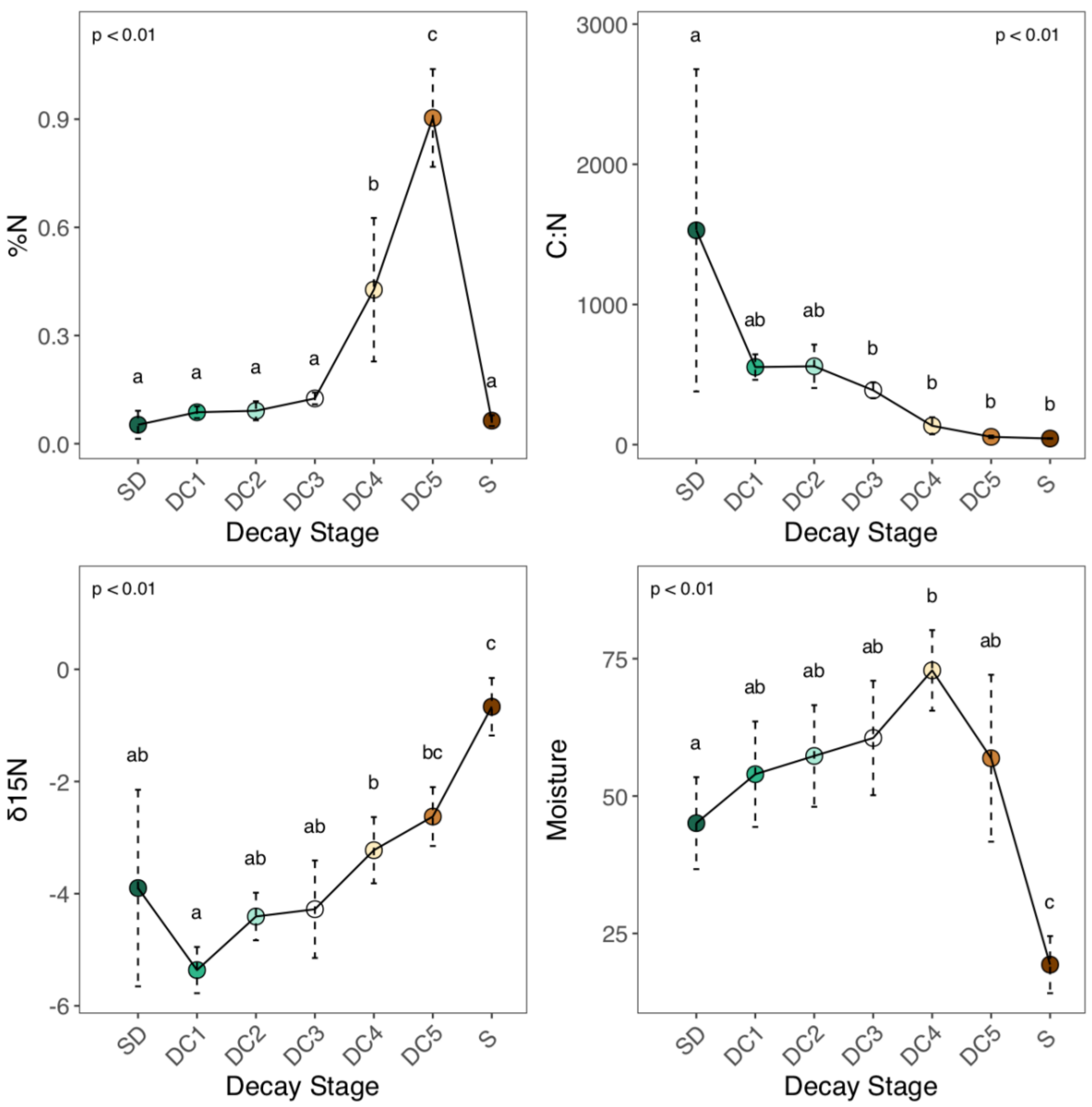

Figure 4.4: Wood chemical composition across CWD decay classes from standing dead to soil. Moisture content , %N and δ15N all tend to increase in later decay stages, while C:N decreases. (SD=standing dead, DC1=decay class 1, DC2=decay class 2, DC3=decay class 3, DC4=decay class 4, DC5=decay class 5). N=27 (4 per decay stage of log, 3 soil). Error bars represent standard deviation.

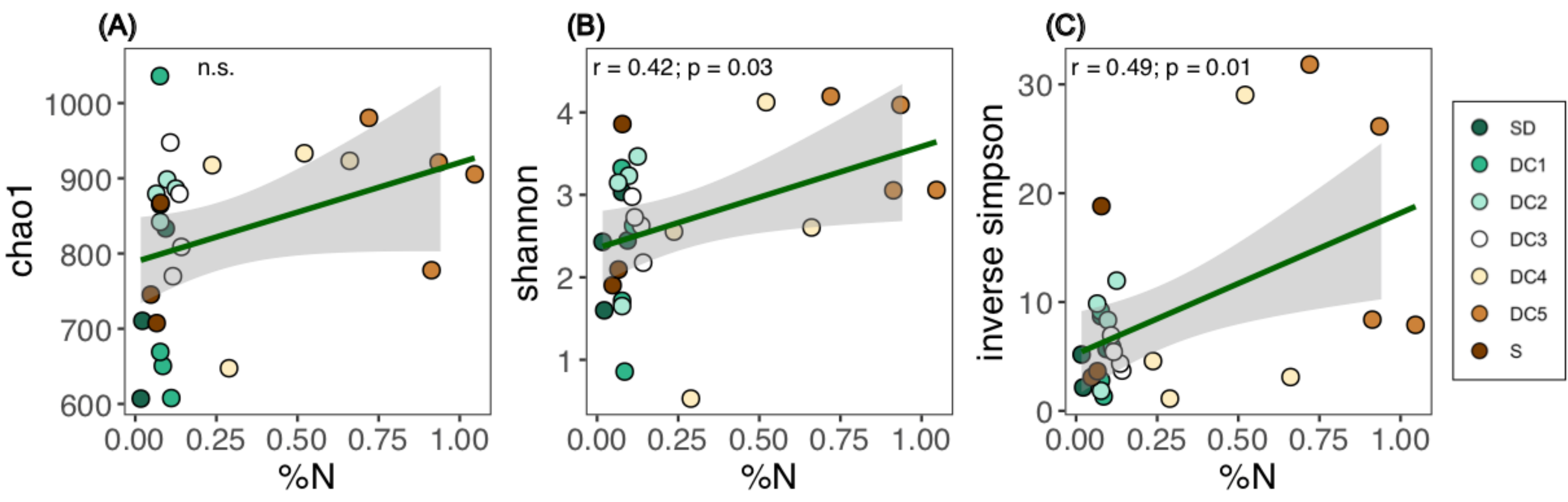

Figure 4.5: Correlation of fungal diversity indices in CWD stages and soil against the %N ratio of the wood. (SD=standing dead, DC1=decay class 1, DC2=decay class 2, DC3=decay class 3, DC4=decay class 4, DC5=decay class 5). N=27 (4 per decay stage of log, 3 soil). n.s. = non significant

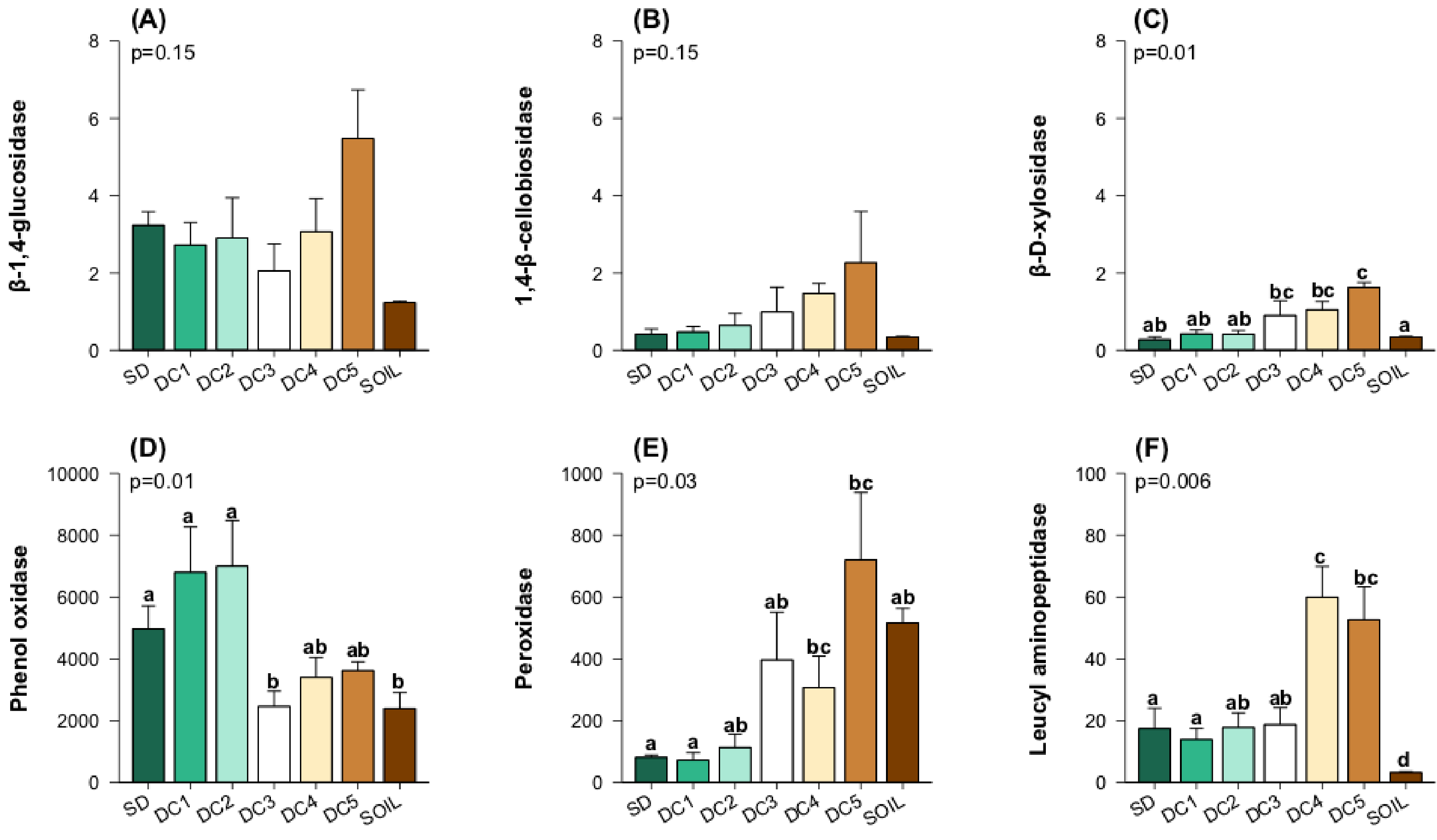

Figure 4.6: Enzyme activity associated with cellulose ((A) β-1,4-glucosidase, (B) 1,4- β-cellobiosidase), hemicellulose ((C) β-D-xylosidase), lignin ((D) phenol oxidase and (E) peroxidase), and polypeptide breakdown ((F) leucyl aminopeptidase) expressed as mUnits per g dry weight of substrate. Results are presented as means and standard error for standing dead wood (SD), all five decay classes (DC1-DC5), and soil. p-values are from Krustal-Wallis tests, and bars marked with the same letter did not differ statistically (Dunn's test for *post hoc* comparisons with α=0.05).

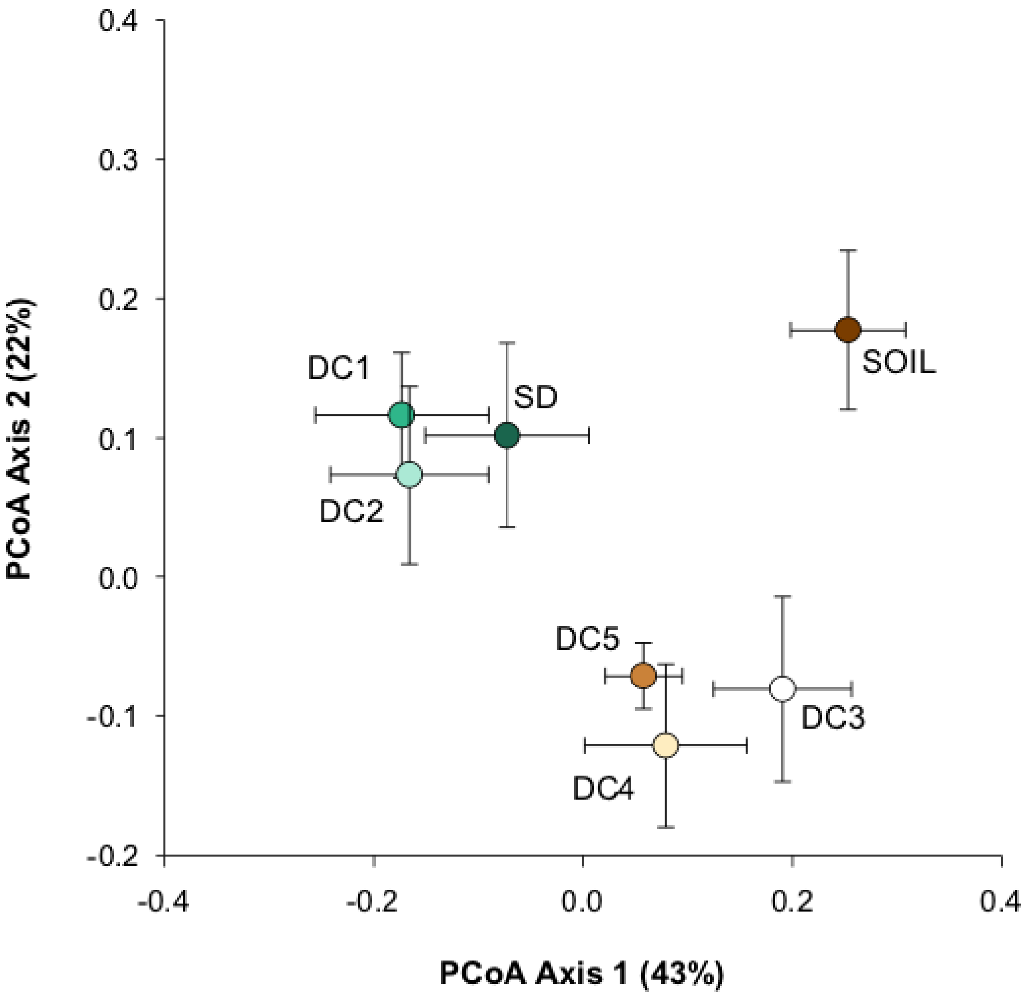

Figure 4.7: Principal Coordinate Analysis (PCoA) applied to the enzyme data using the Bray-Curtis coefficient. Results are presented as means (points) and standard error. Three general groupings are evident corresponding to early decay stages (SD (standing dead), DC1, and DC2), late decay stages (DC3-DC5), and soil. The PCoA explains 65% of the total variation in enzyme activity.

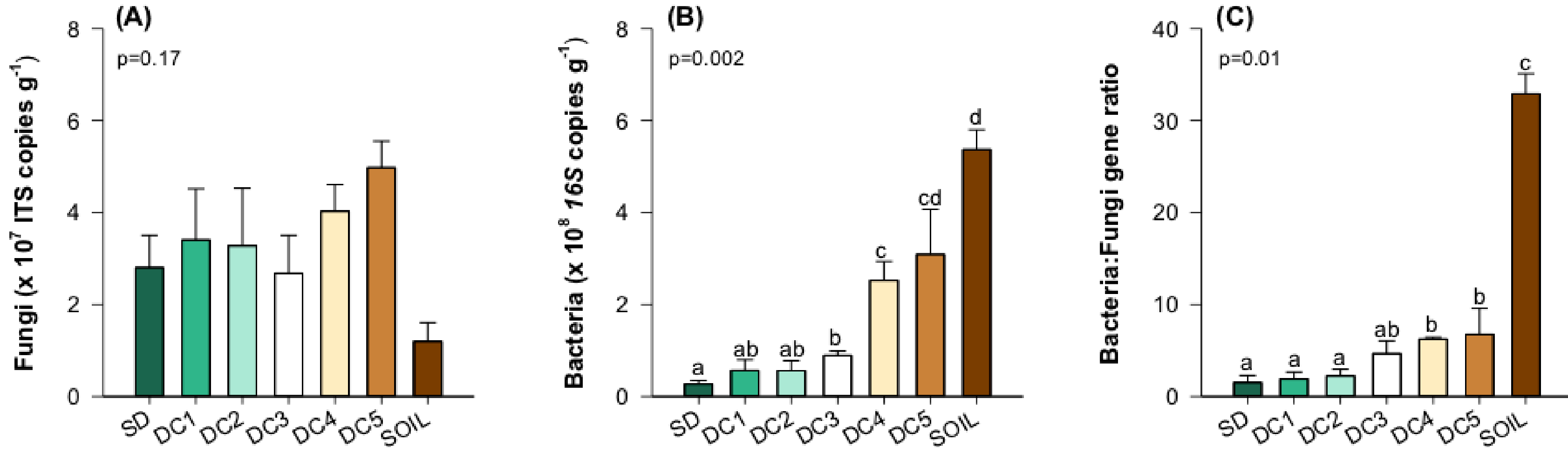

Figure 4.8: (A) Fungal ITS and (B) bacterial *16S rRNA* gene abundances reported as copies per g dry weight of substrate, along with (C) bacterial-to-fungal gene ratios. Results are presented as means and standard error for standing dead wood (SD), all five decay classes (DC1-DC5), and soil. p-values are from Krustal-Wallis tests, and bars marked with the same letter did not differ statistically (Dunn's test for *post hoc* comparisons with α=0.05).

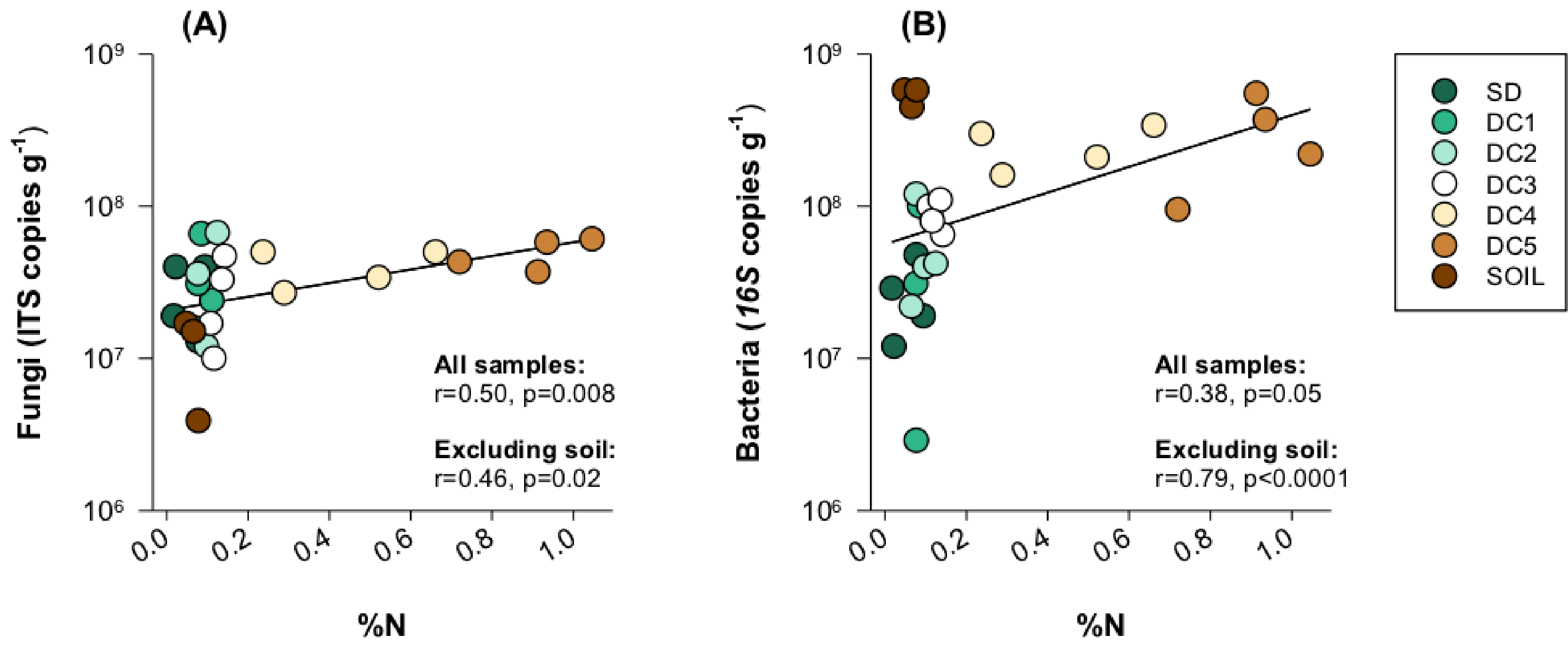

Figure 4.9: Positive correlation of %N with abundance of both (A) fungal ITS and (B) bacterial *16S* genes, expressed as gene copies per g dry weight of substrate. (SD=standing dead, DC1=decay class 1, DC2=decay class 2, DC3=decay class 3, DC4=decay class 4, DC5=decay class 5). N=27 (4 per decay stage of log, 3 soil).

Chapter 5: Conclusion

5.1 Summary of Experiments

Both anthropogenic and natural disturbances drive global change by altering the distributions, abundances, functions, and interactions of organisms with each other and the abiotic environment (Hassett and Zak, 2005; Holden and Treseder, 2013; Neary et al., 1999a). These perturbations in forest ecosystems potentially have serious implications for global C cycles due to the large C stocks in soils and CWD (Magnússon et al., 2016; Sanderman et al., 2018; Scharlemann et al., 2014). Minor alterations to microbial communities that regulate C fluxes between the atmosphere and terrestrial systems could lead to sizeable changes in the amount of sequestered C stored in forests (Jomura et al., 2007; Xu and Shang, 2016). Current literature suggests that lumber harvesting and fire effects on soil microbial communities are transient, but more research is needed to address the short- and long-term effects of fire on microbial communities (Hart et al., 2005). Unravelling the past, present, and future effects of disturbances on microbial communities and ecosystems processes is essential as we look to moderate human influence moving forward.

In this dissertation I combined a series of observational and manipulative experiments to analyze fungal community responses to historical and predicted future disturbances to temperate forest ecosystems. Specifically, I inquired whether or not soil fungal communities follow patterns in diversity and productivity predicted by the intermediate disturbance hypothesis. To understand historical disturbances, I first investigated the short-term effects of an experimental cut and burn on soil fungal communities. I assessed the effects of the cut + burn by mapping the variation in disturbance severity and linking those severities to changes in fungal structure and function by comparing and contrasting pre- and post-disturbance community composition and enzymatic activity. I then examined long-term effects of lumber harvesting and burning on fungal communities by utilizing a series of established cut + burn plots comprising a 100+ year

chronosequence. I assessed successional trajectories of fungal communities by comparing fungal diversity, abundances, and enzymatic activity in separate aged stands. Finally, I explore fungal succession in *Populus grandidentata* CWD from standing dead to incorporation of woody necromass into soil. I assessed how patterns in fungal decomposer communities in CWD differ across stages of decay, and whether those shifts in fungal communities co-varied with substrate chemistry, decompositional stage, or co-occurring bacterial communities.

Chapter II: Short-term effects of clear-cutting and burning on fungal community structure and function in mineral soils

In this chapter I explored how fungal communities in mineral soils are affected by an implemented cut + burn manipulation in a 130-year old mixed hardwood forest, and how variation in disturbance severity within a 1-ha plot structured communities. The results revealed differential effects of varying degrees of disturbance severity at the scale of meters. Areas of high disturbance severity, consisting of cutting + high intensity burning, lent support for the IDH with emphatic decreases in both fungal diversity, abundances, and enzymatic activity from pre-disturbance conditions. These community changes were accompanied by stark changes to soil physio-chemical properties with steep increases in pH, soil moisture, and CA_{2+}. On the other hand, moderate disturbances, consisting of cutting + marginal to no burning, primarily led to an increase in fungal diversity and abundance.

It is also important to note that pooling all disturbed soils led to differential results at the plot level for abiotic and biotic properties. Plot lever differences in abiotic conditions between pre- and post- cut+burn soils were primarily driven by the high severity disturbance areas. Conversely, trends in diversity at the plot level were driven by areas of low to moderate disturbance. The results of this chapter offer support for microbial communities adhering to the IDH, and also highlight the importance of the scale of sampling and offer insight into potentially missed trends in the variable effects of disturbance at the scale of meters.

Chapter III: Decadal Trends of Fungal Succession in Mineral Soils Following Clear-Cutting and Burning of a Mixed Hardwood Forest

In Chapter III I utilized an existing cut + burn chronosequence to examine whether successional patterns in soil fungal communities adhere to trends predicted by the IDH. I first noted that plant community diversity did in fact follow predicted patters with a peak in diversity at intermediate levels of succession. However, I did not find support evidence that either fungal community diversity or abundance peaked in mid-successional stands. To the contrary, fungal diversity in the middle-aged stand was lower than both the youngest and oldest stand. I posit that the result was primarily driven by a high abundance of EM that was accompanied by a particularly high relative abundance of oaks and pines compared to the other experimental plots. When trends were analyzed without the plot with high oak abundance, fungal diversity was seen to decrease with successional stage.

Contrasting with trends in diversity, microbial abundances were observed to increase with stand age, reflecting similar trends of primary producer productivity previously reported in similar forests. This research highlights the importance of decadal studies and highlights the potential for stochasticity in community assembly as I detected a higher than expected relative abundance of oaks and assoc❤itated EM in mid-successional stages. Additionally, this study offers insight into the decadal legacy of disturbances on soil fungal diversity and abundance.

Chapter IV: Fungal Succession of Populus grandidentata (Bigtooth Aspen) During Wood Decomposition

In Chapter IV, I investigated patterns of fungal decomposers in CWD of a primary successional species. *Populus grandidentata*, that is currently being replaced in north temperate forests. In this study I showed that fungal communities are dynamic throughout a continuum of decay stages, beginning in standing dead and continuing to shift into incorporation of woody necromass into soil. Early decay stages were defined by ascomycete-dominated communities with a high representation of saprotrophic yeasts in the Saccharomycetales. The Saccharomycetales were accompanied by a strong representation of non-filamentous basidiomycetous yeasts. These yeasts were supplanted in mid and late decay stages by a diverse conglomeration of Agaricales

that are often associated with wood decay. I also noted the infiltration of ECM in late decay stages that I attribute to the foraging for N-rich substrates made available my decomposers in early successional stages.

Importantly, nitrogen was shown to be an important driver of fungal enzymatic activity. Additionally, I noted a correlation with bacterial and fungal community composition and abundance along the decay continuum. I did not however detect support for the IDH, as neither fungal diversity or abundance differed between decay stages. Notably, I show that fungal community structure changes substantially and in a functionally predictable way over the course of wood decomposition despite no changes in overall diversity.

5.2 Synthesis & Future Directions

Disturbances have been repeatedly shown to alter species abundances, distributions, and functionality through direct and indirect effects (González-Pérez et al., 2004; Hassett and Zak, 2005; Holden and Treseder, 2013). Fungal communities have shown a particular sensitivity to clear-cutting and fire induced mortality, as well as resulting perturbations to soil temperatures (Frey et al., 2008), moisture (Manzoni et al., 2012), and nutrient availability (Allison et al., 2010). My dissertation offers further support for the importance of disturbances in structuring soil fungal communities. Notably, my work highlights the importance of spatial variability in disturbance severity and long-term legacies on fungal successional trajectories. My second chapter illustrates the differential effects of detected trends when analyzing pooled vs un-pooled samples within a plot. The high variability in disturbance severity and fungal responses within a single 1-ha plot underlines the difficulty in trying to generalize observed trends to stand, landscape, or regional levels. My results are in line with previous studies suggesting that factors controlling the spatial distribution of soil microbial communities (Zhao et al., 2009) and soil physio-chemical properties (Mueller and Schmit, 2007; Nave et al., 2017) are very complex making scaling difficult. Much progress has recently been made by methods using mathematical techniques such as continuous wavelet transformations (CWT) and Hilbert-Huang transformations (HHT) that break spatial signals into multiple lower resolution levels, but further

research is needed using real-world soil measurements to determine widescale applicability (Biswas, 2018).

My dissertation also addresses the applicability of Odum's intermediate succession hypothesis on productivity (Odum, 1969) and the IDH on diversity (Connell, 1978) to soil fungal communities. My dissertation provides no support for fungal communities adhering to the predicted trends in diversity and productivity. In Chapter II I observed decreases in diversity, abundance and enzymatic activity in high severity burn areas within the cut + burn plot. I also noted an increase in diversity and abundance of fungal communities in areas of low to moderate disturbance within the plot. Additionally, long term trajectories observed in Chapter III did not align with predicted trends. Even with the mid-successional plot with exceptionally low diversity removed from analyses I detected a decline in fungal diversity and an increase in microbial biomass from young to old stands. The observations of increasing microbial biomass are more in line with recent studies indicating sustained productivity in late successional stands due to increased resource use efficiency attributed to increased canopy structural complexity (Gough et al., 2013; Hardiman et al., 2013). Meanwhile, successional trends in CWD along a decay gradient supported neither hypothesis as neither fungal diversity or abundance was seen to differ between stages of decay.

Understanding the factors contributing to microbial community structure and function in space and time remains a critical focus to study under current and predicted scenarios of climate change. Overall, the work described here offers further support for the importance of disturbances in structuring soil fungal communities. Notably, my work highlights the importance of spatial variability in disturbance severity and long-term legacies on fungal composition, activity, and successional trajectories.

5.3 References

Adamčík, S., Buyck, B., 2011. The Species of Russula Subsection Xerampelinae Described. Cryptogam. Mycol. 32, 63–81. https://doi.org/10.7872/crym.v32.iss1.2012.063

Allison, S.D., McGuire, K.L., Treseder, K.K., 2010. Resistance of microbial and soil properties to warming treatment seven years after boreal fire. Soil Biol. Biochem. 42, 1872–1878. https://doi.org/10.1016/j.soilbio.2010.07.011

Baldrian, P., Kolařík, M., Štursová, M., Kopecký, J., Valášková, V., Větrovský, T., Žifčáková, L., Šnajdr, J., Rídl, J., Vlček, Č., Voříšková, J., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. ISME J. 6, 248–258. https://doi.org/10.1038/ismej.2011.95

Baldrian, P., Šnajdr, J., Merhautová, V., Dobiášová, P., Cajthaml, T., Valášková, V., 2013. Responses of the extracellular enzyme activities in hardwood forest to soil temperature and seasonality and the potential effects of climate change. Soil Biol. Biochem. 56, 60–68. https://doi.org/10.1016/j.soilbio.2012.01.020

Barg, A.K., Edmonds, R.L., 1999. Influence of partial cutting on site microclimate, soil nitrogen dynamics, and microbial biomass in Douglas-fir stands in western Washington. Can. J. For. Res. 29, 705–713. https://doi.org/10.1139/x99-045

Belillas, C.M., Feller, M.C., 1998. Relationships between fire severity and atmospheric and leaching nutrient losses in British Columbia's coastal western hemlock zone forests. Int. J. Wildl. Fire 8, 87–101.

Biswas, A., 2018. Scale–location specific soil spatial variability: A comparison of continuous wavelet transform and Hilbert–Huang transform. Catena 160, 24–31. https://doi.org/10.1016/j.catena.2017.08.019

Boddy, L., Watkinson, S., 1994. Wood decomposition, higher fungi, and their role in nutrient redistribution. Can. J. Bot. 73.

Brais, S., Camire, C., Pare, D., 1995. Impacts of whole-tree harvesting and winter windrowing on soil pH and base status of clayey sites of northwestern Quebec. Can. J. For. Res. 25.

Brais, S., Paré, D., Lierman, C., 2006. Tree bole mineralization rates of four species of the Canadian eastern boreal ... Can. J. For. Res. 36.

Buyck, B., Mitchell, D., Parrent, J., 2006. Russula parvovirescens sp. nov., a common but ignored species in the eastern United States . Mycologia 98, 612–615. https://doi.org/10.1080/15572536.2006.11832664

Byrd, K.B., Parker, V.T., Vogler, D.R., Cullings, K.W., 2000. The influence of clear-cutting on ectomycorrhizal fungus diversity in a lodgepole pine (Pinus centorta) stand, Yellowstone National Park, Wyoming, and Gallatin National Forest, Montana. Can. J. Bot. 78, 149–156. https://doi.org/10.1139/b99-171

Campbell, G.S., Jungbauer, J.D., Bristow, K.L., Hungerford, R.D., 1995. Soil temperature and water content beneath a surface fire. Soil Sci. 6, 363–374.

Catford, J.A., Daehler, C.C., Murphy, H.T., Sheppard, A.W., Hardesty, B.D., Westcott, D.A., Rejmánek, M., Bellingham, P.J., Pergl, J., Horvitz, C.C., Hulme, P.E., 2012. The intermediate disturbance hypothesis and plant invasions: Implications for species richness and management. Perspect. Plant Ecol. Evol. Syst. 14, 231–241. https://doi.org/10.1016/j.ppees.2011.12.002

Certini, G., 2005. Effects of fire on properties of forest soils: A review. Oecologia. https://doi.org/10.1007/s00442-004-1788-8

Chapin, F.S., Matson, P.A., Mooney, H., 2002. Principles of Ecosystem Ecology.
Chapman, S.K., Langley, J. a, Hart, S.C., Koch, G.W., 2005. Plants actively control nitrogen cycling: uncorking the microbail bottleneck. New Phytol. 169, 27–34.
Claridge, A.W., Trappe, J.M., Hansen, K., 2009. Do fungi have a role as soil stabilizers and remediators after forest fire? For. Ecol. Manage. 257, 1063–1069. https://doi.org/10.1016/j.foreco.2008.11.011
Connell, J.H., 1978. Diversity in tropical rain forests and coral reefs. Science (80-.). 199, 1302–1310. https://doi.org/10.1126/science.199.4335.1302
Cortina, J., Vallejo, V.R., 1994. Effects of clearfelling on forest floor accumulation and litter decomposition in a radiata pine plantation. For. Ecol. Manage. 70, 299–310. https://doi.org/10.1016/0378-1127(94)90095-7
Covington, W.W., 1981. Changes in Forest Floor Organic Matter and Nutrient Content Following Clear Cutting in Northern Hardwoods. Ecol. Soc. Am. 62, 41–48.
Czimczik, C.I., Masiello, C.A., 2007. Controls on black carbon storage in soils. Global Biogeochem. Cycles 21. https://doi.org/10.1029/2006GB002798
Debano, L.F., 2000. The role of fire and soil heating on water repellency in wildland environments: A review, in: Journal of Hydrology. pp. 195–206. https://doi.org/10.1016/S0022-1694(00)00194-3
DeBano, L.F., Neary, D.G., Ffolliott, P.F., 1998. Fire’s Effects on Ecosystems. John Wiley and Sons, New YOrk.
Deka, H.K., Mishra, R.R., 1983. The effect of slash burning on soil microflora. Plant Soil 73, 167–175. https://doi.org/10.1007/BF02197713
Dooley, S.R., Treseder, K.K., 2012. The effect of fire on microbial biomass: A meta-analysis of field studies. Biogeochemistry 109, 49–61. https://doi.org/10.1007/s10533-011-9633-8
Everett, R.L., Sharrow, S.H., 1985. Response of grass species to tree harvesting in singleleaf pinyon- Utah juniper stands. Res. Pap. - US Dep. Agric. For. Serv. 45. https://doi.org/10.5962/bhl.title.69006
Fahey, T.J., Arthur, M.A., 1994. Studies of Root Decomposition Following Harvest of a Northern. For. Sci. 40, 618–629.
Floudas, D., Binder, M., Riley, R., Barry, K., Blanchette, R., Henrissat, B., Martínez, A.T., Otillar, R., Spatafora, J.W., Yadav, J.S., Coutinho, P.M., Vries, R.P. De, Ferreira, P., Findley, K., Foster, B., 2012. Lignin Decomposition Reconstructed from 31 Fungal Genomes. Science (80-.). 336, 1715–1719.
Freeman, S., Horowitz, S., Sharon, A., 2001. Pathogenic and nonpathogenic lifestyles in Colletotrichum acutatum from strawberry and other plants. Phytopathology 91, 986–992. https://doi.org/10.1094/PHYTO.2001.91.10.986
Frey, S.D., 2019. Mycorrhizal Fungi as Mediators of Soil Organic Matter Dynamics. Annu. Rev. Ecol. Evol. Syst. 50, 237–259. https://doi.org/10.1146/annurev-ecolsys-110617-062331
Frey, S.D., Drijber, R., Smith, H., Melillo, J., 2008. Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biol. Biochem. 40, 2904–2907. https://doi.org/10.1016/j.soilbio.2008.07.020
Fukatsu, T., Sato, H., Kuriyama, H., 1997. Isolation, Inoculation to Insect Host, and Molecular Phylogeny of an Entomogenous Fungus Paecilomyces tenuipes. J. Invertebr. Pathol. 70, 203–208. https://doi.org/10.1006/jipa.1997.4696
Fukuzawa, K., Shibata, H., Takagi, K., Nomura, M., Kurima, N., Fukazawa, T., Satoh, F., Sasa,

K., 2006. Effects of clear-cutting on nitrogen leaching and fine root dynamics in a cool-temperate forested watershed in northern Japan. For. Ecol. Manage. 225, 257–261. https://doi.org/10.1016/j.foreco.2006.01.001
Gadgil, R.L., Gadgil, P.D., 1971. Mycorrhiza and litter decomposition. Nature 233, 133. https://doi.org/10.1038/233133a0
Gholz, H.L., Perry, C.S., Cropper, W.P., Hendry, L.C., 1985. Litterfall, decomposition, and nitrogen and phosphorus dynamics in a chronosequence of slash pine (Pinus elliottii) plantations. For. Sci. 31, 463–478. https://doi.org/10.1093/forestscience/31.2.463
Giovannini, G., Lucc, 1983. Effect of fire on hydrophobic and cementing substances of soil aggregates. Soil Sci. 136, 231–236.
Giovannini, G., Lucchesi, S., 1997. Modifications induced in soil physico-chemical parameters by experimental fires at different intensities. Soil Sci. 162, 479–486.
Godbout, C., Fortin, J.A., 1985. Synthesized ectomycorrhizae of aspen: fungal genus level of structural characterization. Can. J. Bot. 63, 252–262. https://doi.org/10.1139/b85-029
González-Pérez, J.A., González-Vila, F.J., Almendros, G., Knicker, H., 2004. The effect of fire on soil organic matter--a review. Environ. Int. 30, 855–870. https://doi.org/10.1016/j.envint.2004.02.003
Gough, C.M., Hardiman, B.S., Nave, L.E., Bohrer, G., Kyle, D., Vogel, C.S., Nadelhoffer, K.J., Curtis, P.S., Gough, C., Hardiman, B.S., Nave, L., Bohrer, G., Maurer, K.D., Vogel, C.S., Nadelhoffer, K.J., Curtis, P.S., 2013. Sustained carbon uptake and storage following moderate disturbance in a Great Lakes forest 23, 1202–1215.
Gough, C.M., Vogel, C.S., Harrold, K.H., George, K., Curtis, P.S., 2007. The legacy of harvest and fire on ecosystem carbon storage in a north temperate forest. Glob. Chang. Biol. https://doi.org/10.1111/j.1365-2486.2007.01406.x
Gough, C.M., Vogel, C.S., Schmid, H.P., Curtis, P.S., 2008. Controls on Annual Forest Carbon Storage: Lessons from the Past and Predictions for the Future. Bioscience. https://doi.org/10.1641/B580708
Gul, S., Whalen, J.K., Thomas, B.W., Sachdeva, V., Deng, H., 2015. Physico-chemical properties and microbial responses in biochar-amended soils: Mechanisms and future directions. Agric. Ecosyst. Environ. https://doi.org/10.1016/j.agee.2015.03.015
Hamman, S.T., Burke, I.C., Stromberger, M.E., 2007a. Relationships between microbial community structure and soil environmental conditions in a recently burned system. Soil Biol. Biochem. https://doi.org/10.1016/j.soilbio.2007.01.018
Hamman, S.T., Burke, I.C., Stromberger, M.E., 2007b. Relationships between microbial community structure and soil environmental conditions in a recently burned system. Soil Biol. Biochem. 39, 1703–1711. https://doi.org/10.1016/j.soilbio.2007.01.018
Hardiman, B.S., Gough, C.M., Halperin, A., Hofmeister, K.L., Nave, L.E., Bohrer, G., Curtis, P.S., 2013. Maintaining high rates of carbon storage in old forests: A mechanism linking canopy structure to forest function. For. Ecol. Manage. 298, 111–119. https://doi.org/10.1016/j.foreco.2013.02.031
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. For. Ecol. Manage. https://doi.org/10.1016/j.foreco.2005.08.012
Hart, S.C., Firestone, M.K., Paul, E.A., 1991. Decomposition and nutrient dynamics of pondersosa pine needles in a Mediterranean-type climate. Can. J. For. Res. 22, 306–314.

https://doi.org/10.16309/j.cnki.issn.1007-1776.2003.03.004
Harvey, A.E., Jurgensen, M.F., Larsen, M.J., 1980. Clearcut harvesting and ectomycorrhizae: survival of activity on residual roots and influence on a bordering forest stand in western Montana. Can. J. Bot. 10, 300–303.
Hassett, J.E., Zak, D.R., 2005. Aspen Harvest Intensity Decreases Microbial Biomass, Extracellular Enzyme Activity, and Soil Nitrogen Cycling. Soil Sci. Soc. Am. J. 69, 227. https://doi.org/10.2136/sssaj2005.0227
Hatch, A.B., C.T., H., 1933. Some Hymenomycetes forming mycorrhizae with Pinus strobus. J. Arnold Arbor. 14, 324–334.
Högberg, M.N., Bååth, E., Nordgren, A., Arnebrant, K., Högberg, P., 2003. Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs - A hypothesis based on field observations in boreal forest. New Phytol. 160, 225–238. https://doi.org/10.1046/j.1469-8137.2003.00867.x
Holden, S.R., Treseder, K.K., 2013. A meta-analysis of soil microbial biomass responses to forest disturbances. Front. Microbiol. 4. https://doi.org/10.3389/fmicb.2013.00163
Hu, Z., Xu, C., McDowell, N.G., Johnson, D.J., Wang, M., Luo, Y., Zhou, X., Huang, Z., 2017. Linking microbial community composition to C loss rates during wood decomposition. Soil Biol. Biochem. 104, 108–116. https://doi.org/10.1016/j.soilbio.2016.10.017
Ice, G.G., Neary, D.G., Adams, P.W., 2004. Effects of Wildfire on Processes. J. For. 102, 16–20.
Jensen, M., Michelsen, A., Gashaw, M., 2001. Responses in plant, soil inorganic and microbial nutrient pools to experimental fire, ash and biomass addition in a woodland savanna. Oecologia 128, 85–93. https://doi.org/10.1007/s004420000627
Jiménez Esquilín, A.E., Stromberger, M.E., Massman, W.J., Frank, J.M., Shepperd, W.D., 2007. Microbial community structure and activity in a Colorado Rocky Mountain forest soil scarred by slash pile burning. Soil Biol. Biochem. https://doi.org/10.1016/j.soilbio.2006.12.020
Johnson, C.E., Johnson, A.H., Siccama, T.G., 1991. Whole-Tree Clear-Cutting Effects on Exchangeable Cations and Soil Acidity. Soil Sci. Soc. Am. J. 55, 502. https://doi.org/10.2136/sssaj1991.03615995005500020035x
Jomura, M., Kominami, Y., Tamai, K., Miyama, T., Goto, Y., Dannoura, M., Kanazawa, Y., 2007. The carbon budget of coarse woody debris in a temperate broad-leaved secondary forest in Japan. Tellus, Ser. B Chem. Phys. Meteorol. 59, 211–222. https://doi.org/10.1111/j.1600-0889.2006.00234.x
Kara, O., Bolat, I., 2009. Short-term effects of wildfire on microbial biomass and abundance in black pine plantation soils in Turkey. Ecol. Indic. 9, 1151–1155. https://doi.org/10.1016/j.ecolind.2009.01.002
Kardol, P., Cornips, N.J., van Kempen, M.M.L., Bakx-Schotman, J.M.T., van der Putten, W.H., 2007. Microbe-Mediated Plant-Soil Feedback Causes Historical Contingency Effects in Plant Community Assembly. Ecol. Monogr. 77, 147–162.
Kirk, J.L., Beaudette, L.A., Hart, M., Moutoglis, P., Klironomos, J.N., Lee, H., Trevors, J.T., 2004. Methods of studying soil microbial diversity. J. Microbiol. Methods 58, 169–188. https://doi.org/10.1016/j.mimet.2004.04.006
Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by 14C labeling. Soil Biol. Biochem. 41, 210–219. https://doi.org/10.1016/j.soilbio.2008.10.016

Kyaschenko, J., Clemmensen, K.E., Hagenbo, A., Karltun, E., Lindahl, B.D., 2017. Shift in fungal communities and associated enzyme activities along an age gradient of managed Pinus sylvestris stands. ISME J. 11, 863–874. https://doi.org/10.1038/ismej.2016.184

Lapin, M., Barnes, B. V, 1995. Society for Conservation Biology Using the Landscape Ecosystem Approach to Assess Species and Ecosystem Diversity Using the Landscape Ecosystem Approach to Assess Species and Ecosystem Diversity. Conserv. Biol. 9, 1148–1158.

Lewandowski, T.E., Forrester, J.A., Mladenoff, D.J., D'Amato, A.W., Palik, B.J., 2016. Response of the soil microbial community and soil nutrient bioavailability to biomass harvesting and reserve tree retention in northern Minnesota aspen-dominated forests. Appl. Soil Ecol. 99, 110–117. https://doi.org/10.1016/j.apsoil.2015.11.001

Likens, G.E., Bormann, F.H., Johnson, N.M., Fisher, D.W., Robert, S., 2010. Effects of Forest Cutting and Herbicide Treatment on Nutrient Budgets in the Hubbard Published by : Ecological Society of America. America (NY). 40, 23–47.

Little, L.R., Maun, M.A., 1996. The Ammophila Problem Revisited : A Role for Mycorrhizal Fungi. Br. Ecol. Soc. 84, 1–7.

López-Mondéjar, R., Zühlke, D., Becher, D., Riedel, K., Baldrian, P., 2016. Cellulose and hemicellulose decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems. Sci. Rep. 6, 1–12. https://doi.org/10.1038/srep25279

Lundgren, B., 1982. Bacteria in a pine forest soil as affected by clear-cutting. Soil Biol. Biochem. 14, 537–542. https://doi.org/10.1016/0038-0717(82)90083-9

Magnússon, R., Tietema, A., Cornelissen, J.H.C., Hefting, M.M., Kalbitz, K., 2016. Tamm Review: Sequestration of carbon from coarse woody debris in forest soils. For. Ecol. Manage. 377, 1–15. https://doi.org/10.1016/j.foreco.2016.06.033

Manzoni, S., Schimel, J.P., Porporato, A., Ecology, S., April, N., Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress : results from a meta-analysis. Ecology 93, 930–938.

Margalef, O., Sardans, J., Fernández-Martínez, M., Molowny-Horas, R., Janssens, I.A., Ciais, P., Goll, D., Richter, A., Obersteiner, M., Asensio, D., Peñuelas, J., 2017. Global patterns of phosphatase activity in natural soils. Sci. Rep. 7, 1–13. https://doi.org/10.1038/s41598-017-01418-8

Martín-Pinto, P., Vaquerizo, H., Peñalver, F., Olaizola, J., Oria-De-Rueda, J.A., 2006. Early effects of a wildfire on the diversity and production of fungal communities in Mediterranean vegetation types dominated by Cistus ladanifer and Pinus pinaster in Spain. For. Ecol. Manage. https://doi.org/10.1016/j.foreco.2006.01.006

McGuire, K.L., Treseder, K.K., 2010. Microbial communities and their relevance for ecosystem models: Decomposition as a case study. Soil Biol. Biochem. 42, 529–535. https://doi.org/10.1016/j.soilbio.2009.11.016

Morris, M.H., Smith, M.E., Rizzo, D.M., Rejmánek, M., Bledsoe, C.S., 2008. Contrasting ectomycorrhizal fungal communities on the roots of co-occurring oaks (Quercus spp.) in a California woodland. New Phytol. 178, 167–176. https://doi.org/10.1111/j.1469-8137.2007.02348.x

Morrison, E.W., Frey, S.D., Sadowsky, J.J., van Diepen, L.T.A., Thomas, W.K., Pringle, A., 2016. Chronic nitrogen additions fundamentally restructure the soil fungal community in a temperate forest. Fungal Ecol. 23, 48–57. https://doi.org/10.1016/j.funeco.2016.05.011

Morrissey, E.M., Berrier, D.J., Neubauer, S.C., Franklin, R.B., 2014. Using microbial communities and extracellular enzymes to link soil organic matter characteristics to greenhouse gas production in a tidal freshwater wetland. Biogeochemistry 117, 473–490. https://doi.org/10.1007/s10533-013-9894-5

Mueller, G.M., Schmit, J.P., 2007. Fungal biodiversity: What do we know? What can we predict? Biodivers. Conserv. 16, 1–5. https://doi.org/10.1007/s10531-006-9117-7

Nave, L.E., Gough, C.M., Maurer, K.D., Bohrer, G., Hardiman, B.S., Le Moine, J., Munoz, A.B., Nadelhoffer, K.J., Sparks, J.P., Strahm, B.D., Vogel, C.S., Curtis, P.S., 2011. Disturbance and the resilience of coupled carbon and nitrogen cycling in a north temperate forest. J. Geophys. Res. Biogeosciences 116. https://doi.org/10.1029/2011JG001758

Nave, L.E., Gough, C.M., Perry, C.H., Hofmeister, K.L., Le Moine, J.M., Domke, G.M., Swanston, C.W., Nadelhoffer, K.J., 2017. Physiographic factors underlie rates of biomass production during succession in Great Lakes forest landscapes. For. Ecol. Manage. 397, 157–173. https://doi.org/10.1016/j.foreco.2017.04.040

Nave, L.E., Le Moine, J.M., Gough, C.M., Nadelhoffer, K.J., 2019. Multidecadal trajectories of soil chemistry and nutrient availability following cutting vs. Burning disturbances in upper great lakes forests. Can. J. For. Res. 49, 731–742. https://doi.org/10.1139/cjfr-2018-0211

Nave, L.E., Sparks, J.P., Le Moine, J., Hardiman, B.S., Strahm, B.D., Curtis, P.S., 2014. Changes in soil nitrogen cycling in northern temperate forest ecosystem during succession. Biogeochemistry 121, 471–488. https://doi.org/10.1007/s

Neary, D.G., Klopatek, C.C., DeBano, L.F., Ffolliott, P.F., 1999a. Fire effects on belowground sustainability: A review and synthesis, in: Forest Ecology and Management. https://doi.org/10.1016/S0378-1127(99)00032-8

Neary, D.G., Klopatek, C.C., DeBano, L.F., Ffolliott, P.F., 1999b. Fire effects on belowground sustainability: A review and synthesis, in: Forest Ecology and Management. pp. 51–71. https://doi.org/10.1016/S0378-1127(99)00032-8

O'Connell, A.M., McCaw, W.L., 1997. Prescribed burning of thinning slash in regrowth stands of karri (Eucalyptus diversicolor) 2. Nitrogen budgets in pre- and post-burn fuel. Int. J. Wildl. Fire 7, 41–49. https://doi.org/10.1071/WF9970041

Odum, E.P., 1969. The Strategy of Ecosystem Development. Science (80-.). 164, 262–270.

Özkan, U., Gökbulak, F., 2017. Effect of vegetation change from forest to herbaceous vegetation cover on soil moisture and temperature regimes and soil water chemistry. Catena 149, 158–166. https://doi.org/10.1016/j.catena.2016.09.017

Palese, A.M., Giovannini, G., Lucchesi, S., Dumontet, S., Perucci, P., 2004. Effect of fire on soil C, N and microbial Biomass. Agronomie 24, 47–53. https://doi.org/10.1051/agro

Pearsall, D.R., 1985. Landscape ecosystems of the University of Michigan Biological Station: ecosystem diversity and ground-cover diversity. University of Michigan, Ann Arbor.

Pellitier, P.T., Zak, D.R., 2018. Ectomycorrhizal fungi and the enzymatic liberation of nitrogen from soil organic matter: why evolutionary history matters. New Phytol. 217, 68–73. https://doi.org/10.1111/nph.14598

Pietikainen, J., Fritze, H., 1995. Clear-cutting and prescribed burning in coniferous forest: comparision of effects on soil fungal and total microbial biomass, respiration activity and nitrification. Soil Biol. Biochem. 27, 1995.

Ramanathan, V., Carmichael, G., 2008. Global and regional climate changes due to black carbon. Nat. Geosci. https://doi.org/10.1038/ngeo156

Reazin, C., Morris, S., Smith, J.E., Cowan, A.D., Jumpponen, A., 2016. Fires of differing intensities rapidly select distinct soil fungal communities in a Northwest US ponderosa pine forest ecosystem. For. Ecol. Manage. 377, 118–127. https://doi.org/10.1016/j.foreco.2016.07.002

Rincón, A., Pueyo, J.J., 2010. Effect of fire severity and site slope on diversity and structure of the ectomycorrhizal fungal community associated with post-fire regenerated Pinus pinaster Ait. seedlings. For. Ecol. Manage. 260, 361–369. https://doi.org/10.1016/j.foreco.2010.04.028

Sanderman, J., Hengl, T., Fiske, G.J., 2018. Soil carbon debt of 12,000 years of human land use. Proc. Natl. Acad. Sci. U. S. A. 115, E1700. https://doi.org/10.1073/pnas.1800925115

Scharlemann, J.P.W., Tanner, E.V.J., Hiederer, R., Kapos, V., 2014. Global soil carbon: Understanding and managing the largest terrestrial carbon pool. Carbon Manag. 5, 81–91. https://doi.org/10.4155/cmt.13.77

Schmidt, M.W.I., Noack, A.G., 2000. Black carbon in soils and sediments: Analysis, distribution, implications, and current challenges. Global Biogeochem. Cycles. https://doi.org/10.1029/1999GB001208

Sikes, B.A., Maherali, H., Klironomos, J.N., 2012. Arbuscular mycorrhizal fungal communities change among three stages of primary sand dune succession but do not alter plant growth. Oikos 121, 1791–1800. https://doi.org/10.1111/j.1600-0706.2012.20160.x

Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol. Biochem. 42, 391–404. https://doi.org/10.1016/j.soilbio.2009.10.014

Sinsabaugh, R.L., Linkins, A.E., 1990. Enzymic and chemical analysis of particulate organic matter from a boreal river. Freshw. Biol. 23, 301–309. https://doi.org/10.1111/j.1365-2427.1990.tb00273.x

Smith, S., Read, D., 2008. Mycorrhizal Symbiosis, Third. ed. Elsevier Ltd, New York.

Soil Survey Staff, 1991. Soil Survey of Cheboygan, Michigan.

Spatafora, J.W., Blackwell, M., 1993. Molecular systematics of unitunicate perithecial ascomycetes: The clavicipitales-hypocreales connection. Mycologia 85, 919–922. https://doi.org/10.1080/00275514.1993.12026353

Štursová, M., Žifčáková, L., Leigh, M.B., Burgess, R., Baldrian, P., 2012. Cellulose utilization in forest litter and soil: Identification of bacterial and fungal decomposers. FEMS Microbiol. Ecol. 80, 735–746. https://doi.org/10.1111/j.1574-6941.2012.01343.x

Talbot, J.M., Bruns, T.D., Smith, D.P., Branco, S., Glassman, S.I., Erlandson, S., Vilgalys, R., Peay, K.G., 2013. Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. Soil Biol. Biochem. 57, 282–291. https://doi.org/10.1016/j.soilbio.2012.10.004

Taylor, D.L., Hollingsworth, T.N., McFarland, J.W., Lennon, N.J., Nusbaum, C., Ruess, R.W., 2014. A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning. Ecol. Monogr. 84, 3–20. https://doi.org/10.1890/12-1693.1

Turner, B.L., Condron, L.M., 2013. Pedogenesis, nutrient dynamics, and ecosystem development: The legacy of T.W. Walker and J.K. Syers. Plant Soil 367, 1–10. https://doi.org/10.1007/s11104-013-1750-9

Urich, T., Lanzen, A., Qi, J., Huson, D.H., Schleper, C., Schuster, S.C., 2008. Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. PLoS One 3, e2527. https://doi.org/10.1371/journal.pone.0002527

Vázquez, D.P., Aizen, M.A., 2004. Asymmetric Specialization : A Pervasive Feature of Plant-Pollinator Interactions. Ecology 85, 1251–1257.
Verma, S., Jayakumar, S., 2012. Impact of forest fire on physical, chemical and biological properties of soil: A. Proc. Int. Acad. … 2, 168–176.
Vermeulen, S., Bossio, D., Lehmann, J., Luu, P., Paustian, K., Webb, C., Augé, F., Bacudo, I., Baedeker, T., Havemann, T., Jones, C., King, R., Reddy, M., Sunga, I., Von Unger, M., Warnken, M., 2019. A global agenda for collective action on soil carbon. Nat. Sustain. 2, 2–4. https://doi.org/10.1038/s41893-018-0212-z
Wan, S., Hui, D., Luo, Y., Applications, E., Oct, N., 2001. Fire Effects on Nitrogen Pools and Dynamics in Terrestrial Ecosystems : A Meta-Analysis. Ecol. Appl. 11, 1349–1365.
Wang, W., Li, Y., Wang, H., Zu, Y., 2014. Differences in the activities of eight enzymes from ten soil fungi and their possible influences on the surface structure, functional groups, and element composition of soil colloids. PLoS One 9. https://doi.org/10.1371/journal.pone.0111740
Wicklow, D.T., 1975. Fire as an Environmental Cue Initiating Ascomycete Development in a Tallgrass Prairie. Mycologia 67, 852–862. https://doi.org/10.1080/00275514.1975.12019813
Wicklow, D.T., Hirschfield, B.J., 1979. Competitive Hierarchy in Post-Fire Ascomycetes. Mycologia 71, 47–54. https://doi.org/10.1080/00275514.1979.12020986
Widden, P., Parkinson, D., 1974. The effects of a forest fire on soil microfungi. Soil Biol. Biochem. 7, 125–138.
Xu, M., Shang, H., 2016. Contribution of soil respiration to the global carbon equation. J. Plant Physiol. 203, 16–28. https://doi.org/10.1016/j.jplph.2016.08.007
Zak, D.R., Pellitier, P.T., Argiroff, W.A., Castillo, B., James, T.Y., Nave, L.E., Averill, C., Beidler, K. V., Bhatnagar, J., Blesh, J., Classen, A.T., Craig, M., Fernandez, C.W., Gundersen, P., Johansen, R., Koide, R.T., Lilleskov, E.A., Lindahl, B.D., Nadelhoffer, K.J., Phillips, R.P., Tunlid, A., 2019. Exploring the role of ectomycorrhizal fungi in soil carbon dynamics. New Phytol. 223, 33–39. https://doi.org/10.1111/nph.15679
Zhao, X., Wang, Q., Kakubari, Y., 2009. Stand-scale spatial patterns of soil microbial biomass in natural cold-temperate beech forests along an elevation gradient. Soil Biol. Biochem. 41, 1466–1474. https://doi.org/10.1016/j.soilbio.2009.03.028

Appendix A Supplementary Figures & Table for Chapter 2

Table A 1. Community data of the harvested overstory tree community including total counts of stems >8cm DBH, species specific basal areas per m2 and percent relative abundance of canopy basal area.

Species	Stem counts	Basal Area (m2/ha)	Percent Relative Abundane
Acer rubrum	97	1.75	5.9
Betula papyrifera	5	0.09	0.3
Pinus banksiana	2	0.11	0.4
Pinus resinosa	224	10.14	34.0
Pinus strobus	107	2.92	9.8
Populus grandidentata	56	2.8	9.4
Quercus rubra	667	12.03	40.3

Table A 2. Juvenile and sapling tree community data including juvenile stem counts, sapling stem counts and sapling relative abundance based on stem counts.

Species	Juvenile Stem Count	Juvenile % Relative Abundance	Seedling Stem Count	Seedling % Relative Abundance
Abies alsamea	0	0	2	<0.1
Acer pennsylvanicum	0	0	3	<0.1
Acer rubrum	82	17.4	6161	53.6
Acer spicatum	0	0	3	<0.1
Fagus grandifolia	4	<0.1	192	1.7
Pinus banksiana	0	0	4	<0.1
Pinus resinosa	9	0.1	297	2.6
Pinus strobus	358	76.0	2223	19.3
Populus grandidentata	0	0	9	0.1
Quercus rubra	18	<0.1	2607	22.7

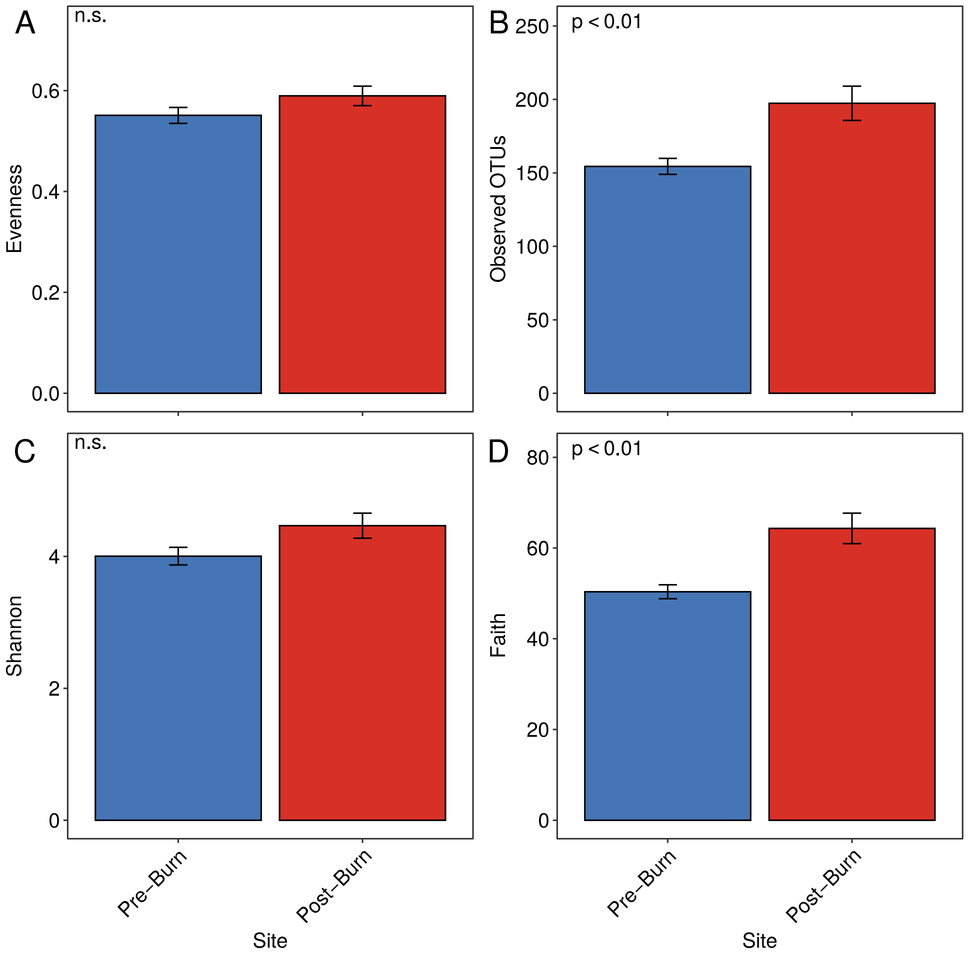

Figure A 1. Mean measures of diversity indices in pre-disturbance (blue bars) and post-disturbance fungal communities. Paired t-tests on the indices indicate there are no differences in species (A) evenness or (C) richness (evenness + abundance). There were significantly more (B) OTUs and an increase in (D) the Faith index measuring phylogenetic diversity. Whiskers represent standard deviations from the mean.

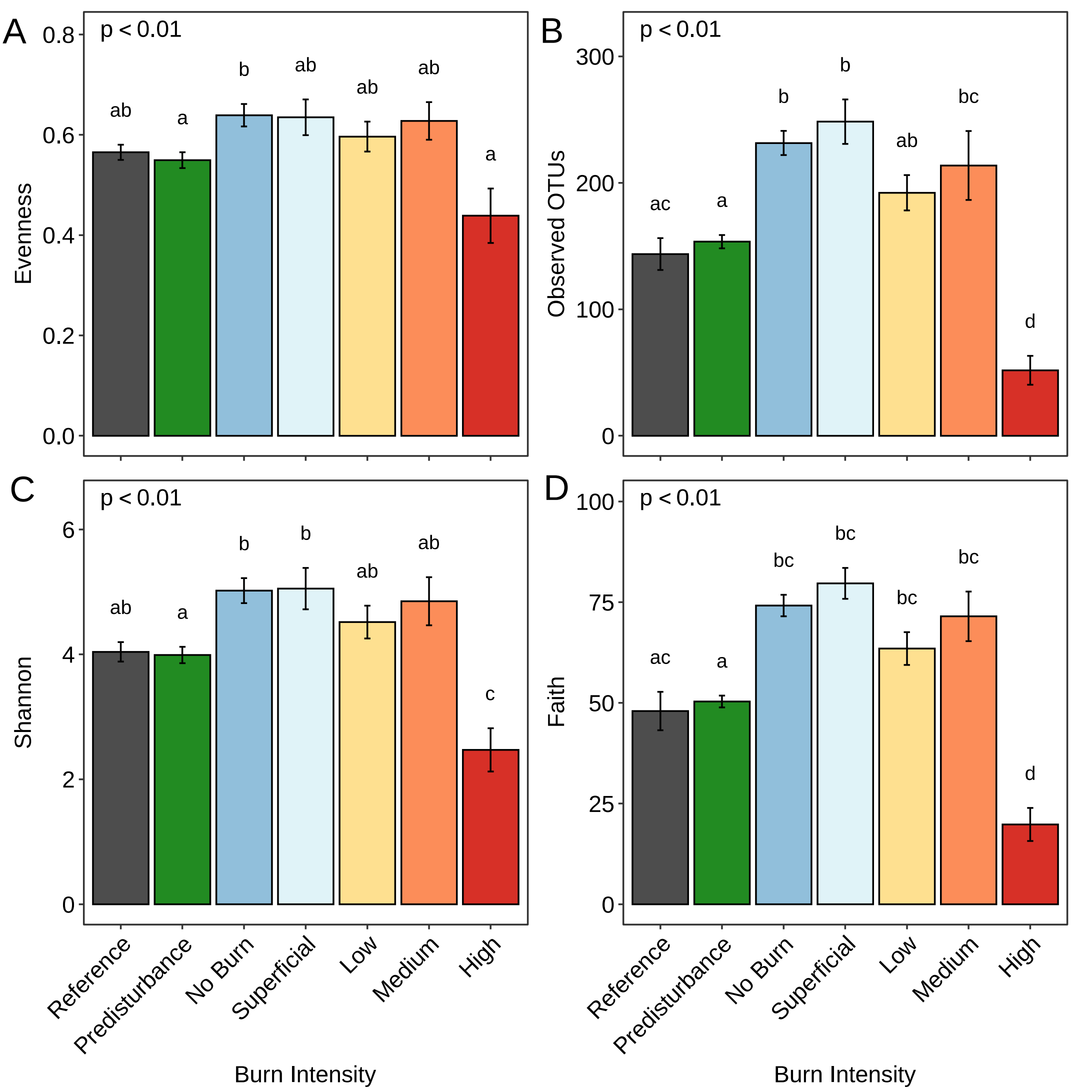

Figure A 2. Mean measures of diversity indices in reference (grey), pre-disturbance (green), cut + no burn (dark blue), cut + superficial burn (light blue), cut + low burn (yellow), cut + medium burn (orange) and cut + high burn (red) fungal communities. An ANOVA and Tukey post-hoc analyses on (A) evenness, (B) observed OTUS, (C) richness (evenness + abundance) and (D) Faith phylogenetic index indicate an enrichment from the pre-disturbance levels when experiencing a cut + no through medium burn. Cut + high burn severity disturbance resulted in decreased observed OTUS, species richness and phylogenetic diversity. Whiskers represent standard deviations from the mean.

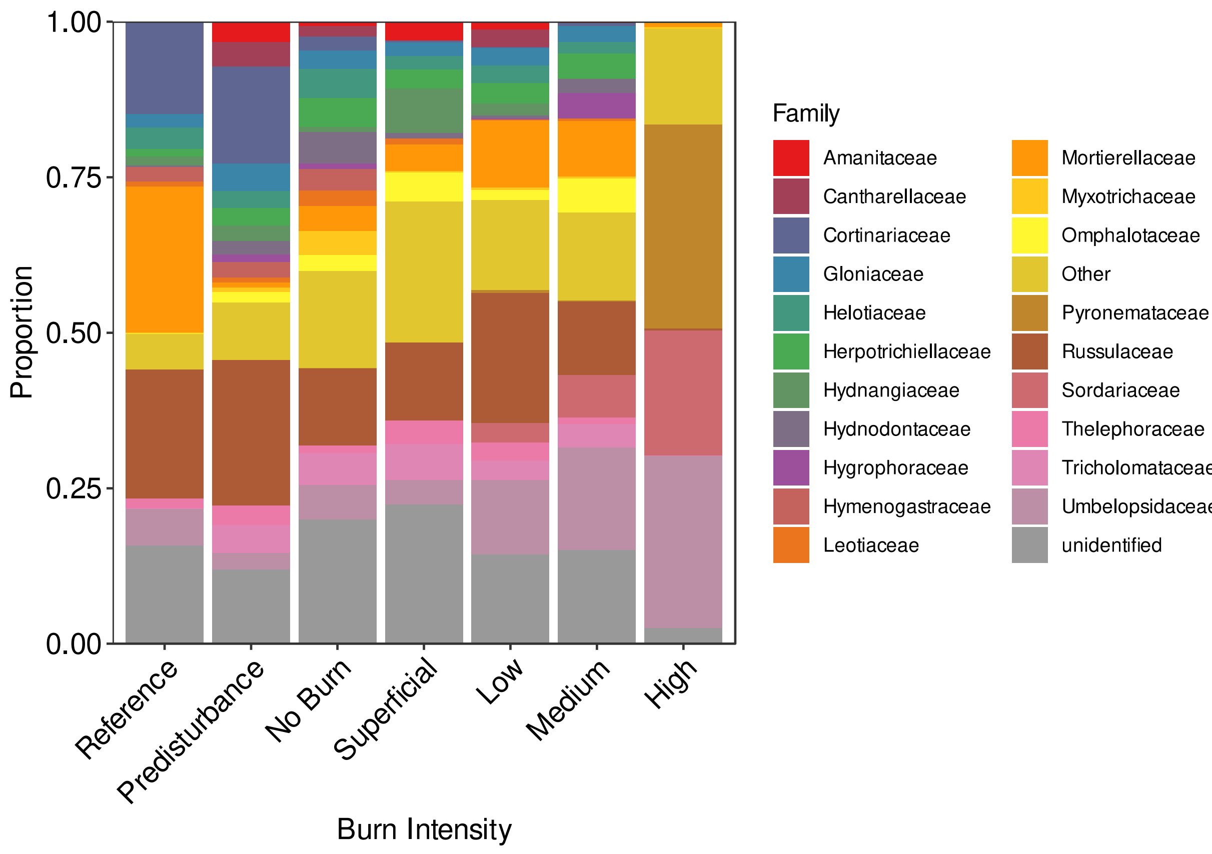

Figure A 3. Taxonomic relative abundances of fungal DNA sequences at the family level. Fungi are classified at a 99% confidence. Other includes all families that accounted for less than 1% of overall abundances. Unidentified include al fungi that could not be classified past the kingdom.

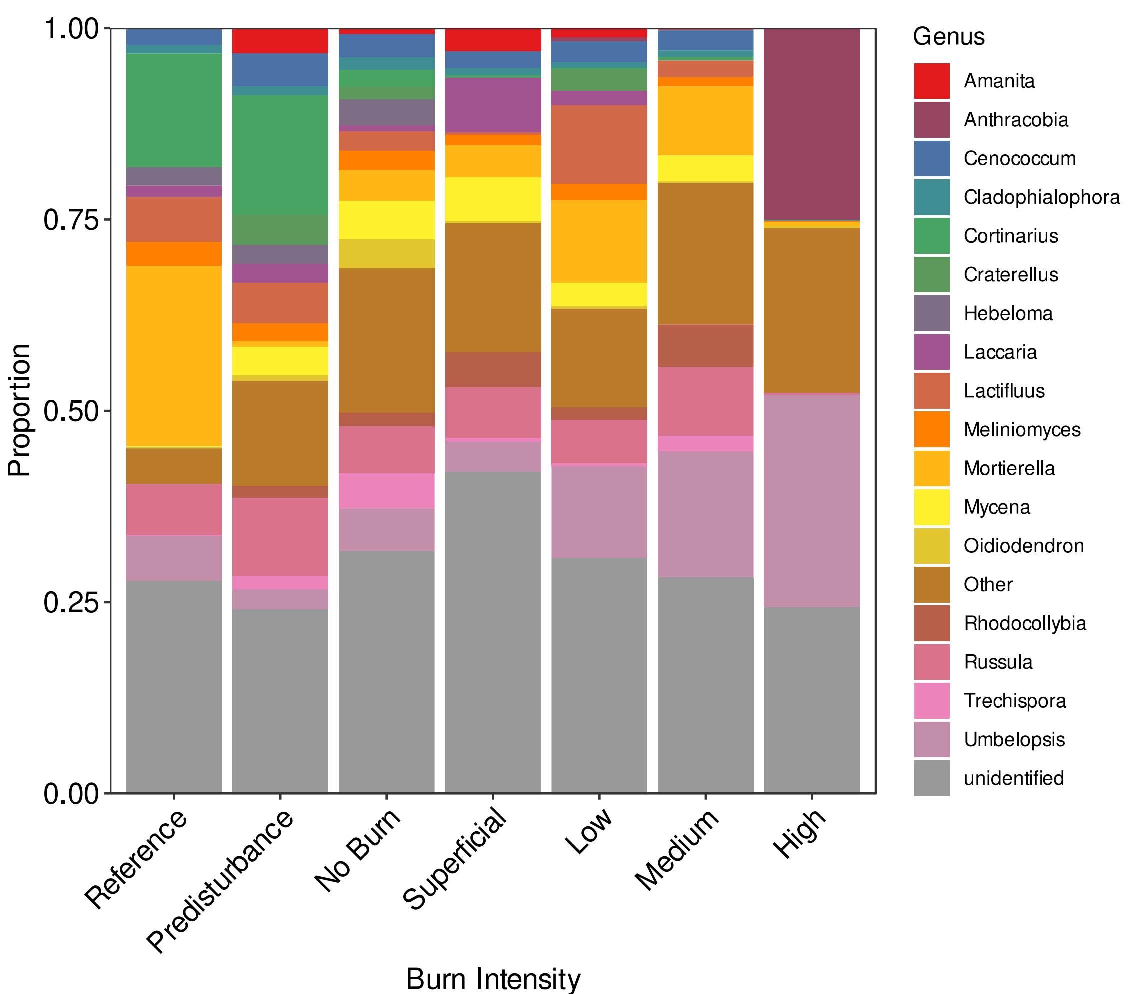

Figure A 4. Taxonomic relative abundances of fungi at the genus level. Fungi are classified at a 99% confidence. Other includes all families that accounted for less than 1% of overall abundances. Unidentified include al fungi that could not be classified past the kingdom.

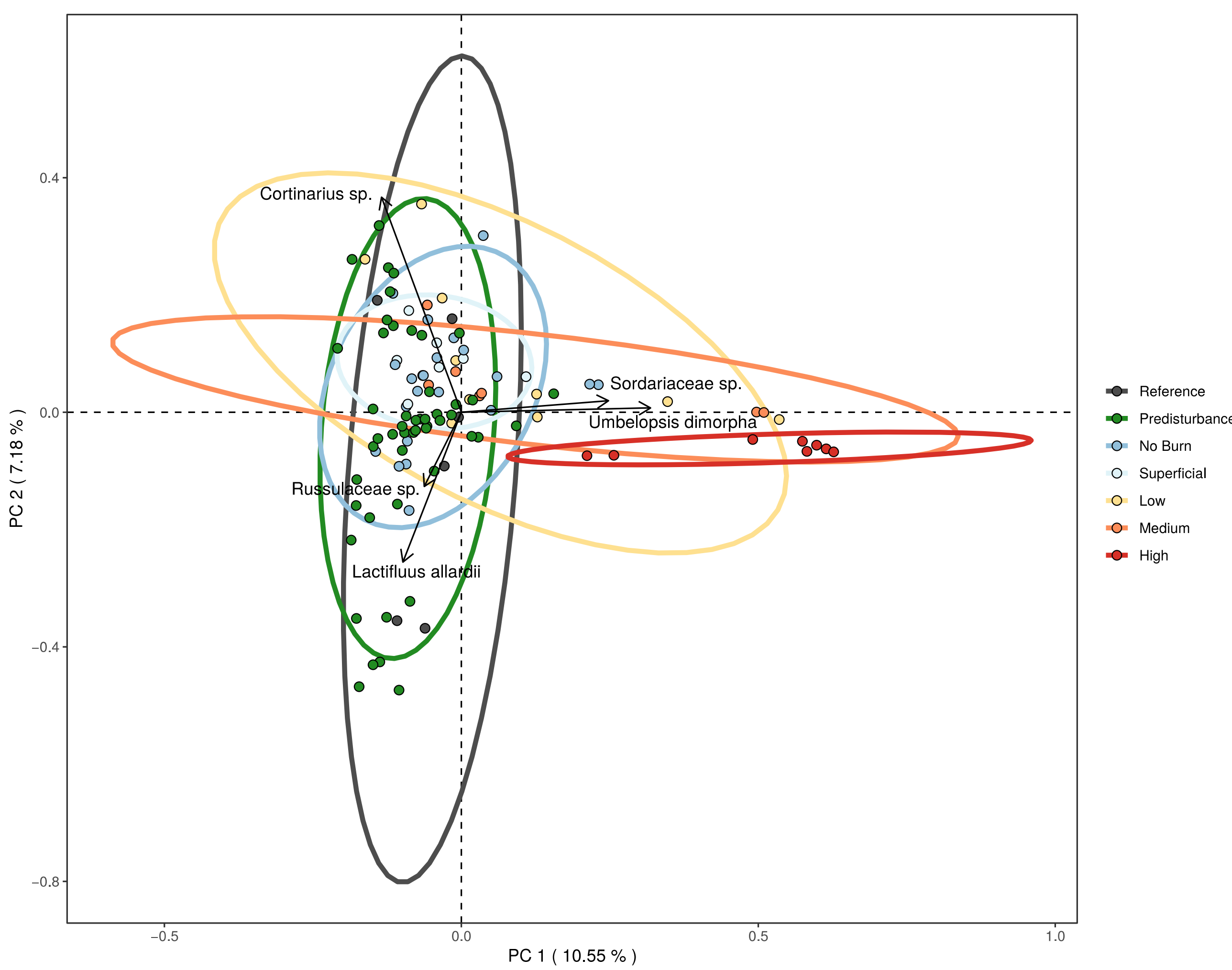

Figure A 5. Principal coordinates analysis (PCoA) of fungal communities in soils from reference (grey), pre-disturbance (green), cut + no burn (dark blue), superficial burn (light blue), low burn (yellow), medium burn (orange) and high burn (red) Bray-curtis abundances using ThetaYC calculator of dissimilarity. A Mantel test indicated significant community differences based on disturbance severities ($p<0.05$). Ellipses signify a 95% confidence interval of distributions for the varying disturbances. The PCoA explains XXX% of the total variation in fungal community dissimilarity at the family level. The top 5 OTUs responsible for community dissimilarity are represented by vectors.

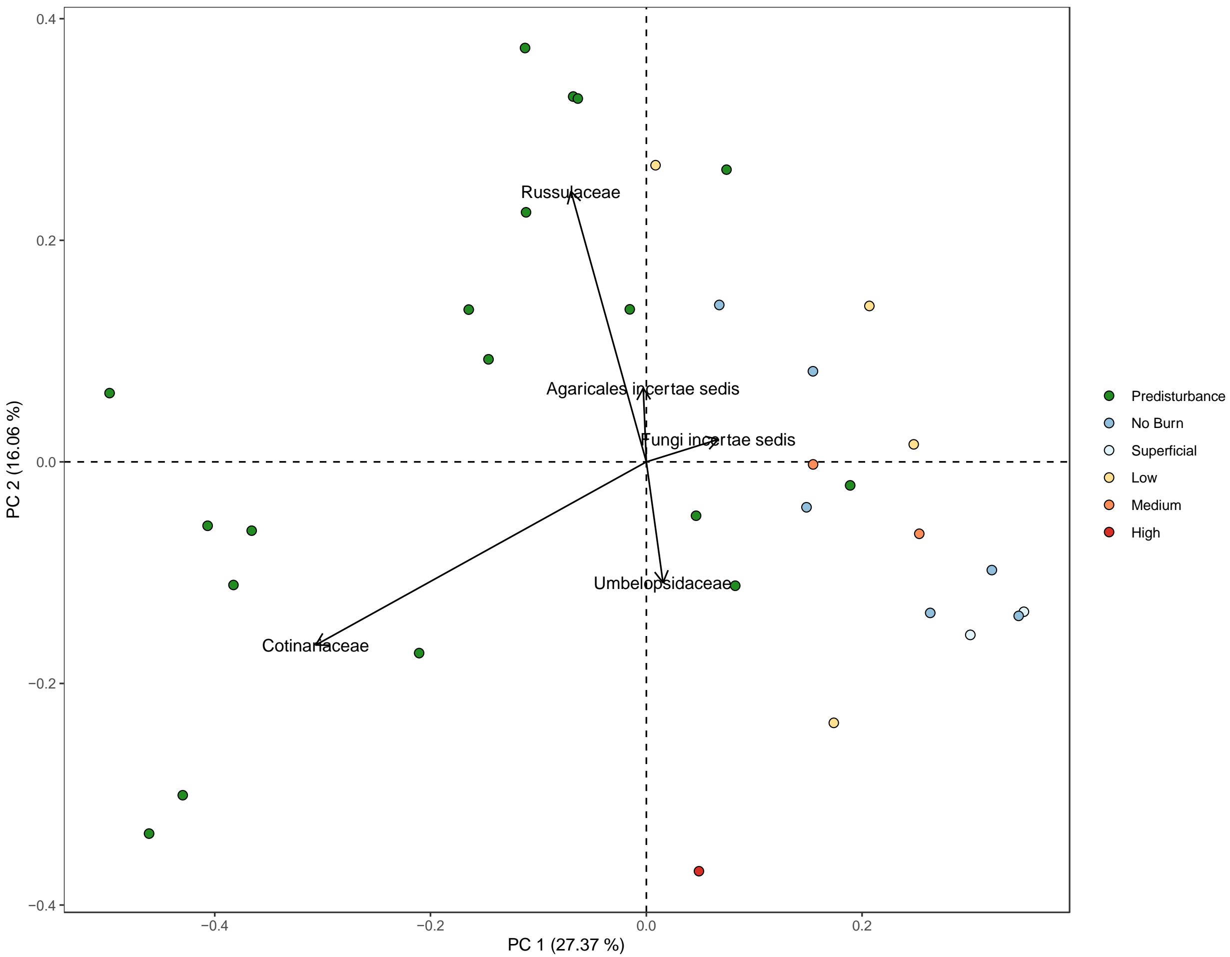

Figure A 6. Principal coordinates analysis (PCoA) of fungal community RNA in soils from reference (grey), pre-disturbance (green), cut + no burn (dark blue), superficial burn (light blue), low burn (yellow), medium burn (orange) and high burn (red) Bray-curtis abundances using ThetaYC calculator of dissimilarity. The PCoA explains ~43% of the total variation in fungal community dissimilarity at the family level. The top 5 families responsible for community dissimilarity are represented by vectors.

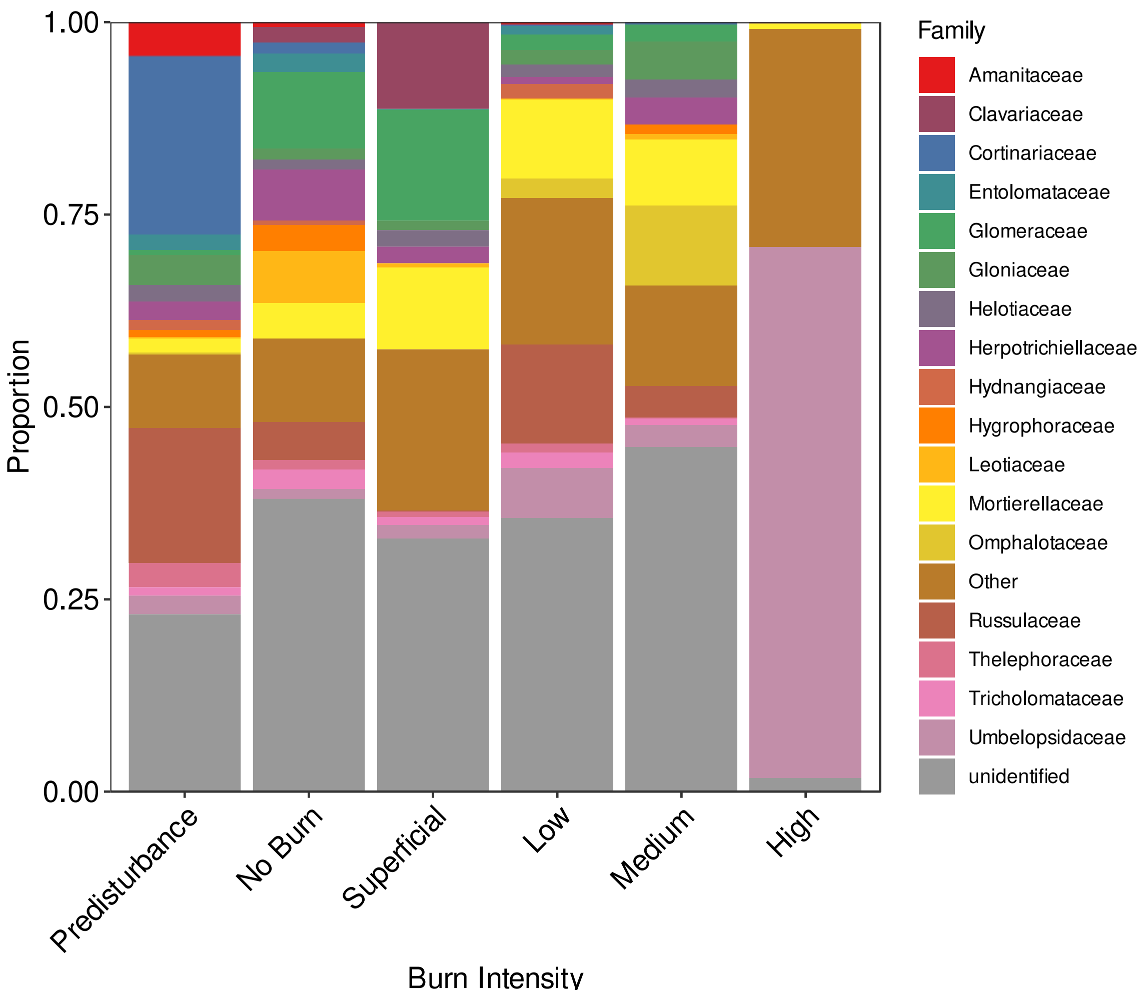

Figure A 7. Taxonomic relative abundances of fungal RNA sequences at the family level. Fungi are classified at a 99% confidence. Other includes all families that accounted for less than 1% of overall abundances. Unidentified include al fungi that could not be classified past the kingdom.

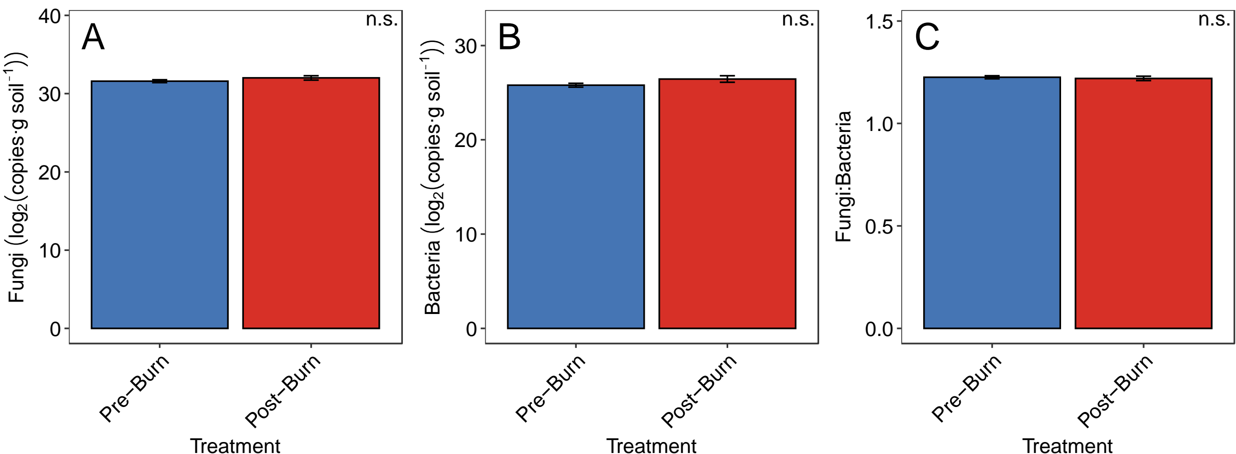

Figure A 8. fungal and bacterial abundances from gene copies pre- and post-cut +burn. A) Fungal ITS and (B) bacterial *16S rRNA* gene abundances reported as copies per g dry weight of substrate, along with (C) bacterial-to-fungal gene ratios. Results are presented as means + S.E. for pre and post-disturbance communities. Non-significant analyses are signified with by n.s..

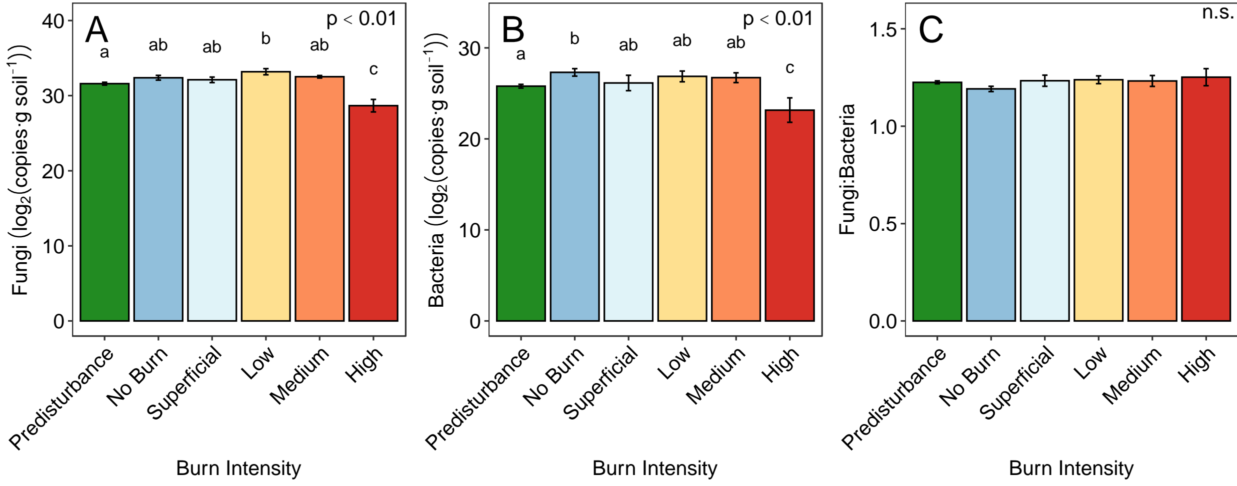

Figure A 9. Fungal and bacterial abundances from gene copies by disturbance severity. A) Fungal ITS and (B) bacterial *16S rRNA* gene abundances reported as copies per g dry weight of subtrate, along with (C) bacterial-to-fungal gene ratios. Results are presented as means + S.E. for pre-disturbance and the varying disturbance severities. Means marked with the same letter did not differ statistically (Krustal-Wallis tests followed by Dunn's *post hoc* with α=0.05).

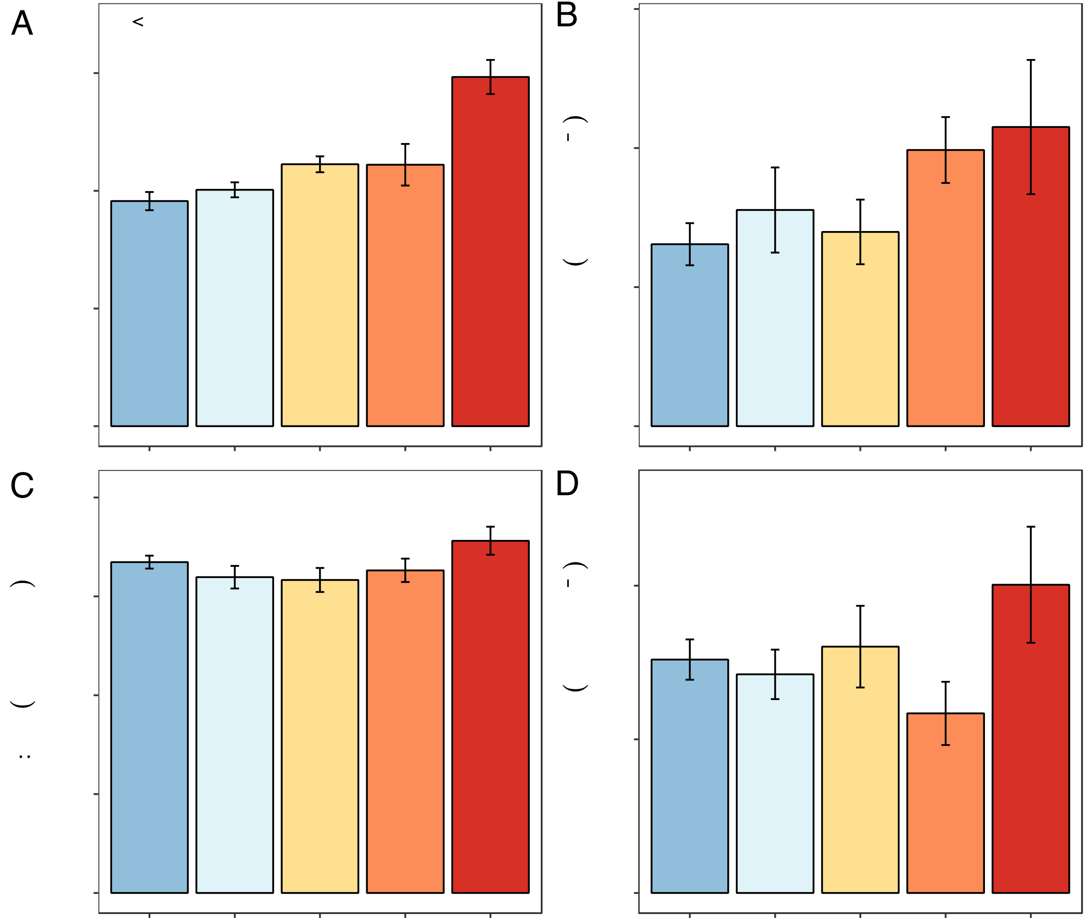

Figure A 10. Mean measures of (A) pH, (B) ECEC, (C) wet:dry ratios and (D) bulk density responses due to cut and burn treatments. Cut + no burn = dark blue, cut + superficial burn = light blue, cut + low burn = yellow, cut + medium burn = orange and cut + high burn = red. An ANOVA and Tukey post-hoc analyses revealed a difference in high burn severity pH relative to other disturbance severities. Whiskers indicate standard errors. Significant post-hoc results are indicated with differing letters above bars.

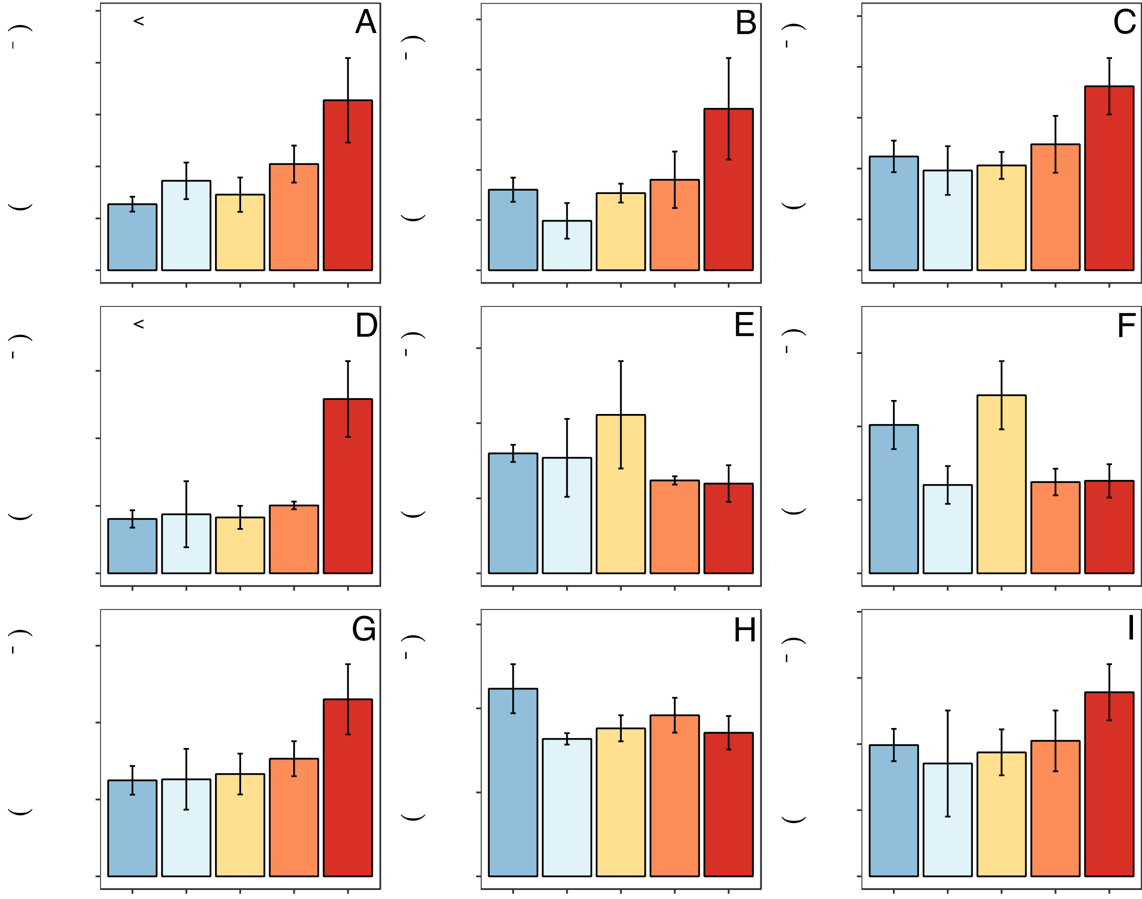

Figure A 11. Mean measures of cation and anion ((A) CA^{2+}, (B) K^{+}, (C) Mg^{2+}, (D) P^{3-}, (E) Cu^{+}, (F) Fe^{2+}, (G) Mn^{2+}, (H) Na^{+} and (I) S^{2-}) by disturbance severity. Cut + no burn = dark blue, cut + superficial burn = light blue, cut + low burn = yellow, cut + medium burn = orange and cut + high burn = red. ANOVAs and Tukey post-hoc analyses revealed a difference in high burn severity Ca^{2+} (A) and P^{3-} (D) relative to other disturbance severities. While not significant K^{+}, Mg^{2+}, Mn^{2+} also showed elevated levels in high severity locations. Whiskers indicate standard errors. Significant post-hoc results are indicated with differing letters above bars.

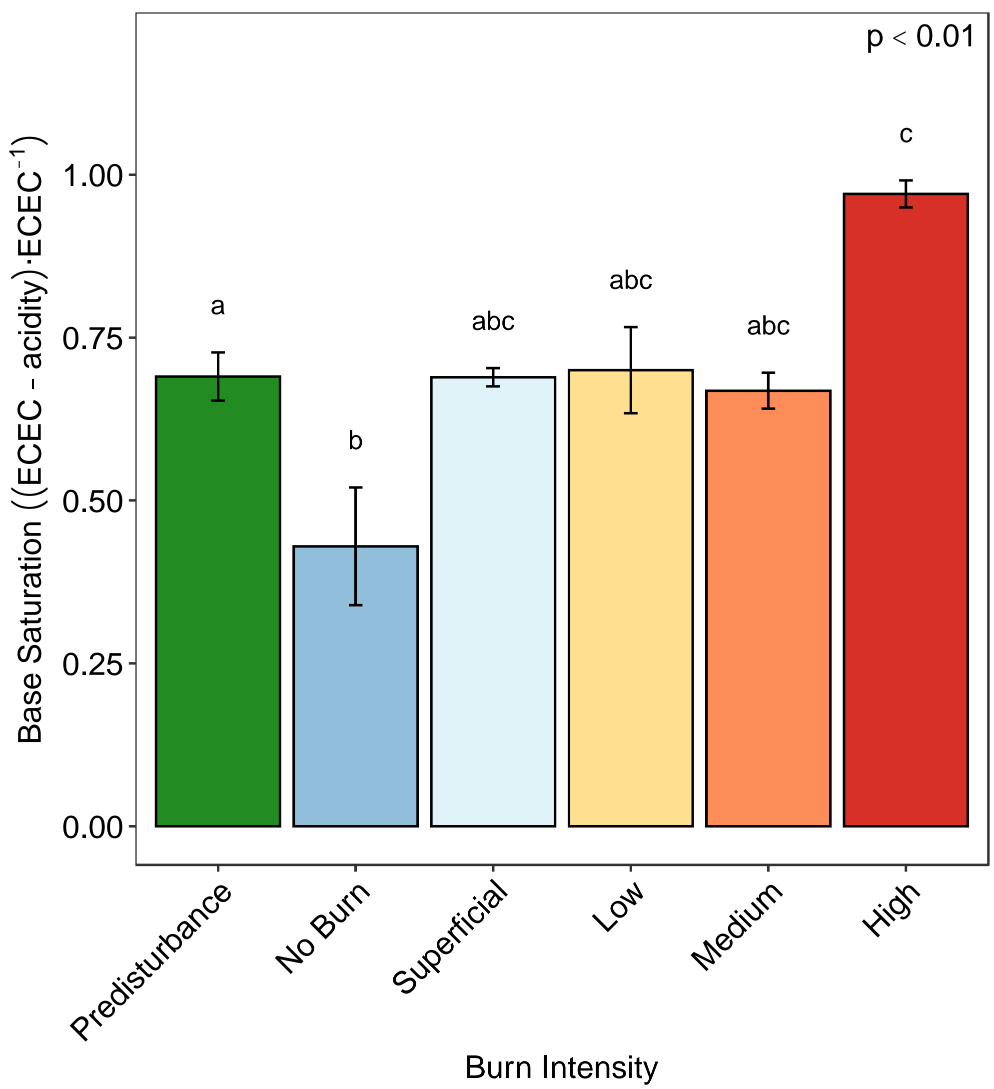

Figure A 12. Mean measures of base saturation by disturbance severity. Cut + no burn = dark blue, cut + superficial burn = light blue, cut + low burn = yellow, cut + medium burn = orange and cut + high burn = red. cut + no burn showed reduced base saturation. High severity burn had elevated base saturation relative to pre-disturbance and less severe treatments. Whiskers indicate standard errors. Significant post-hoc results are indicated with differing letters above bars.

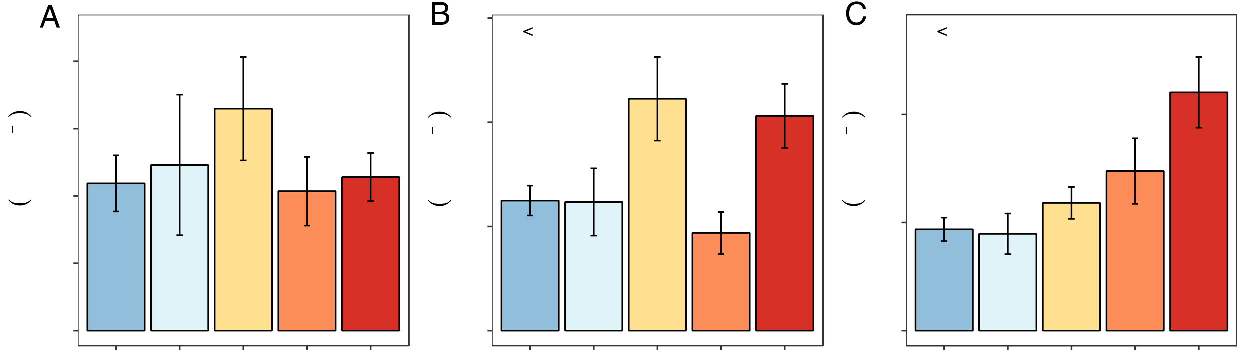

Figure A 13. Mean measures of mean availability of soil nutrients A) NO_3, B) NH_4 and C) PO_4 responses due to cut and burn treatments. Cut + no burn = dark blue, cut + superficial burn = light blue, cut + low burn = yellow, cut + medium burn = orange and cut + high burn = red. ANOVAs and Tukey post-hoc analyses revealed differences in available NH_4 and PO_4 with elevated availability in high burn severity locations. Whiskers indicate standard errors. Significant post-hoc results are indicated with differing letters above bars.

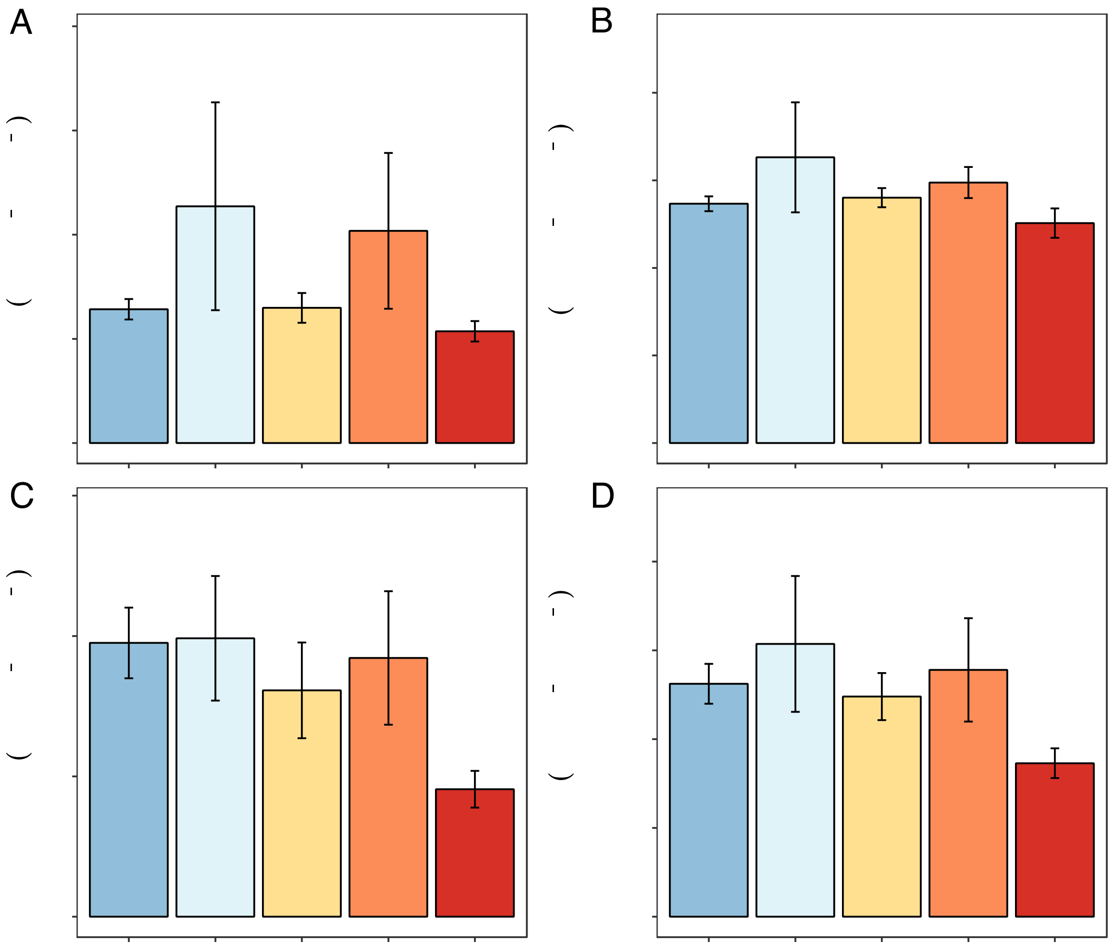

Figure A 14. Mean measures of mean availability of extracellular enzyme activity of (A)Nag, (B)alpha glucosidase, (C) alkaline phosphatase and (D) phenol oxidase in mineral soil of varying disturbance severities. Cut + no burn = dark blue, cut + superficial burn = light blue, cut + low burn = yellow, cut + medium burn = orange and cut + high burn = red. ANOVAs show no differences in any EEA across disturbance severities. Whiskers indicate standard errors.

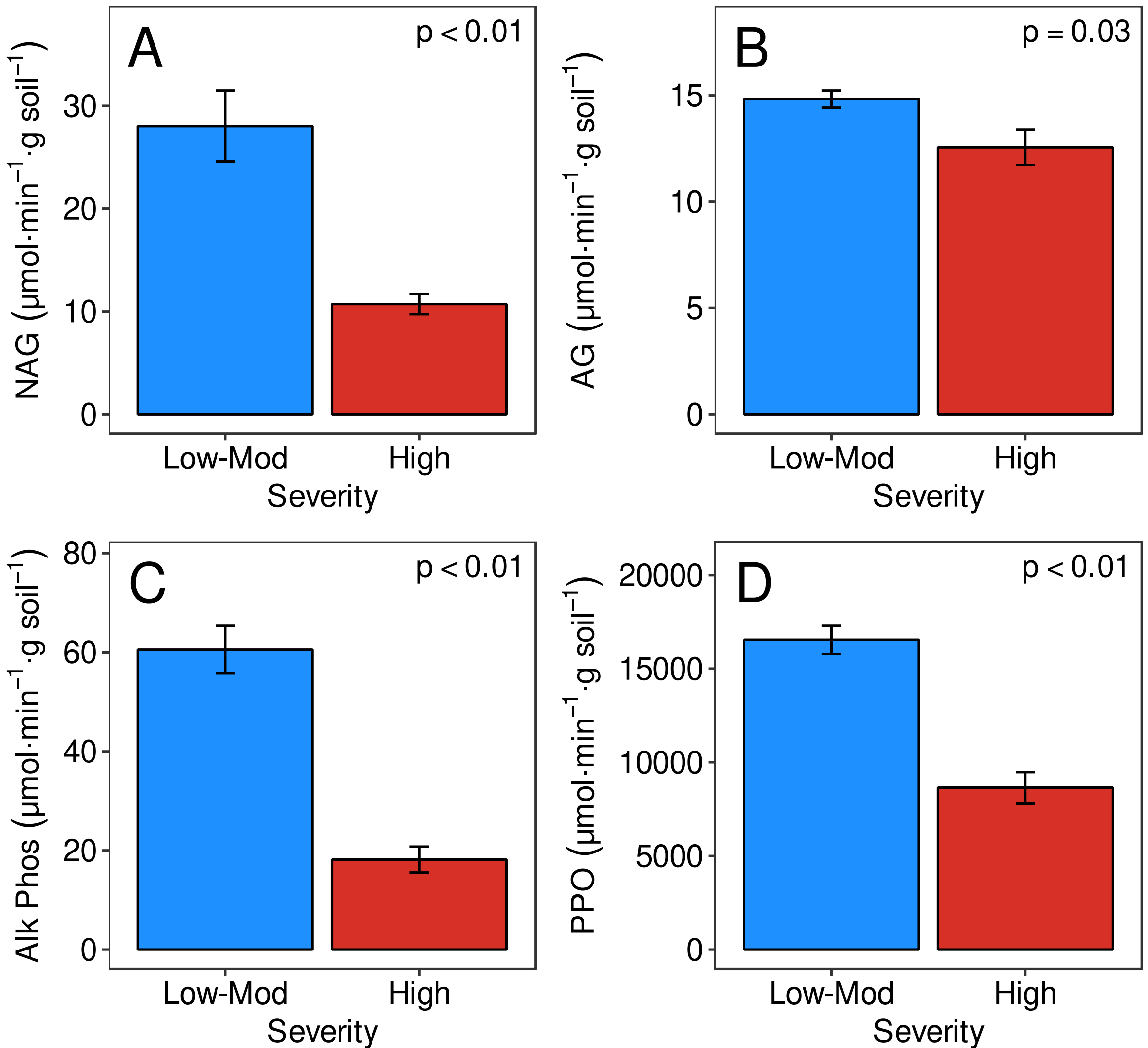

Figure A 15. Measures of mean availability of extracellular enzyme activity of (A) N-acetyl glutamate (B) alpha glucosidase, (C) alkaline phosphatase and (D) phenol oxidase in mineral soil of high severity vs all other disturbances pooled. Cut + no burn - cut+moderate burn = blue, cut + high burn = red

Appendix B Supplementary Figures & Tables for Chapter 3

Table B 1. The six enzymes measured, their abbreviations, target molecules, and enzyme commission numbers (E.C. Number).

Enzyme	Abbreviation	Target molecule	E.C. Number
β-1,4-glucosidase	BG	Cellulose	3.2.1.21
Alpha-glucosidase	AG	Starch	3.2.1.2
N-Acetylglutamate	NAG	Chitinase	2.3.1.1
Alkaline phosphatase	Alk	xylan	3.1.3.1
Phenol oxidase	PHEN_OX	Lignin	1.10.3.2
Peroxidase	PER	Hydrogen Peroxide	1.11.1.7

Table B 2. Mean EEA (µmmol/min/g) of oxidative and hydrolytic enzymes in varying aged stands. Average EEA of Alpha glucosidase (AG) increased in older stands (79 and 104 year old. An * indicates significant differences in activity.

Stand Age (years)	NAG	AG *	BG	ALK	PHEN-OX	PER
104	2.8 (±0.6)	3.0 (± 0.2)	1.9 (± 0.3)	5.5 (± 2.9)	631.3 (± 452.7)	1616.0 (± 964.2)
79	2.6 (± 1.2)	2.1 (± 0.6)	2.1 (± 0.8)	4.9 (± 4.5)	615.0 (± 491.3)	1425.9 (± 961.65)
67	3.2 (± 2.6)	1.6 (± 0.2)	2.2 (± 1.2)	5.0 (± 2.4)	481.4 (± 233.3)	1223.3 (± 1091.9)
61	2.1 (± 2.2)	1.7 (± 1.7)	1.8 (± 1.8)	3.8 (± 3.8)	405.2 (± 407.4)	1066.6 (± 1027.4)
35	2.8 (± 2.9)	1.7 (± 0.1)	1.6 (± 0.2)	5.8 (± 2.5)	479.9 (± 177.1)	1296.3 (± 811.7)
17	3.5 (± 2.5)	1.9 (± 0.1)	1.9 (± 0.4)	7.0 (± 4.9)	518.3 (± 436.3)	387.5 (± 243.5)

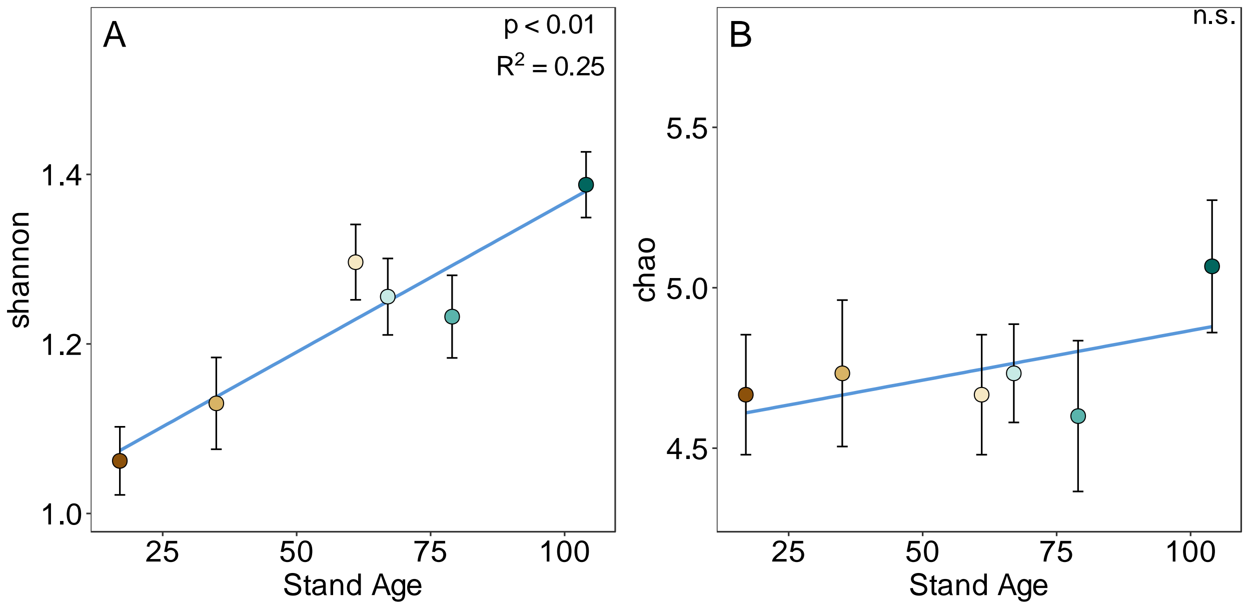

Figure B 1. Diversity indices of sorted Oi horizon leaf litter across varying age chronosequence stands from 17-year-old stands to 104 year old stands. Stand ages: 17 year (red), 35 year (orange), 61 year (yellow), 67 year (light blue), 79 (blue), 104 year (dark blue). The shaded band is the 95% confidence interval of the linear model.

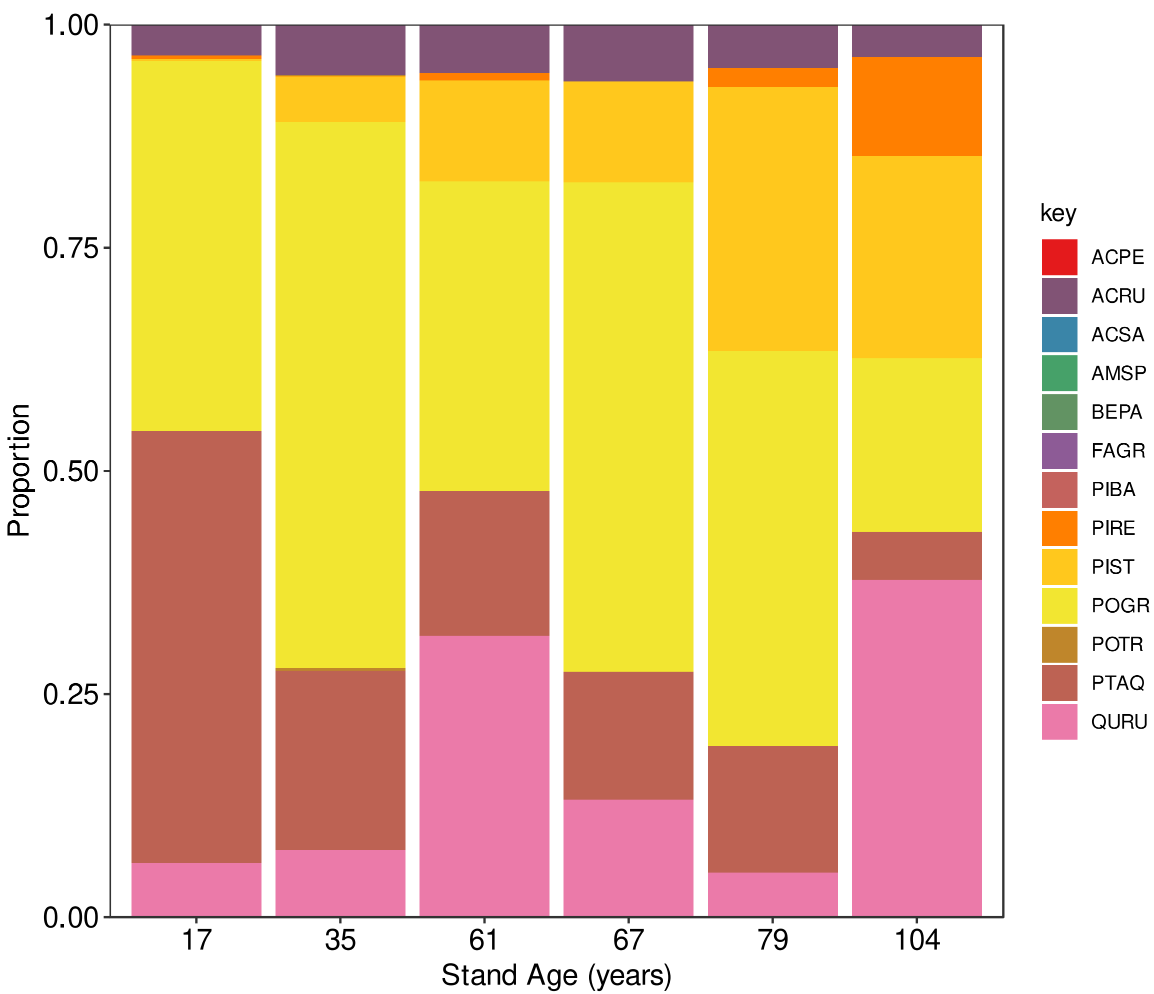

Figure B 2. Relative abundances of leaf litter in the Oi horizons. Legend indicates latin binomials of species: ACPE (*Acer pennsylvaticum)*, ACRU (*Acer rubrum*), ACSA (Acer saccharum), AMSP (A*melanchier arborea*), BEPA (*Betula papyrifera*), FAGR (*Fagus grandidentata*), PIBA (*Pinus banksiana*), PIRE (*Pinus resinosa*), PIST (*Pinus strobus*), POGR (*Populus grandidentata*), POTR (*Populus tremuloides*), PTAO (*Pteridium aqualinum*), QURU (*Quercus rubra*).

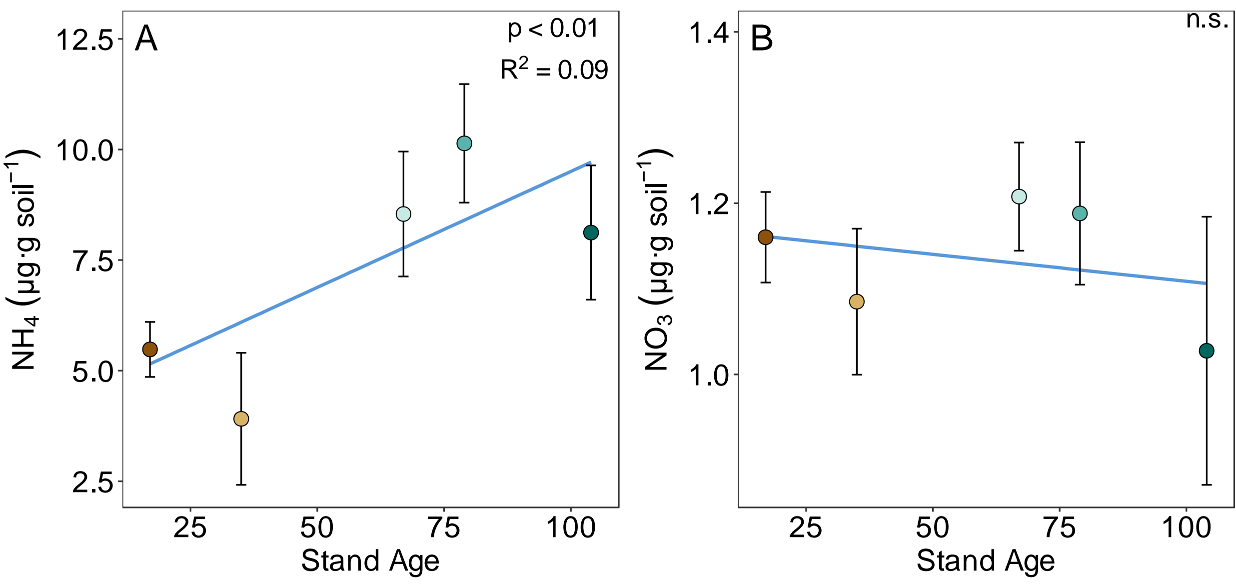

Figure B 3. Available mineralized N in A horizon soil without 1954. A) Available NH_4 increases with stand age ($p<0.01$, $r^2=0.09$) B) No differences were detected in available NO_3 across stands. Means are represented by closed circles and vertical hashed lines indicate ±1 S.E. Shaded bands indicate 95% confidence intervals.

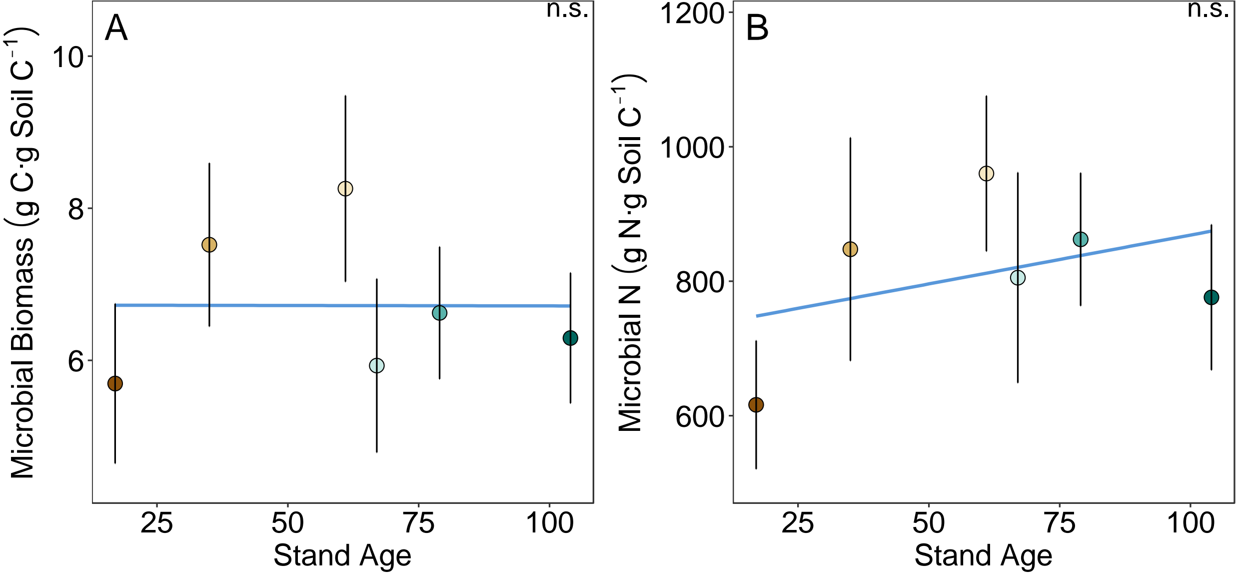

Figure B 4. Relationships between microbial C and N per gram soil C. There is no relationship between microbial biomass indices per gram C across stands. Indicates no difference in substrate quality across stands. Stand ages: 17 year (red), 35 year (orange), 61 year (yellow), 67 year (light blue), 79 (blue), 104 year (dark blue).

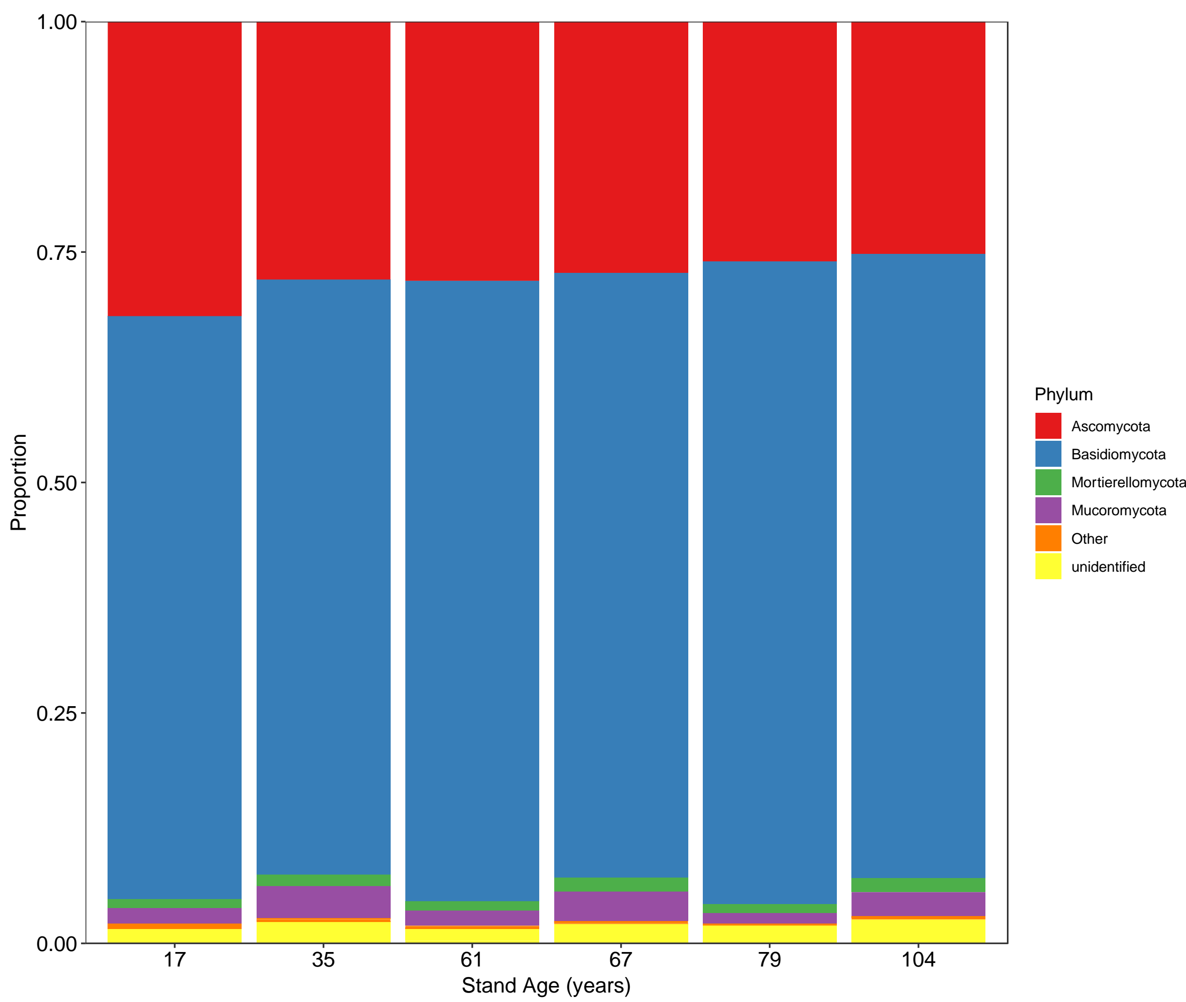

Figure B 5. Relative abundances of OTUs in fungal phyla across varying aged stands. All phyla that individually accounted for <1% of total relative abundance in each stand were pooled as "other".

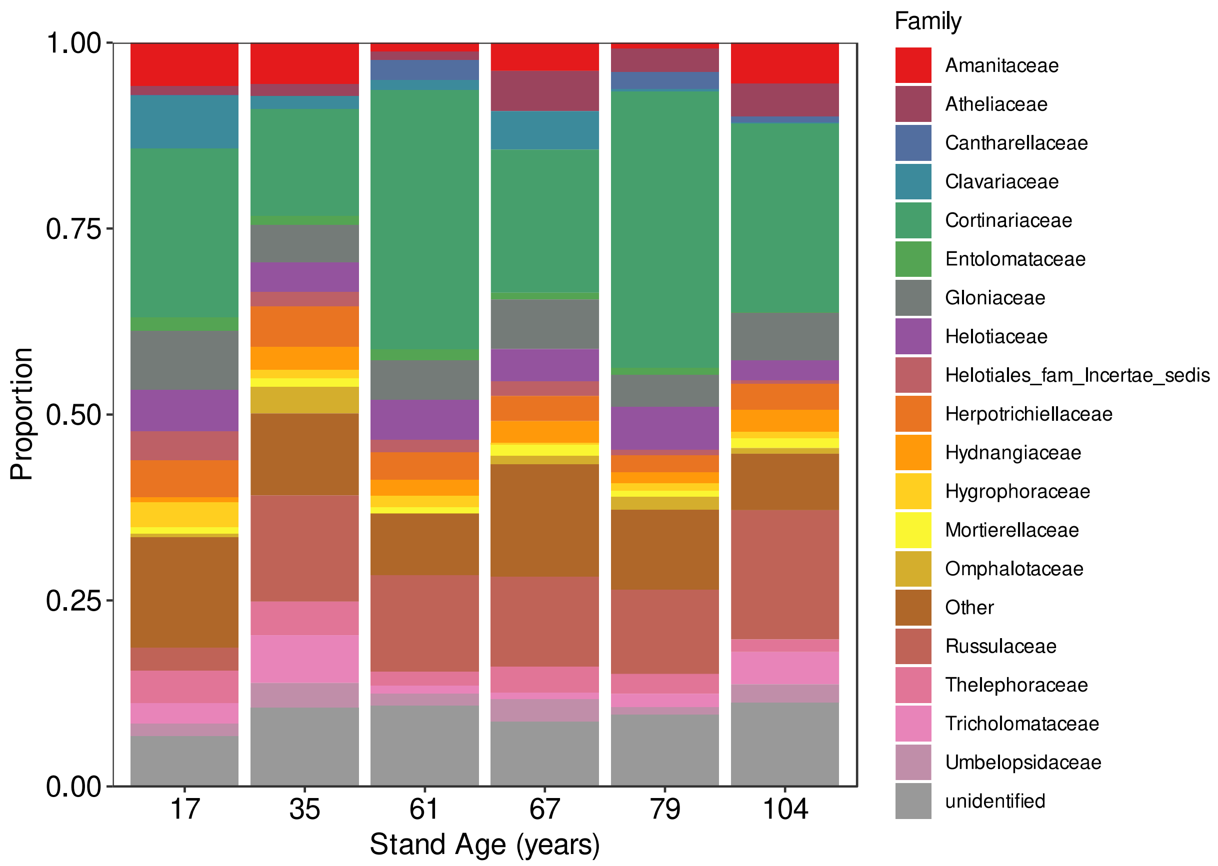

Figure 6. Relative abundances of OTUs in fungal families across varying aged stands. All families that individually accounted for <1% of total relative abundance in each stand were pooled as “other”.

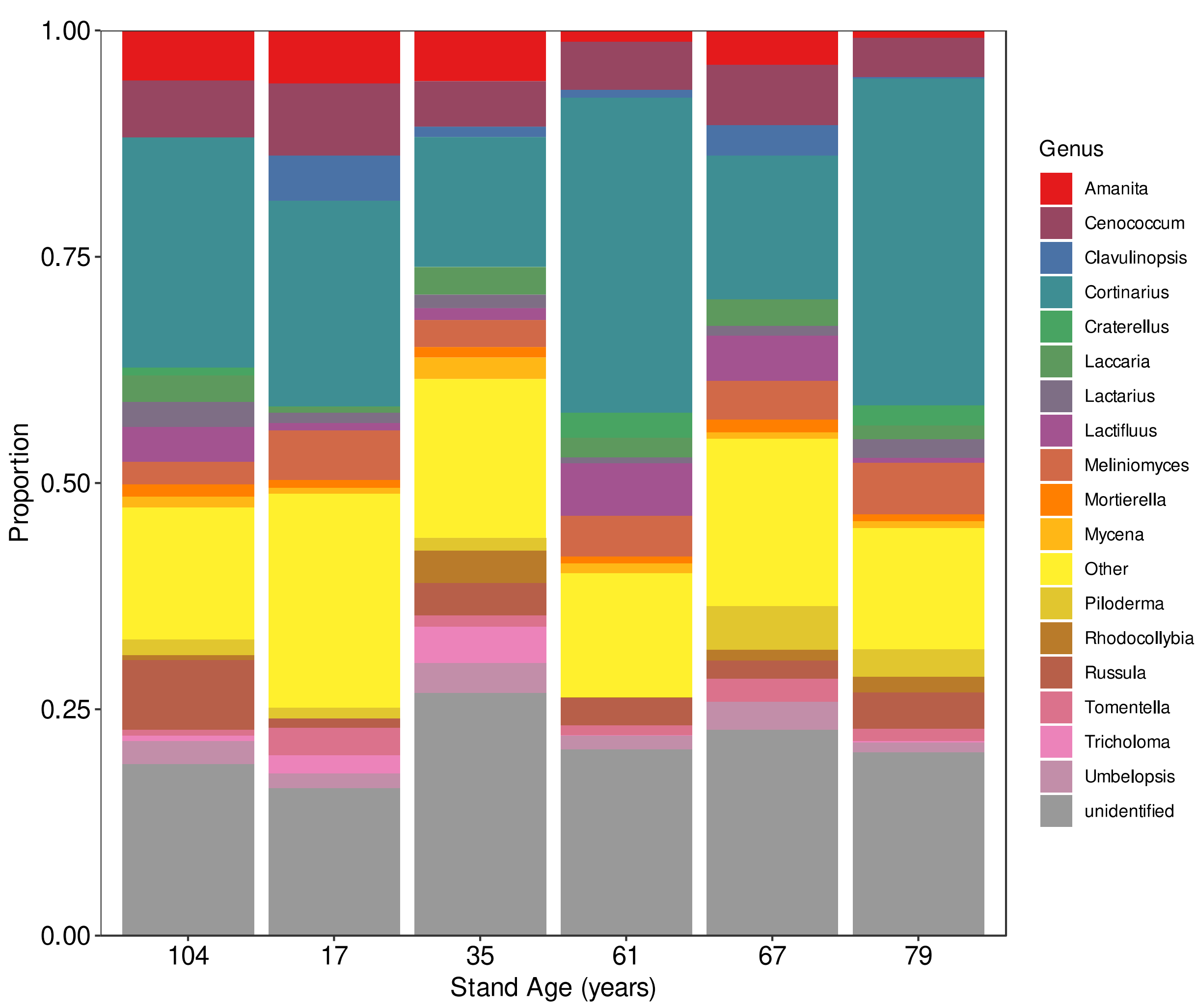

Figure B 7. Relative abundances of OTUs in fungal genera across varying aged stands. All genera that individually accounted for <1% of total relative abundance in each stand were pooled as “other”.

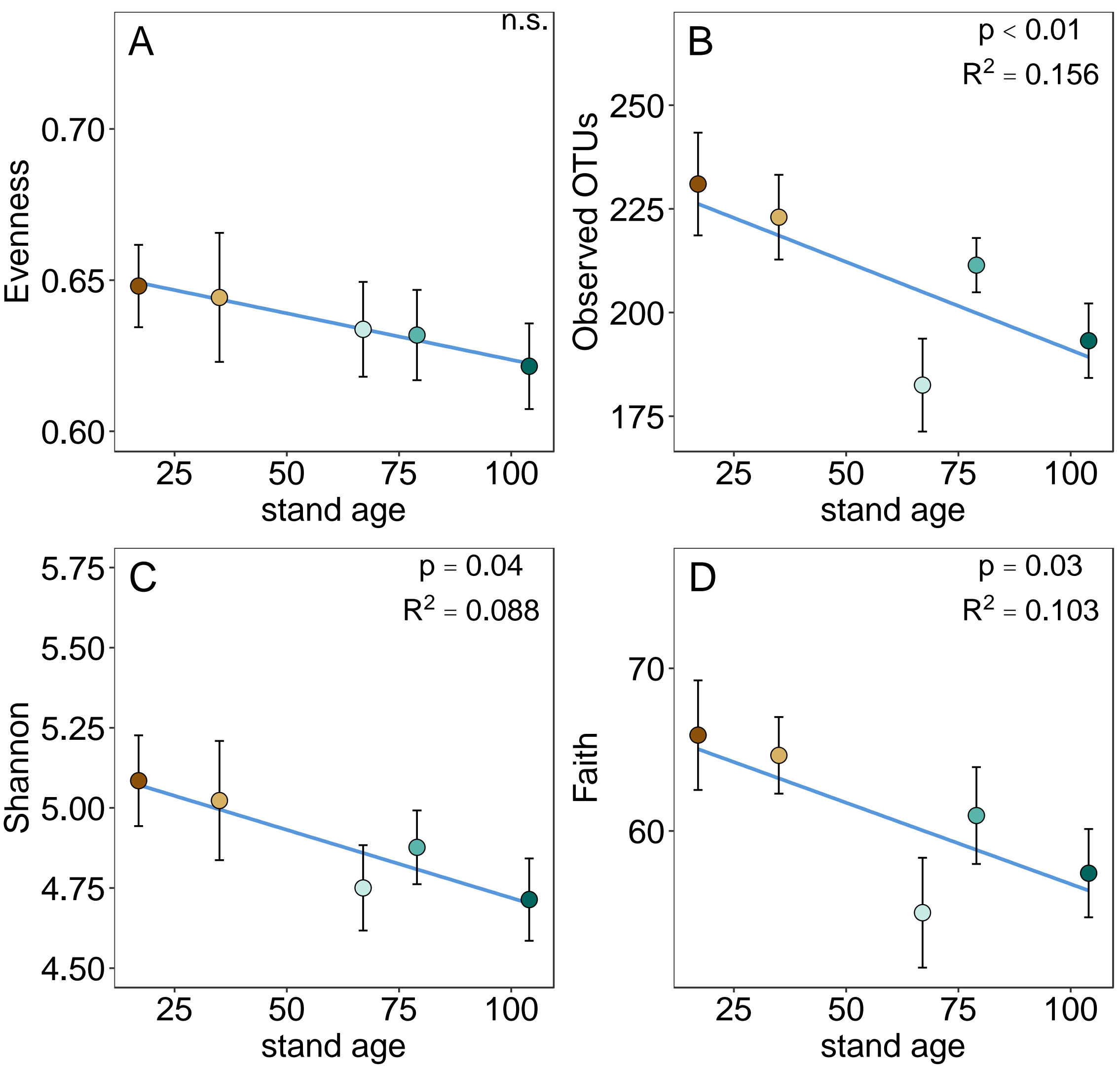

Figure B 8. Diversity indices across varying age chronosequence stands exluding the 61-year old stand (1954). Stand ages: 17 year (red), 35 year (orange), 67 year (light blue), 79 (blue), 104 year (dark blue). The shaded band is the 95% confidence interval of the linear model

Appendix C Supplementary Figures & Table for Chapter 4

Table C 1: Coarse woody debris classification guide adapted from the Forest Inventory and Analysis National Core Field Guide (2018). Note: Decay class 5 logs are difficult to identify as they blend into the duff, litter, and soil layers. The decay class 5 logs in our study were identified using tags from an older study.

Coarse Woody Debris Decay Classification		
Decay Class	Structural Integrity	Texture of Rotten Portions
Decay Class 1	Sound, freshly fallen, Intact logs, bark still intact	Intact, no rot; conks of stem decay absent
Decay Class 2	Sound, log holds it shape, bark mostly intact	Mostly intact; sapwood partly soft (starting to decay) but cannot be pulled apart by hand
Decay Class 3	Log becoming soft, Heartwood sound; Bark beginning to sluff off,	Sapwood can be pulled apart by hand or sapwood absent, large pieces
Decay Class 4	Heartwood rotten; Bark gone, log no longer holds shape, establishment of mosses and herbaceous plants	Soft, sapwood mostly absent, can be pulled apart easily by hand, small pieces; A metal pin can be pushed into heartwood
Decay Class 5	None, Piece no longer maintains its shape, log spreads out on ground, substrate for plants	Soft, very small pieces, can crumble when dry

Table C 2: The six enzymes measured, target molecules, and enzyme commission numbers (E.C. Number).

Enzyme	Target	E.C. Number
β-1,4-glucosidase	Cellulose	3.2.1.21
1,4- β-cellobiosidase	Cellulose	3.2.1.91
β-D-xylosidase	Hemicellulose	3.2.1.37
Leucyl aminopeptidase	Polypeptides	3.4.11.1
Phenol oxidase	Lignin	1.10.3.2
Peroxidase	Lignin	1.11.1.7

Table C 3: Total number of observed OTUs and calculations of sequence coverage by sample. (SD=standing dead, DC1=decay class 1, DC2=decay class 2, DC3=decay class 3, DC4=decay class 4, DC5=decay class 5). N=27 (4 per decay stage of log, 3 soil).

Sample	Sequence coverage (%)	Number of OTUs	Treatment
SD	99.72	511	Standing Dead
SD	99.69	552	Standing Dead
SD	99.71	630	Standing Dead
SD	99.77	454	Standing Dead
DC1	99.64	741	Decay Class 1
DC1	99.75	371	Decay Class 1
DC1	99.80	457	Decay Class 1
DC1	99.75	456	Decay Class 1
DC2	99.70	530	Decay Class 2
DC2	99.71	628	Decay Class 2
DC2	99.68	604	Decay Class 2
DC2	99.68	659	Decay Class 2
DC3	99.70	579	Decay Class 3
DC3	99.67	667	Decay Class 3
DC3	99.69	632	Decay Class 3
DC3	99.71	545	Decay Class 3
DC4	99.67	583	Decay Class 4
DC4	99.74	435	Decay Class 4
DC4	99.66	700	Decay Class 4
DC4	99.66	662	Decay Class 4
DC5	99.68	730	Decay Class 5
DC5	99.68	809	Decay Class 5
DC5	99.66	654	Decay Class 5
DC5	99.74	559	Decay Class 5
S	99.75	520	Soil
S	99.75	513	Soil
S	99.69	710	Soil

Table C 4: Twenty most abundant fungal OTUs identified by matching sequences to the UNITE fungal database.

OTU	# of sequences	Guild	Taxon
Otu00001	148821	Wood Saprotroph	Rigidoporus corticola
Otu00002	142937	Undefined Saprotroph	Saccharomycetales unclassified
Otu00003	138975	Undefined Saprotroph	Scheffersomyces shehatae
Otu00004	118428	Undefined Saprotroph	Rhodotorula lignophila
Otu00005	116735	Undefined Saprotroph	Pichia sp
Otu00006	109789	Wood Saprotroph	Trichaptum biforme
Otu00007	84795	Undefined Saprotroph	Basidiodendron sp
Otu00008	84265	Ectomycorrhizal	Russula sp
Otu00009	73083	Wood Saprotroph	Phlebia centrifuga
Otu00010	69490	Wood Saprotroph	Trechispora sp
Otu00011	68481	Undefined Saprotroph	Candida boleticola
Otu00012	68229	Wood Saprotroph	Perenniporia
Otu00013	59905	Wood Saprotroph	Phlebia fuscoatra
Otu00014	58969	Undefined Saprotroph	Saccharomycete
Otu00015	54601	Endophyte	Leptodontidium
Otu00016	53112	Wood Saprotroph	Bjerkandera
Otu00017	50915	Wood Saprotroph	Aporpium macroporum
Otu00018	48536	Undefined Saprotroph	Candida ergatensis
Otu00019	47133	Wood Saprotroph	Phanochaete sordida
Otu00020	46914	Ectomycorrhizal	Piloderma sphaerosporum

Table C 5: Correlations comparing activity of individual enzymes versus %N indicates a significant increase for all enzymes except phenol oxidase.

Enzyme	r	P value
β-1,4-glucosidase	0.58	0.002
1,4- β-cellobiosidase	0.52	0.01
β-D-xylosidase	0.73	<0.0001
Leucyl aminopeptidase	0.69	<0.0001
Phenol oxidase	-0.21	0.29
Peroxidase	0.52	0.01

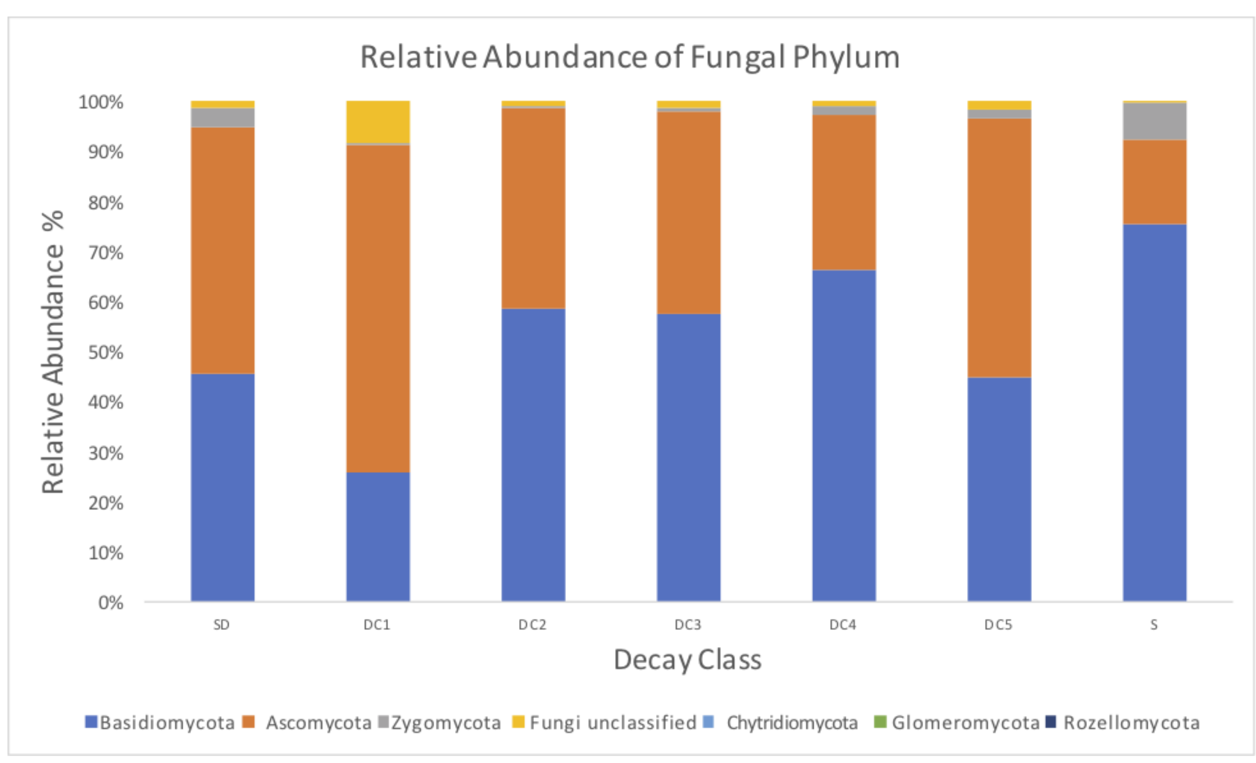

Figure C 1: The average relative abundances (as % of classified sequences) of the dominant fungal phyla across CWD decay classes from standing dead to soil. (SD=standing dead, DC1=decay class 1, DC2=decay class 2, DC3=decay class 3, DC4=decay class 4, DC5=decay class 5). N=27 (4 per decay stage of log, 3 soil).

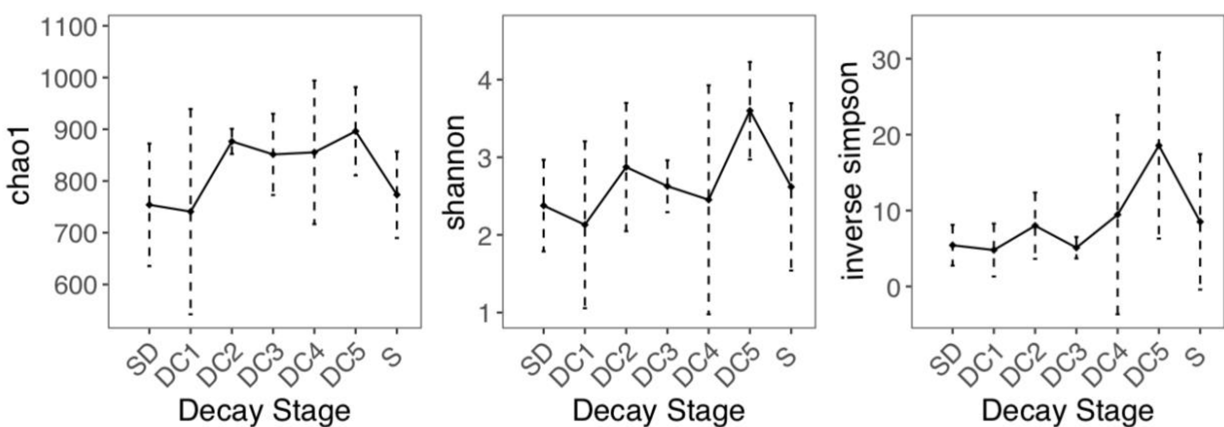

Figure C 2: Diversity indices across CWD decay stages from standing dead to soil. No significant differences were found in richness based on abundances (chao) or evenness and richness (npshannon and invsimpson) indices within wood or between wood and soil. Hashed whiskers represent standard deviation. (SD=standing dead, DC1=decay class 1, DC2=decay class 2, DC3=decay class 3, DC4=decay class 4, DC5=decay class 5). N=27 (4 per decay stage of log, 3 soil).

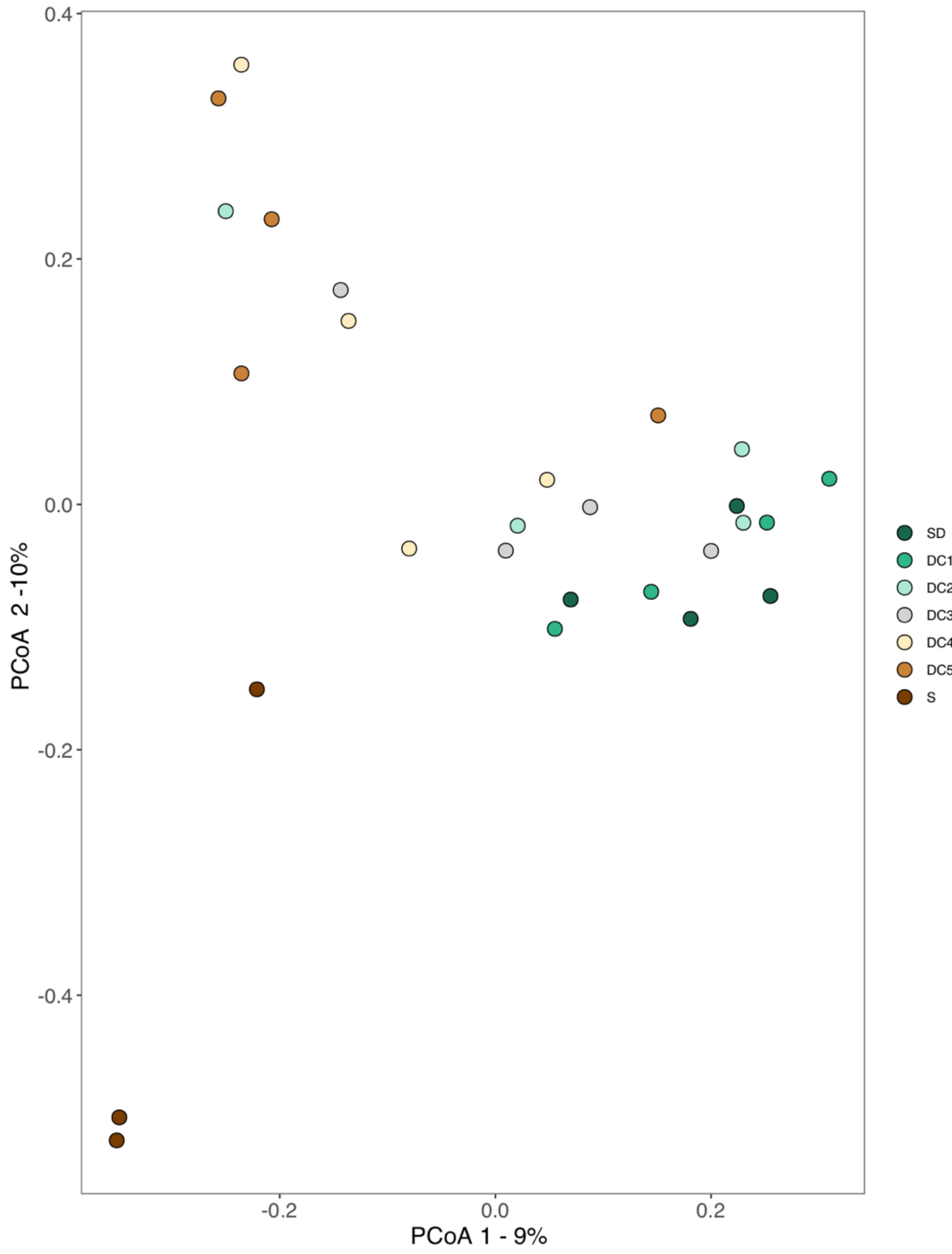

Figure C 3: Principal coordinates analysis (PCoA) of fungal communities in soil and wood decay stage samples based on Bray-curtis abundance using ThetaYC calculator of dissimilarity. The PCoA explains 19% of the total variation in fungal community dissimilarity. (SD=standing dead, DC1=decay class 1, DC2=decay class 2, DC3=decay class 3, DC4=decay class 4, DC5=decay class 5). N=27 (4 per decay stage of log, 3 soil).