Assessment of forest biomass management practices through fungal community sampling: wood-inhabiting fruiting body surveys and DNA-based analyses of wood stakes in a western conifer forest of North America

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Kymberly R. Draeger

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The dissertation is approved by the following members of the Final Oral Committee:
Glen R. Stanosz, Professor, Forest and Wildlife Ecology
Daniel L. Lindner, Research Plant Pathologist, USDA Forest Service
Eric Kruger, Professor, Forest and Wildlife Ecology
Thea Whitman, Assistant Professor, Soil Science
Paul Koch, Assistant Professor, Plant Pathology

Abstract

Assessment of forest biomass management practices through fungal community sampling: wood-inhabiting fruiting body surveys and DNA-based analyses of wood stakes in a western conifer forest of North America

Kymberly R. Draeger

Under the supervision of Professor Glen R. Stanosz

University of Wisconsin-Madison

To assess the effects of biomass harvesting treatments and compensatory soil amendments on wood-inhabiting fungal communities, two methods of fungal sampling (collection of fruiting bodies and DNA-based methods) were used to analyze changes in wood-inhabiting fungal communities in a conifer-dominated forest in western North America. Plots were established with biomass retention at four levels and with four soil amendment treatments.

Fruiting body surveys of woody substrates yielded 1,002 observations over 3 years, comprising 129 mostly basidiomycete species. Community composition of fungal fruiting bodies was significantly influenced by biomass treatments in 2014 (p = 0.003), while there was no significant effect of soil amendment treatments relative to community composition.

DNA-based methods, analyzing wood stakes on or within soil, revealed 2,316 different operational taxonomic units (OTUs) of mostly ascomycete species. Fungal community composition within wood stakes as indicated by high-throughput amplicon sequencing was

influenced by biomass treatments all 3 years ($p < 0.04$), while soil amendments were found to significantly influence fungal community composition in 2015 and 2017 ($p < 0.04$).

The two sampling methods revealed different fungal communities. Fruiting body surveys sampled a range of wood substrates and identified important forest pathogens. DNA-based methods produced more data, identified more OTUs ($p < 0.001$) and were more sensitive to treatments, especially soil amendment treatments. Both methods had advantages and disadvantages, and success with each method depends on the study, the questions being asked, and the researcher's skills. The potential for biomass harvesting and soil amendments to alter fungal communities in conifer-dominated forests of western North America has implications for forest health and productivity. Within this limited study, removal of thinned woody biomass did not negatively influence fungal community compostion.

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General Introduction

Western United States wildfires and woody biomass. Most forest ecosystems in the Rocky Mountain region evolved with fire disturbances (Arno, 2000; Cooper 1991; Keane, 2002; Veblen et al., 2000). Fire suppression implemented in the early 1900s, coupled with timber management practices, have unquestionably changed the historical trajectory of the forests (Arno, 2000; Cooper 1991; Keane, 2002). Woody biomass includes the living trees and woody plants, as well as dead limbs, tops, and other woody parts that accumulate naturally or result from forest management activities such as thinning (USDA Forest Service, 2013). Without periodic fires, overstocked forests with excess biomass have been subjected to high intensity fires (Keyes and O'Hara, 2002). High-intensity fires lead to crown torching, soil scorching, and increased mortality to plants and soil organisms (Arno, 2000). Timber harvesting practices contribute to fuel loads by leaving behind less profitable logging residues such as dead and rotten trees, non-commercial species, and small trees that are not economical for transport or manufacturing (Keyes and O'Hara, 2002; Perlack et al., 2005). As part of the 2002 President's Healthy Forests Initiative, the United States Department of Agriculture (USDA), Department of Interior (DOI), and Council on Environmental Quality recommended that forestland areas exceeding prescribed or recommended stocking densities be thinned or treated to reduce woody biomass and fuel loads (Perlack et al., 2005; U.S. Department of Energy et al., 2011). Despite the ecological and silvicultural benefits of thinning, the current cost of biomass removal often exceeds its monetary value (McElliogott et al., 2011). Therefore, forest managers in the intermountain west are eager for incentives to develop viable bioenergy markets.

Biofuels. Woody biomass can be a feedstock for the production of a variety of biofuels, including bio-oil. Heating biomass in an oxygen-deprived environment leads to incomplete combustion (pyrolysis). When the resulting vapors are condensed, the subsequent byproducts are liquid bio-oil, aerosols, and char (Mohan, et al., 2006). Bio-oil can easily be upgraded into a "drop-in" fuel (Mohan et al., 2006) that can be blended with other petroleum products, transported via current pipeline infrastructure, and utilized by existing equipment (Richard, 2010). The biochar byproduct, a recalcitrant black carbon functionally similar to charcoal, has been recommended for application to agricultural and forest soils to improve soil quality, enhance soil organic matter content, and sequester carbon (McElligott et al., 2011; Page-Dumroese et al., 2009). While other biofuel possibilities exist, bio-oil is most appropriate for efficient Western coniferous biomass utilization (Mohan et al., 2006).

Policies supporting bioenergy production. North American woody biomass is currently being sold internationally to countries whose commitments to renewable energy fuels have surpassed their local supply (Heinimo and Junginger, 2009). A major market is western Europe because of the European Union's goal of a 20% renewable energy portfolio (European Commission, 2014). By 2020, the EU expects to import between 15-30 million tonnes (16.8-33.6 million U.S. tons) of dried wood biomass as pellets for large-scale co-firing in their industrial sector (European Commission, 2014).

In 2011, the U.S. consumed about 130 million dry tons of forest biomass for energy production annually (U.S. Department of Energy et al., 2011). To address the sustainability of United States energy supplies, Congress established goals requiring over 1 billion dry tons of biomass feedstock annually to supplement the nation's power, transportation fuel, and chemical

production needs by 2030 (USDA Forest Service, 2013). The Energy Independence and Security Act of 2007 set goals to produce seven times more biofuels, 36 billion gallons, by 2022 (U.S. Department of Energy et al., 2011).

Recognizing the potential for the increased economic value in woody biomass feedstocks, the Department of Energy (DOE), DOI, and USDA announced an initiative in the Memorandum of Understanding on Policy Principles for Woody Biomass Utilization for Restoration and Fuel Treatment on Forests, Woodlands, and Rangelands, encouraging the utilization of woody biomass rather than reduction through prescribed burning or other on-site disposal methods (Norton et al., 2003). These policies prompted concerns over the unknown impacts of such removal operations on forest sustainability, highlighting the need for more information on the effects of biomass removal from forest systems (Kimmins, 1997; U.S. Department of Energy et al., 2011).

Sustainable forestry and biomass harvesting guidelines. Without a sustainable forest there cannot be a sustainable harvest yield (Maser, 1989). The goals of sustainable forestry are to meet the needs of the present *and* allow for a continuous supply of forest products for future generations while ensuring the protection of habitat, water resources, and genetic diversity (Fox, 2000). Gifford Pinchot, the first chief of the U.S. Forest Service, believed that forests should be managed for, "…the greatest good of the greatest number in the long run," (USDA Forest Service, 2016).

In order to address the sustainability of forest resources, biomass harvesting guidelines have already been established for many states, regions, and countries; however, Idaho has yet to develop such guidelines. Biomass harvesting guidelines incorporate silvicultural guidelines and

forestry best management practices (BMPs) specific to site location, tree species, soil types, and wildlife habitat (Forest Guild Biomass Working Group, 2010). All sustainable woody biomass harvesting guidelines include the retention of downed dead woody materials.

Fungal biodiversity. Some fungi parasitize and kill living hosts. For example, the root rotting pathogen *Heterobasidion annosum* (Fr.) Bref. sensu lato causes expanding mortality centers, and results in estimated lost profits of 790 million euro per year (~900 million U.S. dollars) in the European Union (Garbelotto and Gonthier, 2013). *Phellinus weirii* (Murill) Gilb. causes laminated or yellowing root and butt rot and is especially damaging to fir and hemlock trees, which allows tolerant and root rot resistant pine species to succeed in the forest ecosystem (Hansen and Goheen, 2000). Another root pathogen, *Armillaria solidipes* Peck has responded to fertilization with increased hyphal growth and increased incidence of infection of Douglas-fir [*Pseudotsuga menziesii* (Mirbel) Franco] (Entry et al., 1991).

Dead wood provides the base of the food chain for microbes, invertebrates, small mammals, amphibians, birds, and larger vertebrates (Ferris and Humphrey, 1999; Lonsdale et al., 2007). The ecology of dead wood relies on wood-decaying fungi to modify the physiochemical composition of the wood to create available resources for other functional groups (Lonsdale et al., 2007; Rayner and Boddy, 1988). White and brown rot fungi are the only organisms capable of substantial and efficient decay of wood's recalcitrant lignocellulosic structures (Hammel, 1997; Rayner and Boddy, 1988). Therefore, fungi are directly responsible for the degradation, decomposition, and recycling of wood carbon, nutrients, and energy into soil organic matter and humus (Ferris and Humphrey, 1999; Hammell, 1997; Lonsdale et al., 2007; Rayner and Boddy, 1988).

Water-limited sites in the western United States are especially reliant on soil organic matter to maintain site productivity by enhancing water retention and availability, support seedling regeneration, and facilitate mutualistic mycorrhizal associations (Harvey et al., 1987). The importance of ectomycorrhizal (EM) fungi in forest ecosystems is highlighted by the estimate that 2,000 species of EM fungi associate with Douglas-fir, and about 72% of those fungi can associate with multiple plant hosts (Horton et al., 2005). Due to their ubiquitous nature, fungi have been used as indicators of ecosystem biodiversity, community composition, as well as conservation and management practices (Bader et al., 1995; Ferris and Humphrey, 1999; Lindner et al., 2006; Lonsdale et al., 2007; Purahong et al., 2014). Understanding how biomass harvesting affects wood-inhabiting fungal communities is critical for assessing the environmental sustainability of such harvests.

Biomass harvesting will likely affect the fungi that depend on wood substrates for habitat. Bader et al. (1995), Norden et al. (2004), and Rydin et al. (1997) have established that reductions in the quality and quantity of woody debris due to logging and management practices have an adverse effect on the richness and abundance of fungi. Whereas some fungal species require large-diameter, well-decayed woody debris, other fungal species inhabit fine woody debris (Bader et al., 1995; Lindner et al., 2006; Lonsdale et al., 2007; Rydin et al., 1997). Fungal communities are dynamic, successional, and can be niche specific (Rajala et al., 2012). Therefore, reductions in wood substrates will not only affect the primary colonizing fungi, but those organisms that would have followed in succession.

Globally, biodiversity has been identified as integral to ecosystem function, stability, and sustainability (Heywood, 1995). The individual traits and interactions of species contribute to maintaining the functioning and stability of ecosystems and biogeochemical cycles (Loreau et

al., 2001). Even when high diversity is not found to be critical for maintaining ecosystem processes under constant environmental conditions, it is hypothesized that biodiversity provides a buffer against environmental fluctuations and insures ecosystems against declines in functionality (Yachi and Loreau, 1999). Because different species respond differently to various fluctuations, the species that may appear to be functionally redundant for an ecosystem process at a given time are no longer redundant through time (Yachi and Loreau, 1999). Additionally, aesthetic, cultural, and economic reasons also contribute to the global desire to conserve biodiversity (Heywood, 1995; Loreau et al., 2001).

Fungal species diversity was assessed in the current study. Species diversity is often evaluated through species richness, indicating how many different kinds of organisms occur. Species richness is the most widely adopted diversity index (Magurran, 1988), the most appropriate and relevant measurement for fungal biodiversity, and the most used factor for evaluating fungal biodiversity (Zak and Willig, 2004). However, species that use down woody debris change as decay progresses, and due to this succession, species composition may vary while richness remains the same (Bunnell and Houde, 2010). Therefore, this research also utilized the fungal community composition, or the community of species present, to assess treatment effects.

Fungal community sampling. Prior to the development of molecular methods, fungal diversity studies were limited by microscopic identification of reproductive fruiting structures or identification of cultures isolated from wood, soil, etc. However, despite the presence of its mycelium or spores, a fungus might not produce a fruiting body, and culture methods tend to favor rapidly growing fungal species that grow on common culture media (Anderson and

Cairney, 2004; Peay et al., 2008; Smith and Onions, 1994). Hawksworth (1991) reported that only about 17% of known fungi are capable of growing in culture. Therefore, both methods are limited in assessing environmental fungal diversity.

Molecular approaches targeting the nuclear ribosomal RNA gene cluster are now the standard technique used for environmental fungal identifications and phylogenetic studies (Bridge and Spooner, 2001; Brundrett, 2002; Bruns et al., 1991; Gardes and Bruns, 1993; Horton and Bruns, 2001; Peay et al., 2008). Specifically, the Internal Transcribed Spacer (ITS) regions within the RNA gene cluster has been identified as the universal DNA barcode marker for fungi (Ihrmark et al., 2012; Schoch et al., 2012). Parts of the ITS region are extremely conserved and Polymerase Chain Reaction (PCR) amplifications of the ITS region are highly reliable even with small amounts of DNA (Gardes and Bruns, 1993; Peay et al., 2008). There are many published ITS sequences available online for comparison (Peay et al., 2008; Schoch et al., 2012), and most importantly, fungal specific PCR primers have the ability to preferentially amplify fungal sequences in mixed samples of plant, animal, prokaryotic, and fungal DNA (Berbee and Taylor, 2001).

High-throughput amplicon sequencing (HTAS), also referred to as next generation sequencing, has revolutionized ecological, population, and conservation genetic studies by generating huge amounts of sequence data on non-model organisms in a cost effective and timely manner (Ekblom and Galindo, 2011). In a 4-hour run, the Life Technologies Ion Personal Genome Machine (PGM) can generate between 60 megabases (Mb) and 2 gigabases (Gb) of sequence data (Life Technologies, 2014), compared with the 50,000 base pairs generated using traditional Sanger sequencing technologies (Peay et al., 2008). Studies that incorporate HTAS sequencing technologies will continue to dominate in fields such as microbial ecology.

Fungal assessments in western North America. Studies of fungal biodiversity and community composition in the intermountain west are rare and much remains unknown regarding the role of fungi in dry forest ecosystems. The current lack of information pertaining to wood-inhabiting fungi in western North America represents a significant knowledge gap that will undoubtedly limit the effectiveness of any biomass harvesting guidelines that do not fully consider the predominance and significance of fungi to the sustainability of these ecosystems. In this study, we utilized above-ground fruiting body surveys and DNA-based methods to assess fungal communities within a standardized wood stake decomposition protocol that has been employed in various forest ecosystems (Finer, et al., 2016; Jurgensen et al., 2006).

Goals and objectives. The overall goal of this project was to contribute to the understanding of fungal responses to forest management practices in western North America. The forest management practices in this study included biomass harvesting and retention treatments, along with compensatory soil amendments that included fertilizer and biochar. This research was designed to assess the sustainability and environmental impacts of removing woody biomass from western coniferous forests to be used as feedstock for biofuel production.

The objective of Chapter 1 was to assess wood-inhabiting fungal biodiversity relative to treatment plots by identifying wood-inhabiting fruiting bodies using morphological examination and DNA analyses. Fruiting bodies were collected from many different substrates, from twigs to small-and large-diameter logs, to stumps in the fall of 2014-2016. Two null hypotheses were tested: (1) that biomass harvesting and retention does not influence above-ground wood-inhabiting fruiting body diversity and community composition results, and (2) that compensatory

soil amendments do not influence above-ground wood-inhabiting fruiting body diversity and community composition results. Community composition refers to the species present.

The objective of Chapter 2 was to determine the presence of fungi inhabiting wood stakes inserted in treatment plots using High-throughput amplicon sequencing (HTAS). Aspen (*Populus tremuloides* Michx.) and loblolly pine (*Pinus taeda* L.) wood stakes were affixed to the soil surface and positioned within the mineral soil layers. Wood stakes were retrieved in the springs of 2015-2017, and drill shavings were collected for DNA extraction and sequencing. The two null hypotheses tested were: (1) that biomass harvesting and retention treatments do not influence wood stake-inhabiting fungal diversity and community composition results, and (2) that compensatory soil amendments do not influence wood stake-inhabiting fungal diversity and community composition results.

The objective of Chapter 3 was to compare the benefits and biases of assessing fungal communities based on above-ground wood-inhabiting fruiting body survey data vs. HTAS data to analyze effects in treatment plots. Both sampling methods utilized permutational multivariate analysis of variance (PERMANOVA) tests to assess effects of biomass harvesting and retention treatments, along with compensatory soil amendment treatments. The null hypothesis tested was that methods of sampling and analysis do not influence fungal community composition results.

References

Adams, R.I., Miletto, M., Taylor, J.W., Bruns, T.D., 2013. The diversity and distribution of fungi on residential surfaces. PLoS One 8: 1–9.

Anderson, I.C., Cairney, J.W.G., 2004. Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. Environ. Microbiol. 6: 769–79.

Arno, S.F., 2000. Fire in Western Forest Ecosystems, in: Brown, J.K., Smith, J.K. (Eds.), Wildland Fire in Ecosystems: Effects of Fire on Flora. USDA Forest Service Gen. Tech.Rep. RMRS-FTR-42-Vol. 2: 97–120.

Arnold, A.E., 2007. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. Fungal Biol. Rev. 21: 51–66.

Bader, P., Jansson, S., Jonsson, B.G., 1995. Wood-inhabiting fungi and substratum decline in selectively logged boreal spruce forests. Biol. Conserv. 72: 355–362.

Berbee, M.L., Taylor, J.W., 2001. Fungal Molecular Evolution: Gene Trees and Geologic Time, in: Esser, K., Lemke, P.A. (Eds.), The Mycota: A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research, VII: Systematics and Evolution, Part B. Springer, p. 229–245.

Brazee, N.J., Lindner, D.L., D'Amato, A.W., Fraver, S., Forrester, J. A., Mladenoff, D.J., 2014. Disturbance and diversity of wood-inhabiting fungi: effects of canopy gaps and downed woody debris. Biodivers. Conserv. 23: 2155–2172.

Bridge, P., Spooner, B., 2001. Soil fungi : diversity and detection. Plant and Soil 232: 147–154.

Brundrett, M.C., 2002. Coevolution of roots and mycorrhizas of land plants. New Phytol. 154: 275–304.

Bruns, T.D., White, T.J., Taylor, J.W., 1991. FUNGAL MOLECULAR SYSTEMATICS. Annu. Rev. Ecol. Syst. 22: 525–564.

Bunnell, F.L., Houde, I., 2010. Down wood and biodiversity – implications to forest practices. Environmental Review 18: 397-421.

Cooper, S.V., Neiman, K.E., Roberts, D.W., 1991. Forest habitat types of Northern Idaho: A Second approximation. General Technical Report INT-236. Ogden, UT: U.S. Department of Agriculture, Forest Service. Intermountain Research Station, p. 1-152.

Dean, A., Voss, D., 1999. Design and Analysis of Experiments. Springer: 675-685.

Ekblom, R., Galindo, J., 2011. Applications of next generation sequencing in molecular ecology of non-model organisms. Heredity (Edinb). 107: 1–15.

Entry, J.A., Cromack, K., Jr., Kelsey, R. G., Martin, N.E., 1991. Response of Douglas-fir to infection by *Armillaria ostoyae* after thinning or thinning plus fertilization. Phytopathology 81: 682-689.

European Commission., 2014. State of play on the sustainability of solid and gaseous biomass used for electricity heating and cooling in the EU. SWD(2014), p. 1-259.

Everett, R.L., Schellhaas, R., Keenum, D., Spurbeck, D., Ohlson, P., 2000. Fire history in the ponderosa pine/Douglas-fir forests on the east slope of the Washington Cascades. For. Ecol. Manage. 129: 207–225.

Ferris, R., Humphrey, J.W., 1999. A review of potential biodiversity indicators for application in British forests. Forestry 72: 314–328.

Finer, L., Jurgensen, M., Palviainen, M., Piirainen, S., Page-Dumroese, D., 2016. Does clear-cut harvesting accelerate initial wood decomposition? A five-year study with standard wood material. Forest Ecology and Management 372: 10-18.

Forest Guild Biomass Working Group, 2010. "Forest biomass retention and harvesting guidelines for the Northeast." Forest Guild, Santa Fe, New Mexico. 17 March 2016. <http://www.forestguild.org/publications/research/2012/FG_Biomass_Guidelines_SE.pdf>

Fox, T.R., 2000. Sustained productivity in intensively managed forest plantations. For. Ecol. Manage. 138: 187–202.

Gardes, M., Bruns, T., 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol. Ecol. 2: 113–118.

Hammell, K.E., 1997. Fungal Degradation of Lignin, in: Cadisch, G., Giller, K.E. (Eds.), Driven by Nature: Plant Litter Quality and Decomposition. CAB International.

Hansen, E.M., Goheen, E.M., 2000. *Phellinus weirii* and other native root pathogens as determinants of forest structure and process in western North America. Annu. Rev. Phytopathol. 38: 515-539.

Harvey, A.E., Jurgensen, M.F., Larsen, M.J., Graham, R.T., 1987. Relationships among soil microsite, ectomycorrhizae, and natural conifer regeneration of old-growth forests in western Montana. Can. J. For. Res. 17: 58–62.

Hawksworth, D.L., 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycological Research 105 (12): 1422-1432.

Heywood, V.H. (ed), 1995. The global biodiversity assessement. United Nations Environmental Programme. Cambridge University Press, Cambridge. p 1-1140.

Heinimo, J., Junginger, M., 2009. Production and trading of biomass for energy - An overview of the global status. Biomass and Bioenergy 33: 1310-1320.

Horton, T., Bruns, T., 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Mol. Ecol. 10: 1855–1871.

Horton, T.R., Molina, R., Hood, K., 2005. Douglas-fir ectomycorrhizae in 40- and 400-year-old stands: mycobiont availability to late successional western hemlock. Mycorrhiza 15: 393-403.

Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal ITS2 region--evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol. Ecol. 82: 666–77.

Jurgensen, M., Reed, D., Page-Dumroese, D., Laks, P., Collins, A., Mroz, G., Degórski, M., 2006. Wood strength loss as a measure of decomposition in northern forest mineral soil. Eur. J. Soil Biol. 42: 23–31.

Keane, R.E., Ryan, K.C., Veblen, T.T., Allen, C.D., Logan, J., Hawkes, B., 2002. Cascading effects of fire exclusion in the Rocky Mountain ecosystems: a literature review. General Technical Report. RMRS-GTR-91. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, p. 1-24.

Keyes, C., O'Hara, K., 2002. Quantifying stand targets for silvicultural prevention of crown fires. West. J. Appl. For. 17: 101–109.

Life Technologies, 2014. "ION PGM System Specifications." Life Technologies. 17 March 2016. <http://www.lifetechnologies.com/us/en/home/life-science/sequencing/next-generation-sequencing/ion-torrent-next-generation-sequencing-workflow/ion-torrent-next-generation-sequencing-run-sequence/ion-pgm-system-for-next-generation-sequencing/ion-pgm-system-specifications.html>.

Lindahl, B, D., Nilsson, R.H., Tedersoo, L., Abarenkov, K., Carlsen, T., Pennanen, T., Stenlid, J., Kauserud, H., 2013. Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. New Phytol. 199: 288–299.

Lindner, D.L., Burdsall Jr., H.H., Stanosz, G.R., 2006. Species diversity of polyporoid and corticioid fungi in northern hardwood forests with differing management histories. Mycologia 98: 195–217.

Lonsdale, D., Pautasso, M., Holdenrieder, O., 2007. Wood-decaying fungi in the forest: conservation needs and management options. Eur. J. For. Res. 127, 1–22.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A, Hooper, D.U., Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D., Wardle, D.A., 2001. Biodiversity and ecosystem function: Current knowledge and future challenges. Science 294 (804): 804-808.

Magurran, A.E., 1988. Ecological diversity and its measurement. Princeton University Press, 61-125.

Mardis, E.R., 2008. The impact of next-generation sequencing technology on genetics. Trends Genet. 24: 133–141.

Maser, C., 1989. Sustainable Forestry. Trumpeter J. Ecosophy 6: 52–54.

Mohan, D., Pittman, C.U., Steele, P.H., 2006. Pyrolysis of Wood/Biomass for Bio-oil: A Critical Review. Energy & Fuels 20: 848–889.

Moore, J., Hanley, D., Chappell, H., Shumway, J., Webster, S.B., Mandzak, J.M., 1998. Fertilizing Eastern Washington coniferous forests. Ext Bull EB1874.

Nordén, B., Ryberg, M., Götmark, F., Olausson, B., 2004. Relative importance of coarse and fine woody debris for the diversity of wood-inhabiting fungi in temperate broadleaf forests. Biol. Conserv. 117: 1–10.

Norton, G., Abraham, S., Veneman, A., 2003. Memorandum of understanding on policy principles for woody biomass utilization for restoration and fuel treatments on forests, woodlands, and rangelands. United States Department of Agriculture, United States Department of energy, and the United States Department of the Interior, p. 1-7.

Page-Dumroese, D., Coleman, M., Jones, G., Venn, T., Kasten Dumroese, R., Anderson, N., Chung, W., Loeffler, D., Archuleta, J., Kimsey, M., Badger, P., Shaw, T., McElligott, K., 2009. Portable in-woods pyrolysis: Using forest biomass to reduce forest fuels, increase soil productivity, and sequester carbon. Paper presented at the North American biochar conference; August 9-12; Boulder, CO. Center for Energy and Environmental Security.

Peay, K.G., Kennedy, P.G., Bruns, T.D., 2008. Fungal Community Ecology. Bioscience 58: 799–810.

Perlack, R., Wright, L., Turhollow, A., Graham, R.L., Stokes, B., Erbach, D., 2005. Biomass as feedstock for a bioenergy and bioproducts industry: the technical feasibility of a billion-ton annual supply. U.S. Department of Energy. http://www.osti.gov/bridge.

Purahong, W., Kahl, T., Schloter, M., Bauhus, J., Buscot, F., Kruger, D., 2014. Comparing fungal richness and community composition in coarse woody debris in Central European beech forests under three types of management. Mycol. Prog. 13: 959–964.

Rajala, T., Peltoniemi, M., Pennanen, T., Makipaa, R., 2012. Fungal community dymanics in relation to substrate quality of decaying Norway Spruce (Picea abies [L.Karst.) logs in boreal forests. FEMS Microbiol Ecol 81: 494-505.

Rayner, A.D.M., Boddy, L., 1988. Fungal Decomposition of Wood: Its Biology and Ecology. John Wiley & Sons Ltd.

Richard, T.L., 2010. Challenges in scaling up biofuels infrastructure. Science 329: 793–6.

Rydin, H., Diekmann, M., Hallingbäck, T., 1997. Biological characteristics, habitat associations, and distribution of macrofungi in Sweden. Conserv. Biol. 11: 628–640.

Schoch, C.L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc. Natl. Acad. Sci. U. S. A. 109: 6241–6246.

Smith, D., Onions, A.H.S., 1994. The preservation and maintenance of living fungi, 2nd ed. CAB International, England.

U.S. Congress, 2000. Biomass research and development act of 2000. US Congr. Washington, DC H.R. 2559, p. 1–12.

U.S. Department of Energy, Perlack, R.D., Stokes, B.J., 2011. U.S. Billion-Ton Update: Biomass Supply for a Bioenergy and Bioproducts Industry. Oak Ridge Natl. Lab. Oak Ridge, TN. ORNL/TM-20, p. 1–227.

USDA Forest Service, 2016. "History and Culture, Your national heritage." U.S. Forest Service. 17 March 2016. <http://www.fs.usda.gov/main/giffordpinchot/learning/history-culture.html>.

USDA Forest Service, 2013. "Woody Biomass Utilization." U.S. Forest Service. 17 March 2016. <http://www.fs.fed.us/woodybiomass/whatis.shtml>.

Veblen, T., Kitzberger, T., Donnegan, J., 2000. Climatic and human influences on fire regimes in ponderosa pine forests in the Colorado Front Range. Ecol. Appl. 10: 1178–1195.

Yachi, S., Loreau, M., 1999. Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. Proc. Natl. Acad. Sci. USA (96): 1463-1468.

Zak, J.C., Willig, M.R., 2004. Fungal Biodiversity Patterns, in: Mueller, G.M., Bills, G.F., Foster, M.S. (Eds.), Biodiversity of Fungi: Inventory and Monitoring Methods. Elsevier, 59-75.

Chapter 1

Influence of biomass harvesting and soil amendments on diversity and community composition of wood-inhabiting fungi in the intermountain region of western North America

Abstract

The suppression of fires in western forests has led to an increase of woody biomass and fuel loads. Legislation and policies promoting the utilization of forest biomass for biofuels has prompted the need to assess the effects of woody biomass harvesting on biodiversity, particularly the organisms that decay wood and recycle nutrients. Therefore, the effects of biomass harvesting and compensatory soil amendments were assessed by sampling above-ground fungal fruiting bodies in a western conifer forest. Fruiting bodies of wood-inhabiting fungi were sampled in 400-m^2 plots that were established with biomass at four levels (three treatments were thinned with removal of all cut material, retention of cut material, and doubling of the cut material, compared to the non-thinned controls) and four soil amendment treatments (fertilizer, biochar, fertilizer and biochar, compared to non-amended controls).

Over 3 years, 1,002 wood-inhabiting fungal fruiting bodies were collected and identified over 3 years, comprising 129 basidiomycete species. Twenty-two species did not match known species descriptions, and over 85% of species were found on moderately decayed wood, decay classes 2 and 3. A number of forest pathogens were identified, as well as a number of species that are described as ‘rare’ and ‘sensitive’ to intensive forest management. Species accumulation curves suggest that even more species are likely to be found with increased sampling intensity.

While no significant differences were observed in richness or diversity indices, the fungal community composition was influenced by treatment effects.

The effect of biomass harvesting significantly influenced the fungal community composition in the first year of sampling ($p = 0.003$). Richness and diversity indices were not normally distributed among plots, likely due to whole plots being sampled for all coarse woody debris following biomass harvesting treatments.

Soil amendments moderately influenced the fungal community composition only in the second year of sampling (2015, $p = 0.064$), with fertilization being the primary driver of dispersion differences ($p < 0.02$). Though there was no significant effect of biochar on the fungal community composition, the biochar amended plots did have the greatest species richness.

Fruiting body surveys relay important information about fungal species composition in relation to forest management, as well as the presence of rare or sensitive species and important forest pathogens.

1.1 Introduction

Many forest ecosystems in the Rocky Mountain region of North America evolved with frequent, low intensity fires that prevented the accumulation of woody biomass (Arno, 2000; Veblen et al., 2000). Woody biomass includes living trees and woody plants, as well as dead limbs, tops, and other woody parts that accumulate naturally or result from forest management activities such as thinning (USDA Forest Service, 2013). Fire suppression has led to overstocked forests that contain excess biomass, increasing the risk for higher intensity fires (Keyes and O'Hara, 2002). Higher-intensity fires lead to crown torching, soil scorching, and increased mortality in plants and soil organisms (Arno, 2000; Keane et al., 2002). A Presidential Initiative in 2002 recommended thinning or other treatments to reduce woody biomass and fuel loads in overstocked forest land (U.S. Department of Energy et al., 2011).

To enhance the sustainability of United States energy supplies, Congress established a goal to utilize over 1 billion dry tons of biomass feedstock annually to supplement the nation's power, transportation fuel, and chemical production needs by 2030 (USDA Forest Service, 2013). Achieving that goal will require a significant increase in utilization of forest biomass above the approximately 130 million dry tons of forest biomass currently consumed for energy production annually (U.S. Department of Energy et al., 2011). The Department of Energy (DOE), Department of Interior (DOI), and the United States Department of Agriculture (USDA) announced an initiative encouraging the utilization of forest biomass, rather than reduction through prescribed burning or other on-site disposal methods, resulting in the potential increased value of woody biomass feedstocks (Norton et al., 2003).

The economic value of woody biomass includes its potential as a feedstock for the production of a variety of biofuels, including bio-oil. While other biofuel possibilities exist, bio-

oil is most appropriate for efficient Western coniferous biomass utilization based on access to current infrastructure (Mohan et al., 2006). An additional benefit to bio-oil production is the byproduct biochar, a recalcitrant black carbon functionally similar to charcoal (Coleman et al., 2010). Biochar as a soil amendment is recommended for application to agricultural and forest soils to improve soil quality, enhance persistent soil organic matter content, and sequester carbon (Page-Dumroese et al., 2016), though there are many caveats to 'improved soil quality'. Thinning forests may also result in ecological and silvicultural benefits; however, the current cost of removal often exceeds the monetary value of the biomass (McElligott et al., 2011). Therefore, forest managers in the intermountain west are eager for incentives to develop viable bioenergy markets (McElligott et al., 2011). Public policies promoting biomass utilization exist, yet concerns over the unknown impacts highlight the need for more information on the effects of biomass removal from forest systems (Kimmins, 1997; U.S. Department of Energy, et al., 2011).

Woody biomass harvest guidelines implemented in some states, regions, and countries include the retention of downed, dead woody materials acting as substrates for fungi with critical roles in healthy forest ecosystems (Stokland et al., 2012). White and brown rot fungi are the only organisms capable of substantial and efficient decay of wood's recalcitrant lignocellulosic structure (Hammel, 1997; Rayner and Boddy, 1988), making resources available for other functional groups (Rayner and Boddy, 1988; Stokland et al., 2012). Wood decay produces soil organic matter, recycles nutrients, and is the basis of the food chain for many microbes, invertebrates, small mammals, amphibians, birds, and larger vertebrates (Ferris and Humphrey, 1999; Lonsdale et al., 2008).

Reduction in quality and quantity of forest woody debris due to past logging and management practices has already been shown to have an adverse effect on the richness and

abundance of fungi (Bader et al., 1995; Norden et al., 2004; Rydin et al., 1997). Some fungal species are found on freshly cut, fine woody debris; others require large-diameter, well-decayed woody substrate (Lindner et al., 2006; Lonsdale et al., 2008; Rydin et al., 1997). Fungal communities are dynamic, successional, and can be niche specific (Rajala et al., 2012). Therefore, fungi have been used as indicators for ecosystem biodiversity, community composition, and conservation and management practices (Bader et al., 1995; Ferris and Humphrey, 1999; Lindner et al., 2006; Lonsdale et al., 2008; Purahong et al., 2014). It is hypothesized that reduction in wood substrates will not only affect the primary colonizing fungi, but those organisms that follow in succession. Understanding how biomass harvesting affects wood-inhabiting fungal communities is critical for assessing the sustainability of the harvests. For example, loss of mutualistic mycorrhizal fungi would be problematic for the regeneration of tree seedlings.

Because studies of fungal biodiversity and community composition in the intermountain west are rare, much remains unknown regarding the role of fungi in dry forest ecosystems. There is limited knowledge of the effects of forest management activities, including biomass harvesting, on the diversity and abundance of wood-inhabiting fungi in dry ecosystems of the intermountain west of North America. The objective of this study was to determine the effects of forest management practices on the abundance and diversity of wood-inhabiting fungal fruiting bodies.

1.2 Materials and Methods

1.2.1. *Design of study area*

Research was conducted at the University of Idaho – Experimental Forest (UIEF, 46.849512, -116.845068) (Fig. 1.1). The elevation at this site ranges from 830-890 meters above sea level, with a 0-15% slope and south-facing aspect. The mean annual temperature was 7.8 ºC (measured between 1981-2010, PRISM Climate Group). The site was previously logged, exposed to periodic burning, and occupied by mixed conifer species grown atop silt-loam soils with a significant (>30 cm) Andisol layer of volcanic ash (Cooper et al. 1991; McDaniel and Hipple, 2010). The UIEF site is classified as a grand fir/ninebark habitat type (Cooper et al., 1991) and is dominated by ponderosa pine (*Pinus ponderosa* Dougl. ex Laws), grand fir [*Abies grandis* (Dougl. ex D. Don) Lindl.], Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco], lodgepole pine (*Pinus contorta* Dougl. ex Loud.), and western larch (*Larix occidentalis* Nutt). UIEF has been used for cattle grazing, recreation, research, and managed for commercial forestry. In the late 1980s, UIEF was harvested as a seed-tree harvest leaving ponderosa pine for forest regeneration. The seed trees were subsequently harvested in 2012. The current study is part of a larger investigation of the effects of forest biomass harvesting practices with regard to whole ecosystem sustainability; therefore, other potentially important responses were quantified, including pre- and post-treatment stand composition (Table 1.1), provided by Sherman et al. (2017).

In 2013, 400-m^2 study plots were established with biomass treatments at four levels and soil amendments at four levels. Treatment combinations (4x4) were randomly assigned and

replicated twice, yielding 32 plots. Experimental sites were named University of Idaho_ Ponderosa Pine (UIPP) and University of Idaho_Mixed Conifer (UIMC), based on their dominant cover type. Biomass treatment plots were established, including non-thinned control and manipulated plots that were thinned from below (i.e. cutting the small trees including those that are either undesirable species, suppressed, having poor form, or are visibly diseased) to a relative basal area of 40%. Retention of the cut biomass resulting from thinning was manipulated. Biomass treatments in these thinned test plots included removal of all cut material (0X), retention of this cut material (1X), and doubling of the amount of this cut material (2X), compared to the non-thinned control (non-thinned). To create 2X plots, material that was cut during thinning was removed from 0X plots and scattered over the 2X plots. Specifications regarding the quantity of biomass thinned and manipulated is described in the pre- and post-treatment site characteristics (Table 1.1). Soil amendments consisted of fertilizer, biochar, fertilizer+biochar, and a non-amended control. The fertilization treatment consisted of urea fertilizer (46-0-0) applied at a rate of 224 kg N ha^{-1}. The biochar was produced from western mixed conifer feedstock in a steam boiler at 980°C; it consists of 25.7% C, 28.6% ash, and has a surface area of 201 m^2 g^{-1}, with an average pH of 7.8. The biochar was added at a rate of 2 Mg ha^{-1} (2.2 tons ha^{-1}).

1.2.2. *Wood-inhabiting fungal fruiting body surveys*

Fruiting bodies of wood-inhabiting fungi were sampled within the 400-m^2 treatment plots. All fine and coarse woody debris and standing trees (living or dead, to a height of 2 m) within each plot were non-destructively inventoried for fruiting bodies during late September and

October of 2014, 2015, and 2016, by turning over every piece of debris. Inventories were conducted in the fall to increase detection of fungal species producing annual (i.e., non persistent) and perennial fruiting bodies.

Fungal fruiting body sampling methods follow established protocols (Mueller et al., 2004). The following characteristics were reported when a fruiting body was encountered: fungal species (when easily identified), substrate type (branch, log, suspended log, snag, stump, and living tree), wood substrate species, diameter class, and decay class following the five-class system of Maser et al. (1979). Dead fruiting bodies were inventoried unless their state of degradation precluded identification.

1.2.3. *Taxa identification*

Fruiting bodies that were not easily identified in the field were collected, dried, and taken to the laboratory for further study. Samples were identified in the lab by microscopic examination of morphological features (Bernicchia et al., 2010; Gilbertson and Ryvarden, 1986; Hjorstam et al., 1987) and/or DNA extraction followed by sequencing of the ITS regions. All DNA extractions, PCR, and sequencing of fruiting bodies followed protocols described by Lindner and Banik (2008). Sanger sequences were generated at the University of Wisconsin – Madison Biotechnology Center and were aligned and edited using Sequencher 4.9 (GeneCodes Corporation). The Basic Local Alignment Search Tool (BLAST) was used to search for similar sequences in GenBank (Schäffer and Aravind, 2001). Specimens not identified on morphological characters were identified using a 97% similarity threshold for species-level identifications, because the use of a 97% similarity cut-off is well supported in the literature for

the ITS regions (Blaalid et al., 2013). When unable to match a species to a known species description, the sample was assigned to the genus it most resembled and given a species number. Dried and identified voucher specimens were deposited in the Center for Forest Mycology Research (CFMR) herbarium at the USDA Forest Service - Forest Products Laboratory (Madison, Wisconsin). Fungal nomenclature was based on Index Fungorum (www.indexfungorum.org) and Mycobank (www.mycobank.org). Sequences were submitted to GenBank, accession SRP154885.

1.2.4. *Fungal biodiversity and community analysis*

Species diversity is often evaluated through species richness, indicating how many different kinds of organisms occur. Species richness is the most widely adopted diversity index (Magurran, 1988), the most appropriate and relevant measurement for fungal biodiversity, and the most used factor for evaluating fungal biodiversity (Zak and Willig, 2004). The use of fungal species richness to represent fungal species diversity is well supported in the literature (Berglund and Jonsson, 2001; Berglund et al., 2005; Bonet et al., 2010; Odor et al., 2006; Olsson and Jonsson, 2010). Though species richness has high sensitivity to sample size, species richness indices have high discriminate ability (Magurran, 1988). Other diversity metrics utilize components of species abundance and evenness; however, that may not be appropriate for fungal diversity assessments because it is impossible to identify discrete fungal individuals in the field. Molina et al. (2001) noted that fungal individuals may be square centimeters to hectares in size, and it is impossible to discern what proportion of the fruiting bodies collected represent genetically identical individuals. Therefore, fungal species richness was the most appropriate

index to test correlations and linear regression models. However, species that use down woody debris change as decay progresses, and due to this succession, species composition may vary while richness remains the same (Bunnell and Houde, 2010).

Sampling intensity was investigated by calculating species accumulation curves (SAC) using Kindt's exact method and plotted with the expected (mean) species richness (Oksanen et al., 2012). Fungal species richness was assessed for normality relative to site, biomass harvesting treatments, and soil amendment additions. For comparison to other published diversity studies, species diversity indices were generated using Shannon's equation (Shannon and Weaver, 1949) and Simpson's equation (Simpson, 1949).

Richness and diversity indices were tested for use as response variables for correlation and regression analyses. Data normality was checked visually with scatter plots, quantile-quantile (Q-Q) plots and Shapiro-Wilk's test (Bock et al., 2007; Dean and Voss, 1999). Regression analyses were used to assess the predictive power of richness and diversity indices from a set of continuous explanatory variables, namely forest metrics assessed on these sites and provided by Sherman et al. (2017), including stand basal area (m^2 ha^{-1}), tree density (trees ha^{-1}), quadratic mean diameter (cm), standard density index (trees ha^{-1}), Curtis's relative density, and coarse woody debris (tons $hectare^{-1}$). Whereas BA of a tree is defined as the cross-sectional area at breast height (Wenger, 1984), total stand BA is related to stand volume and represents a measurement of stand density (Husch et al., 2003). The standard density index by Reineke (1933), based on the relationship between number of trees per acre and their average diameter, was developed for assessing even-aged forests and is directly related to the potential response to release when thinned. A stepwise model selection, performed using the stepAIC() function from the MASS package, was used to assess variable selection for regression analyses (Ripley et al.,

2018). A Mantel test was used to check for autocorrelation (Gardener, 2014). Because richness and diversity indices did not follow assumptions of normality, forest metrics were assessed based on the fungal community composition (which species were present).

Fungal community analyses of the fruiting body community data were conducted using R Statistical Software (R Core Team, 2015) and the Vegan package (Oksanen et al., 2012). To assess the effects of biomass harvesting treatments (0X, 1X, 2X biomass retained, non-thinned control) and compensatory amendments (fertilizer, biochar, fertilizer+biochar, non-amended control) on the fungal community composition, a nonparametric permutational multivariate ANOVA (PERMANOVA) two-way factorial test (Anderson, 2001) based on Raup-Crick dissimilarity matrices was performed by the adonis function in the Vegan package of R. Of over 60 possible measurements of similarity or dissimilarity described (Anderson, 2001), the Raup-Crick distance measure was chosen for its efficacy in community assemblage studies with presence/absence data (Chase et al., 2011; Gardener, 2014; Raup and Crick, 1979). Multivariate dispersion, or variation between groups (Anderson et al., 2006), was tested using the betadisper function in the Vegan package of R to ensure that differences in dispersion were not influencing PERMANOVA significance.

1.3 Results

1.3.1. *Fungal species*

During the three fall sampling periods of 2014, 2015, and 2016, over 1,000 fungal observations were made. An observation was defined as the occurrence of a fruiting body,

greater than 1cm^2, on a substrate. Altogether 129 fungal species were identified, with 22 species that did not match known species descriptions (Appendix 1a). Over 85% of species were found on downed woody material in decay class 2 and 3 (Appendix 1b). The only species observed during the initial stage of decay (decay class 1) were *Cryptoporus volvatus* (Peck) Shear, *Phaeolus schweinitzii* (Fr.) Pat., and *Stereum sanguineolentum* (Alb. & Schwein.) Fr. By decay class 3, fruiting bodies of mycorrhizal fungi, including *Amphinema byssoides* (Pers.) J. Erikss. and *Piloderma spp.,* were more common. Additionally, a number of important forest pathogens were identified (Table 1.2). To determine the adequacy of sampling, species accumulation curves were computed, representing sampling intensity by year (Fig. 1.2) and biomass treatment (Fig. 1.3). No curves level off, indicating more species are likely to be found with more intensive sampling. Species richness was reported, along with Shannon and Simpson's diversity indices relative to site, biomass treatments, and soil amendments (Table 1.3). Species richness and diversity indices were not normally distributed due to observing zero fruiting bodies on some plots while other plots had greater diversity. Even when all 3 years of data were combined, richness values did not normalize (Shapiro-Wilk, $W=0.93$, $p=0.03$).

In 2014, 159 fruiting body observations were recorded, comprising 56 different fungal species. The top five most common species observed were *Coniophora arida* (Fr.) P. Karst, *Cryptoporus volvatus, Tubulicrinis glebulosus* (Fr.) Donk., *Athelia bombacina* (Link) Pers., and *Trichaptum abietinum*. Fruiting bodies included a diverse range of morphologies and diverse phylogenetic groups. Although the fewest observations were recorded in 2014, the species accumulation curve indicated sampling efforts mirror that of 2015, meaning that as more plots were sampled, a similar amount of new species were observed.

In 2015, 221 fruiting body observations were recorded, comprising 60 fungal species. The five most common species observed in 2015 were *Trichaptum abietinum, Coniophora arida, Hyphoderma setigerum* (Fr.) Donk*, Acanthophysellum lividocoeruleum* (P. Karst.) Parmasto*,* and *Gloeophyllum spp.* Two species of *Gloeophyllum* were identified based on ITS sequences, namely *G. abietinum* (Bull.) P. Karst and *G. sepiarium* (Wulfen) P. Karst. Both species are flat to bracket-like with concentric undulations and furrows, found in the same habitats (often growing together), with overlapping microscopic features (Breitenback and Kranzlin, 1986). Therefore, while specimens were collected (each year and within each plot) for molecular identification and herbarium collection, observations were reported as *Gloeophyllum spp.* because not all fruiting bodies were collected within a plot. Both 2014 and 2015 species accumulation curves differ from 2016's species accumulation curve because less species were identified in 2014 and 2015 (Fig.1.2).

In 2016, 622 fruiting body observations were recorded, comprising 89 fungal species. The five most common species observed in 2016 were *Trichaptum abietinum, Coniophora arida, Stereum sanguinolentum, Athelia bombacina,* and *Acanthophysellum lividocoeruleum.* The total species richness curve, representing all the years' data, suggests that while 2016 did have the most observed fruiting bodies, there were species present within the first two years that were not present within the 2016 season (Fig.1.2).

1.3.2. *Biomass Treatments*

Within this limited study, we did not find an overall negative effect of biomass removal. The different biomass treatments had appreciable differences in mean richness values for the

biomass treatments, especially during 2014 (Fig. 1.4). The richness values associated with the biomass treatments: 0X, 1X, 2X, and non-thinned, over all 3 years were 72, 46, 58, and 62, respectively (Fig. 1.3). Because richness and diversity indices were not normally distributed, fungal community composition was utilized for further assessments relative to treatments.

In 2014, the fungal community composition differed significantly as a function of biomass treatment (Table 1.4, PERMANOVA: $F.model_{3,24}$: 2.35, R^2=0.19, $p < 0.01$), with a significant difference in betadispersion, ($F = 3.56$, $p = 0.03$). There was a significant difference in dispersion between the fungal community composition present at the 2X and 1X biomass treatments (Tukey HSD, $p.adj = 0.016$). This result mirrors a visual difference in richness, with the richness of the 1X treatments averaging less than that of the 2X treatment (Fig. 1.4)

By the second and third years after plot establishment, biomass treatments had less significant effects. In 2015, 2 years after the plots were established, there was no significant effect of biomass treatment on the fungal community composition (Table 1.4, $p = 0.21$). By 2016, the biomass treatments had a marginally significant effect on the fungal community composition (PERMANOVA, F.model = 2.12, $R^2 = 0.17$, $p = 0.059$), with no significant difference in betadispersion ($p = 0.59$). Upon visual assessment of richness boxplots (Fig. 1.4), there is similarity with respect to both the medians and ranges of number of species present.

1.3.3. *Soil Amendments*

There were some differences in species richness and fungal community composition due to soil amendments. The number of unique species found within the soil amendment plots were 62, 74, 51, and 65, respectively, in the fertilizer+biochar, biochar, non-amended and fertilizer

amendments. Only in 2015, the second year of sampling, did soil amendments appear to strongly effect on the fungal community composition (Table 1.4, PERMANOVA: $F.model_{3,24} = 1.68$, $R^2 = 0.13$, $p = 0.064$), with a significant difference in dispersion. The fungal community composition of the fertilized soil treatments had significantly different dispersion from the biochar treatments (Tukey HSD, *p.adj* = 0.011), as well as the control non-amended treatments (*p.adj* = 0.02). While there was no significant effect of soil amendment on the fungal community composition in 2016, there was a significant difference in dispersion (F = 3.47, $p = 0.029$). The dispersion of the non-amended control was significantly different from the fertilizer+biochar amendment (Tukey HSD, *p.adj.* = 0.025). The effect of fertilization appears to have the most influence on the fungal community composition, relative to the other amendments.

1.3.4. *Sites*

The sites differed in tree species present pre-and post-thinning (Table 1.1), which likely led to differences in fungal community compositions. UIMC had 102 recorded fungal observations, while UIPP had 81. Though there were some obvious differences between the two sites, including number of fungal observations and mean fungal fruiting body richness and diversity indices (Fig. 1.6), they were not utilized for further analyses because they did not follow the assumptions of normality. All fungal community composition data were assessed through PERMANOVA analyses, and due to site differences, all permutational analyses were constrained by site.

In 2014, 93 fruiting body observations were recorded at UIMC, and 66 fruiting body observations were recorded at UIPP. The effect of site, even with constraint, was significant in

influencing the fungal community composition (PERMANOVA: $F.model_{1,24}$: 2.37, $R^2 = 0.06$, $p = 0.014$), with a significant difference in betadispersion, ($F = 4.01$, $p = 0.05$). There was a significant difference of fungal community composition dispersion at UIPP and UIMC (Tukey HSD, $p.adj = 0.05$).

In 2015, the second year after plots were established, 95 fruiting body observations were recorded at UIMC, with 125 fruiting body observations were recorded at UIPP. While there were differences in fungal observations, the species richness values associated with the two sites were similar (Fig. 1.6). The fungal community composition differed almost significantly as a function of site (Table 1.4, PERMANOVA: $F.model_{1,24} = 3.98$, $R^2 = 0.11$, $p = 0.06$), though with no significant differences in dispersion.

Three years after plot establishments, the differences due to site were less apparent. Exactly 299 fruiting body observations were recorded at UIMC, while 324 fruiting body observations were recorded at UIPP. There was no significant difference in community composition due to site (Table 1.4, $p = 0.212$), though a significant difference in betadispersion was observed ($F = 4.62$, $p = 0.04$).

1.3.5. *Forest Metrics*

Forest metrics describing tree stand characteristics were tested for explanatory power against all 3 years of fungal community data (Table 1.5). In 2014, basal area was found to have significant explanatory power influencing the fungal community composition. In 2015, basal area and standard density index both were found to influence the fungal community. By 2016,

only coarse woody debris, measured in tons per hectare, was a significant factor in explaining the fungal community composition.

1.4 Discussion

1.4.1. *Biomass treatments*

The biomass treatment effects are complex because these treatments change a variety of habitat characteristics; the thinning of the trees changes the amount of coarse woody debris left on the ground and the canopy cover relative to the remaining forest stand. The widely accepted best management practices for stands such as these include thinning of small, undesired species, and the thinned treatments with biomass removal (0X plots) yielded the greatest fungal richness over the 3-year study. However, this increase in plot alpha diversity should not be confused with an increase in beta diversity at the landscape scale, as some fungi respond ubiquitously to available substrates. For example, *Trichaptum abietinum* was the most abundant fungal species observed on plots, regardless of whether new slash substrates were purposefully placed through biomass treatments (1X, 2X) or not (non-thinned, 0X).

Complicating the analyses, the fungal observations were related not only to the amount of fresh biomass as fungal substrates, but also to the living forest's responses to the biomass treatments. In terms of basal area, tree growth was significantly reduced in 2X and non-thinned plots compared with 1X and 0X plots 1 year after plot initiations in 2015 (Sherman et al., 2017). By 2016, non-thinned plots exhibited significant tree mortality, influencing forest metrics especially by reducing stand BA (Sherman et al., 2017). Interestingly, the spreading root

pathogen *Heterobasidion occidentale* was only observed in the non-thinned and 2X biomass plots, which could be due to the fact that it was already present in those plots or could indicate an additional stress factor for trees. Fungal communities are known to be influenced by (i.) tree composition, (ii.) disturbance, and (iii.) soil nutrients (Carroll and Wicklow, 1992); therefore, members in the fungal community are clearly dynamic and do not respond equally to all treatments. For example, in a study assessing fungal richness on islands of old growth spruce in Scandinavia, Berglund and Jonsson (2001) found crust (corticoid) fungal richness to be positively influenced by tree height and number of downed logs per hectare, whereas polyporous fungi were more influenced by tree canopy cover and coarse woody debris (downed log volume per hectare).

1.4.2. *Soil amendments and interactions with thinning*

The results of this study are consistent with previous conclusions that fungal communities are influenced by forest fertilization (Molina et al., 2001). Zheng et al. (2017) attributes fertilization effects to: (i.) N and C mobilization, (ii.) influences by above-ground plant communities, and (iii.) altered soil physiochemical properties. So, while biomass treatments had a significant effect on the fungal community composition, the effects were likely complicated by soil amendments and differed by year.

The soil amendment of biochar did not significantly influence the fungal community composition or indices of fungal diversity. This result is in accordance with Noyce et al. (2015), where the authors conclude biochar additions at 5 Mg ha^{-1} were neither beneficial nor toxic to sequenced soil microbes. The biochar treatment rate in this study was 2 Mg ha^{-1}. Lehmann et al.

(2011) produced a summary table of mechanisms by which microbial abundance is affected by biochar, and the columns related to mycorrhizal fungi and other fungi are dominated by “reaction not known” and “no change”. Li et al. (2018) concluded that there is no consensus regarding the effects of biochar on microbial biomass or fungal abundance and diversity within forested systems, and only when application rates equal or exceed 5 Mg ha^{-1} have significant effects on microbial biomass or community composition been reported.

Research by Teste et al. (2012) that assessed ectomycorrhizal (EM) fungal diversity in relation to thinning and fertilization experiments in pine forests of Alberta also found that fertilization (with 300 kg ha^{-1} N, in the form of urea and NH_4+) reduced EM fungal richness and diversity. Additionally, they identified an interaction effect with tree thinning (to a residual density of 2500 trees ha^{-1}, >1 cm diameter at breast height [DBH], with slash left on site) (Teste et al., 2012). Whereas thinning with fertilization (3 years prior) had decreased EM fungal richness, thinning without fertilization did not significantly reduce richness compared with the non-thinned and non-fertilized control (Teste et al., 2012). Similarly in the current study, while richness differences between the non-thinned and thinned (1X) biomass treatments were evident in the first year, that difference was negligible by the second year (Fig. 1.4). The interaction of thinning (which involves removal of competitive trees in addition to carbon inputs in the form of slash), combined with fertilization, likely explains why the fertilized plots across biomass treatments had large variance and significant differences in beta dispersion. Teste et al. (2012) also found that EM fungal richness was reduced by 16% the first year after fertilization, and 30% 3 years after fertilization, suggesting that, in the current study, variation in the fungal communities due to interactions of biomass and fertilization were still relevant in 2016, though no significant interactions were observed statistically. Additionally, at these plots, soil

amendments did not significantly influence BA growth until 2015-2016, when fertilized plots (fertilizer alone and fertilizer+biochar) had higher BA periodic annual increments (39% and 27%, respectively) compared with the non-amended control plots (Sherman, et al., 2017). So, some soil amendments increased BA growth, while some biomass treatments reduced stand BA growth, and the differences in BA are representative of changing forest stand dynamics with some trees thriving and others diminishing. The fungal communities responded to these changing forest stand dynamics, in an equally complicated manner.

1.4.3. *Forest metrics*

Fungal community composition differed significantly as a function of basal area (BA), standard density index (SDI), and coarse woody debris (tons hectare^{-1}). These forest metrics were designed to describe the stand structure and cannot describe all stand dynamics. For example, Bonet et al. (2010) conducted a study of richness of wild mushrooms in mixed pine forests of the Pyrenes and found stand BA to be an important predictor for mushroom yield. Their maximum mushroom productivity coincided with a basal area of 15-20 m^2 ha^{-1} (Bonet et al., 2010), which was equivalent to the basal area of the non-thinned plots in the current study. Greater stand BA could diminish sunlight reaching the forest floor, thereby influencing micro-niches of water availability; however the metric cannot indicate that association. Likewise, while BA is related to DBH and volume, in a mixed-age stand, it does not specifically describe individual tree sizes, and Odor et al. (2006) found tree size to be the most important factor explaining fungal species richness per tree species. Therefore, because mycorrhizal species are known suppliers of water and nutrients to living plants and trees, and saprophytic fungal species

specialize in colonizing stressed and dying trees for decomposition (Leake et al., 2002), the BA metric alone could not specifically describe the changing fungal community. Given that the goal of this study was not to look specifically at forest metrics, it is not surprising that it was not possible to fully determine the effects of forest metrics in this study. Regarding fungal community composition, there is very little explanatory power with general forest metrics. More sensitive forest metrics would include indices of small and large coarse woody debris, soil moisture, diameter differences, and decay classes of the wood substrates on site. Since most metrics are biomass based, indicating tonnage of timber, such metrics are largely related to tonnage of fungus. However, in this study the goal was focused on assessing fungal community richness and composition, rather than mass or abundance.

1.4.4. *Sampling*

Despite the importance of fungi within the global environment, few assays detailing fungal species richness have been conducted (Lonsdale et al., 2008). In this study, species accumulation curves did not level off suggesting that even more species could be encountered with further sampling. Blaser et al. (2013) identified a similar number of fungal samples (1395 total fungal samples, with 128 species found in coniferous forests) from forests in Germany, and produced comparable curves. In the current study, 129 fungal species were observed within a specific forest ecotype in a limited geographic area, with ~14% that did not match known species descriptions.

In fungal sampling, it is common to have some assayed fungi that do not match known species descriptions (Brazee et al., 2012; Czederpiltz et al., 2004; Lindner et al., 2006). Although

taxonomic knowledge is essential in efforts to conserve biodiversity, only 5-10% of the estimated 1-10 million fungi have been described in scientific literature (Hawksworth, 2001). From this study, fungal sampling, collection, and sequencing led to at least one new species description in *Rhizochaete* (Nakasone et al., 2017), with significant potential for more.

The current study employed 3 years of fruiting body data to make inferences about forest management practices, which is in accordance with the conclusion from Berglund et al. (2005) that wood-inhabiting fungal species diversity assessments should be repeated in at least 2 consecutive years to provide a (potentially) valid reference point for subsequent monitoring. The current study was able to show effects of forest management practices on the fungal community composition and supports others who have applied fungal diversity assays, specifically with polypores and crust fungi, to evaluate forest health and management strategies (Brazee et al., 2012; Lindner et al., 2006; Stokland and Larsson, 2011).

An additional factor not tested within this study was sampling biases among the plots. Within the 0X plots, the slash had been removed, allowing for easy visibility and mobility. Within the 1X and 2X plots, the randomly distributed slash created some visibility and mobility limitations, which this research acknowledges as an unintended sampling bias. Furthermore, though soil moisture data was not available, fungal sampling in 2016 occurred during very wet conditions, which likely contributed to greatest fruiting body occurrences during the last year.

1.4.5. *Dynamic communities of forest biodiversity*

Similar to succession in forestry (Wenger, 1984), fungal succession occurs when the fungal community modifies the physical environment (at the stand level and within specific

substrates, like log decomposition) such that other populations can be established (Frankland, 1992). Some forest fungal pathogens found in this study can kill trees (Filip, et al. 2015; Hagle, 2009; Otrosina and Garbelotto, 2010; Rocky Mountain Region, Forest Health Protection, 2010; Sinclair, et al., 1987). After infection by primary pathogens, other opportunistic fungal pathogens are quick to inhabit the stressed or dying trees. Some fungal pathogens are consistently associated with attack of stressed trees by subcortical insects, or insects that feed in the phloem layer of inner bark (Castello et al., 1976; Persson et al., 2011; Schmidt, 2006; van der Wal et al., 2017). Decomposing woody materials creates habitat for other creatures, as well as cycles the next generation of soil nutrients (Ferris and Humphrey, 1999; Lonsdale et al., 2008). The complexity of changing forest ecosystems compels forest managers to have greater awareness of the roles of fungi because management responsibilities often now expanding beyond management for timber. The University of Idaho Experimental Forest is managed for a variety of uses, and recreational or ecological values may not be tied to the economic value of forest timber (de Groot et al., 2010).

1.5 References

Anderson, M., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46.

Anderson, M.J., Ellingsen, K.E., McArdle, B.H., 2006. Multivariate dispersion as a measure of beta diversity. Ecol. Lett. 9, 683–93.

Aragon, J., 2010. Modelling the production and species richness of wild mushrooms in pine forests of the Central Pyrenees in northeastern Spain. Canadian Journal of Forest Research 40: 347-356.

Arnstadt, T., Hoppe, B., Kahl, T., Kellner, H., Kruger, D., Bauhus, J., Hofrichter, M., 2016. Dynamics of fungal community composition, decomposition, and resulting deadwood properties in logs of *Fagus sylvatica, Picea abies,* and *Pinus sylvestris*. Forest Ecology and Management 382: 129-142.

Arno, S.F., 2000. Fire in Western Forest Ecosystems, in: Brown, J.K., Smith, J.K. (Eds.), Wildland Fire in Ecosystems: Effects of Fire on Flora. USDA Forest Service Gen. Tech.Rep. RMRS-FTR-42-Vol. 2, 97–120.

Bader, P., Jansson, S., Jonsson, B.G., 1995. Wood-inhabiting fungi and substratum decline in selectively logged boreal spruce forests. Biological Conservation 72: 355–362.

Berlund, H., Jonsson, B.G., 2001. Predictability of plant and fungal species richness of old-growth boreal forest islands. Journal of Vegetation Science 12: 857-866.

Berlund, H., Edman, M., Ericson, L., 2005. Temporal variation of wood-fungi diversity in boreal old-growth forests: implications for monitoring. Ecological Applications 15(3): 970-982.

Bernicchia, A., Perez Gorjon, S., Arras, L., 2010. Corticiaceae s.l.: 427 colour potos and 455 drawings of microscopical elements. Alassio, Candusso.

Blaalid R, Kumar S, Nilsson RH, Abarenkov K, Kirk PM, Kauserud H, 2013. ITS1 versus ITS2 as DNA metabarcodes for fungi. Molecular Ecology Resources 13 (2): 218-224.

Blaser, S., Prati, D., Senn-Irlet, B., Fischer, M., 2013. Effects of forest management on the diversity of deadwood-inhabiting fungi in Central European Forests. Forest Ecology and Management 304: 42-48.

Boddy, L., Frankland, J.C., vanWest, P., 2008. Ecology of Saprotrophic Basidiomycetes. The British Mycological Society, Elsevier Ltd.

Bonet, J.A., Palahi, M., Colinas, C., Pukkala, T., Fischer, C.R., Miina, J., Martinez de Aragon, J., 2010. Modelling the production and species richness of wild mushrooms in pine forests of the Central Pyrenees in northeastern Spain. Journal of Forest Research 40: 347-356.

Bock D.E., Velleman, P.F., deVeaux, R.D., 2007. Stats: Modeling the World, 2nd edition. Pearson Education Inc., Boston, MA.

Brazee, N.J., Lindner, D.L., Fraver, S., D'Amato, A.W., Milo, A.M., 2012. Wood-inhabiting, polyporoid fungi in aspen-dominated forests managed for biomass in the U.S. Lake States. Fungal Ecology 5: 600-609.

Breitenbach, J., Kranzlin, F., 1986. Fungi of Switzerland: A contribution to the knowledge of the fungal flora of Switzerland, Volume 2. Verlag Mykologia, Luzern, Switzerland.

Bunnell, F.L., Houde, I., 2010. Down wood and biodiversity – implications to forest practices. Environmental Review 18: 397-421.

Carroll, G.C., Wicklow, D.T. (Eds.), 1992. The Fungal Community: It's organization and role in the ecosystem, 2nd edition. Marcel Dekker, Inc., New York.

Castello, J.D., Shaw, C.G., Furniss, M.M., 1976. Isolation of *Cryptoporus volvatus* and *Fomes pinicola* from *Dendroctonus pseudotsugae*. Phytopathology 66:1431-1434.

Chase J.M., Kraft, N.J.B., Smith, K.G., Vellend, M., Inouye, B.D., 2011. Using null models to disentangle variation in community dissimilarity from variation in α-diversity. Ecosphere 2(2):article 24, 1-11.

Coleman, M., Page-Dumroese, D., Archuleta, J., Badger, P., Chung, W., Venn, T., Loeffler, D., Jones, G., McElligott, K., 2010. Can portable pyrolysis units make biomass utilization affordable while using bio-char to enhance soil productivity and sequester carbon?, in: Jain, T.B., Graham, R.T., Sandquist, J. (Eds.), Integrated management of carbon sequestration and biomass utilization opportunities in a changing climate: Proceedings of the 2009 National Silviculture Workshop; 2009 June 15-18; Boise, ID. Proceedings RMRS-P-61. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, pp. 159-168.

Cooper, S.V., Neiman, K.E., Roberts, D.W., 1991. Forest habitat types of Northern Idaho: A second approximation. United States Department of Agriculture, Forest Service, Intermountain Research Station, General Technical Report INT-236.

Curtis, R.O., 1982. A simple index of stand density for Douglas-fir. Forest Science 28: 92-94.

Czederpiltz, D.L.L., Wikler, K., Radmacher, M.R., Volk, T.J., Hadar, Y., Micales, J., 2004. Biodiversity of wood-inhabiting fungi in Isreali pine forests. Fungi in forest ecosystems: systematics, diversity, and ecology. Memoirs of the New York Botanical Garden: 89: 191-202.

Dean, A., Voss, D., 1999. Design and Analysis of Experiments. Springer-Verlag, New York, Inc., New York.

de Groot, R.S., Alkemade, R., Braat, L., Hein, L., Willeman, L., 2010. Challenges in integrating the concept of ecosystem services and values in landscape planning, management and decision making. Ecological Complexity 7: 260-272.

Ferguson, B.A., Dreisbach, T.A., Parks, C.G., Filip, G.M., Schmitt, C.L., 2003. Coarse-scale population structyure of pathogenic *Armillaria* species in a mixed-conifer forest in the Blue Mountains of northeast Oregon. Canadian Journal of Forest Research 33: 312-326.

Ferris, R., Humphrey, J.W., 1999. A review of potential biodiversity indicators for application in British forests. Forestry 72, 314–328.

Filip, G.M., Bronson, J.J., Chadwick, K.L., Filip, J.B., Frankel, S.J., Goheen, D.J., Goheen, E.M., Mori, S.R., Saavedra, A.L., 2015. Precommercial thinning in mixed-species conifer plantations affected by Armillaria and Heterobasidion root diseases in West-Central Oregon and Washington: 30-Year Results. Forest Science 61(5): 914-925.

Forest Guild Biomass Working Group, 2012. "Forest biomass retention and harvesting guidelines for the Southeast." Forest Guild, Santa Fe, New Mexico. http://www.forestguild.org/publications/research/2012/FG_Biomass_Guidelines_SE.pdf (accessed 22 February 2018).

Frankland, J.C., 1992. Mechanisms in Fungal Succession. In Carroll, G.C., Wicklow, D.T. (Eds.), 1992. The Fungal Community: It's organization and role in the ecosystem, 2nd edition. Marcel Dekker, Inc., New York: 383-401.

Gardener, M., 2014. Community ecology: analytical methods using R and Excel. Pelagic Publishing, Exeter, UK.

Gilbertson, R.L., Ryvarden, L., 1986. North American Polypores, Volumes 1-2. Fungiflora, Oslow, Norway.

Hagle, S.K., 2009. Laminated root rot ecology and management. Chapter 11.2. Forest insect and disease management guide for the northern and central Rocky Mountains. USDA Forest Service, Northern Region, State and Private Forestry, p 1-20. Accessed (15 May 2018) https://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5187461.pdf

Hammell, K.E., 1997. Fungal Degradation of Lignin, in: Cadisch, G., Giller, K.E. (Eds.), Driven by Nature: Plant Litter Quality and Decomposition. CAB International, Wallingford, pp. 33-45.

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. Advanced Ecological Research 15: 133-302.

Hawksworth, D.L., 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycological Research 105 (12): 1422-1432.

Hjorstam, K., Larsson, K.-H., Ryvarden, L., 1987. The Corticiaceae of North Europe, Volumes 1-8. Fungiflora, Oslow, Norway.

Husch, B. Beers, T.W., Kershaw, Jr., J.A., 2003. Forest Mensuration, 4th edition. John Wiley & Sons, Inc., Hoboken, New Jersey.

Keane, R.E., Ryan, K.C., Veblen, T.T., Allen, C.D., Logan, J., Hawkes, B., 2002. Cascading effects of fire exclusion in the Rocky Mountain ecosystems: a literature review. General Technical Report. RMRS-GTR-91. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, 1-24.

Keyes, C., O'Hara, K., 2002. Quantifying stand targets for silvicultural prevention of crown fires. West. J. Appl. For. 17, 101–109.

Kimmins, J.P. (Hamish), 1997. Biodiversity and its relationship to ecosystem health and integrity. The Forestry Chronicle 73 (2): 229-232.

Leake, J.R., Donnelly, D.P., Boddy, L., 2002. Interactions between ecto-mycorrhizal and saprophytic fungi. In: van der Heijden, M.G.A., Sanders, I.R. (eds.) Mycorrhizal Ecology. Ecological Studies (Analysis and Synthesis), volume 157. Springer, Berlin, Heidelberg.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota – A review. Soil Biology & Biochemistry 43: 1812-1836.

Li, Y., Hu, S., Chen, J., Muller, K., Li, Y., Fu, W., Lin, Z., Wang, H., 2018. Effects of biochar application in forest ecosystems on soil properties and greenhouse gas emissions: a review. Journal of Soils and Sediments. Doi.org/10.1007/s11368-017-1906-y.

Lindner, D.L., Burdsall Jr., H.H., Stanosz, G.R., 2006. Species diversity of polyporoid and corticioid fungi in northern hardwood forests with differing management histories. Mycologia 98, 195–217.

Lindner, D.L., Banik, M.T., 2008. Molecular phylogeny of *Laetiporus* and other brown rot polypore genera in North America. Mycologia 100, 417–430.

Lonsdale, D., Pautasso, M., Holdenrieder, O., 2008. Wood-decaying fungi in the forest: conservation needs and management options. Eur. J. For. Res. 127, 1–22. DOI 10.1007/s10342-007-0182-6.

Magurran, A.E., 1988. Ecological diversity and its measurement. Princeton University Press, 61-125.

Maser, C., Anderson, R.G., Cromack, K., Williiams, J.T., Martin, R.E., 1979. Dead and down woody materia. Wildlife habitats in managed forests of the Blue Mountains of Oregon and Washington. Agric. Handb. 553, 78–95.

McDaniel, P. A., Hipple, K.W., 2010. Mineralogy of loess and volcanic ash eolian mantles in Pacific Northwest (USA) landscapes. Geoderma 154, 438–446.

Molina R., Pilz, D., Smith, J., Dunhan, S., Dreisbach, T., O'Dell, T., Castellano, M., 2001. Conservation and management of forest fungi in the Pacific Northwestern United States: an integrated ecosystem approach. British Mycological Society Symposium Series 22: 19-63.

McElligott, K., Page-Dumroese, D., Coleman, M., 2011. Bioenergy production systems and biochar application in forests: Potential for renewable energy, soil enhancement, and carbon sequestration. Res. Note RMRS-RN-46. Fort Collins, CO; U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. https://www.fs.fed.us/rm/pubs/rmrs_rn046.pdf (accessed 22 February 2018).

Mohan, D., Pittman, C.U., Steele, P.H., 2006. Pyrolysis of wood/biomass for bio-oil: a critical review. Energy & Fuels 20, 848–889.

Mueller, G.M., Schmit, J.P., Huhndorf, S.M., Vyvarden, L., O'Dell, T.E., Lodge, D.J., Leacock, P.R., Mata, M., Umana, L., Wu, Q., Czederpiltz, D.L., 2004. Recommended protocols for sampling macrofungi, in: Mueller, G.M., Bills, G.F., Foster, M.S. (Eds.), Biodiversity of Fungi: Inventory and Monitoring Methods. Elsevier, 168–172.

Nakasone, K.K., Draeger, K.R., Ortiz-Santana, B., 2017. A contribution to the taxonomy of Rhizochaete (Polyporales, Basidiomycota). Cryptogamie, Mycologie 38 (1); 1-20.

Nordén, B., Ryberg, M., Götmark, F., Olausson, B., 2004. Relative importance of coarse and fine woody debris for the diversity of wood-inhabiting fungi in temperate broadleaf forests. Biol. Conserv. 117, 1–10.

Norton, G., Abraham, S., Veneman, A., 2003. Memorandum of understanding on policy principles for woody biomass utilization for restoration and fuel treatments on forests, woodlands, and rangelands. United States Department of Agriculture, United States Department of energy, and the United States Department of the Interior. https://www.fs.fed.us/woodybiomass/documents/BiomassMOU_060303_final_web.pdf (accessed 22 February 2018).

Noyce, G.L., Basiliko, N., Fulthorpe, R., Sackett, T.E., Thomas, S.C., 2015. Soil microbial respons over 2 years following biochar addition to a north temperate forest. Biology and Fertility of Soils 51 (6): 649-659.

Odor, P., Heilmann-Clausen, J., Christensen, M., Aude, E., van Dort, K.W., Piltaver, A., Siller, I., Veerkamp, M.T., Walleyn, R., Standovar, T., van Hees, A.F.M., Kosec, J., Matocec, N., Kraigher, H., Grebenc, T., 2006. Diversity of dead wood inhabiting fungi and bryophytes in semi-natural beech forests in Europe. Biological Conservation 131: 58-71.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2012. Vegan: Community Ecology Package.

Olsson, J., Jonsson, B.G., 2010. Restoration fire and wood-inhabiting fungi in a Sweedish *Pinus sylvestris* forest. Forest Ecology and Management 259: 1971-1980.

Otrosina W.J., Garbelotto, M., 2010. *Heterobasidion occidentale sp. nov.* and *Heterobasidion irregulare* nom. nov.: A disposition of North American *Heterobasidion* biological species. Fungal Biology 114(1): 16-25.

Page-Dumrose, D.S., Coleman, M.D., Thomas, S.C., 2016. Opportunities and uses of biochar on forest sites in North America, in: Bruckman, V.J., Varol, E.A., Uzun, B.B., Liu, J. (Eds.), Biochar: A regional supply chain approach in view of climate change mitigation., Cambridge University Press, 315-335.

Persson, Y., Ihrmark, K., Stenlid, J., 2011. Do bark beetles facilitate the establishment of rot fungi in Norway spruce? Fungal Ecology 4: 262-269.

Purahong, W., Kahl, T., Schloter, M., Bauhus, J., Buscot, F., Kruger, D., 2014. Comparing fungal richness and community composition in coarse woody debris in Central European beech forests under three types of management. Mycological Progress 13, 959–964.

PRISM Climate Group, Oregon State University, http: //prism.oregonstate.edu, created 4 Feb 2004. Accessed 26 Feb 2018.

Rajala, T., Peltoniemi, M., Pennanen, T., Makipaa, R., 2012. Fungal community dynamics in relation to substrate quality of decaying Norway Spruce (Picea abies [L.Karst.) logs in boreal forests. FEMS Microbiology Ecology 81, 494-505.

Raup, D.M., Crick, R.E., 1979. Measurement of faunal similarity in paleontology. Journal of Paleontology 53 (5): 1213-1227.

Rayner, A.D.M., Boddy, L., 1988. Fungal Decomposition of Wood: Its Biology and Ecology. John Wiley & Sons Ltd., New York.

Reineke, L.H., 1933. Perfecting a stand-density index for even-aged forests. Journal of Agricultural Research 46 (7): 627-638.

Ripley, B., Venables, B., Bates, D.M., Hornik, K., Gebhardt, Al, Firth, D., Package 'MASS'. Accessed (03 May 2018) https://cran.r-project.org/web/packages/MASS/MASS.pdf.

Rocky Mountain Region, Forest Health Protection. 2010. Field guide to diseases & insects of the Rocky Mountain Region. Gen. Tech. Rep. RMRS-GTR-241 Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, 1-336.

Rydin, H., Diekmann, M., Hallingbäck, T., 1997. Biological characteristics, habitat associations, and distribution of macrofungi in Sweden. Conservation Biology 11, 628–640.

Schäffer, A., Aravind, L., 2001. Improving the accuracy of PSI-BLAST protein database searches with composition-based statistics and other refinements. Nucleic Acids Research 29, 2994–3005.

Shannon, C.E., Weaver, W., 1949. The mathematical theory of communication. University of Illinois Press, Urbana, 1-117.

Sherman, L.A., Page-Dumroese, D.S., Coleman, M.D., 2017. Idaho forest growth response to post-thinning energy biomass removal and complementary soil amendments. GCB Bioenergy, 1-16. DOI: 10.1111/gcbb.12486.

Sinclair, W.A., Lyon, H.H., Johnson, W.T., 1987. Diseases of Trees and Shrubs. Cornell University Press, Ithaca, New York.

Simpson, E.H., 1949. Measurement of Diversity. Nature 163: 688.

Stokland, J.R., Siitonen, J., Gunnar Jonsson, B., 2012. Biodiversity in Dead Wood. Cambridge University Press, New York.

Stokland J.N., Larsson, K.H., 2011. Legacies from natural forest dynamics: Different effects of forest management on wood-inhabiting fungi in pine and spruce forests. Forest Ecology and Management 261 (11): 1707-1721.

Teste, F.P., Lieffers, V.J., Strelkov, S., 2012. Ectomycorrhizal community responses to intensive forest management: thinning alters impacts of fertilization. Plant and Soil 360: 333-347.

U.S. Department of Energy, Perlack, R.D., Stokes, B.J., 2011. U.S. Billion-Ton Update: Biomass Supply for a Bioenergy and Bioproducts Industry, ORNL/TM-2011/224, Oak Ridge Natl. Lab. Oak Ridge, TN. pp. 1–227. https://www1.eere.energy.gov/bioenergy/pdfs/billion_ton_update.pdf (accessed 22 February 2018).

USDA Forest Service, 2013. Woody Biomass Utilization. http://www.fs.fed.us/woodybiomass/whatis.shtml (accessed 22 February 2018).

Van der Wal, A., Gunnewiek, P.K., de Hollander, M., de Boer, W., 2017. Fungal diversity and potential tree pathogens in decaying logs and stumps. Forest Ecology and Management 406: 266-273.

Veblen, T., Kitzberger, T., Donnegan, J., 2000. Climatic and human influences on fire regimes in ponderosa pine forests in the Colorado Front Range. Ecol. Appl. 10, 1178–1195.

Wenger, K. (Ed.), 1984. Forestry Handbook, 2nd Edition. Edited for the Society of American Foresters, Wiley-Interscience Publication, New York.

Zak, J.C., Willig, M.R., 2004. Fungal Biodiversity Patterns, in: Mueller, G.M., Bills, G.F., Foster, M.S. (Eds.), Biodiversity of Fungi: Inventory and Monitoring Methods. Elsevier, 59-75.

Zheng, Y., Hu, H.W., Guo, L.D., Anderson, I.C., Powell, J.R., 2017. Dryland forest management alters fungal community composition and decouples assembly of root- and soil-associated fungal communities. Soil Biology & Biochemistry 109: 14-22.

Table 1.1. Stand structure and composition at the two sites located at the University of Idaho Experimental Forest, named UI_Ponderosa Pine (UIPP) and UI_Mixed Conifer (UIMC). Pretreatment characteristics were measured in 2012, and post-treatment characteristics were measured in 2013.

	TPH (trees ha^{-1})	QMD (cm)	BA (m^2 ha^{-1})	SDI (trees ha^{-1})	RD (Curtis)	Species Distribution % BA				
						DF	GF	LP	PP	WL
Pretreatment site characteristics										
UIMC	1613	12	17	484	34	12	27	28	15	18
UIPP	1513	11	15	395	31	8	0	1	91	0
Post-treatment site characteristics (thinned plots)										
UIMC	371	13	5	127	10	17	27	26	21	9
UIPP	375	14	6	144	11	9	0	2	90	0

TPH, trees per hectare; QMD, quadratic mean diameter; BA, basal area; SDI, standard density index (Reineke, 1933); RD, Curtis's relative density (Curtis, 1982); DF, Douglas-fir; GF, grand fir, LP, lodgepole pine; PP, ponderosa pine; WL, western larch. Table is modified from Sherman, et al. (2017).

Table 1.2. Selected pathogens found during fall fruiting body surveys of the University of Idaho Experimental Forest (2014-2016), and their associated diseases. Disease and decay descriptions follow Rocky Mountain Region, Forest Health Protection (2010), Sinclair et al. (1987), and Schmidt (2006). Nomenclature follows www.indexfungorum.org.

Pathogen	Disease
Armillaria ostoyae (Romagn.) Herink	Root Rot
Coniophora spp.	Root and Butt Rot
Cryptoporus volvatus (Peck) Shear	Gray-Brown Sap Rot
Dichomitus squalens (P. Karst) D.A. Reid	Red Ray Rot
Heterobasidion occidentale Otrosina & Garbel.	Root and Butt Rot
Phaeolus schweinitzii (Fr.) Pat.	Root and Butt Rot
Stereum sanguinolentum (Alb. & Schwein.) Fr.	Red Heart Rot
Trichaptum abietinum (Pers.) Ryvarden	Red Streak

Table 1.3. Species Richness, Shannon, and Simpson's diversity indices by plot, site, biomass treatment and soil amendment from fruiting body surveys in 2014, 2015, and 2016. Plots were initiated in spring of 2014, and fungal surveys were conducted in fall. Biomass treatments included a non-thinned control, thinning with no slash retained (0X), thinning with normal slash retention (1X), and thinning with double slash retention (2X). Soil amendments included a non-amended control, biochar, fertilizer, and fertilizer+biochar (Fert+Biochar). *Richness and diversity indices were not normally distributed.

			2014			2015			2016		
Site	Biomass Treatment	Soil Amendment	Species Richness	Shannon Diversity	Simpson's Diversity	Species Richness	Shannon Diversity	Simpson's Diversity	Species Richness	Shannon Diversity	Simpson's Diversity
UIPP	0X	Biochar	4	1.39	0.75	6	1.68	0.79	7	1.86	0.83
UIPP	0X	Fert+Biochar	6	1.79	0.83	3	1.04	0.63	11	2.04	0.84
UIPP	0X	Fertilizer	4	1.39	0.75	7	1.89	0.84	8	1.89	0.82
UIPP	0X	Non-amended	5	1.55	0.78	5	1.56	0.78	7	1.69	0.77
UIPP	1X	Biochar	3	1.10	0.67	0	0.00	1.00	6	1.48	0.73
UIPP	1X	Fert+Biochar	0	0.00	1.00	4	1.26	0.69	9	1.84	0.79
UIPP	1X	Fertilizer	2	0.69	0.50	4	1.24	0.67	11	2.23	0.88
UIPP	1X	Non-amended	3	1.10	0.67	3	1.10	0.67	10	2.06	0.84
UIPP	2X	Biochar	4	1.39	0.75	9	1.97	0.82	12	2.27	0.88
UIPP	2X	Fert+Biochar	3	1.10	0.67	6	1.73	0.81	7	1.67	0.78
UIPP	2X	Fertilizer	3	1.10	0.67	7	1.68	0.78	11	2.11	0.86
UIPP	2X	Non-amended	3	0.95	0.56	10	2.05	0.85	8	1.94	0.84
UIPP	Non-thinned	Biochar	5	1.61	0.80	5	1.56	0.78	9	2.14	0.88
UIPP	Non-thinned	Fert+Biochar	2	0.69	0.50	2	0.69	0.50	7	1.85	0.83
UIPP	Non-thinned	Fertilizer	6	1.79	0.83	3	1.10	0.67	11	2.34	0.89
UIPP	Non-thinned	Non-amended	8	2.04	0.86	5	1.43	0.72	8	1.98	0.85
UIMC	0X	Biochar	6	1.55	0.78	6	1.75	0.82	10	2.15	0.86
UIMC	0X	Fert+Biochar	3	1.10	0.67	3	1.04	0.63	13	2.48	0.91
UIMC	0X	Fertilizer	12	2.35	0.90	4	1.39	0.75	20	2.89	0.94
UIMC	0X	Non-amended	12	2.35	0.90	7	1.91	0.84	16	2.60	0.90
UIMC	1X	Biochar	0	0.00	1.00	6	1.75	0.82	14	2.25	0.84
UIMC	1X	Fert+Biochar	3	1.04	0.63	5	1.61	0.80	10	2.05	0.86
UIMC	1X	Fertilizer	1	0.00	0.00	3	1.10	0.67	7	1.63	0.78
UIMC	1X	Non-amended	0	0.00	1.00	3	1.10	0.67	14	2.49	0.90
UIMC	2X	Biochar	6	1.50	0.70	5	1.56	0.78	10	2.21	0.88
UIMC	2X	Fert+Biochar	3	1.01	0.61	5	1.39	0.69	13	2.26	0.85
UIMC	2X	Fertilizer	4	1.33	0.72	3	1.01	0.61	7	2.04	0.86
UIMC	2X	Non-amended	6	1.75	0.82	5	1.46	0.74	11	2.23	0.87
UIMC	Non-thinned	Biochar	7	1.91	0.84	5	1.56	0.78	9	1.99	0.84
UIMC	Non-thinned	Fert+Biochar	5	1.55	0.78	7	1.91	0.84	13	2.35	0.88
UIMC	Non-thinned	Fertilizer	5	1.55	0.78	4	1.24	0.67	7	1.95	0.86
UIMC	Non-thinned	Non-amended	5	1.61	0.80	5	1.61	0.80	11	2.18	0.86

Table 1.4. Results of permutational multivariate analysis of variance (PERMANOVA) of fungal community composition in 2014, 2015, and 2016 relative to site (UIPP, UIMC), biomass treatments (0X, 1X, 2X biomass retained, non-thinned control), and soil amendments (biochar, fertilizer+biochar, fertilizer, non-amended control). Betadispersion was also reported. Plots were established in Spring of 2014, and fungal surveys were accomplished in Fall. The effects of site and biomass treatment were significant in 2014 and 2016.

	PERMANOVA				Betadispersion		
	DF	F Model	R^2	*p*	*DF*	F	*p*
2014							
Site	1,24	2.37	0.06	0.014	1,30	4.01	0.05
Biomass Treatment	3,24	2.35	0.19	0.003	3,28	3.56	0.03
Soil Amendment	3,24	1.18	0.09	0.301	3,28	0.37	0.77
2015							
Site	1,24	3.98	0.11	0.067	1,30	2.06	0.16
Biomass Treatment	3,24	1.32	0.11	0.213	3,28	0.57	0.64
Soil Amendment	3,24	1.68	0.13	0.064	3,28	4.91	0.007
2016							
Site	1,24	3.97	0.11	0.212	1,30	4.62	0.04
Biomass Treatment	3,24	2.12	0.17	0.059	3,28	0.65	0.59
Soil Amendment	3,24	0.71	0.06	0.675	3,28	2.62	0.070

Table 1.5. Results of significant permutational multivariate analyses of variance (PERMANOVA) of fungal community composition in 2014, 2015, and 2016 relative to forest composition metrics. Plots were established in spring of 2014. Fungal surveys were accomplished in fall.

	PERMANOVA			
	DF	F.Model	R^2	*p*
2014				
Basal Area	1,25	2.08	0.07	0.054
2015				
Basal Area	1,25	2.74	0.08	0.008
Standard Density Index	1,25	3.28	0.09	0.026
2016				
Coarse Woody Debris (tons hectare^{-1})	1,25	1.88	0.05	0.08

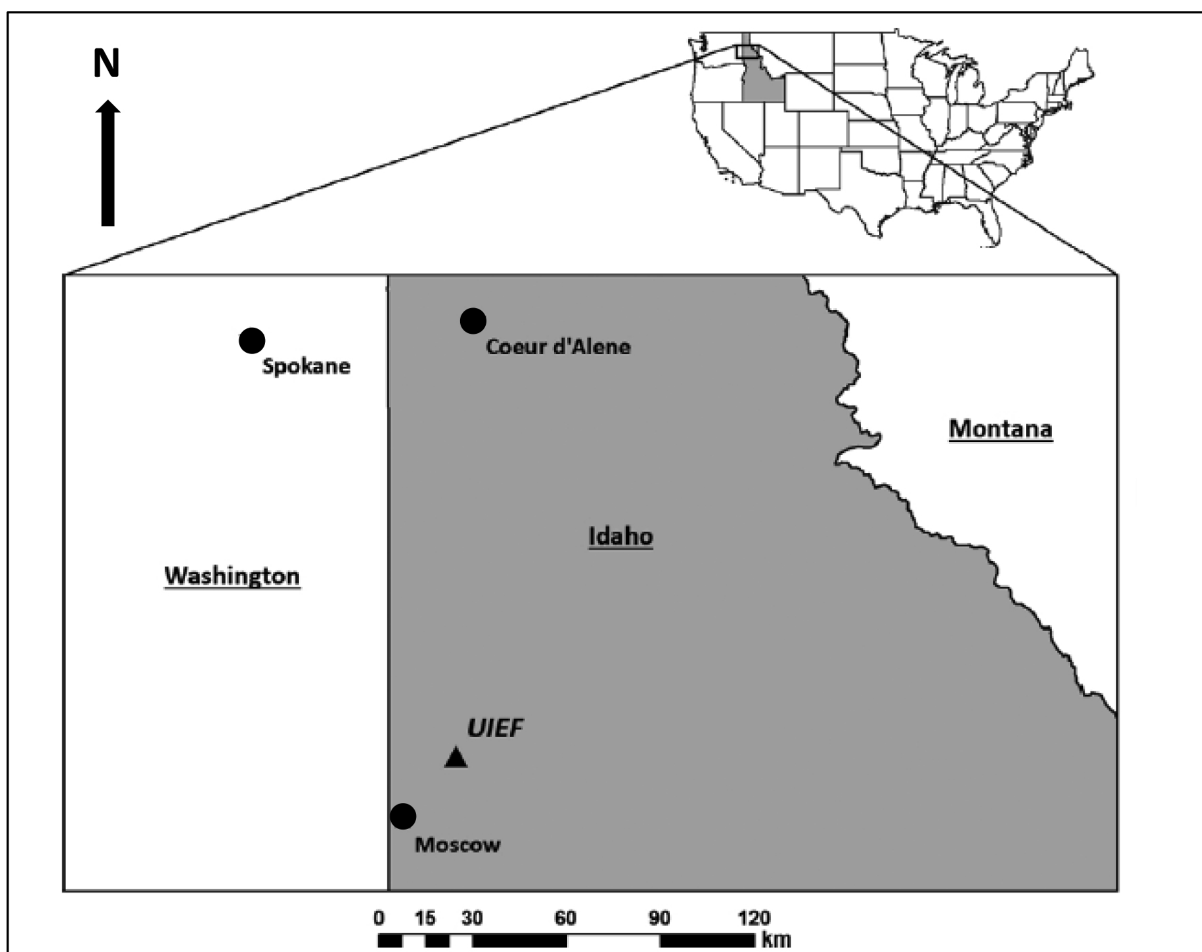

Fig. 1.1. Location map of study area in northern Idaho, USA. The triangle indicates University of Idaho Experimental Forest (UIEF) site, and circles represent major cities (modified from Sherman et al., 2017).

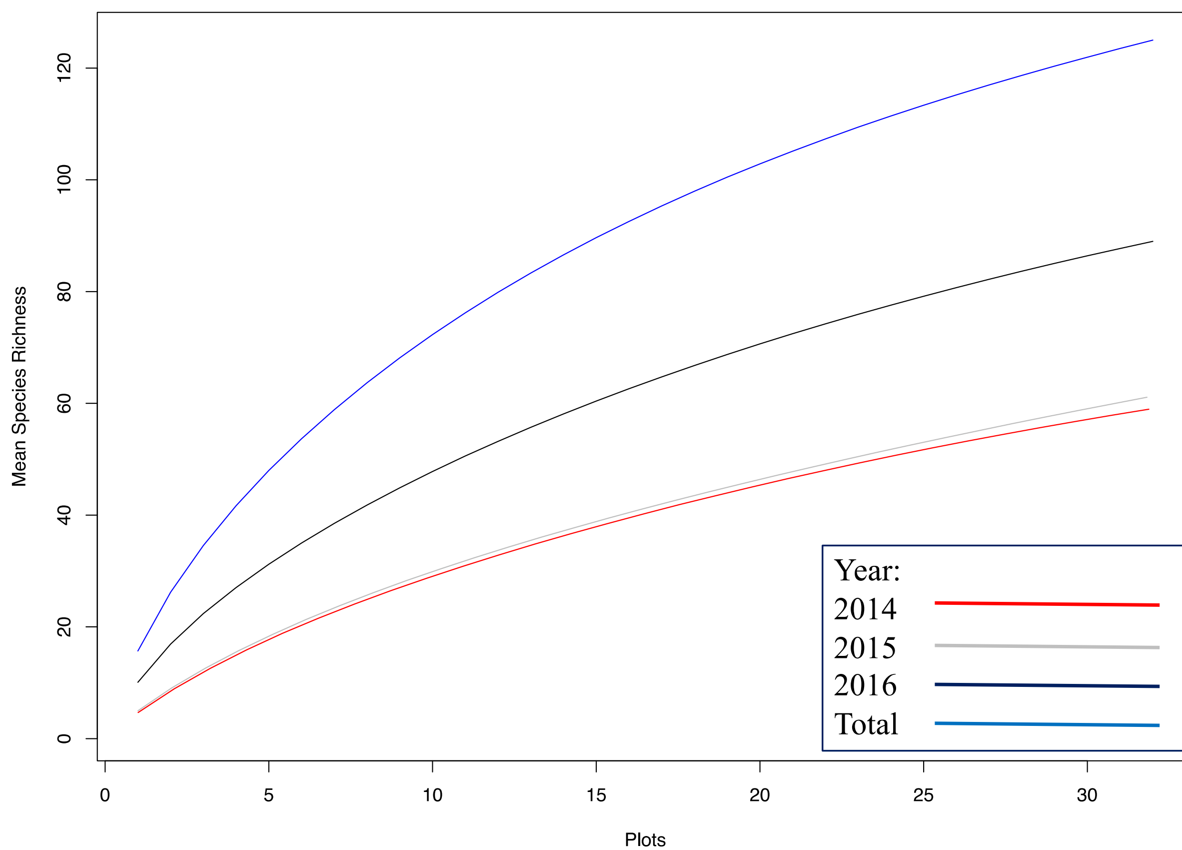

Fig. 1.2. Species accumulation curves of mean fungal species richness from each year (2014, 2015, 2016, and in total), relative to the number of sampled plots. Plots were established in spring of 2014, and fungal surveys were conducted in fall in 20 x 20 m^2 plots. The data from 2015 (gray line) is obscured by data from 2014 (red line).

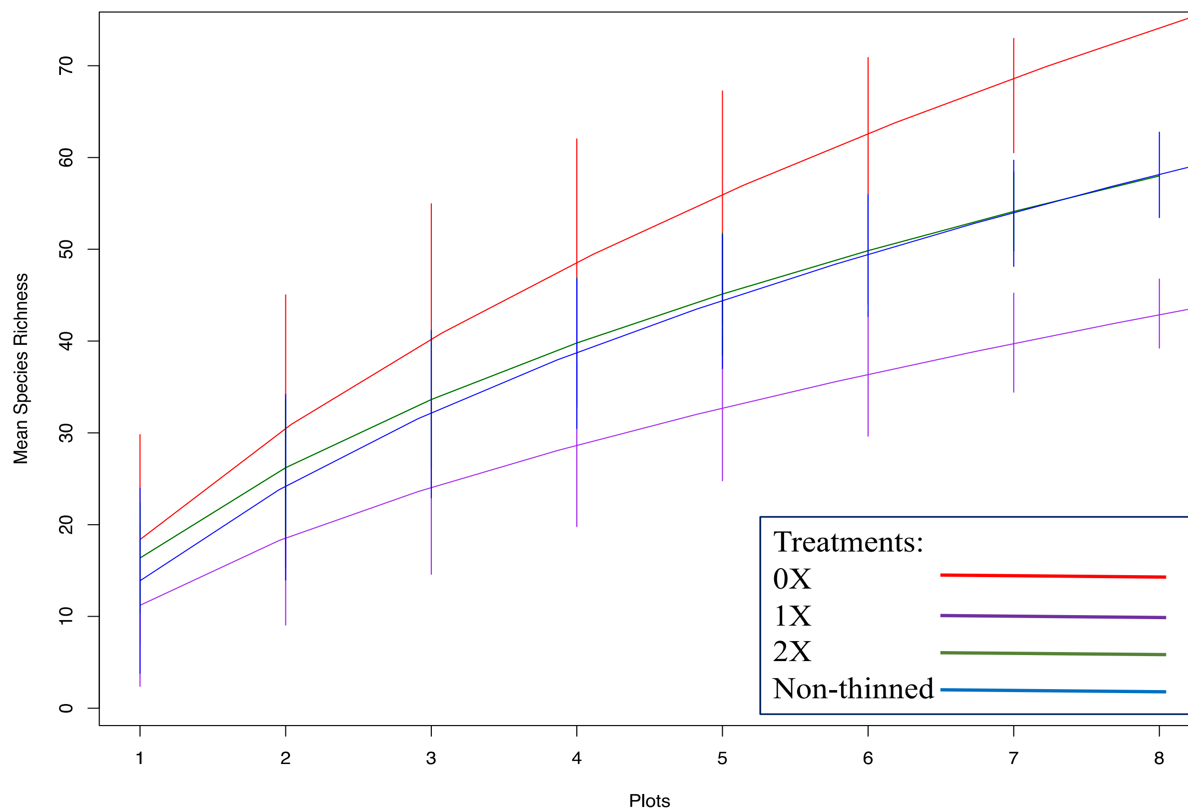

Fig. 1.3. Species accumulation curves of mean fungal species richness from each biomass treatment [a non-thinned control, thinning with no slash retained (0X), thinning with normal slash retention (1X), and thinning with double slash retention (2X)], relative to the number of sampled plots. Plots were established in spring of 2014, and fungal surveys were conducted in fall, over three years, in 20 x 20 m^2 plots. The data relative to the 2X biomass treatments (dark green line) is obscured by data from the non-thinned treatments (blue line). Confidence intervals at 95% are shown to illustrate the range of variation within treatments, over three years.

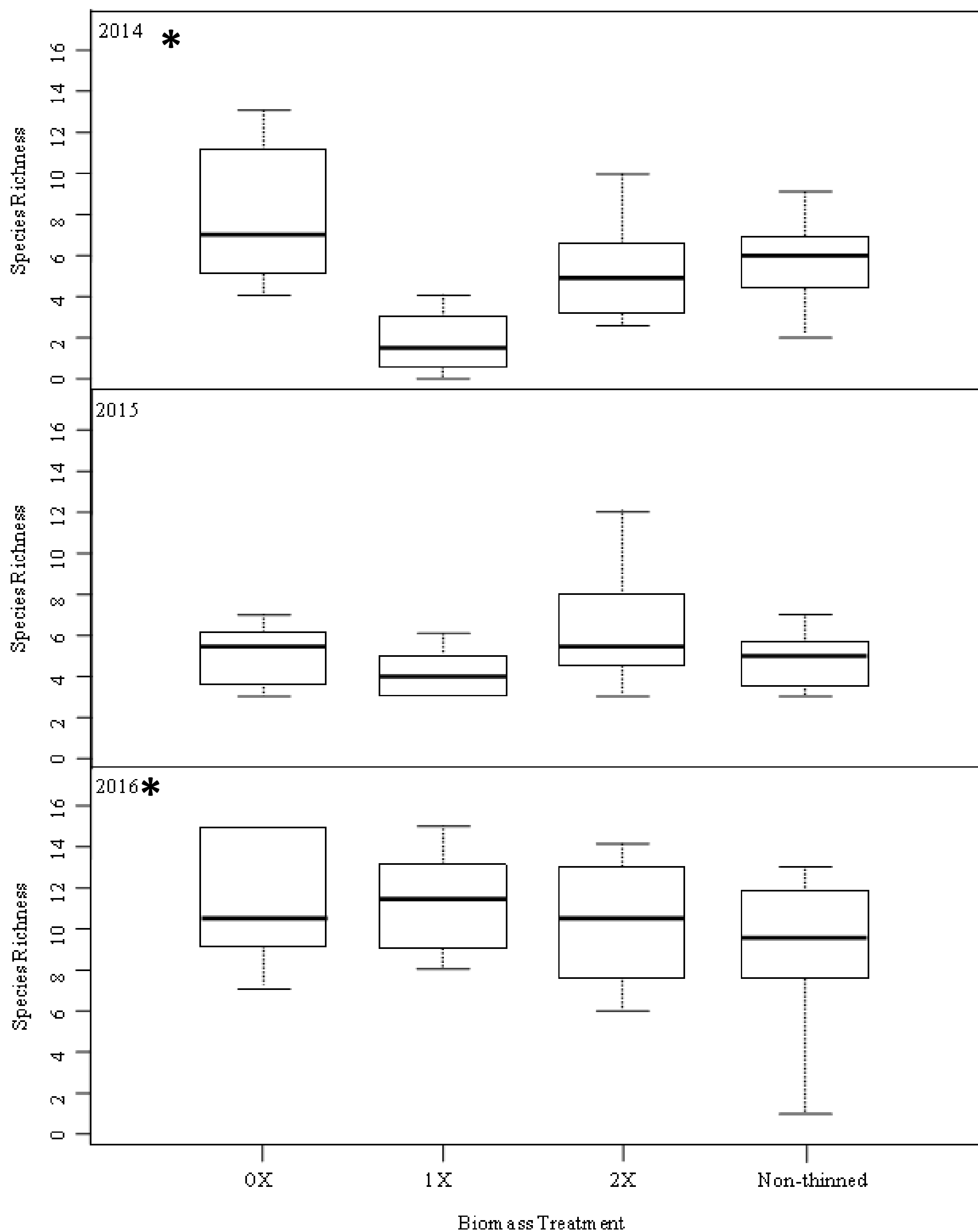

Fig. 1.4. Boxplots of fungal fruiting body species richness (number of species present) by biomass treatment plots (0X = no slash, 1X = normal slash, 2X = double slash, non-thinned = control), from 2014, 2015, and 2016. Plots were established in Spring of 2014, and fruiting body surveys were conducted in fall. *The fungal community composition differed significantly due to biomass treatment in 2014 and 2016 (PERMANOVA, $p < 0.05$).

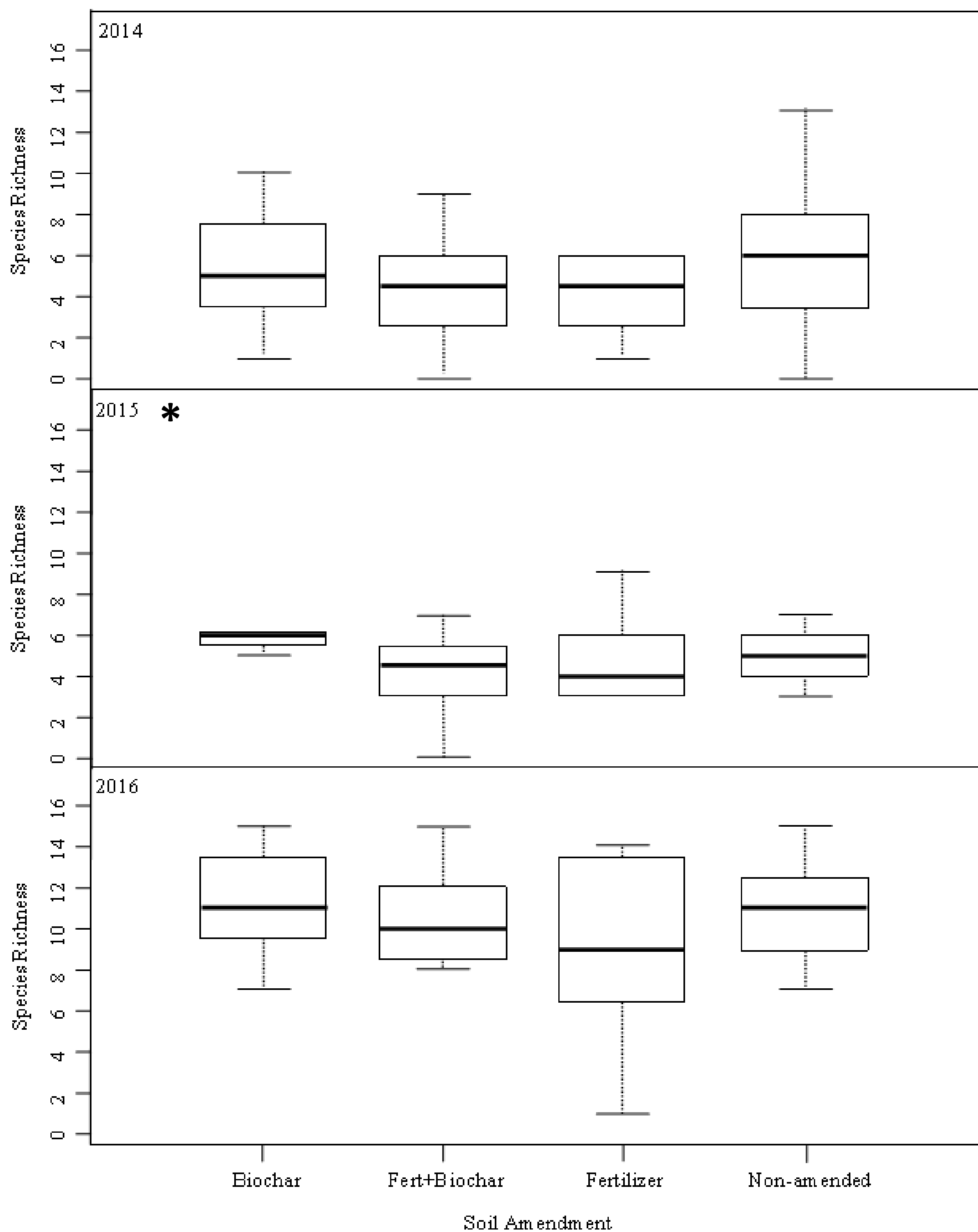

Fig. 1.5. Boxplots of fungal fruiting body species richness (number of species present) by soil amendment (biochar, fert+biochar = fertilizer+biochar, fertilizer, and non-amended control) from 2014, 2015, and 2016. Plots were established in spring of 2014, and fruiting body surveys were conducted in fall. *Only in 2015 were fungal communities almost significantly different due to soil amendment (PERMANOVA, $p = 0.064$).

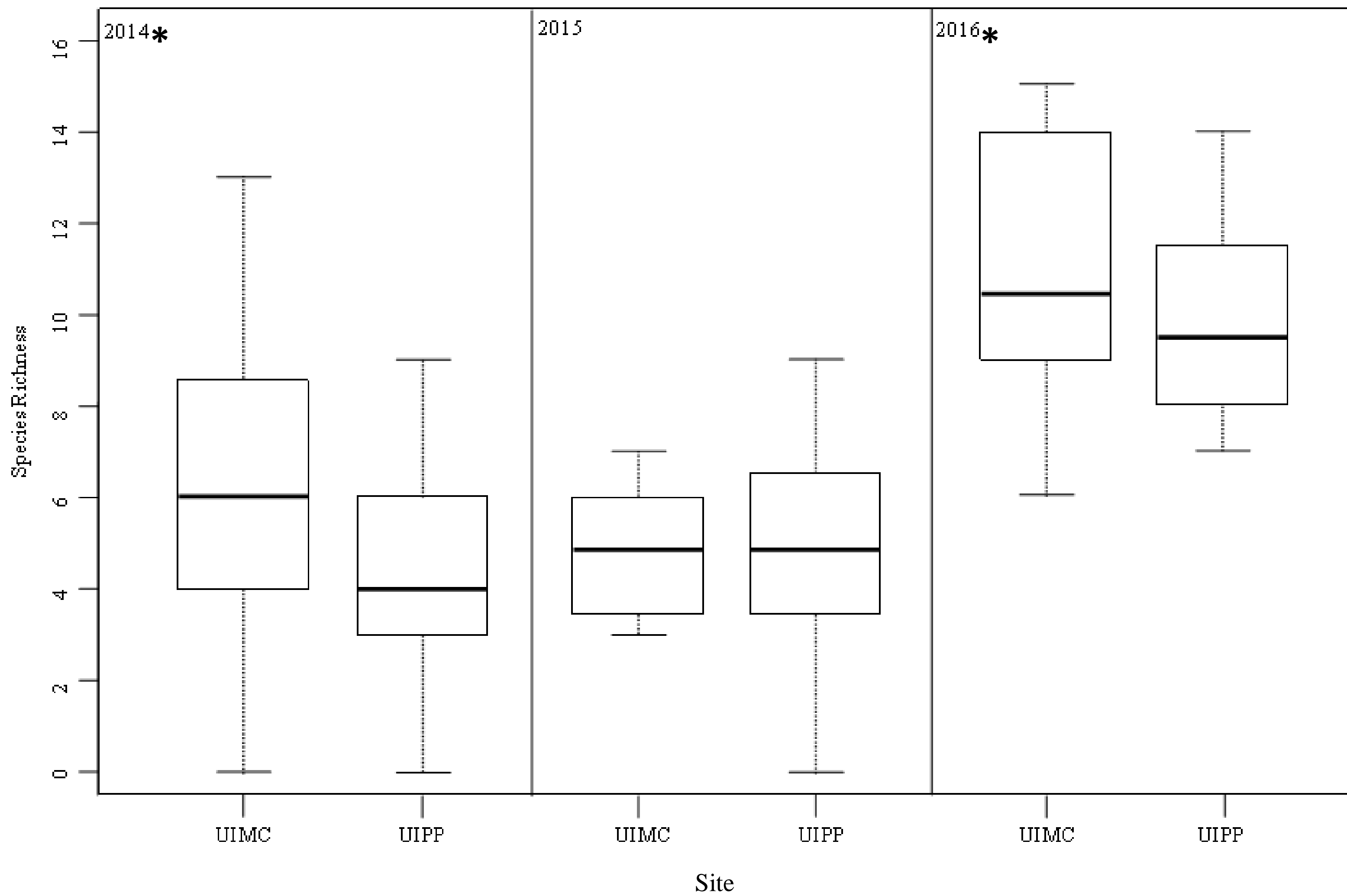

Fig. 1.6. Boxplots of fungal fruiting body species richness (number of species present) by site (University of Idaho_Mixed Conifer = UIMC, University of Idaho_Ponderosa Pine = UIPP), from 2014, 2015, and 2016. Plots were established in Spring of 2014, and fruiting body surveys were conducted in Fall. *In 2014 and 2016, the fungal community composition differed significantly due to site (PERMANOVA, p > 0.03).

Chapter 2

Effects of forest management practices on wood-stake colonizing fungal diversity and community composition in western North America

Abstract

The effects of biomass harvesting and compensatory soil amendments were assessed by sequencing fungal DNA. Standardized wood stakes of loblolly pine and aspen were inserted into the mineral soil and affixed on the soil surface litter layer. These were retrieved yearly for three years (spring 2015-2017). Stakes were drilled to produce wood shavings, which were used for extraction of fungal DNA.

Wood stakes were placed in 400-m^2 plots established with varying levels of biomass retention (removal of cut material, retention of cut material, doubling of the cut material, and non-thinned controls) and varying soil amendments (fertilizer, biochar, fertilizer and biochar, non-amended control). The 16 treatments were repeated at two sites, yielding 32 treatment plots.

After 3 years of sampling, over 2,000 different operational taxonomic units (OTUs) were identified through high-throughput amplicon sequencing (HTAS). Over 50% of identified taxa were assigned to the phylum Ascomycota (most abundant orders: *Helotiales, Chaetothriales*, and Coniochaetales) with 33% to Basisidiomycota. The species of stake (aspen or pine) and wood stake location (surface or within the mineral layer) were highly significant in influencing both fungal richness and the fungal community composition ($p < 0.01$).

Biomass treatments significantly influenced the fungal communities present within surface stakes every year of data collection ($p < 0.05$), whereas only in the second year (2016)

was biomass treatment significant in influencing the fungal community composition of the stakes in the mineral soil ($p < 0.05$). Initially the doubled biomass treatment had fewer fungal OTUs, but by the third year the double biomass treatment had the greatest OTU richness.

Soil amendments also significantly influenced the fungal community compositions, with complicated results. Fungal colonization of both aspen and pine surface stakes were influenced by soil amendments in the first year ($p < 0.01$), whereas only surface aspen and mineral pine stakes were influenced by soil amendments in the second year ($p < 0.05$). By the third year, both pine and aspen surface stakes had significant differences in fungal community composition due to soil amendments ($p < 0.05$). During the first 2 years, fertilized plots yielded lower OTU richness than non-amended sites, but after 3 years, fertilized plots yielded greater OTU richness within surface stakes.

2.1 Introduction

In the past, frequent, low intensity fires reduced both the production and accumulation of woody biomass in many forest ecosystems of the Rocky Mountain region (Arno, 2000; Veblen et al., 2000). Fire suppression has led to overstocked forests that also contain excess woody debris, increasing the risk for higher intensity fires (Keyes and O'Hara, 2002). To reduce woody biomass and fuel loads in overstocked forest stands, as well as to address uncertainty regarding the sustainability of U.S. energy supplies, interest in biomass harvesting and alternative energies have increased (US DOE, 2011).

Living trees and other woody plants, as well as dead limbs, tops, and foliage that accumulate naturally or result from forest management activities such as thinning (USDA Forest Service, 2013), comprise biomass that can be a feedstock for the production of a variety of biofuels. Although other biofuel possibilities exist, bio-oil is most appropriate for efficient Western coniferous biomass utilization based on access to existing infrastructure (Mohan et al., 2006). An additional potential benefit is the byproduct of bio-oil manufacture, biochar, a recalcitrant black carbon functionally similar to charcoal (Bruckman, et al., 2016; Coleman et al., 2010). Biochar has been recommended for application to agricultural and forest soils to improve soil quality, enhance soil organic matter content, and sequester carbon (Page-Dumroese et al., 2009). Whereas many states have adopted biomass harvest guidelines, field sites where this research was conducted are located in Idaho where guidelines have yet to be established.

Woody biomass harvest guidelines include the retention of downed, dead woody materials that are substrates for fungi with critical roles in healthy forest ecosystems (Stokland et al., 2012). White and brown rot fungi are the only organisms capable of substantial and efficient

decay of wood's recalcitrant lignocellulosic structure (Hammel, 1997; Rayner and Boddy, 1988), making resources available for other functional groups (Rayner and Boddy, 1988; Stokland et al., 2012). Wood decay by fungi provides persistent soil organic matter, recycles nutrients, and is the basis of the food web of microbes, invertebrates, small mammals, amphibians, birds, and larger vertebrates (Ferris and Humphrey, 1999; Lonsdale et al., 2008; McComb and Lindenmayer, 1999). Soils can harbor diverse and abundant communities of fungi, including those that migrate to and utilize above-ground dead wood resources; some fungi can additionally share those nutrient resources in a mutualistic relationship with plants and other tree species (Baldrian et al., 2012; Boddy et al., 2008; Cooke and Rayner, 1984).

Prior to the development of molecular methods, studies of fungal diversity and community composition were limited to identification of fruiting bodies or strains cultured on laboratory media; however not all fungi produce visible, persistent fruiting bodies or can be grown in culture (Anderson and Cairney, 2004; Peay et al., 2008; Smith and Onions, 1994). Today, the standard molecular approach used for environmental fungal identifications and phylogenetic studies targets the nuclear ribosomal RNA gene cluster (Bridge and Spooner, 2001; Brundrett, 2002; Bruns et al., 1991; Gardes and Bruns, 1993; Horton and Bruns, 2001; Peay et al., 2008). The Internal Transcribed Spacer (ITS) regions within the RNA gene cluster have been identified as the universal DNA barcode marker for fungi (Ihrmark et al., 2012; Schoch et al., 2012). Parts of the ITS region are extremely conserved and Polymerase Chain Reaction (PCR) amplifications of the ITS region are highly reliable even with small amounts of DNA (Gardes and Bruns, 1993; Peay et al., 2008). There are many published ITS sequences available online for comparison (Peay et al., 2008; Schoch et al., 2012), and most importantly, fungal specific PCR primers have the ability to preferentially amplify fungal sequences in mixed samples of

plant, animal, prokaryotic, and fungal DNA (Berbee and Taylor, 2001). This research targeted the ITS2 region of the ribosomal RNA genes.

High-throughput amplicon sequencing (HTAS), sometimes referred to as next generation sequencing (NGS), has revolutionized ecological, population, and conservation genetic studies by generating vast amounts of sequence data from non-model organisms in a cost-effective and timely manner (Ekblom and Galindo, 2011). In a 4-hour run, the Life Technologies Ion Personal Genome Machine (PGM) can generate between 60 megabases (Mb) and 2 gigabases (Gb) of sequence data (Life Technologies, 2014), as opposed to 50,000 base pairs generated using traditional Sanger sequencing technologies (Peay et al., 2008). Therefore, studies that incorporate HTAS sequencing technologies will continue to dominate in fields such as microbial ecology.

Studies of fungal biodiversity and community composition in the intermountain west are rare and much remains unknown regarding the role of fungi in dry forest ecosystems. The current lack of information pertaining to wood-inhabiting and soil-forming fungi in western North America represents a significant knowledge gap that will undoubtedly limit the effectiveness of any biomass harvesting guidelines that do not fully consider the predominance and significance of fungi to the sustainability of these ecosystems. In this study, we utilized a standardized wood stake decomposition protocol that has been employed in various forest ecosystems (Finer, et al., 2016; Jurgensen et al., 2006). This was combined with HTAS to produce results that contribute to the global database of fungal diversity and community composition in wood. Our objective was to determine the presence of wood-stake colonizing fungi with DNA sequence analyses, in order to test the null hypotheses that forest biomass management practices do not influence wood-stake colonizing fungal diversity and community composition.

2.2 Material and methods

2.2.1. *Field study location and design*

Research was conducted at the University of Idaho – Experimental Forest (UIEF, 46.849512, -116.845068). The elevation at this site ranges from 830-890 meters above sea level, with a 0-15% slope and south-facing aspect. The mean annual temperature was 7.8 ºC (measured between 1981 - 2010, PRISM Climate Group). The stand was previously logged, exposed to periodic burning, and occupied by mixed conifer species grown atop silt-loam soils with a significant (>30 cm) Andisol layer of volcanic ash (McDaniel and Hipple, 2010). The UIEF site may be classified as a grand fir/ninebark habitat type (Cooper et al., 1991) and is dominated by ponderosa pine (*Pinus ponderosa* Dougl. ex Laws), grand fir [*Abies grandis* (Dougl. ex D. Don) Lindl.], Douglas-fir [*Pseudotsuga menziesii* (Mirbel) Franco], lodgepole pine (*Pinus contorta* Dougl. ex Loud.), and western larch (*Larix occidentalis* Nutt). UIEF has been used for cattle grazing, recreation, research, and managed for commercial forestry. This study is part of a larger study designed to assess the effects of forest biomass harvesting practices with regard to whole ecosystem sustainability; therefore, other potentially important responses were quantified, including post-treatment stand composition (Sherman et al., 2017), as well as soil metrics (Dumroese et al., unpublished).

In 2013, (400-m^2) study plots were established with biomass treatments at four levels and soil amendments at four levels. Treatment combinations (4x4) were randomly assigned and replicated twice, to yield 32 plots. Experimental sites were named UIEF_ Ponderosa Pine (UIPP), and UIEF_Mixed Conifer (UIMC). Biomass treatment plots were established, including

non-thinned control and manipulated plots that were thinned from below (i.e. cutting the small trees including those that are either undesirable species, suppressed, having poor form, or are visibly diseased) to a relative basal area density of 40%. Retention of the cut biomass resulting from thinning was manipulated. Biomass treatments in these thinned test plots included removal of all cut material (0X), retention of this cut material (1X), and doubling of the amount of this cut material (2X), compared to the non-thinned control (non-thinned). To create 2X plots, material that was cut during thinning was removed from 0X plots and scattered over the 2X plots. Soil amendments consisted of fertilizer, biochar (2 Mg ha^{-1}), fertilizer+biochar, and a non-amended control. Urea fertilizer (46-0-0) was applied at a rate of 224 kg N ha^{-1}. Fertilizer treatments were applied to balance tree nutrient requirements because nitrogen is considered the main limiting nutrient in western forest soils (Coleman et al., 2014). The biochar was produced from Western mixed conifer feedstock in a steam boiler at 980 ℃; it consists of 25.7% C, 28.6% ash, and has a surface area of 201 m^2 g^{-1}, with an average pH of 7.8.

2.2.2. *Soil and forest metrics*

Post-treatment soil surveys were conducted in 2017 and provided by Dumroese et al. (unpublished). Forest soils were assessed at the organic forest floor horizon and within the mineral soils. Each forest floor sample consisted of all organic matter down to the mineral soil that was collected within a 30 cm diameter circle. Three forest floor samples were taken per plot, therefore forest floor metrics (% OM, pH, % C and % N) are the average of three samples. The mineral soil analyses were conducted on the mineral soil, after the forest floor layers had been removed. Mineral soil sample cores were obtained from two depths (0-10 cm and 10-20 cm), and

the mineral soil metrics (soil dry weight, rock weight, root weight, % OM, pH, % C and % N) are the average of three samples per plot.

Forest metrics were provided by Sherman et al. (2017), including stand basal area (m^2 ha^{-1}), tree density (trees ha^{-1}), quadratic mean diameter (cm), standard density index (trees ha^{-1}), Curtis's relative density, and coarse woody debris (tons $hectare^{-1}$). Whereas BA of a tree is defined as the cross-sectional area at breast height (Wenger, 1984), total stand BA is related to stand volume and represents a measurement of stand density (Husch et al., 2003). The standard density index by Reineke (1933), based on the relationship between number of trees per acre and their average diameter, was developed for assessing even-aged forests and is directly related to the potential response to release when thinned.

2.2.3. *Wood stake decomposition and colonization*

Using protocols described by Jurgensen et al. (2006), loblolly pine (*Pinus taeda* L.) and aspen (*Populus tremuloides* Michx.) stakes (2.5 x 2.5 x 30 cm) were inserted vertically to a depth of 30 cm in the mineral soil of each treatment plot. Additional pine and aspen stakes (2.5 x 2.5 x 15 cm) were affixed on the surface of the litter layer with large metal staples. Installation occurred in May of 2014. After 1 year, and continuing every year for 3 years, five aspen and five pine stakes were removed from both the mineral soil and litter layer location of each plot in the spring.

Fungi that colonized the wood stakes were identified using DNA-based methods described by Lindner et al. (2011). Wood shavings from which fungal DNA was later extracted were obtained within 24 hours of stake collection. Shavings were collected using DNA-sterile, 4-

mm-diameter drill bits that were inserted to an approximate depth of 1 cm at two points on the stakes. The points were approximately 1 cm from each cut end of the stake. Because the stakes removed from the mineral soil had been inserted vertically, the drilled shavings from the two points were collected separately, with different sterile drill bits, and labeled "mineral_top" (referring to the portion of the stake closest to the soil surface) and "mineral_bottom" (referring to the portion of the stake that was deepest in the mineral soil). With no difference in depth, drilled shavings from the two points of each surface stake were combined, using the same sterilized drill bit for each end. All shavings were collected in 2 mL strip tubes in 96-well format, covered with filter-sterilized cell lysis solution (CLS) (Lindner et al., 2011) and frozen at -20C until DNA was extracted.

Because the five aspen and five pine stake replicates within each treatment plot represents sub-sampling (i.e. not independent), the cell lysis solution (CLS) containing wood shavings from the aspen stakes (and pine stakes, respectively) was pooled prior to extraction (Appendix 2). Pooled wood shaving samples were created by pooling all 5 wood stakes for a given year, within each treatment, from a specific stake location (surface, mineral_top, mineral_bottom).

HTAS was performed on an Ion Torrent PGM following manufacturer's instructions and Lindahl (2013). DNA was extracted and cleaned from the wood stake shavings using GeneClean III kits (Qbiogene), which were previously found to be reliable and cost effective for the recovery of high quality genomic fungal DNA from wood samples (Lindner et al., 2011). Tagged and barcoded primers fITS7 (Ihrmark et al., 2012) and ITS4 were used for the PCR reaction. Following DNA amplification, Zymo Research's Select-A-Size DNA Clean & Concentrator spin columns were used to clean the resulting PCR product. Equimolar proportions of DNA were

templated in an emulsion PCR step where the product was attached and amplified onto the Ion Sphere Particles (ISPs). Sequencing of the cleaned, template positive ISPs was performed using the Ion PGM Hi-Q sequencing kit according to manufacturer's recommendations.

Sequence reads obtained from the PGM were trimmed, filtered, clustered, and assigned to operational taxonomic units (OTUs) with USEARCH and VSEARCH, facilitated by the AMPtk program (Palmer, 2016). Taxa were assigned using UTAX Classifier and USEARCH global, also scripted through AMPtk (Palmer, 2016). Sequence reads were archived with the National Center for Biotechnology Information, accession SRP154885.

2.2.4. *Fungal diversity and community composition analysis*

Effects of site, biomass treatments, and soil amendments on fungal species diversity were evaluated by year, stake species (aspen and pine), and stake location on or in the soil (surface, mineral_top, and mineral_bottom). Richness (number of different taxa that occurred) was used as the measure of diversity (Magurran, 1988; Zak and Willig, 2004). Fungal OTU richness was assessed relative to year, site, biomass harvesting treatments, and soil amendment additions through analysis of variance (ANOVA). Significant differences were analyzed with Tukey's Honest Significant Difference (HSD) test.

Fungal richness indices from the 2017 datasets were tested for use as response variables for correlation and regression analyses. Data normality was checked visually with scatter plots, quantile-quantile (Q-Q) plots and Shapiro-Wilk's test (Bock et al., 2007; Dean and Voss, 1999). Regression analyses were used to assess the predictive power of richness indices from a set of continuous explanatory variables, namely post-treatment soil and forest metrics assessed on these

sites in 2017. Given the three layers of soil analyses, (1) the forest floor samples were assessed relative to the fungi within surface stakes; (2) the 0-10 cm mineral soil samples were assessed relative to the fungi within mineral_top stakes; and (3) the 10-20 cm mineral soil samples were assessed relative to the fungi within mineral_bottom stakes.

Fungal community composition analyses of the wood stake data were conducted using R Statistical Software (R Core Team, 2015), the Vegan package (Oksanen et al., 2012) and phyloseq (McMurdie and Holmes, 2013). To assess the effects of biomass treatments (0X, 1X, 2X biomass retained, non-thinned control) and compensatory amendments (fertilizer, biochar, fertilizer+biochar, non-amended control) on the fungal community composition, a nonparametric permutational multivariate ANOVA (PERMANOVA) test (Anderson, 2001) based on Raup-Crick dissimilarity matrices was performed by the adonis function in the Vegan package of R. The multivariate PERMANOVA test was designed for assessment of multivariate data in response to complex experimental designs, with no assumptions of multivariate normality (Anderson, 2017). This type of analysis is common within ecological studies (Rajala et al. 2012), and is especially useful for analyzing community ecology data within the framework of experimental designs that were established with other priorities. The PERMANOVA test examined the OTUs within the whole community composition – assessing where OTUs overlapped and differed – to determine significant categorical treatment effects that could not be found using richness values alone, and has been used previously to assess the effects of different forest management practices on wood-inhabiting fungal community compositions (Hartmann et al., 2012; Purahong et al., 2014). To ensure significant PERMANOVA results are not due to differences in dispersion, the Betadisper function was used to test for multivariate homogeneity of group dispersions or variation between groups (Anderson et al., 2006). Additionally, the

fungal community composition in 2017 was assessed relative to the measured soil metrics from these plots using the same PERMANOVA test. Nonparametric multidimensional scaling (NMDS) was performed with the Vegan package of R, using the metaMDS function (Oksanen et al., 2018) to visualize the fungal communities in ordination space. Of over 60 possible measurements of similarity or dissimilarity described, the Raup-Crick distance measure was chosen for its efficacy in community assemblage studies with presence/absence data (Anderson, 2001; Chase et al., 2011; Gardener, 2014; Raup and Crick, 1979).

2.3 Results

2.3.1. *Fungal OTUs*

Wood shavings from stakes from all plots (n = 32) at the University of Idaho Experimental Forest were analyzed over 3 years, yielding a large and robust dataset (n = 559 pooled wood shaving samples). Over 2,000 different OTUs were identified through HTAS. Over 50% of identified taxa were assigned to phylum Ascomycota, with ~33% to phylum Basidiomycota. The order of the most abundant identified taxa was *Helotiales* (saprobic/parasitic fungi) at 16%, followed by *Agaricales* (gilled mushrooms) at ~6% of the total. Although many OTUs were representatives of saprobic genera and species, approximately ¼ of the OTUs could not be assigned to the level of order, and about ¾ of the OTUs could not be assigned to a particular species. The identity of the 20 most common OTUs is reported (Table 2.1).

2.3.2. *Wood stake species*

The species of stake (aspen or pine) had a significant effect on fungal OTU richness and fungal community composition. Fungal OTU richness differed significantly between aspen and pine stakes (Fig. 2.1), with mean OTU richness of about 54 for aspen stakes and 80 for pine stakes (ANOVA: $F_{1,557} = 107.4$, $p < 0.01$). PERMANOVA testing of the fungal community composition substantiated the differences noted between aspen and pine stakes ($F.model_{1,557} = 13.2$, $p < 0.01$). Ordination showed that the fungal communities present on pine rarely overlap with the communities present on aspen wood (Fig. 2.2); therefore, the different wood stake species promoted different fungal communities in addition to significant differences in species richness.

2.3.3. *Wood stake location*

The location of wood stake (surface, mineral_top, mineral_bottom) yielded significant differences in fungal OTU richness (Fig. 2.1, ANOVA: $F_{2,556} = 34.16$, $p < 0.01$). Tukey HSD post-hoc analysis of fungal OTU richness indicated significant differences among the surface and both the mineral_top and mineral_bottom locations; whereas the mean number of OTUs of surface stakes was about 82, the number of OTUs from the mineral stakes (top and bottom) were about 23 OTUs less (p.adj. < 0.001). There was no significant difference in mean OTU richness between the mineral_top and mineral_bottom portions of the stakes (p.adj. $= 0.98$). The fungal community composition was also influenced by stake location (PERMANOVA: $F.model_{2,556} = 564.67$, $p < 0.01$), and ordination of fungal community composition by stake location yielded

clear separation between the surface communities and the mineral soil communities (Fig. 2.3). Visually, the fungal communities present at the surface have greater separation in ordination space from the communities present within the mineral soil, whereas there is overlap in the fungal community compositions within the mineral_top and mineral_bottom stake locations.

2.3.4. *Year and site*

The year of wood stake collection significantly influenced the fungal community composition (PERMANOVA $F.model_{2,556} = 28.24$, $p < 0.01$), with no significant variation of group dispersions. Mean richness among years varied only slightly (from 71 mean OTUs in 2015 to 63 mean OTUs in 2017) (Fig. 2.1), and analysis of variance found no significant difference in OTU richness (ANOVA: $F_{2,556} = 2.373$, $p = 0.09$). Upon ordination, there is considerable overlap of the fungal community compositions by year (Fig. 2.4). Because year, species of wood stake, and location of wood stake within the soil were not randomly assigned, the data were separated by year, species of stake, and stake location for further analyses.

Due to site differences, the analyses were constrained by site. Even with blocking, site was a significant factor affecting the fungal community composition within surface stakes, the mineral_top pine stakes, and the mineral_bottom aspen stakes (PERMANOVA, $p < 0.05$), though no significant difference in OTU richness by site was observed for either aspen or pine stakes.

2.3.5. *Biomass treatment*

Surface Stakes

Biomass treatments significantly influenced the fungal communities present within surface stakes every year of data collection (Table 2.2, PERMANOVA, $p < 0.05$). Within the first year after the treatment plots were established (2015), biomass treatment had significant effects on the fungal community composition within both pine and aspen surface stakes (Table 2.2, PERMANOVA: $p < 0.01$), with no significant differences in dispersion among groups. Fungal OTU richness of pine and aspen stakes were assessed relative to biomass treatment, though no significant effects on richness were found (ANOVA: $p > 0.1$). The mean fungal OTU richness of pine surface stakes varied only slightly between 106 and 110 OTUs for all biomass treatments. Within the aspen surface stakes, the fungal OTU richness was highest for 0X and 1X, with mean OTU richness values of about 84; whereas mean OTU richness for 2X and non-thinned plots was about 71.

By the second year (2016), biomass treatment had a significant influence on the fungal community composition within surface stakes (Table 2.2, PERMANOVA: $p < 0.05$), though no significant differences in fungal OTU richness were found relative to biomass treatment. Within the surface pine stakes, there was a significant difference in fungal community composition dispersion between biomass treatment groups (Betadisper: $F_{3,28} = 9.55$, $p < 0.001$). The dispersion of 2X treatments were significantly different from 0X (Tukey HSD, p.adj. = 0.026); non-thinned treatments were significantly different from 0X (p.adj. = 0.026); 2X treatments were significantly different from 1X (p.adj. = 0.001); and non-thinned treatments were significantly

different from 1X (*p*.adj. = 0.001). The 1X treatments were not significantly different from the 0X treatments, and the non-thinned treatments were not significantly different from 2X treatments ($p > 0.05$). Surface aspen stakes did not have significant differences in community dispersion among treatments.

Three years after sites were established (2017), only surface stakes had significant differences in fungal community composition due to biomass treatments (Table 2.2, PERMANOVA: $p < 0.05$), with no significant differences in mean fungal OTU richness. Within surface stakes, mean OTU richness values for all biomass treatments varied between 55-61 OTUs for aspen stakes. Whereas in past years the 2X biomass treatment had some of the lowest OTU richness values, the 2X biomass treatment in 2017 had the highest mean fungal OTU richness at 84, followed by 0X, non-thinned, and 1X treatments having 83, 76, and 74 OTUs, respectively.

Mineral Stakes

Only in the second year (2016) was biomass treatment a significant factor influencing the fungal community composition of the mineral stakes (Table 2.2, PERMANOVA, $p < 0.05$). Within the mineral_top of the pine stakes, the mean fungal OTU richness for non-thinned plots was 90, whereas the other biomass treatments plots had a mean OTU richness less than 75. Within the mineral_bottom of the pine stakes, the mean fungal OTU richness was highest for 2X plots at 74; with 0X, 1X and non-thinned plots having mean OTU richness values of 62- 68. Similarly, within the mineral_bottom of the aspen stakes, the mean fungal OTU richness was highest for 2X plots at 49; with 0X, 1X, and non-thinned plots having mean OTU richness values

of 34 - 45. Analyses of the fungal communities at the bottom of the aspen mineral stake found the only significant interaction between the site and biomass treatment (PERMANOVA: F.model$_{3,17}$ = 3.11, $p = 0.049$). Within this dataset, the lowest OTU richness was found at UIPP - 1X sites with 11 and 12 OTUs found within individual treatment plots. The highest OTU richness was found at UIMC - 2X sites, with >60 OTUs within individual treatment plots.

2.3.6. *Soil Amendment*

One year after plot establishments (2015), soil amendment had significant effects on the fungal community composition within both pine and aspen surface stakes (Table 2.2, PERMANOVA: $p < 0.01$), with no significant differences in dispersion between groups. Within the surface aspen stakes, the type of soil amendment did significantly influence the fungal OTU richness (ANOVA: $F_{3,22} = 3.22$, $p = 0.042$). A Tukey HSD post-hoc analysis of fungal OTU richness found non-amended sites to be almost significantly different from the fertilized sites, where mean OTU richness of the non-amended plots was around 90, the fertilized plots had about 37 fewer OTUs (*p*.adj = 0.058). Similarly, the fertilizer+biochar plots had about 34 less OTUs than the non-amended plots (*p*.adj. = 0.075). There were no significant differences in mean OTU richness between the other soil amendment treatments. Fungal OTU richness was not influenced by soil amendments within the surface pine stakes (ANOVA: $p > 0.1$),

Two years later, by 2016, soil amendments significantly influenced the fungal community composition of aspen stakes (Table 2.2, PERMANOVA: $p < 0.05$). There were no significant differences in fungal OTU richness relative to the soil amendments within the surface_aspen stakes, though the non-amended control plots had the highest mean OTU richness

at 83 (with biochar, fertilizer+biochar, and fertilizer plots having 75, 68, and 78 OTUs, respectively). PERMANOVA testing of the effects of soil amendment on the fungal communities within the surface aspen stakes did find a significant difference in fungal community composition ($F.model_{3,23} = 5.85$, $p = 0.026$), with no significant differences in dispersion.

By 2017, both pine and aspen surface stakes had significant differences in fungal community composition due to soil amendments (Table 2.2, PERMANOVA: $p < 0.05$), with no significant differences in mean fungal OTU richness. Mean richness values within the surface aspen stakes were similar for all soil amendment treatments (biochar, non-amended, fertilizer+biochar, fertilizer, at 72, 56, 61, and 57 OTUs, respectively). Interestingly, whereas fertilizer treatments had decreased fungal OTU richness in the previous years, in 2017, the fertilized treatments had the highest mean fungal OTU richness at 85 (followed by biochar, non-amended, and fertilizer+biochar treatments having 81, 78, and 76 OTUs, respectively).

2.3.7. *Soil and forest metrics*

Because 2017 was the final year of wood stake collection, soil and forest metrics were collected. Within the surface aspen stakes, the fungal community composition was significantly influenced by pH (Table 2.3, PERMANOVA, $F.model_{1,16} = 3.93$, $p = 0.047$), % Carbon ($F.model_{1,16} = 5.19$, $p = 0.026$), and % Nitrogen ($F.model_{1,16} = 7.19$, $p = 0.013$). Within the surface pine stakes, the fungal community composition was significantly influenced by pH (PERMANOVA, $F.model_{1,14} = 5.43$, $p = 0.029$) and % Nitrogen ($F.model_{1,14} = 8.12$, $p = 0.018$). The forest metrics did not significantly influence the fungal community composition, richness, or

variance within either the surface pine or surface aspen stakes. Forest and soil metrics did not significantly influence fungal community composition or the fungal OTU richness within the mineral stakes of pine or aspen.

2.4 Discussion

2.4.1. *Biomass treatments*

Within this limited study, we did not find an overall negative effect of biomass removal. The significant differences in fungal community composition and fungal OTU richness due to biomass treatment were less evident over time. Similar results were obtained in a thinning experiment in a German pine stand conducted by Maassen et al. (2006). Microbial biomass, basal respiration, and enzyme activities in the soil were found to not differ significantly between thinned and non-thinned treatments after 5 years. Maassen et al. (2006) observed changes in the microbial structure through phospholipid fatty acid (PLFA) assessments of prokaryotes and eukaryotes. Hartman et al. (2012) did notice effects of whole-tree timber harvesting on fungal community composition in soils 10 years after harvesting in a Canadian forest (through DNA-based fungal assessments), with organic matter removal and soil compaction as the driving factors influencing their analyses. Soil compaction was less of an issue on our plots, as thinning and biomass manipulation were conducted by crews on foot, dragging the debris with pulleys and ropes. The nature of biomass treatment effects on the fungal community composition changed over time. Initially the 2X treatment stakes had less fungal colonization in early years

compared with the non-thinned treatments; by the third year, the 2X treatment plots did foster suitable habitat for a richer diversity of decomposition fungi.

2.4.2. *Soil amendments*

Soil amendments had a significant effect on fungal community composition, though the results are complicated. The wood stakes within fertilized plots yielded significantly lower fungal OTU richness within the first two years, but after 3 years, fertilized plots yielded higher OTU richness within surface pine stakes. The significant soil metrics associated with variance in fungal community composition were pH, %N, and % C, and those metrics were likely influenced by the biomass treatments (as noted above) and the soil amendments added within treatment plots. Not only does fertilization change the carbon to nitrogen balance within the soil environment, urea-based fertilizers have been shown to increase the measured pH of aqueous solutions (Bull et al., 1964). The significance of pH is also related to fungal succession through wood substrates, as fungi are known to change the pH of wood substrates during decomposition (Rayner and Boddy, 1988).

Fertilization has already been shown to decrease ectomycorrhization of roots and species richness of ectomycorrhizal fungi (Brunner, 2004; Morrison et al., 2016). The importance of ectomycorrhizal (EM) fungi in forest ecosystems can be highlighted with the fact that Douglas-fir is estimated to associate with 2,000 species of EM fungi, and about 72% of those fungi can associate with multiple plant hosts (Horton et al., 2005). Identification of known ectomycorrhizal fungi was limited in this study by the comparative lack of sequence data for ectomycorrhizal species that produce fruiting bodies on wood.

Whereas nitrogen may be a limiting factor to tree growth, it's influence on wood decay and decomposition is complicated (Fog, 1988). Fertilization, in the form of ammonium nitrate, has been found to increase the relative abundance of saprotrophs (Morrison et al., 2016), altering the community composition of fungi and decreasing relative abundance of lignolytic fungi, or the fungi that can decompose lignin (Entwistle et al., 2018). Entry et al. (1991) found second-growth stands of Douglas-fir had higher incidence of infection by the root pathogen *Armillaria ostoyae* (Romagn.) Herink when thinned and fertilized with urea (360 kg N $hectare^{-1}$), compared with stands that were just thinned. The fertilization rate in this study was 224 kg N ha^{-1}. Regardless, fertilization continues to be a regular practice, and fertilization on these specific plots resulted in a significant increase in basal area growth and total volume growth of plot trees (Sherman et al., 2017).

Adding biochar as a soil amendment did not significantly influence the fungal community composition or indices of fungal diversity. This result is in accordance with Noyce et al. (2015), where the authors assessed soil chemical properties and the molecular microbial community composition over 2 years following a 5 Mg ha^{-1} biochar treatment in a mixed-deciduous forest in Ontario. They found no significant difference in pH, organic C, organic N, or microbial biomass due to biochar additions and concluded that biochar additions at 5 Mg ha^{-1} were neither beneficial nor toxic to soil microbes (Noyce et al., 2015). The biochar treatment rate in this study was 2 Mg ha^{-1}. Additionally, biochar applications have been found to increase soil pH within agricultural and forest soils, which Li et al. (2018) postulates could be due to the alkaline nature of the biochar itself, as well as by binding free Al^{3+} ions to the char surface, increasing the abundance of soil exchangeable base cations, which increases soil base saturation, raising pH. With significant differences in biochar and the ecosystems in which it has been applied (e.g. agricultural fields vs.

forest plots), Li et al. (2018) concluded that there is no consensus regarding the effects of biochar on microbial biomass or fungal abundance and diversity.

2.4.3. *Effect of wood species and stake location relative to the forest floor*

In this study, differences in fungal community composition and fungal OTU richness were significantly associated with the different species of wood stake deployed into or on top of the soil. Rajala et al. (2012) investigated the fungal communities inhabiting logs in a boreal forest in southern Finland using denaturing gradient gel electrophoresis fingerprinting and sequencing (DGGE) (Rajala et al., 2012). Wood from different tree species were found to harbor significantly different fungal communities in a mixed conifer-deciduous forest environment (Rajala et al., 2012). Eighteen species of wood-inhabiting fungi were found within pine logs, and 13 species of fungi were found within aspen logs (Rajala et al., 2012). In contrast, use of HTAS allowed identification of a much greater number of OTUs (mean fungal OTU richness was 54 in aspen stakes and 80 in pine stakes), even though the total volume of aspen and pine stakes used in the current study was much less than that of the logs sampled in Rajala et al. The difference in fungal species present within the pine wood could be due to the fact that aspen wood is not a naturally occurring substrate within these plots at the UIEF. In a standardized wood stake decomposition study conducted in a mixed conifer-deciduous forest in Finland, Finer et al. (2016) found aspen stakes decomposed faster than pine stakes within the first few years after wood stake deployment. Aspen wood has higher concentrations of carbohydrates and nitrogen, with lower lignin concentrations than coniferous wood (Finer, et al., 2016; Strukelj et al., 2013). Strukelj et al. (2013) found naturally lower lignin concentrations in deciduous tree

species facilitated faster decomposition to very low wood densities, whereas increased lignin and alkyl carbon compounds within the conifer stakes led to slower decomposition over a greater period of time. In this study the pine stakes had greater richness than the aspen stakes, a result that is consistent with Strukelj et al. (2013), where the authors postulated that such differences are due to increased stable carbon compounds and lignin concentrations in the decomposing coniferous wood, promoting increased decomposer diversity and carbon retention.

Stake location (within the mineral soil or on the litter surface) strongly influenced the fungal OTU richness and community composition results, reflecting differences in fungi present in different layers of the forest floor and soil. This is in agreement with other studies that found microbial communities in the forest soil are largely different among the litter layer, organic layer, and within the mineral soils and are highly stratified during decomposition (Baldrian et al., 2012; Lindahl et al., 2007; O'Brien et al., 2005). The litter layer of soil contains detritus and decomposing organic matter (in a heterogeneous mix of substrates, nutrients, moisture, and temperatures), and other studies identified saprophytic species predominating in the litter layer (Baldrian et al., 2012; Lindahl et al., 2007; O'Brien et al., 2005). In the current study, the surface stakes exhibited the greatest species richness, which is consistent with previous reports that the litter horizon had higher fungal diversity (Baldrian et al., 2012; O'Brien et al., 2005). After sampling soils within a coniferous forest in central Europe, Baldrian et al. (2012) showed that the litter layer contains a higher proportion of microorganisms expressing exocellulase sequences when compared to the more homogeneous organic horizon, with the deepest soil horizon samples exhibiting the lowest fungal richness. Since the majority of the slash and organic materials are within the litter horizon, it makes sense that the microbes emitting enzymes to degrade lignocellulosic materials would be significantly more abundant in this layer.

2.4.4. *Fungal communities and OTU analyses*

Results of this study are consistent with other HTAS studies of fungal communities from coniferous forests, in which a large proportion of the sequences were not identifiable to the species level (Baldrian et al., 2012; Lindahl et al., 2007; O'Brien et al., 2005). OTU identification was limited by the general lack of published sequences of known and described taxa in sequence repositories. Lack of well-characterized sequences in public databases stems from the lack of trained taxonomists available to properly describe and submit taxonomic data with the submitted sequences, as well as the microscopic and sometimes non-culturable nature of these organisms. The dominance of OTUs in the phylum *Ascomycota* is also in accordance with others who have sampled wood substrates (Baldrian et al., 2012; Hartman et al., 2012; Lindahl et al., 2007; O'Brien et al., 2005).

The OTUs assigned species names tended to be taxa that have been researched more extensively, usually conspicuous fungi that are easily observed in the field or are known pathogens and ubiquitous decomposers. For example, *Sydowia polyspora* (Bref. & Tavel) E. Mull. was frequently observed in the current study and is a wound pathogen associated with needle necrosis on true fir species (Talgo et al., 2010), and this organism illustrates the complex roles fungi play in the environment with other organisms. On pines, *S. polyspora* has been associated with a gall midge (*Cecidomyia*) that burrows at the base of pine needles. On Douglas-fir, this pathogen invades the carcasses of aphids (*Aphidoidea)*, using them as a food base before penetrating the stomata of the needles (Peace, 1962).

Analysis of variance (ANOVA) of OTU richness did identify striking differences among some treatments. With large fungal community datasets, like this study with over 500

individually sequenced samples comprising over 2,000 OTUs, it is difficult to be aware of all changes in OTU composition. Observed OTU richness values allowed the communities to be analyzed in a simplistic manner, with common statistical tools (Dean and Voss, 1999). However, species richness indices can only indicate the number of taxa present, and it is already known that species that use downed woody debris change as decay progresses; therefore, due to this succession, species composition may vary while richness indices remain the same (Bunnell and Houde, 2010). Additionally, fungal species richness indices do not always follow normal distributions, and assumptions of the statistical tests cannot always be met.

2.4.5. *Forest management practices*

Management at the University of Idaho Experimental Forest includes use of commercial forest management practices. Thinning with removal of material was the recommended Silviculture and Best Management Practice (BMP) for similarly overstocked sites in Washington and Oregon (Powell, 1999). Sherman et al. (2017) concluded that biomass removal at these sites had no significant effect on plot tree growth compared to normal biomass retention; however tree growth was less in high biomass retention plots (2X) compared to normal biomass retention (1X) plots after 3 years.

While the increased carbon to nitrogen (C:N) ratios of the additional slash on the soil surface has been shown to promote saprotrophic fungal communities (Lindahl et al., 2007), the removal of the trees also influenced the fungal community associated with the living trees. Results of this study complement the findings of others (Kohout et al., 2017; Rajala et al., 2012) who concluded that fungal communities are dynamic, successive, and can be niche specific.

After biomass plot initiation, the fungal communities responded to the disturbance and additional lignocellulosic materials available, with 0X and non-thinned plots exhibiting more OTU richness than others. As decomposition increased, C:N ratios decreased due to respiratory removal of C in combination with retention of N within the litter and the associative microbial community (Lindahl et al., 2007). Initially, less free nitrogen is available in the soil environment because it is tied up in microbial biomass; however late successional mycorrhizal fungi found deeper within the mineral soils eventually increase C:N ratios as organic matter ages, by selective removal and transfer of N from the soil through mycorrhizal fungi to their host plants (Hogberg et al., 1999). Therefore, it should not be surprising that soil metrics of % C and % N were found to be significantly related to variance in fungal community composition throughout time.

The current study has demonstrated a linkage between forest management practices and fungal biodiversity. Silviculture treatments in the western United States include thinning to supress pathogens, reduce competition, and to remediate past fire-supressing management practices (Arno, 2000; Cooper 1991; Keane, 2002). Above-ground forest community dynamics and management practices influence the below-ground fungal community composition, which can influence feedback mechanisms that can be measured above-ground. For example, decay of wood that leads to suitable habitat for mycorrhizal species enables greater nutrient and water uptake by plants that can be measured in tree growth (Rayner and Boddy, 1988). Therefore, this research emphasizes the importance of collaboration of scientists with forest managers to make informed decisions about sustainable forestry and biodiversity. The strength of fungal biodiversity assessments to indicate effects of silvicultural treatments is underscored. The sequenced fungal OTU community was remarkably more diverse and sensitive to treatments than the trees, understory plants, or insects (Sherman et al., 2017). Soil quality indicators are

measurements that can represent the conditions of the system and are sensitive to change. Therefore, as sequencing costs continue to fall, fungal OTU assays should be considered as important soil quality indicators because they can provide important information regarding soil biodiversity, cycling of carbon and other nutrients, in addition to identifying microbial indicators of soil quality (Doran and Parkin, 1994). By sequencing a greater range of wood substrates on the plots, fungal taxa representing all stages of wood decomposition and plant mycorrhizal associations would present the most complete indicators of soil quality.

2.5 References

Anderson, I.C., Cairney, J.W.G., 2004. Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. Environ. Microbiol. 6, 769–79.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46.

Anderson, M.J., 2017. Permutational Multivariate Analysis of Variance (PERMANOVA). Wiley Statistics Reference Online, 1. John Wiley & Sons, Ltd. DOI: 10.1002/9781118445112.stat0784.

Anderson, M.J., Ellingsen, K.E., McArdle, B.H., 2006. Multivariate dispersion as a measure of beta diversity. Ecol. Lett. 9, 683–93.

Arno, S.F., 2000. Fire in Western Forest Ecosystems, in: Brown, J.K., Smith, J.K. (Eds.), Wildland Fire in Ecosystems: Effects of Fire on Flora. USDA Forest Service Gen. Tech.Rep. RMRS-FTR-42-Vol. 2, 97–120.

Bader, P., Jansson, S., Jonsson, B.G., 1995. Wood-inhabiting fungi and substratum decline in selectively logged boreal spruce forests. Biol. Conserv. 72, 355–362.

Baldrian, P., Kolarik, M., Stursova, M., Kopecky, J., Valaskova, V., Vetrovsky, T., Zifcakova, L., Snajdr, J., Ridl, J., Vlcek, C., Voriskova, J., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. International Society for Microbial Ecology (6) 248-258.

Berbee, M.L., Taylor, J.W., 2001. Fungal Molecular Evolution: Gene Trees and Geologic Time, in: Esser, K., Lemke, P.A. (Eds.), The Mycota: A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research, VII: Systematics and Evolution, Part B. Springer, 229–245.

Bock D.E., Velleman, P.F., deVeaux, R.D., 2007. Stats: Modeling the World, 2nd edition. Pearson Education Inc., Boston, MA.

Boddy, L., Frankland, J.C., vanWest, P., 2008. Ecology of Saprotrophic Basidiomycetes. The British Mycological Society, Elsevier Ltd.

Bridge, P., Spooner, B., 2001. Soil fungi : diversity and detection. Plant Soil 232, 147–154.

Bruckman, V.J., Apaydin Varol, E., Uzun, B.B., Liu, J., 2016. Biochar: a regional supply chain approach in view of climate change mitigation. Cambridge Univeristy Press.

Bruns, T.D., White, T.J., Taylor, J.W., 1991. Fungal molecular systematics. Annu. Rev. Ecol. Syst. 22, 525–564.

Brunner, I. 2004. Increasing the nitrogen load reduces ectomycorrhization and alters element concentration in Norway spruce seedlings. In, Cripps, CL, ed. Fungi in Forest Ecosystems: Systematics, Diversity and Ecology. The New York Botanical Garden, volume 89, New York. P.235-242.

Bull, H.B., Breese, K., Ferguson, G.L., Swenson, C.A., 1964. The pH of urea solutions. Archives of Biochemistry and Biophysics 104 (2): 297-304.

Bunnell, F.L., Houde, I., 2010. Down wood and biodiversity – implications to forest practices. Environmental Review 18: 397-421.

Cannon, P.F., Kirk, P.M., 2007. Fungal Families of the World. CAB International, Cambridge, MA.

Chase J.M., Kraft, N.J.B., Smith, K.G., Vellend, M., Inouye, B.D., 2011. Using null models to disentangle variation in community dissimilarity from variation in α-diversity. Ecosphere 2(2):article 24, 1-11.

Cooke, M.C., 1876. Mycographia, seu lcones fungorum 1(4) 45-86.

Cooke, R.C., Rayner, A.D.M., 1984. Ecology of saprotrophic fungi. Longman Group Limited, New York.

Cooper, S.V., Neiman, K.E., Roberts, D.W., 1991. Forest habitat types of Northern Idaho: A Second approximation. General Technical Report INT-236. Ogden, UT: U.S. Department of Agriculture, Forest Service. Intermountain Research Station, 1-152.

Dean, A., Voss, D., 1999. Design and Analysis of Experiments. Springer-Verlag, New York, Inc., New York.

Doran, J.W., Parkin, T.B., 1994. Defining and assessing soil quality. In: Doran et al. (eds) Defining Soil Quality for a Sustainable Environment. Soil Science Sosciety of America Special Publication Number 35, Madison, WI, pp. 3-22.

Ekblom, R., Galindo, J., 2011. Applications of next generation sequencing in molecular ecology of non-model organisms. Heredity (Edinb). 107, 1–15.

Entry, J.A., Cromack Jr., K., Kelsey, R.G., Martin, N.E., 1991. Response of Douglas-fir infection by *Armillaria ostoyae* after thinning or thinning plus fertilization. Phytopathology 81: 682-689.

Entwistle, E.M., Zak, D.R., Argiroff, W.A., 2018. Anthropogenic N deposition increases soil C storage by reducing the relative abundance of lignolytic fungi. Ecological Monographs 88(2):1-20.

Fierer, N., Grandy, A.S., Six, J., Paul, E.A., 2009. Searching for unifying principles in soil ecology. Soil Biology and Biochemistry 41: 2249-2256.

Ferris, R., Humphrey, J.W., 1999. A review of potential biodiversity indicators for application in British forests. Forestry 72, 314–328.

Finer, L., Jurgensen, M., Palviainen, M., Piirainen, S., Page-Dumroese, D., 2016. Does clear-cut harvesting accelerate initial wood decomposition? A five-year study with standard wood material. Forest Ecology and Management 372: 10-18.

Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biological Reviews 63: 433-462.

Gardes, M., Bruns, T., 1993. ITS primers with enhanced specificity for basidiomycetes- application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118.

Gardener, M., 2014. Community ecology: analytical methods using R and Excel. Pelagic Publishing, Exeter, UK.

Hammell, K.E., 1997. Fungal Degradation of Lignin, in: Cadisch, G., Giller, K.E. (Eds.), Driven by Nature: Plant Litter Quality and Decomposition. CAB International, Wallingford, 33-45.

Hartman, M., Howes, C.G., VanInsberghe, D., Yu, H., Bachar, D. Christen, R., Nilsson, R.H., Hallam, S.J., Mohn, W.W., 2012. Signficiant and persistent impact of timber harvesting on soil microbial communtiies in Northern coniferous forests. International Society for Microbial Ecology Journal 6: 2199-2218.

Hogberg, P. Hogberg M.N., Quist M.E., Ekblad, A., Nasholm, T., 1999. Nitrogen isotope fractionation during nitrogen uptake by ectomycorrhizal and non-mycorrhizal *Pinus sylvestris*. New Phytologist 142: 569-576.

Holden, S.R., Treseder, K.K., 2013. A meta-analysis of soil microbial biomass responses to forest disturbances. Frontiers in Microbiology(4)163: 1-17.

Horton, T.R., Molina, R., Hood, K., 2005. Douglas-fir ectomycorrhizae in 40- and 400-year-old stands: mycobiont availability to late successional western hemlock. Mycorrhiza 15, 393-403.

Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal ITS2 region--evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiology Ecology 82, 666–77.

Jurgensen, M. Reed, D., Page-Dumroese, D., Laks, P., Collins, A., Mroz, G., Degorski, M., 2006. Wood strength loss as a measure of decomposition in northern forest mineral soil. European Journal of Soil Biology 42: 23-31.

Keyes, C., O'Hara, K., 2002. Quantifying stand targets for silvicultural prevention of crown fires. West. J. Appl. For. 17: 101–109.

Kohout, P., Charvatova, M., Stursova, M., Masinova, T., Tomsovsky, M., Baldrian, P., 2017. Clearcutting alters decomposition processes and initiates complex restructuring of fungal communities in soil and tree roots. ISME Journal, Spring Nature. https://doi.org/10.1038/s41396-017-0027-3. (Accessed 28 May 18)

Lindahl, B.D., Nilsson, R.H., Tedersoo, L., Abarenkov, K., Carlsen, T., Pennanen, T., Stenlid, J., Kauserud, H., 2013. Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. New Phytol. 199, 288–299.

Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Hogberg, P., Stenlid, J., Finlay, R.D., 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytologist 173 (3): 611-620.

Lindner, D.L., Burdsall Jr., H.H., Stanosz, G.R., 2006. Species diversity of polyporoid and corticioid fungi in northern hardwood forests with differing management histories. Mycologia 98, 195–217.

Lindner, D.L., Vasaitis, R., Kubartová, A., Allmér, J., Johannesson, H., Banik, M.T., Stenlid, J., 2011. Initial fungal colonizer affects mass loss and fungal community development in Picea abies logs 6yr after inoculation. Fungal Ecol. 4: 449–460.

Lonsdale, D., Pautasso, M., Holdenrieder, O., 2008. Wood-decaying fungi in the forest: conservation needs and management options. Eur. J. For. Res. 127: 1–22.

Magurran, A.E., 1988. Ecological diversity and its measurement. Princeton University Press, 61-125.

Maassen, S. Fritze, H., Wirth, S. 2006. Response of soil microbial biomass, activities, and community structure at a pine stand in northeastern Germany 5 years after thinning. Canadian Journal of Forest Research 36: 1427-1434.

McComb, W., Lindenmayer, D., 1999. Dying, dead, and down trees. In: Hunter Jr., M.L. (Ed.), Maintaining Biodiversity in Forest Ecosystems. Cambridge university Press, 335-372.

McDaniel, P.A., Hipple, K.W., 2010. Mineralogy of loess and volcanic ash eolian mantles in Pacific Northwest (USA) landscapes. Geoderma 154: 438–446.

McMurdie, P.J., Holmes, S., 2013. Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8 (4): e61217.

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 Ecology 23: 48-57.

Nordén, B., Ryberg, M., Götmark, F., Olausson, B., 2004. Relative importance of coarse and fine woody debris for the diversity of wood-inhabiting fungi in temperate broadleaf forests. Biol. Conserv. 117: 1–10.

Noyce, G.L., Basiliko, N., Fulthorpe, R., Sackett, T.E., Thomas, S.C., 2015. Soil microbial respons over 2 years following biochar addition to a north temperate forest. Biology and Fertility of Soils 51 (6): 649-659.

O'Brien, H.E., Parrent, J.L., Jackson, J.A., Moncalvo, J.M., Vilgalys, R., 2005. Fungal community analysis by large-scale sequencing of environmental samples. Applied and Environmental Microbiology 71 (9): 5544-5550.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2018. Package 'vegan. https://cran.r-project.org/web/packages/vegan/vegan.pdf (accessed 7 March 2018).

Page-Dumroese, D.S., Coleman, M.D., Thomas, S.C., 2016. Opportunities and uses of biochar on forest sites in North America. In: Bruckman, V.J., Apaydin Varol, E., Uzun, B.B., Liu, J. (Eds.), Biochar: a regional supply chain approach in view of climate change mitigation. Cambridge Univeristy Press, 315-335.

Palmer, J., 2017., AMPtk: Amplicon Toolkit for NGS data. https://github.com/nextgenusfs/amptk (accessed 7 March 2018).

Peace, T.R., 1962. Pathology of trees and shrubs: with special reference to Britain. Claredon Press, 1-723.

Peay, K.G., Kennedy, P.G., Bruns, T.D., 2008. Fungal community ecology. Bioscience 58: 799–810.

Powell, D.C., 1999. Suggested stocking levels for forest stands in northeastern Oregon and southeastern Washington: An implementation guide for the Umatilla National Forest. USDA, Forest Service, Pacific Northwest Region, Umatilla National Forest, Technical Publication F14-SO-TP-03-99. https://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5405482.pdf (accessed 07 April 18).

Purahong, W., Kahl, T., Schloter, M., Bauhus, J., Buscot, F., Kruger, D., 2014. Comparing fungal richness and community composition in coarse woody debris in Central European beech forests under three types of management. Mycological Progress 13: 959-964.

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna Austria, https://www.R-project.org/ (accessed 7 March 2018).

Rajala, T., Peltoniemi, M., Pennanen, T., Makipaa, R., 2010. Relationship between wood-inhabiting fungi determined by molecular analysis (denaturing gradient gel electrophoresis) and quality of decaying logs. Canadian Journal of Forest Research 40: 2384-2397.

Raup, D.M., Crick, R.E., 1979. Measurement of faunal similarity in paleontology. Journal of Paleontology 53 (5): 1213-1227.

Rayner, A.D.M., Boddy, L., 1988. Fungal Decomposition of Wood: Its Biology and Ecology. John Wiley & Sons Ltd., New York.

Rydin, H., Diekmann, M., Hallingbäck, T., 1997. Biological characteristics, habitat associations, and distribution of macrofungi in Sweden. Conserv. Biol. 11: 628–640.

Schoch, C.L., Seifert, K. a, Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc. Natl. Acad. Sci. U. S. A. 109: 6241–6246.

Shannon C.E., Weaver, W., 1964. The mathematical thoery of communication. University of Illinois Press, Urbana.

Sherman, L.A., Page-Dumroese, D.S., Coleman, M.D., 2017. Idaho forest growth response to post-thinning energy biomass removal and complementary soil amendments. GCB Bioenergy, 1-16.

Smith, D., Onions, A.H.S., 1994. The preservation and maintenance of living fungi, 2nd ed. CAB International, England.

Stokland, J.R., Siitonen, J., Gunnar Jonsson, B., 2012. Biodiversity in Dead Wood. Cambridge University Press, New York.

Strukelj, M., Brais, S., Quideau, S.A., Angers, V.A., Kebli, H., Drapeau, P., Oh, S.W., 2013. Chemical transformations in downed logs and snags of mixed boreal species during decomposition. Canadian Journal of Forest Research 43: 785-798.

Talgo, V., Chastagner, G., Thomsen, I.M., Riley, K., Lange, K., Klemsdal, S.S., Stensvand, A., 2010. *Sydowia polyspora* associated with current season needle necrosis (CSNN) on true fir (*Abies spp*.). Fungal Biology 114 (7) 545-554.

USDA Forest Service., 2013. Woody Biomass Utilization. http://www.fs.fed.us/woodybiomass/whatis.shtml (accessed 22 February 2018).

USDA Forest Service, Forest Health Protection, Rocky Mountain Region., 2011. Coniophora root and butt rot: Brown rot with gray-brown cords and mycelium. https://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5353724.pdf (accessed 30 March 2018).

U.S. Department of Energy., 2011. U.S. Billion-Ton update: Biomass supply for a bioenergy and bioproducts industry. R.D. Perlack and B.J. Stokes (Leads), ORNL/TM-2011/224. Oak Ridge national Laboratory, Oak Ridge, TN, 1-227. https://www1.eere.energy.gov/bioenergy/pdfs/billion_ton_update.pdf (accessed 22 February 2018).

Veblen, T., Kitzberger, T., Donnegan, J., 2000. Climatic and human influences on fire regimes in ponderosa pine forests in the Colorado Front Range. Ecol. Appl. 10, 1178–1195.

Vralstad, T., Myhre, E., Schumacher, T., 2002. Molecular diversity and phylogenetic affinities of symbiotic root-associated ascomycetes of the Helotiales in burnt and metal polluted habitats. New Phytologist 155: 131-148.

Zak, J.C., Willig, M.R., 2004. Fungal Biodiversity Patterns, in: Mueller, G.M., Bills, G.F., Foster, M.S. (Eds.), Biodiversity of Fungi: Inventory and Monitoring Methods. Elsevier, 59-75.

Table 2.1. Twenty most abundant Operational Taxonomic Units (OTUs) from the University of Idaho Experimental Forest DNA-based dataset generated from drilled wood stakes. Taxonomy was assigned with 97% similarity threshold, and some OTUs could only be confidently assigned to higher-order taxonomic ranks. Ecological descriptions of Families from Cannon and Kirk, 2007.

Phylum	Class	Order	Family	Genus	Species	Ecology[1]
Ascomycota	*Eurotiomycetes*	*Chaetothyriales*	*Herpotrichiellaceae*	*Phialophora*		Sapro/Patho/Symbio-troph
Ascomycota	*Sordariomycetes*	*Coniochaetales*	*Coniochaetaceae*	*Coniochaeta*		Sapro/Patho/Symbio-troph
Ascomycota						Unassigned
Ascomycota	*Leotiomycetes*	*Helotiales*	*Helotiaceae*	*Meliniomyces*		Saprotroph, Symbiotroph
Ascomycota	*Sordariomycetes*	*Hypocreales*	*Hypocreaceae*	*Trichoderma*		Saprotroph
Ascomycota	*Leotiomycetes*	*Helotiales*	*Helotiaceae*	*Collophora*		Saprobic on wood and some fungi
Ascomycota	*Sordariomycetes*	*Coniochaetales*	*Coniochaetaceae*	*Coniochaeta*		Sapro/Patho/Symbio-troph
Ascomycota	*Eurotiomycetes*	*Chaetothyriales*	*Herpotrichiellaceae*	*Rhinocladiella*	*atrovirens*	Wood Saprotroph
Ascomycota	*Dothideomycetes*	*Capnodiales*	*Cladosporiaceae*	*Cladosporium*		Sapro/Patho/Symbio-troph
Ascomycota	*Sordariomycetes*	*Coniochaetales*	*Coniochaetaceae*	*Coniochaeta*		Sapro/Patho/Symbio-troph
Ascomycota	*Leotiomycetes*	*Helotiales*	*Dermataceae*			Saprotroph, Pathotroph
Ascomycota	*Dothideomycetes*	*Dothideales*	*Dothioraceae*	*Sydowia*	*polyspora*	Sapro/Patho/Symbio-troph
Ascomycota	*Leotiomycetes*	*Helotiales*	*Helotiaceae*			Saprobic on wood and some fungi
Ascomycota	*Leotiomycetes*	*Helotiales*	*Helotiaceae*			Saprobic on wood and some fungi
Ascomycota	*Dothideomycetes*	*Pleosporales*				Unassigned
Ascomycota	*Xylonomycetes*	*Xylonomycetales*		*Tromeropsis*	*microtheca*	Unassigned
Ascomycota	*Leotiomycetes*	*Helotiales*	*Helotiaceae*	*Helicodendron*	*websteri*	Saprotroph
Basidiomycota	*Cystobasidiomycetes*					Unassigned
Ascomycota	*Sordariomycetes*	*Chaetosphaeriales*	*Chaetosphaeriaceae*			Saprotroph (wood/plant)
Ascomycota	*Eurotiomycetes*	*Chaetothyriales*	*Herpotrichiellaceae*	*Exophiala*		Saprotroph

[1]Sapro/Patho/Symbio-troph = Saprotroph/Pathotroph/Symbiotroph

Table 2.2. Results of fungal community analyses of DNA-based dataset generated from drilled wood stakes, with Permutational Analysis of Variance (PERMANOVA) tests of alpha diversity. Wood stakes were deployed in 2014, and collected in 2015, 2016, and 2017. Two species of wood stake were used, aspen and pine. Fungal DNA was extracted from drilled shavings from the surface stakes, and from two locations of the vertically inserted mineral stakes, labeled “Mineral_Top” and “Mineral_Bottom”. Two sites (UIPP and UIMC) were assessed for biomass treatment (0X, 1X, and 2X slash, with a non-thinned control) and soil amendment (biochar, fertilizer+biochar, fertilizer, and non-amended control) treatment effects.

Year	Stake Species	Stake Location	Variable	PERMANOVA			
				D.F.	F.Model	R^2	*p* value
2015	Aspen	Surface	Site	1,22	8.36	0.10	0.001
			Biomass Treatment	3,22	6.19	0.22	0.004
			Soil Amendment	3,22	11.73	0.42	0.001
		Mineral_Top	Site	1,23	5.05	0.13	0.16
			Biomass Treatment	3,23	1.59	0.12	0.25
			Soil Amendment	3,23	1.88	0.14	0.18
		Mineral_Bottom	Site	1,23	8.52	0.19	0.15
			Biomass Treatment	3,23	2.35	0.16	0.14
			Soil Amendment	3,23	1.74	0.12	0.25
	Pine	Surface	Site	1,24	26.80	0.21	0.001
			Biomass Treatment	3,24	10.80	0.26	0.002
			Soil Amendment	3, 24	14.50	0.34	0.001
		Mineral_Top	Site	1,23	5.54	0.13	0.091
			Biomass Treatment	3,23	1.98	0.14	0.189
			Soil Amendment	3,23	2.55	0.18	0.091
		Mineral_Bottom	Site	1, 22	9.76	0.23	0.278
			Biomass Treatment	3, 22	1.98	0.14	0.252
			Soil Amendment	3, 22	1.35	0.09	0.386
2016	Aspen	Surface	Site	1,23	5.68	0.10	0.005
			Biomass Treatment	3,23	5.85	0.31	0.005
			Soil Amendment	3,23	3.71	0.19	0.026
		Mineral_Top	Site	1,23	8.18	0.23	0.71
			Biomass Treatment	3,23	1.24	0.11	0.40
			Soil Amendment	3,23	-0.02	-0.001	0.87
		Mineral_Bottom	Site	1,17	10.37	0.19	0.031
			Biomass Treatment	3,17	5.55	0.31	0.006
			Soil Amendment	3,17	0.38	0.02	0.79
			Site*Biomass	3,17	3.11	0.17	0.049
	Pine	Surface	Site	1,24	3.66	0.07	0.031
			Biomass Treatment	3,24	4.33	0.26	0.036
			Soil Amendment	3,24	2.92	0.18	0.091
		Mineral_Top	Site	1,24	14.74	0.22	0.012
			Biomass Treatment	3,24	6.99	0.31	0.006
			Soil Amendment	3,24	2.45	0.11	0.155
		Mineral_Bottom	Site	1,24	21.04	0.33	0.063
			Biomass Treatment	3,24	4.59	0.22	0.029
			Soil Amendment	3,24	1.38	0.06	0.36
2017	Aspen	Surface	Site	1,24	4.17	0.08	0.005
			Biomass Treatment	3,24	4.09	0.23	0.018
			Soil Amendment	3,24	4.39	0.25	0.013
		Mineral_Top	Site	1,22	4.41	0.15	0.732
			Biomass Treatment	3,22	0.40	0.04	0.766
			Soil Amendment	3,22	0.89	0.09	0.546
		Mineral_Bottom	Site	1,24	1.47	0.05	0.924
			Biomass Treatment	3,24	0.57	0.06	0.701
			Soil Amendment	3,24	-0.001	-0.001	0.921
	Pine	Surface	Site	1,22	3.61	0.07	0.003
			Biomass Treatment	3,22	4.02	0.22	0.015
			Soil Amendment	3,22	5.51	0.30	0.003
		Mineral_Top	Site	1,24	4.18	0.11	0.197
			Biomass Treatment	3,24	1.88	0.14	0.169
			Soil Amendment	3,24	1.35	0.11	0.331
		Mineral_Bottom	Site	1,24	6.98	0.21	0.747
			Biomass Treatment	3,24	2.45	0.22	0.148
			Soil Amendment	3,24	-1.52	-0.14	0.999

Table 2.3. Results of fungal community analyses of DNA-based dataset generated from drilled wood surface stakes, with Permutational Analysis of Variance (PERMANOVA) tests of alpha diversity relative to forest floor soil metrics. Surface stakes were deployed in 2014, and collected in 2017. Two species of wood stake were used, aspen and pine. Fungal DNA was extracted from drilled shavings from the surface stakes. Soil metrics include dry forest floor weight (Dry FF wt.) % organic matter (OM), pH, % carbon and % nitrogen.

Stake Location	Variable	D.F.	F.Model	PERMANOVA R2	*p value*
Surface Aspen	Dry FF wt.	1,16	1.87	0.04	0.208
	% OM	1,16	2.18	0.05	0.207
	pH	1,16	3.93	0.09	0.047
	% C	1,16	5.19	0.12	0.026
	% N	1,16	7.19	0.16	0.013
Surface Pine	Dry FF wt.	1,14	1.30	0.03	0.399
	% OM	1,14	2.93	0.07	0.134
	pH	1,14	5.43	0.12	0.029
	% C	1,14	3.51	0.08	0.102
	% N	1,14	8.12	0.18	0.018

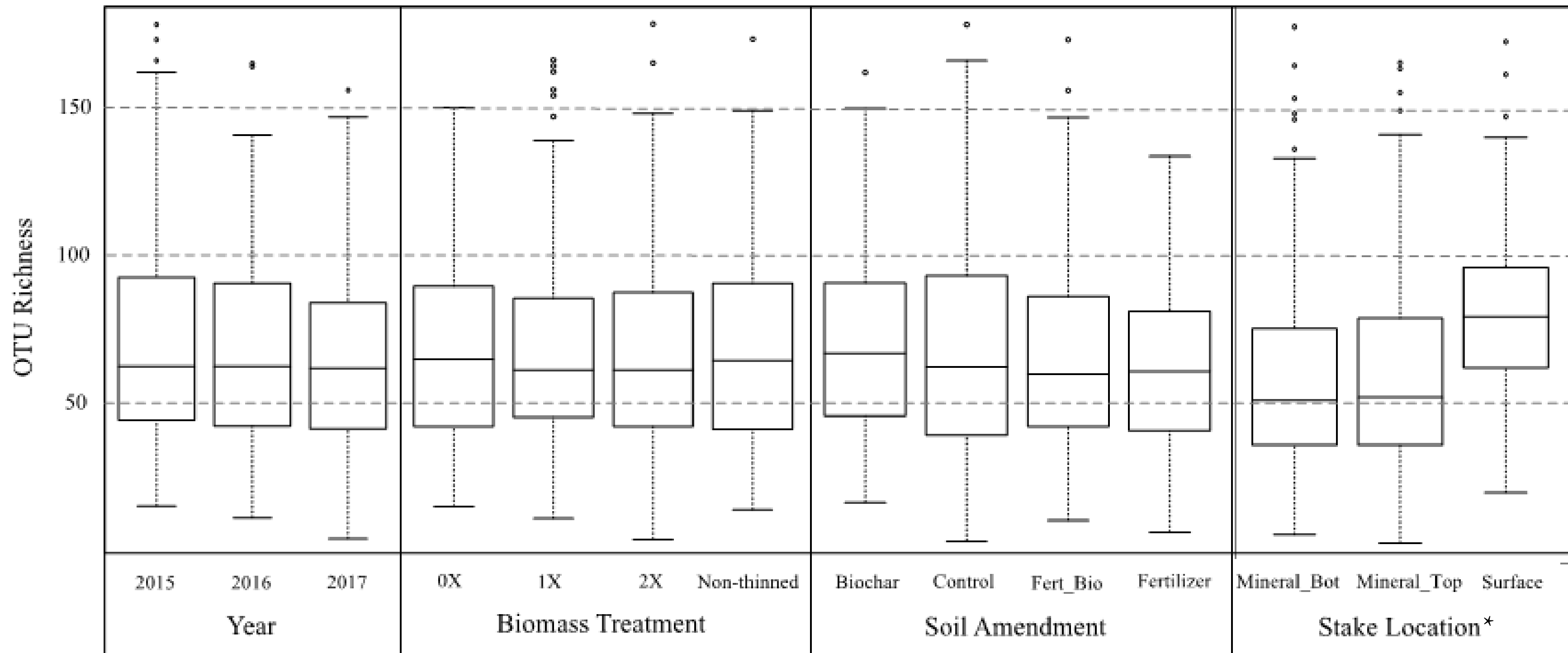

Fig. 2.1. Operational Taxonomic Unit (OTU) richness of University of Idaho Experimental Forest's DNA-based dataset generated from drilled wood stakes, showing differences between years, biomass treatments (0X = no slash), soil amendment treatments (Control = non-amended, Fert_Bio = Fertilizer+Biochar), and stake location* (Mineral_Bot = fungal community found at the bottom of the 30 cm mineral stake) over all 3 years. *Indicates significant difference in richness (ANOVA, $p < 0.05$). Wood stakes were first inserted into the soil in 2014, therefore this dataset represents the fungal community after 1 year, 2 years, and 3 years of colonization and decomposition (n = 559).

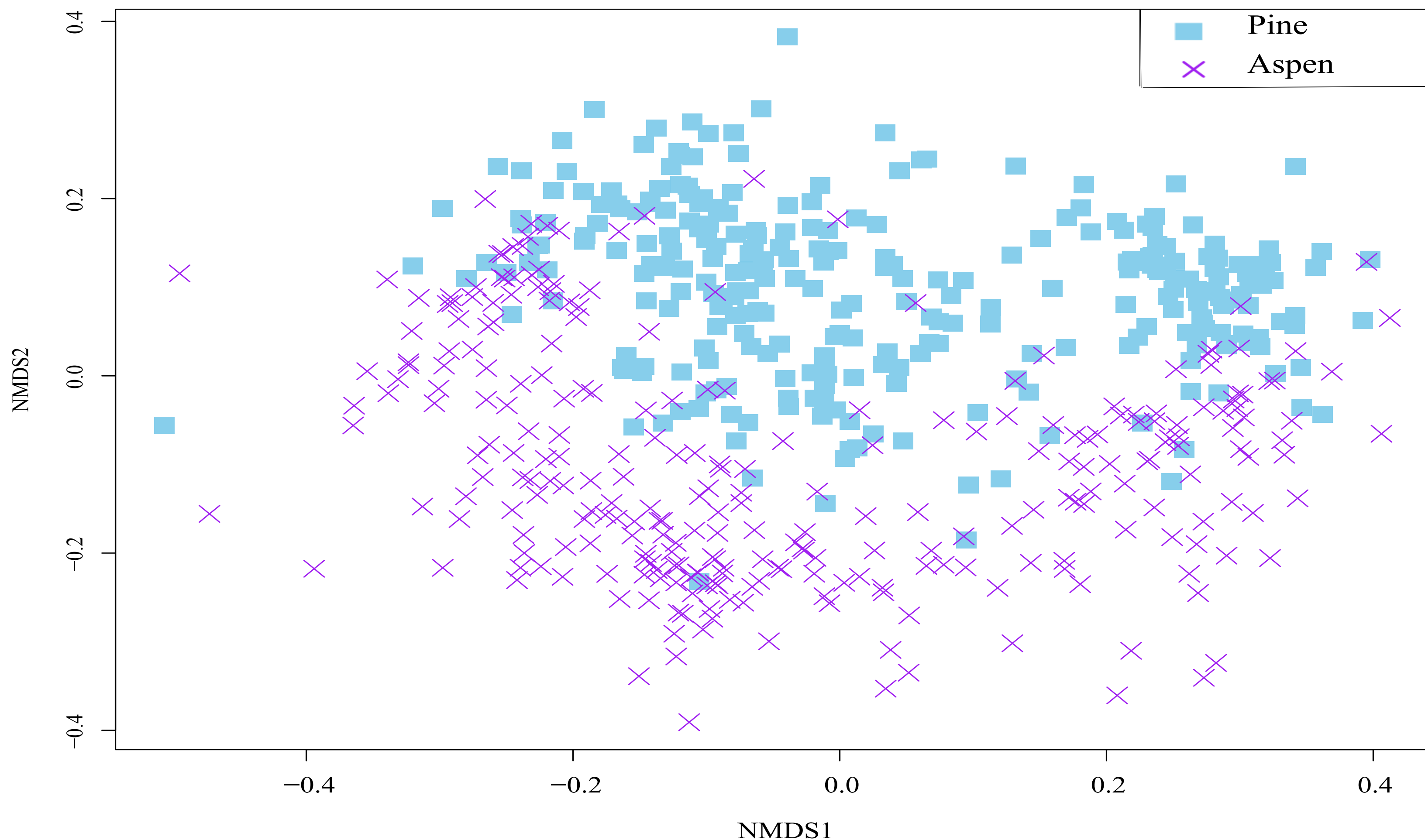

Fig. 2.2. Nonparametric multidimensional scaling (NMDS) ordinations of fungal community data from the DNA-based dataset (ITS2) generated from drilled wood stakes, showing the significant effects of stake species (PERMANOVA: $p < 0.01$) over all three years. Plots configured in three dimensions with Raup-Crick dissimilarities. Stress = 0.16. Wood stakes were loblolly pine (*Pinus taeda* L.) and aspen (*Populus tremuloides* Michx.). Fungal DNA was extracted from drill shavings from the pine and aspen stakes.

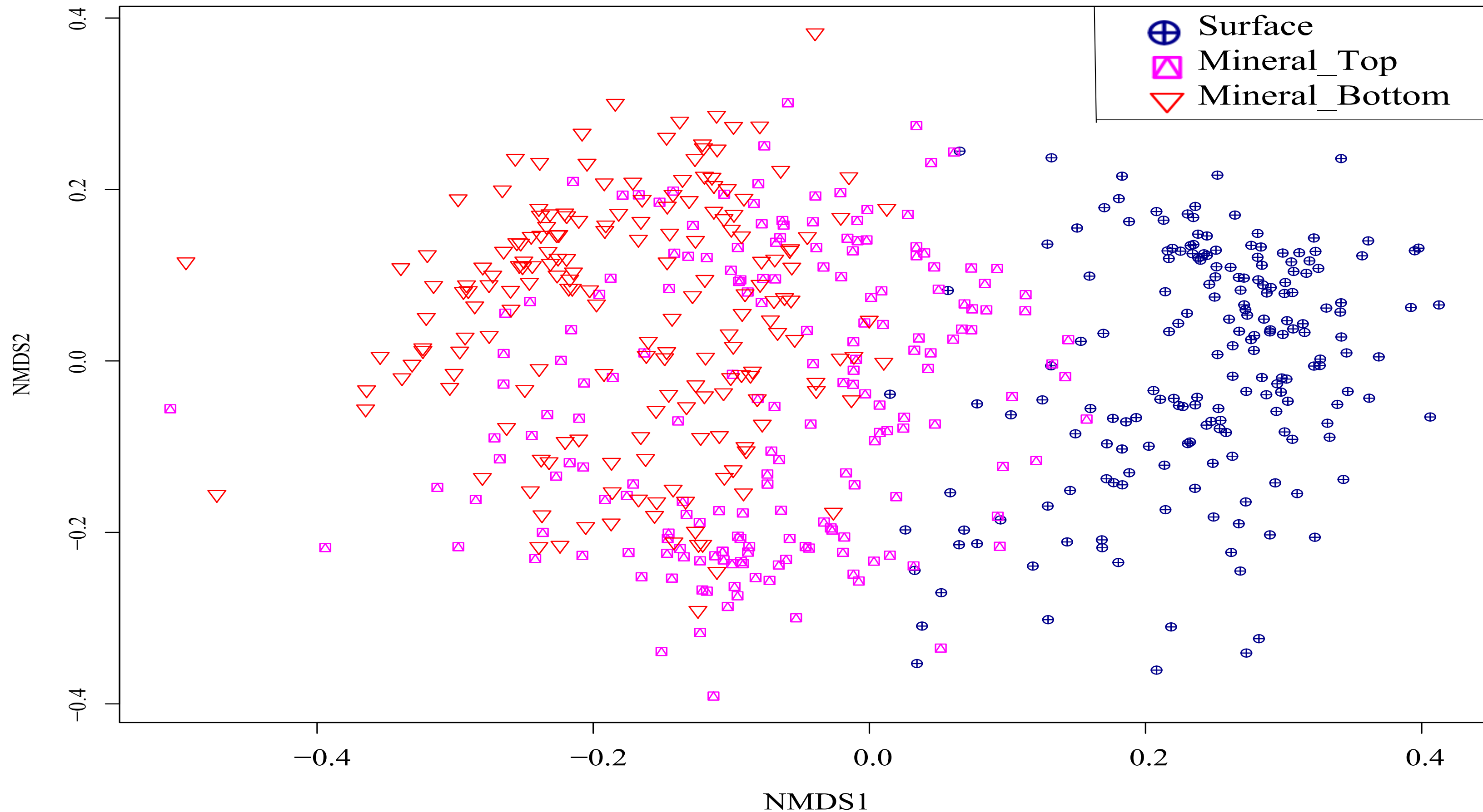

Fig. 2.3. Nonparametric multidimensional scaling (NMDS) ordinations of fungal community data from DNA-based dataset (ITS2) generated from drilled wood stakes, showing the significant effects of stake location (PERMANOVA: $p < 0.01$) over all three years. Plots configured in three dimensions with Raup-Crick dissimilarities. Stress = 0.16. Two species of stakes were used (aspen and pine). Also, two lengths of wood stake were used. Both species of wood stakes were inserted within the mineral soil (2.5 x 2.5 x 30 cm) and stapled to the litter surface (2.5 x 2.5 x 15 cm). Fungal DNA was extracted from drill shavings from the surface stake and from two locations of the vertically inserted mineral stake, labeled “Mineral_Top” and “Mineral_Bottom”.

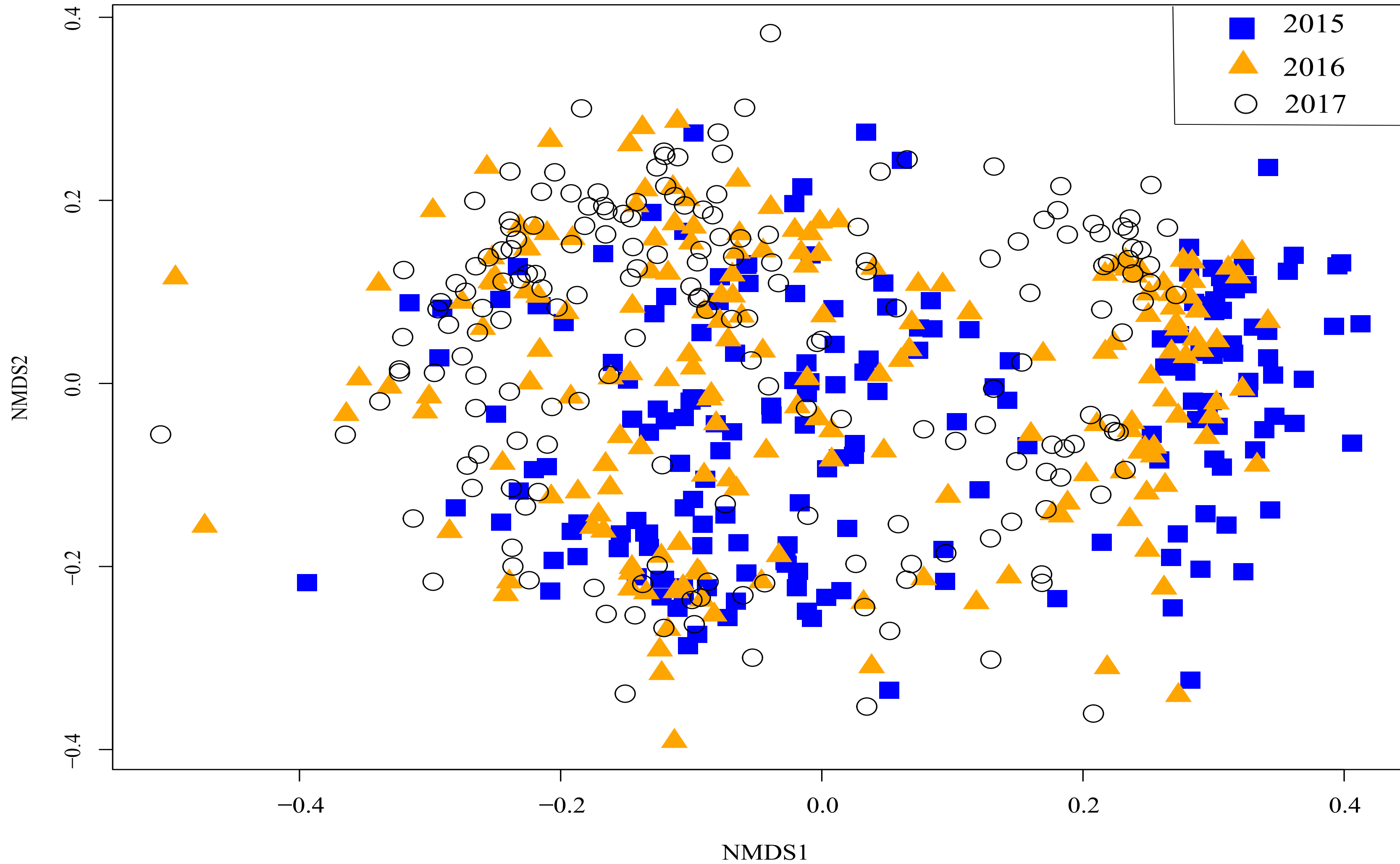

Fig. 2.4. Nonparametric multidimensional scaling (NMDS) ordinations of fungal community data from DNA-based dataset (ITS2) generated from drilled wood stakes, showing the significant effects of year (PERMANOVA: $p < 0.01$). Plots configured in three dimensions with Raup-Crick dissimilarities. Stress = 0.16. Wood stakes were inserted within the mineral soil and on the litter surface in 2014, and removed in years 2015, 2016, and 2017. Fungal DNA was extracted from drill shavings.

Chapter 3

A comparison of fungal sampling methods to reveal community differences due to forest biomass harvesting and compensatory soil amendment treatments in a western conifer forest

Abstract

Two different methods of fungal community sampling (collection of fruiting bodies vs. DNA-based methods) were compared for their ability to track changes in wood-inhabiting fungal communities caused by different biomass harvesting treatments and compensatory soil amendments in a conifer dominated forest in western North America. Wood-inhabiting fruiting body surveys were conducted in the fall of 2014-2016. DNA-based surveys were conducted by affixing wood stakes to the soil surface and retrieving them yearly for 3 years (2015-2017). The stakes were drilled to produce wood shavings that were used for extraction of fungal DNA.

Research was conducted on sites occupied by mixed conifer species. Plots were established with biomass retention at four levels (removal of all cut material, retention of cut material, doubling of cut material, and non-thinned controls) and four different soil amendment treatments (fertilizer, biochar, fertilizer and biochar, and non-amended controls). The 16 treatment combinations were repeated at two sites, yielding 32 treatment plots of 400-m^2 each.

The fruiting body surveys yielded over 1,000 observations over 3 years. In total, 129 mostly basidiomycete fungal species were identified, with 22 species that did not match known species descriptions. Fruiting bodies were collected from many different substrates in various stages of decay, with the majority of species found on substrates that were moderately decayed. The fungal community composition as indicated by fruiting bodies was significantly influenced by biomass treatments in 2014

($p = 0.003$), while there was no significant effect of soil amendment treatments relative to the community composition of fungal fruiting bodies.

DNA-based methods revealed 2,316 different operational taxonomic units (OTUs), of mostly microscopic ascomycete species. Fungal community composition as indicated by DNA was influenced by biomass treatments all three years ($p < 0.04$), while soil amendments significantly influenced the DNA-based fungal community composition in 2015 and 2017 ($p < 0.04$).

Not surprisingly, the two sampling methods revealed very different fungal communities. Fungal fruiting body surveys sampled a range of wood substrates and identified important forest pathogens, whereas sequencing did not. DNA-based methods produced more data and associated OTUs than fruiting body surveys ($p < 0.001$), with very little overlap of OTUs between methods (23 OTUs or 1% common to both methods). DNA-based sampling of wood stakes was more sensitive to treatments, especially soil amendment treatments, though the wood stakes represented only one type of woody debris. Both methods had advantages and disadvantages, and success with each method will depend on the study, the questions being asked, and the researcher's skills.

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Given the importance of fungi to healthy ecosystems for nutrient acquisition and turnover (Berg and McClaugherty 2003; Ferris and Humphrey, 1999; Hammell, 1997; Harley, 1971; Hudson, 1986; Lonsdale et al., 2007; Martinez et al. 2005; Rayner and Boddy, 1988), fungi are often sampled to assess the effects of human actions relative to ecosystem functions (Bader et al., 1995; Lindner et al., 2006; Lonsdale et al., 2007; Purahong et al., 2014; Stokland and Larsson, 2011). In particular, one important question that is currently an issue is how forest management in western North America affects fungal communities. Given that hardly any information exists regarding the best way to survey fungi to answer these kinds of questions, little is known about how sampling methods may affect the findings. Therefore, this study was conducted to assess whether forest management affected fungal communities, and which methods of fungal sampling were most appropriate to answering questions relative to sustainable forest management.

The goals of sustainable management of forests include meeting present material needs and allowing a continuous supply of forest products for future generations, protecting habitat, water resources, and genetic diversity (Fox, 2000). Biomass includes the living trees and other woody plants, as well as dead limbs, tops, and foliage naturally present or resulting from forest management activities such as thinning (USDA Forest Service, 2013). Because of past fire-suppression policies and practices, excess biomass has accumulated in forests of the western United States (Arno, 2000; Keyes and O’Hara, 2002). Overstocked forests have been subject to high-intensity wildfires (Keyes and O’Hara, 2002), resulting in crown torching, soil scorching, and increased mortality of plants and soil organisms (Arno, 2000; Keane, 2002). To enhance the sustainability of United States energy supplies, Department of Energy (DOE), Department of

Interior (DOI), and the United States Department of Agriculture (USDA) announced an initiative encouraging the utilization of forest biomass, rather than reduction through prescribed burning or other on-site disposal methods (Norton et al., 2003).

Woody biomass has economic value as a potential feedstock for the production of a variety of biofuels, including bio-oil. While other biofuel possibilities exist, bio-oil is most appropriate for efficient Western coniferous biomass utilization (Mohan et al., 2006). An additional potential benefit is the byproduct of bio-oil manufacture, biochar, a recalcitrant black carbon functionally similar to charcoal (Coleman, et al., 2010). Biochar has been recommended for application to agricultural and forest soils to improve soil quality, enhance soil organic matter content, and sequester carbon (Page-Dumroese et al., 2016). Although thinning forests may also yield ecological and silvicultural benefits, the current cost of removal often exceeds the monetary value of the biomass (McElligott et al., 2011). Therefore, forest managers in the intermountain west are eager for incentives to develop viable bioenergy markets (McElligott et al., 2011). Public policies and bioenergy markets promoting biomass utilization have raised concerns over the unknown impacts and highlight the need for more information on the effects of biomass removal from forest systems (Kimmins, 1997; U.S. Department of Energy et al., 2011).

Woody biomass harvest guidelines implemented in some states include the retention of downed, dead woody materials that are substrates for fungi with critical roles in healthy forest ecosystems (Stokland et al., 2012). White and brown rot fungi are the only organisms capable of substantial and efficient decay of wood’s recalcitrant lignocellulosic structure (Hammel, 1997; Rayner and Boddy, 1988), thereby making resources available for other functional groups (Cooke and Rayner, 1984; Rayner and Boddy, 1988; Stokland et al., 2012). Wood decay provides soil organic matter, recycles nutrients, and is the basis of the food chain for microbes,

invertebrates, small mammals, amphibians, birds, and larger vertebrates (Ferris and Humphrey, 1999; Lonsdale et al., 2008; McComb and Lindenmayer, 1999).

Alterations in the quality and reductions in quantity of woody debris due to logging and other forest management practices have already been shown to adversely affect richness and abundance of fungi (Bader et al., 1995; Norden et al., 2004; Rydin et al., 1997). Some fungal species are found on freshly cut, fine woody debris; others require large-diameter, well-decayed woody substrate (Lindner et al., 2006; Lonsdale et al., 2008; Rydin et al., 1997). Fungal communities are dynamic, successional, and individual species can be niche specific (Rajala et al., 2012). Therefore, fungi influenced by forest conservation and management practices have been used as indicators of ecosystem biodiversity and community composition (Bader et al., 1995; Ferris and Humphrey, 1999; Lindner et al., 2006; Lonsdale et al., 2008; Purahong et al., 2014). It is hypothesized that reductions in woody substrates will not only affect the primary colonizing fungi, but the diverse array of other organisms that would have followed in succession. Therefore, understanding how biomass harvesting affects wood-inhabiting fungal communities is critical for assessing the environmental sustainability of such harvests. For example, loss of mycorrhizal fungi would be problematic for the regeneration of tree seedlings.

Valuable species assemblage lists (Schmit and Lodge 2005) have been produced via classical studies of fungal diversity that resulted from identification of fruiting bodies or strains cultured on laboratory media. A major advantage of these traditional methods is that specialized and expensive materials and equipment are generally not required. In addition, collections of fruiting bodies are housed in herbaria and fungal cultures may be maintained for many years, allowing researchers to utilize these materials during subsequent analyses. However, not all fungi produce visible, persistent fruiting bodies or can be grown in culture (Anderson and

Cairney, 2004; Peay et al., 2008; Smith and Onions, 1994). With the development of molecular methods some of these limitations can now be overcome. However, as the number of species for which DNA sequences are available continues to increase, lists resulting from molecular-based studies increasingly differ from those produced using classical methods.

A standard molecular approach used for environmental fungal identifications and phylogenetic studies targets the nuclear ribosomal RNA gene cluster (Bridge and Spooner, 2001; Bruns et al., 1991; Peay et al., 2008). Specifically, the Internal Transcribed Spacer (ITS) regions within the RNA gene cluster have been identified as the universal DNA barcode marker for fungi (Gardes and Bruns, 1993; Ihrmark et al., 2012; Schoch et al., 2012). Parts of the ITS region are highly conserved and Polymerase Chain Reaction (PCR) amplifications of the ITS region are highly reliable even with small amounts of DNA (Gardes and Bruns, 1993; Peay et al., 2008). There are many published ITS sequences available online for comparison (Peay et al., 2008; Schoch et al., 2012), and most importantly, with fungal-specific primers, it is possible to identify fungal sequences in mixed samples of plant, animal, prokaryotic, and fungal DNA (Berbee and Taylor, 2001).

High-throughput amplicon sequencing (HTAS), or next generation sequencing (NGS), has revolutionized ecological, population, and conservation genetic studies by generating huge amounts of sequence data on non-model organisms in a cost-effective and timely manner (Ekblom and Galindo, 2011). In a 4-hour run, the Life Technologies Ion Torrent Personal Genome Machine (PGM) can generate between 60 megabases (Mb) and 2 gigabases (Gb) (Life Technologies, 2014), versus 50,000 base pairs generated using traditional Sanger sequencing technologies (Peay et al., 2008). Therefore, studies that incorporate NGS sequencing technologies to determine community compositions will continue to be the norm.

Knowledge of the effects of forest management activities including biomass harvesting on diversity and abundance of fungi in dry ecosystems of the intermountain west of North America is limited. The current lack of information pertaining to wood-inhabiting and soil-forming fungi in western North America potentially limits the effectiveness of any biomass harvesting guidelines that do not fully consider the predominance and significance of fungi to the sustainability of these ecosystems. The objectives of this study were to compare potential benefits and biases of characterizing fungal communities based on analysis of: (i) fruiting body survey data, and (ii) HTAS (DNA-based) data, as influenced by manipulation of biomass in Idaho forests.

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3.2.1. *Study area and experimental design*

Research was conducted at the University of Idaho – Experimental Forest (UIEF, 46.849512, -116.845068). The elevation at this site ranges from 830-890 meters above sea level, with a 0-15% slope and south-facing aspect. The mean annual temperature was 7.8 ºC (measured between 1981-2010, PRISM Climate Group). The stand had been previously logged and exposed to periodic burning, and was at study inception occupied by mixed conifer species grown atop silt-loam soils with a significant (>30 cm) Andisol layer of volcanic ash (Cooper et al. 1991; McDaniel and Hipple, 2010). The UIEF site is classified as a grand fir/ninebark habitat type (Cooper et al., 1991) and is dominated by ponderosa pine (*Pinus ponderosa* Dougl. ex Laws), grand fir [*Abies grandis* (Dougl. ex D. Don) Lindl.], Douglas-fir [*Pseudotsuga menziesii*

(Mirbel) Franco], lodgepole pine (*Pinus contorta* Dougl. ex Loud.), and western larch (*Larix occidentalis* Nutt). UIEF has been used for cattle grazing, recreation, research, and managed for commercial forestry.

In 2013, (400-m^2) study plots were established with biomass treatments at four levels and soil amendments at four levels. Treatment combinations (4x4) were randomly assigned and replicated twice, for a total of 32 plots. Experimental sites were named UIEF_ Ponderosa Pine (UIPP), and UIEF_Mixed Conifer (UIMC). Biomass treatment plots were established, including non-thinned control and manipulated plots that were thinned to remove trees of lower crown positions, and also those of undesirable species, poor form, or were visibly diseased, to a relative density of 40%, which is about 200 trees per hectare for stands with an average diameter at breast height (DBH) of 12 cm (Powell, 1999). Different amounts of biomass were retained to influence a biological response range. Biomass treatments in these thinned test plots included removal of all material cut during the thinning (0X), retention of this cut material (1X), and doubling of the amount of this cut material (2X), compared to the non-thinned control (non-thinned). To create doubled biomass (2X) plots, material that was cut during thinning was removed from 0X plots and randomly scattered over the 2X plots. Soil amendments consisted of fertilizer, biochar (2 Mg ha^{-1}), fertilizer+biochar, and a non-amended control. Urea fertilizer (46-0-0) was applied at a rate of 224 kg N ha^{-1}. The biochar was produced from Western mixed conifer feedstock in a steam boiler at 980 ºC; it consists of 25.7% C, 28.6% ash, and has a surface area of 201 m^2 g^{-1}, with an average pH of 7.8.

3.2.2. Classical fungal fruiting body surveys and taxa identification

Fungi were inventoried within the 400 m^2 soil amendment treatment plots. All fine and coarse woody debris and standing trees (living or dead, to a height of 2m) within each plot were visually inspected for fungal fruiting bodies during late September and October of 2014, 2015, and 2016. Inventories were conducted in the fall to increase detection of fungal species producing ephemeral, annual fruiting bodies, in addition to persistent, perennial fruiting bodies.

Fungal fruiting body sampling methods follow established protocols (Mueller et al., 2004). The following characteristics were reported for each fruiting body when encountered: fungal species (when easily identified), substrate type (branch, log, suspended log, snag, stump, and living tree), wood substrate species, diameter class, and decay class following the five-class system of Maser et al. (1979). Dead fruiting bodies were inventoried unless their state of degradation precluded identification.

Fruiting bodies that were not easily identified in the field were collected, dried, and taken to the laboratory for further study. Samples were identified in the lab by microscopic examination of morphological features (Bernicchia et al., 2010; Gilbertson and Ryvarden, 1986; Hjorstam, et al., 1987) and/or DNA extraction followed by sequencing of the ITS regions. All DNA extractions, PCR, and sequencing protocols for fruiting bodies follow protocols described by Lindner and Banik (2008). Sanger sequences were generated at the University of Wisconsin – Madison Biotechnology Center, aligned and edited using Sequencher 4.9 (GeneCodes Corporation). The Basic Local Alignment Search Tool (BLAST) was used to search for similar sequences in GenBank (Schäffer and Aravind, 2001) and unknown specimens were identified using a 97% similarity threshold for species-level identifications. The use of a 97% similarity

cut-off is well supported in the literature for the ITS regions (Blaalid, et al. 2013). Dried and identified voucher specimens were deposited in the Center for Forest Mycology Research (CFMR) herbarium at the USDA Forest Service - Forest Products Laboratory (Madison, Wisconsin). Fungal nomenclature was based on Index Fungorum (www.indexfungorum.org) and Mycobank (www.mycobank.org).

3.2.3. Molecular fungal identification from colonized wood stakes

Potential for treatments to influence woody substrate decomposition and colonization by fungi was evaluated. Using protocols described by Jurgensen et al. (2006), loblolly pine (*Pinus taeda* L.) stakes (2.5 x 2.5 x 15 cm) were affixed on the surface of the litter layer with large metal staples. Installation occurred in May of 2014. After 1 year, and continuing every year for 2 years, five pine stakes were removed from both the mineral soil and litter layer location of each plot in the spring, when soil moisture is highest due to snow melt.

Fungi inhabiting the wood stakes were identified using DNA-based methods described by Lindner et al. (2011). Wood shavings from which fungal DNA was later extracted were obtained within 24 hours of stake collection. Sterile, 4-mm-diameter drill bits were inserted 1 cm deep at two points, or on each end, of the collected stakes. Drill bits were sterilized with a bleach solution and flame dried with alcohol. Fungal DNA was extracted from shavings produced by drilling into the wood stakes, and drill shavings from the two points of each surface stake were pooled in equal amounts. All shavings were collected in 2 mL strip tubes in 96-well format, covered with filter-sterilized cell lysis solution (CLS) (Lindner et al., 2011) and frozen at -20C until DNA was extracted. Because the five pine stake replicates within each treatment plot

represents sub-sampling (i.e. they likely were not independent replicates), the cell lysis solution (CLS) containing wood shavings from the pine stakes was pooled prior to extraction (Appendix 2). Pooled wood shavings were created by pooling all five wood stakes for a given year within each treatment. The DNA was cleaned using GeneClean III kits (Qbiogene), that were previously found to be reliable and cost effective for the recovery of high quality genomic fungal DNA from wood samples (Lindner et al., 2011).

The procedures for high-throughput sequencing followed the Ion PGM manufacturer's instructions and Lindahl (2013). DNA was extracted from the drilled wood stake shavings using the tagged and barcoded primers fITS7 (Ihrmark et al., 2012) and ITS4 for the PCR reaction, targeting the ITS2 region of the ribosomal RNA gene. Two mock communities were used as controls: a fungal mock community and an artificial mock community (Palmer et al., 2018). Following DNA amplification, Zymo Research's Select-A-Size DNA Clean & Concentrator spin columns were used to clean the resulting PCR product. Equimolar proportions of DNA were templated in an emulsion PCR step where the product was attached and amplified onto the Ion Sphere Particles (ISPs). Sequencing of the cleaned, template positive ISPs was performed using the Ion PGM Hi-Q sequencing kit according to manufacturer's recommendations. "

Sequence reads obtained from the PGM were trimmed, filtered, clustered, and assigned operational taxonomic units (OTUs) with USEARCH and VSEARCH, facilitated by the AMPtk program (Palmer, 2016). Taxa were assigned using UTAX Classifier and USEARCH global, also scripted through AMPtk (Palmer, 2016). Sequence reads were submitted to GenBank and archived with the National Center for Biotechnology Information, accession SRP154885.

3.2.4. Fungal biodiversity and community analyses

Fungal community analyses were conducted using R Statistical Software (R Core Team, 2015), the vegan package (Oksanen et al., 2018) and phyloseq (McMurdie and Holmes, 2013). Species richness was assessed relative to site, biomass treatment, soil amendment treatments, and sampling method with an analysis of variance (ANOVA) test. In comparing the two fungal sampling methods, a nonparametric permutational multivariate ANOVA (PERMANOVA) test (Anderson, 2001) was performed by the adonis function in the vegan package of R to test for fungal community differences relative to site, biomass treatment, and soil amendment treatments. Multivariate dispersion, or variation between groups (Anderson et al., 2006), was tested using the betadisper function in the Vegan package of R to ensure significant treatment effects were not due to differences in community dispersion.

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3.3.1. *Fruiting body surveys*

The above-ground fruiting body surveys yielded over 1,000 observations over 3 years. An observation was defined as the occurrence of a fruiting body, greater than 1 cm^2, on a substrate. Altogether, 129 mostly basidiomycete fungal species were identified fruiting in the fall, with 22 species that did not match known species descriptions. The 15 most common species identified with fruiting body surveys are reported in Table 3.1. Fruiting bodies were collected from many different substrates, from twigs to small- and large-diameter logs, to

stumps. Those substrates were also in various stages of decay, from recently killed wood through humus, with over 85% of species found on substrates in decay classes 2 and 3 (Maser et al., 1979). Fruiting bodies of fungi that do not appear to deeply penetrate substrates (e.g., *Athelia spp*., Eriksson and Ryvarden, 1973) were found alongside forest pathogens that colonize extensively through wood to cause root or heart rots (e.g *Armillaria ostoyae* [Romagn.] Herink, *Phaeolus schweinitzii* [Fr.] Pat., and *Heterobasidion occidentale* Otrosina & Garbel.). The fruiting body surveys identified a few species that may be rare in the intermountain west including *Trechispora farinacea* (Pers.) Liberta, *Leptosporomyces septentrionalis* (J. Erikss.) Krieglst., and *Phlebia centrifuga* P. Karst, which were only found on well-decayed wood substrates. Additionally, *Phaeolus schweinitzii* and *Antrodia carbonica* (Overh.) Ryvarden were only found on large diameter logs, of which there were few on the plots.

The fruiting body surveys revealed significant biomass treatment effects only in the first year (Table 3.2: PERMANOVA, $p < 0.003$). During the fall 2014, fruiting body-based fungal community composition differed significantly as a function of biomass treatment (PERMANOVA, $p < 0.01$), with a significant difference in betadispersion ($F = 3.56$, $p = 0.03$). There was a significant difference in dispersion between the fungal community composition present at the 2X and 1X biomass treatments (Tukey HSD, $p.adj = 0.016$). Total species richness for 2014 was 59 fungal species, with no significant differences in richness found due to biomass treatments. In the third year, biomass treatment was a moderately significant factor influencing the 2016 fungal community composition (PERMANOVA, $p = 0.059$), with no significant differences in betadispersion ($p = 0.59$). Fruiting body surveys in fall 2016 yielded no significant differences in fungal richness due to biomass treatment, with 90 fungal species observed that year overall.

The effects of soil amendments were less recognizable with fruiting body surveys. Only in the second year (fall 2015) did soil amendments appear to influence the fungal community composition (PERMANOVA, $p = 0.064$), with a significant difference in community dispersion (betadisper, $F_{3,28} = 4.91$, $p = 0.007$). The beta dispersion of fungal community compositions relative to fertilized soil amendments was significantly different from both the biochar treatments (Tukey HSD, *p.adj.* = 0.011), as well as the non-amended treatments (*p.adj* = 0.02).

3.3.2. *Fungal DNA assessments of pine stakes*

The fungal DNA extracted from pine stakes revealed 2,316 different operational taxonomic units (OTUs), of mostly microscopic ascomycete species, the majority of which could not be assigned to a particular species. The 15 most common OTUs identified with DNA assessments of drilled pine stakes are reported in Table 3.1.

Biomass treatment effects obtained using the surface pine stakes were significant each year (Table 3.2: PERMANOVA, $p < 0.035$). In the first spring after plot establishment (2015), the pine stakes had significant differences in fungal community composition due to biomass treatments (PERMANOVA, $p < 0.035$), with no significant differences in betadispersion ($p > 0.1$). The biomass treatments had minimal differences in mean OTU richness, ranging from 106 - 110 (ANOVA, $p > 0.9$). Again in the second year, the pine stakes (spring 2016) showed significant differences in fungal community composition due to biomass treatment (PERMANOVA, $p = 0.035$), with significant differences in beta dispersion (betadisper, F = 9.54, $p < 0.001$). The 2X plots had significantly different dispersion from 0X plots (Tukey HSD, *p.adj* = 0.026), non-thinned plots had significantly different dispersion from 0X plots (*p.adj* = 0.025),

2X plots had significantly different dispersion from 1X plots (*p.adj* < 0.001), and non-thinned plots had significantly different dispersion from 1X plots (*p.adj* < 0.001). In the second year, the mean OTU richness due to biomass treatment ranged from 81 for 1X plots to 95 in non-thinned plots, with no significant differences in richness. In the third year, wood stake analyses in the spring of 2017 also showed that fungal community composition was significantly influenced by biomass treatments (PERMANOVA, $p < 0.007$). The differences due to biomass treatment ranged from 72 OTUs on 1X plots to 84 OTUs on 2X plots, again with no significant differences in OTU richness.

Similar to the results of biomass treatments, the fungal community composition within drilled pine stakes reflected significant effects of soil amendment treatment in the first (2015) and third year (2017) assessments (Table 3.2: PERMANOVA, $p < 0.05$), with no significant differences due to betadispersion ($p > 0.1$). Though there was no significant difference in OTU richness due to soil amendments in the first year (ANOVA, $p = 0.162$), mean OTU richness from soil amendment treatment plots varied from 88 OTUs on the biochar plots to 125 OTUs on the fertilizer+biochar plots. During the third year, the mean richness between soil amendments ranged from 76 OTUs with the fertilizer+biochar to 85 OTUs with fertilizer plots, with no significant differences due to soil amendment in that year (ANOVA, $p = 0.75$).

3.3.3. *Sampling differences*

The two sampling methods revealed different fungal communities. Notably, there were significant differences in species richness relative to the two sampling methods, where drilled pine stakes sampling produced more sequence data and associated OTUs than fruiting body

surveys (Fig. 3.1, ANOVA, $F_{1,188} = 999.68$, $p < 0.001$). Sequencing of fungal DNA from wood stakes revealed an average of 86 more OTUs than fruiting body surveys per plot (Tukey HSD, $p < 0.001$). The 15 most common species identified using both methods are reported in Table 3.1. The 262 fruiting bodies from which DNA was obtained and sequenced represented ~ 60% of total fungal fruiting body community. Yet only 23 OTUs, or 1% of the 2,333 different fungal OTUs identified from pine stakes matched the DNA from these fruiting bodies to 97% similarity. The overlapping fungal OTUs identified using both fruiting body surveys and wood stake methods are listed in Table 3.3

The DNA assessments of drilled pine stakes consistently revealed more significant differences in fungal community composition due to biomass treatments and soil amendments. Whereas fruiting body surveys only found significant differences due to biomass treatments in the first and third years, the DNA assessments of pine stakes found significant differences due to biomass treatments all three years (Table 3.2). Likewise, although fruiting body survey results did not indicate a significant difference in fungal community composition due to soil amendment treatments, DNA assessments of drilled pine stakes demonstrated significant differences due to soil amendment treatments in the first and third years (Table 3.2).

3.4 Discussion

3.4.1. *Overlapping fungal communities*

Differences in fungal communities revealed using these two methods are consistent with previous investigations in other forest ecosystems. Rajala et al. (2012) conducted polypore

fruiting body surveys on logs from a semi-natural forest in southern Finland and then analyzed the fungal DNA in samples obtained by drilling those logs. In that study, only one overlapping species was detected using both methods. Gardes and Bruns (1996) also attempted to correspond the above-and below-ground ectomycorrhizal community using fruiting body surveys and molecular methods (sequencing the ITS region and use of restriction enzymes). Both studies concluded that there was no correspondence between number of species found as fruiting bodies and OTUs identified via molecular techniques (Gardes and Bruns, 1996; Rajala et al. 2012). In the current study, some fruiting bodies did not produce a usable sequence, and some fruiting body tissue samples amplified DNA from microscopic fungal contaminants or antagonists (e.g. *Trichoderma spp*.) as opposed to DNA from the fruiting body. There was not a complete set of ITS sequences representing all fungal fruiting bodies for comparison, which reduced the number of possible fungal OTUs in common. Not all fungi produce a usable ITS sequence; this result is universal and not limited to this study.

3.4.2. *Molecular identification of species assemblages*

The use of the standardized wood stake protocol was beneficial in that it produced a dataset for comparing results with those of other studies. While DNA analyses of drilled pine stakes produced more OTUs, the pine stakes themselves are a relatively homogeneous substrate. Specifically, the kiln-dried pine stakes represent small diameter, freshly killed, decorticated wood substrates. The pine stakes did not typically rot past decay stage 3 in the 3-year study. Therefore, the species assemblage list from wood stake analyses represents only one type of wood substrate.

Though many scientists are already familiar with the tools and procedures of DNA extraction, amplification, and even next-generation sequencing (Baldrian et al., 2012; Lindahl et al., 2007; O'Brien et al., 2005), the DNA-based survey produced many OTUs that were not known at the species level. This result is consistent with those from other HTAS studies of fungal communities of coniferous forests, with a large proportion of the sequences not identified to the species level (Baldrian et al., 2012; Lindahl et al., 2007; O'Brien et al., 2005). Rajala et al. (2012) explain that the fungal identities are not known because the taxon has not yet been described. Although many researchers have the ability to produce sequences, there is often a lack of nomenclatural expertise needed to assign formal Latin names to unidentified sequences. Additionally, many fungi that are microscopic or hard to culture have been overlooked in an organismal size bias. Therefore, because they are bound by granting agencies to publish their findings, they have no choice but to publish as "Unknown fungus 1" (Rajala et al., 2012).

High-throughput amplification of the fungal ITS region produces a large amount of data, which must be carefully assessed relative to evaluations of abundance and rare species. It is already known that DNA extraction efficiencies differ for different taxa and/or cell types (i.e. hyphae, spores, fruiting bodies, etc.) (Vesty et al., 2017). PCR biases are already known to exist (Bidartondo and Gardes, 2007; Palmer et al. 2018), and a single isolate may have multiple ITS sequences due to intragenomic variability (Lindner and Banik, 2011). So taxa that appear to be rare within these datasets may just be a result of poor extraction and amplification. The use of a "biological mock" community composed of fungal DNA from fruiting bodies, spores, and/or hyphae provides a measure of success of extraction, PCR and sequencing; in addition, the use of a spike-in "synthetic mock" community allows for the comparison of design, processing, and results between sequencing runs (Palmer et al., 2018). Both types of mock communities

(biologically based and synthetic) were utilized in this study, thus providing a check for both community composition and experimental process. Because these mock communities were used only as a control or processing check, their community results were omitted. With these controls, the DNA extractions from pine stakes produced more sequence data and associated OTUs than fruiting body surveys, which provided more statistical power to discern effects of biomass and soil amendment manipulations.

3.4.3. *Fruiting body surveys*

Fruiting body surveys yield a list of species, whereas molecular studies create a list of OTUs (Schmit and Lodge, 2005). Although the taxonomy of different fungal groups continues to change due to molecular methods, a species assemblage list can be useful to scientists that recognize family trends (e.g. brown rot vs. white rot). Unfortunately, it is difficult to become proficient in mycological taxonomy because the groupings are now based on a combination of macroscopic characters, microscopic morphological differences, and DNA sequences. In the past, all polypores used to be grouped into three main genera (*Fomes*, *Polyporous*, and *Poria*), while currently there are now over 100 genera of polyporoid fungi (Volk, 2000). Therefore, for successful fruiting body survey analyses, a great deal of specialization, knowledge, and training is required.

Fruiting body surveys, which include collection of specimens that are deposited in mycological herbaria, provide materials that subsequent researchers can reference in both taxonomic and habitat studies. For example, a collection of a *Rhizochaete* species allowed Nakasone et al. (2017) to circumscribe and reach new conclusions about the genus by adding

new species and detailing the extent of the genus. Collectors of fruiting bodies also record substrates, portions of which are often physically attached and provide valuable information. For example, *Athelia psychrophila* (Stalpers and R.P. de Vries) P.M. Kirk has been detected by sequencing DNA extracted from asexual mycelia on decaying carrots (de Vries et al., 2008), and also apples and pears (Wenneker et al., 2017). But because the species description is based upon a preserved sexual fruiting body (a thin crust) of *Athelia psychrophila* collected from a decaying conifer log, very different and valuable natural substrate and ecosystem habitat information was known. Similarly, *A. psychrophila* was found during the current study growing on conifer logs >13 cm, of decay class 2 and 3. Fruiting bodies with substrate data and associative molecular data from this study are an important contribution to support future research both within and beyond the forest ecosystems (Glaeser et al., 2013).

Fruiting body surveys can be used to identify rare species, and rare species are likely to be found associated with rare substrates. Similar to the results of Blaser et al. (2013), the fruiting bodies encountered in the current study were not uniformly distributed among different decay classes, with over 85% of species found on downed woody material in decay class 2 and 3. Forest management practices have been known to reduce the quantity and change the quality of wood substrates, and therefore large-diameter, highly decomposed wood substrates are largely reduced (Bader et al., 1995; Lonsdale et al., 2007). In this study, *Phlebia centrifuga* was identified as a rare species, found only on large-diameter logs. This species was also identified as a "rare" and "sensitive species" in Norway, due to its preference for large diameter logs, which are reduced due to logging and forest management practices (Stokland and Larsson, 2011). Conversely, the fungus *Leptosporomyces septentrionalis* (found within this study) was also identified as a "rare" and "sensitive species" in Norway, though due to its preference for

well decayed wood substrates (Stokland and Larsson, 2011). Though no official red list for American fungi currently exists, as it does in Europe, unofficial red lists for America have been proposed (Brazee et al., 2012). The benefit of sampling whole ecosystems is that research is not limited to the chosen "bait" substrates. Because there was no standardization of the wood substrates that were on the plots at study inception, apart from the biomass treatment manipulations of quantity, this likely led to a high degree of variance in quality of substrates among plots.

In the current study, fruiting body surveys also identified important plant and forest pathogens. Interestingly, important root and butt pathogens like *Armillaria ostoyae, Heterobasidion occidentale,* and *Phaeolus schweinitzii,* were only observed with fruiting body surveys. *Stereum sanguinolentum,* a primary colonizing heart rot fungus, was the only species of plant pathogen observed with both fruiting body surveys and wood stake sequencing with 97% sequence similarity.

3.4.4. *Benefits of both methods of fungal community assessment*

While both methods of fungal community assessment were able to discern effects of biomass treatment, and to some degree soil amendments, they told two separate stories. The fruiting body surveys identified macroscopic fungi related to mycorrhizal symbionts, forest pathogens, and saprophytic decay species. The DNA-based analyses identified mostly microscopic fungi that are mainly known to inhabit dead and decaying wood. Additionally, the differences in results may be interesting to different groups of researchers. This research suggests that the methods work together to tell a more complete story of fungal colonization

within forest ecosystems. Largely, the type of survey conducted by future researchers will be determined by the researcher's tools, budget, expertise, and the research questions to be addressed.

Fruiting body surveys are cheaper, can identify rare or endangered fungal species, and were most successful in identifying important forest pathogens. Because fruiting body surveys utilize basic tools comprising microscopic analyses, taxonomic guides, and Sanger sequencing (Mueller et al., 2004), the costs tend to be reduced, though the researcher's time can be extensive. As fruiting bodies represent the stage of sexual recombination and reproduction, fruiting bodies can also yield information about both fungal conservation and genetic differences within an environment (Rayner and Boddy, 1988). However, fruiting body surveys require a tremendous amount of field and microscopy experience for successful completion, and use of statistical analyses to determine treatment effects with fruiting body datasets tends to be more difficult.

Although more expensive (Kircher and Kelso, 2010; Peay et al., 2008), the benefit inherent in use of molecular techniques is that many researchers can perform the associated laboratory procedures to rapidly produce reliable data. Performing DNA extractions and sequencing reactions requires less field experience; therefore, this method may be preferable to ecologists with little mycological field experience. Additionally, soil scientists concerned with nutrient turnover may be less concerned with fruiting body surveys and may value the ease with which molecular data can provide many OTUs. The DNA analyses of wood stakes was more sensitive to treatments, especially the soil amendments, yielding greater statistical power to answer complex questions.

Therefore, depending on the study and the questions being answered, fruiting bodies may be useful in some situations while molecular techniques may be useful in other contexts. For example, forest managers with limited budgets may interested in identifying the forest diseases, and therefore fruiting body surveys may be most appropriate. In contrast, research scientists looking for sensitive microbial responses to treatments may be more interested in the utility of molecular techniques. The explosion of DNA techniques has increased the number of tools in the "toolbox"; therefore, ecologists are now capable to answering complex questions that may extend beyond the realm of their original training. However, fruiting body surveys yield a great deal of information, and this work indicates that fruiting body surveys should not be ignored simply because of the ease and speed with which HTAS data can be produced.

3.5 References

Anderson IC, Cairney JWG. 2004. Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. Environmental Microbiology 6: 769–79.

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32–46.

Anderson MJ, Ellingsen KE, McArdle BH. 2006. Multivariate dispersion as a measure of beta diversity. Ecology Letters 9: 683–93.

Arno SF. 2000. Fire in Western Forest Ecosystems, in: Brown JK, Smith JK, eds., Wildland Fire in Ecosystems: Effects of Fire on Flora. USDA Forest Service Gen. Tech.Rep. RMRS-FTR-42-Vol. 2: 97–120.

Bader P, Jansson S, Jonsson BG. 1995. Wood-inhabiting fungi and substratum decline in selectively logged boreal spruce forests. Biological Conservation 72: 355–362.

Baldrian P, Kolarik M, Stursova M, Kopecky J, Valaskova V, Vetrovsky T, Zifcakova L, Snajdr J, Ridl J, Vlcek C, Voriskova, J, 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. International Society for Microbial Ecology 6: 248-258.

Berbee ML, Taylor JW. 2001. Fungal Molecular Evolution: Gene Trees and Geologic Time. In: Esser K, Lemke PA, eds. The Mycota: A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research, VII: Systematics and Evolution, Part B. Springer, p. 229–245.

Bernicchia A, Perez Gorjon S, Arras L. 2010. Corticiaceae s.l.: 427 colour potos and 455 drawings of microscopical elements. Alassio, Candusso.

Bidartondo JI, Gardes M., 2007. In: Dighton J, White JF, Oudemans P, eds. The fungal community: its organization and role in the ecosystem. New York, NY: Taylor & Francis Group. p. 215-239.

Blaalid R, Kumar S, Nilsson RH, Abarenkov K, Kirk PM, Kauserud H, 2013. ITS1 versus ITS2 as DNA metabarcodes for fungi. Molecular Ecology Resources 13 (2): 218-224.

Brazee NJ, Lindner DL, Fraver S, D’Amato AW, Milo AM, 2012. Wood-inhabiting, polyporoid fungi in aspen-dominated forests managed for biomass in the U.S. Lake States. Fungal Ecology 5: 600-609.

Bridge P, Spooner B. 2001. Soil fungi: diversity and detection. Plant and Soil 232: 147–154.

Bruns, T.D., White, T.J., Taylor, J.W., 1991. Fungal molecular systematics. Annual Review of Ecology, Evolution and Systematics 22: 525–564.

Chase JM, Kraft NJB, Smith KG, Vellend M, Inouye BD. 2011. Using null models to disentangle variation in community dissimilarity from variation in α-diversity. Ecosphere 2, 1-11.

Coleman M, Page-Dumroese D, Archuleta J, Badger P, Chung W, Venn T, Loeffler D, Jones G, McElligott K. 2010. Can portable pyrolysis units make biomass utilization affordable while using bio-char to enhance soil productivity and sequester carbon?, In: Jain TB, Graham RT, Sandquist, J, eds. Integrated management of carbon sequestration and biomass utilization opportunities in a changing climate: Proceedings of the 2009 National Silviculture Workshop; 2009 June 15-18; Boise, ID. Proceedings RMRS-P-61. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, p. 159-168.

Cooke RC, Rayner ADM. 1984. Ecology of saprotrophic fungi. Longman Group Limited, New York.

Cooper SV, Neiman KE, Roberts DW. 1991. Forest habitat types of Northern Idaho: A Second approximation. General Technical Report INT-236. Ogden, UT: U.S. Department of Agriculture, Forest Service. Intermountain Research Station, p. 1-152.

Ekblom R, Galindo J. 2011. Applications of next generation sequencing in molecular ecology of non-model organisms. Heredity (Edinb). 107: 1–15.

Eriksson J, Ryvarden L. 1973. The Corticiaceae of North Europe, Volume 2. Fungiflora, Oslow, Norway, p. 96-98.

Ferris R, Humphrey JW. 1999. A review of potential biodiversity indicators for application in British forests. Forestry 72: 314–328.

Fox TR. 2000. Sustained productivity in intensively managed forest plantations. Forest Ecology and Management 138: 187–202.

Gardener, M., 2014. Community ecology: analytical methods using R and Excel. Pelagic Publishing, Exeter, UK.

Gardes M, Bruns T, 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118.

Gardes M, Bruns TD, 1996. Community structure of ectomycorrhizal fungi in a Pinus muricata forest: above- and below-ground views. Canadian Journal of Botany 74: 1572-1583.

Gilbertson RL, Ryvarden L, 1986. North American Polypores, Volumes 1-2. Fungiflora, Oslow, Norway.

Glaeser JA, Nakasone KK, Lodge DJ, Ortiz-Santana B, Lindner DL, 2013. The culture collection and herbarium of the Center for Forest Mycology Research: A national Resource. In: Browning, J, Palacios, P. (comps.) Proceedings of the Western International Forest Disease Work Conference: 2012 October 8-12, 2012. Tahoe City, CA, p. 123-129. Accessed (16 May 2018) https://www.fs.fed.us/nrs/pubs/jrnl/2013/nrs_2013_glaeser_002.pdf

Hammell KE. 1997. Fungal Degradation of Lignin. In: Cadisch G, Giller KE, eds. Driven by Nature: Plant Litter Quality and Decomposition. CAB International, Wallingford: 33-45.

Hjorstam K, Larsson KH, Ryvarden L. 1987. The Corticiaceae of North Europe Volumes 1-8. Fungiflora, Oslow, Norway.

Ihrmark K, Bödeker ITM, Cruz-Martinez K, Friberg H, Kubartova A, Schenck J, Strid Y, Stenlid J, Brandström-Durling M, Clemmensen KE, Lindahl BD. 2012. New primers to amplify the fungal ITS2 region--evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiology Ecology 82: 666–77.

Jurgensen M, Reed D, Page-Dumroese D, Laks P, Collins A, Mroz G, Degórski M. 2006. Wood strength loss as a measure of decomposition in northern forest mineral soil. European Journal of Soil Biology 42: 23–31.

Keane RE, Ryan KC, Veblen TT, Allen CD, Logan J, Hawkes B. 2002. Cascading effects of fire exclusion in the Rocky Mountain ecosystems: a literature review. General Technical Report. RMRS-GTR-91. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, p. 1-24.

Keyes C, O'Hara K. 2002. Quantifying stand targets for silvicultural prevention of crown fires. Western Journal of Applied Forestry 17: 101–109.

Kimmins JP (H). 1997. Biodiversity and its relationship to ecosystem health and integrity. The Forestry Chronicle 73: 229-232.

Kircher M, Kelso J. 2010. High-throughput DNA sequencing – concepts and limitations. Bioessays 32: 524-536.

Kirk PM, 2014. Nomenclature Novelties. Index Fungorum 120: 1. Accessed (22 May 2018) http://www.indexfungorum.org/Publications/Index%20Fungorum%20no.120.pdf

Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Hogberg P, Stenlid J, Finlay, RD, 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New phytologist 173: 611-620.

Mueller GM, Schmit JP, Huhndorf SM, Vyvarden L, O'Dell TE, Lodge DJ, Leacock PR, Mata M, Umana L, Wu Q, Czederpiltz DL. 2004. Recommended Protocols for Sampling Macrofungi. In: Mueller GM, Bills GF, Foster MS, eds. Biodiversity of Fungi: Inventory and Monitoring Methods. Elsevier, p. 168–172.

Nordén B, Ryberg M, Götmark F, Olausson B. 2004. Relative importance of coarse and fine woody debris for the diversity of wood-inhabiting fungi in temperate broadleaf forests. Biological Conservation 117: 1–10.

Norton G, Abraham S, Veneman A. 2003. Memorandum of understanding on policy principles for woody biomass utilization for restoration and fuel treatments on forests, woodlands, and rangelands. United States Department of Agriculture, United States Department of energy, and the United States Department of the Interior. https://www.fs.fed.us/woodybiomass/documents/BiomassMOU_060303_final_web.pdf (accessed 22 February 2018).

O'Brien HE, Parrent JL, Jackson JA, Moncalvo JM, Vilgalys R, 2005. Fungal community analysis by large-scale sequencing of environmental samples. Applied and Environmental Microbiology 71 (9): 5544-5550.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H. 2018. Package 'vegan. https://cran.r-project.org/web/packages/vegan/vegan.pdf (accessed 7 March 2018).

Page-Dumrose DS, Coleman MD, Thomas SC. 2016. Opportunities and uses of biochar on forest sites in North America. In: Bruckman VJ, Varol EA, Uzun BB, Liu J, eds. Biochar: A regional supply chain approach in view of climate change mitigation., Cambridge University Press, p. 315-335.

Palmer J. 2017. AMPtk: Amplicon Toolkit for NGS data. https://github.com/nextgenusfs/amptk (accessed 7 March 2018).

Palmer, JM, Jusino MA, Banik MT, Lindner DL. 2018. Non-biological synthetic spike-in controls and the AMPtk software pipeline improve mycobiome data. PeerJ 6:e4925. https://doi.org/10.7717/peerj.4925

Peay KG, Kennedy PG, Bruns TD. 2008. Fungal Community Ecology. Bioscience 58: 799–810.

Purahong W, Kahl T, Schloter M, Bauhus J, Buscot F, Kruger D. 2014. Comparing fungal richness and community composition in coarse woody debris in Central European beech forests under three types of management. Mycological Progress 13: 959–964.

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna Austria, https://www.R-project.org/ (accessed 7 March 2018).

Rajala T, Peltoniemi M, Pennanen T, Makipaa R 2012. Fungal community dynamics in relation to substrate quality of decaying Norway Spruce (Picea abies [L.Karst.) logs in boreal forests. FEMS Microbiology Ecology 81: 494-505.

Rayner ADM, Boddy L. 1988. Fungal Decomposition of Wood: Its Biology and Ecology. John Wiley & Sons Ltd., New York.

Rydin H, Diekmann M, Hallingbäck T. 1997. Biological characteristics, habitat associations, and distribution of macrofungi in Sweden. Conservation Biology 11: 628–640.

Schäffer A, Aravind L. 2001. Improving the accuracy of PSI-BLAST protein database searches with composition-based statistics and other refinements. Nucleic Acids Research 29: 2994–3005.

Schmidt JP, Lodge DJ. 2007. Classical methods and modern analysis for studying fungal diversity. In: Dighton J, White JF, Oudemans P, eds. The fungal community: its organization and role in the ecosystem. New York, NY: Taylor & Francis Group. p.193-214.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academies of Sciences U. S. A. 109, 6241–6246.

Shannon CE, Weaver W. 1949. The mathematical theory of communication. University of Illinois Press, Urbana.

Smith D, Onions AHS. 1994. The preservation and maintenance of living fungi, 2nd ed. CAB International, England.

Stokland J, Siitonen J, Gunnar Jonsson B, 2012. Biodiversity in Dead Wood. Cambridge University Press, New York.

Stokland JN, Larsson K-H, 2011. Legacies from natural forest dynamics: Different effects of forest management on wood-inhabiting fungi in pine and spruce forests. Forest Ecology and Management 261(11): 1707-1720.

USDA Forest Service. 2013. Woody Biomass Utilization. http://www.fs.fed.us/woodybiomass/whatis.shtml (accessed 22 February 2018).

U.S. Department of Energy, Perlack RD, Stokes BJ. 2011. U.S. Billion-Ton Update: Biomass Supply for a Bioenergy and Bioproducts Industry, ORNL/TM-2011/224, Oak Ridge Natl. Lab. Oak Ridge, TN. p. 1–227. https://www1.eere.energy.gov/bioenergy/pdfs/billion_ton_update.pdf (accessed 22 February 2018).

Vesty A, Biswas K, Taylor MW, Gear K, Douglas RG. 2017. Evaluating the impact of DNA extraction method on the representation of human oral bacterial and fungal communities. PLOS ONE 12 (1): e0169877.

Volk T. 2000. Polypore primer: an introduction to the characters used to identify poroid wood decay fungi. McIlvainea 14 (2): 74-82.

Table 3.1. Fifteen most abundant fungal operational taxonomic units (OTUs) identified through fruiting body surveys and high-throughput sequencing of extracted DNA from drilled pine stakes, from plots at the University of Idaho Experimental Forest over 3 years of data collection (fall 2014- spring 2017). The third column shows the top OTUs solely from the phylum Basidiomycota. Taxonomy was assigned with 97% similarity threshold, and some OTUs could only be confidently assigned to higher order taxonomic ranks.

OTUs of fruiting body surveys	**OTUs of pine stakes**	**Basidiomycete OTUs of pine stakes**
Trichaptum abietinum	*Sydowia polyspora*	*Xeromphalina setulipes*
Coniophora arida	*Coniochaeta*^{G}	*Hypochnicium geogenium*
Athelia bombacina	*Coniochaeta*^{G}	*Mycena*^{G}
Stereum sanguinolentum	*Coniochaeta*^{G}	*Mycena robusta*
Acanthophysellum lividocoeruleum	*Meliniomyces*^{G}	*Pholiota*^{G}
Hyphoderma setigerum	*Collophora*^{G}	*Coniophora arida*
Gloeophyllum sp.1	*Ascomycota*^{P}	*Sphaerobolus stellatus*
Tubulicrinis glebulosus	*Xeromphalina setulipes*	*Pholiota*^{G}
Dacrymyces chrysospermus	*Hypochnicium geogenium*	*Mycena*^{G}
Cryptoporus volvatus	*Helotiales*^{O}	*Mycena clavicularis*
Hypochnicium albostramineum	*Mycena*^{G}	*Mycena*^{G}
Cinereomyces lindbladii	*Mycena robusta*	*Corticiaceae*^{F}
Athelia psychrophila	*Pholiota*^{G}	*Sebacinales*^{O}
Botryobasidium subcoronatum	*Coniophora arida*^{G}	*Xylodon borealis*
Amphinema byssoides	*Scleroconidioma*^{G}	*Mycena*^{G}

^{G} Closest matching taxonomy to Genus
^{O} Closest matching taxonomy to Order
^{F} Closest matching taxonomy to Family
^{P} Closest matching taxonomy to Phylum

Table 3.2. Results of permutational multivariate analysis of variance (PERMANOVA) comparing fruiting body fungal community composition (in fall of 2014, 2015, and 2016) with the operational taxonomic unit (OTU) community composition identified through DNA analyses of drilled pine stakes (retrieved in the spring of 2015, 2016, and 2017) at the University of Idaho Experimental Forest over three years of data collection. Fungal community composition was assessed relative to site (UIPP, UIMC), biomass treatments (0X, 1X, 2X biomass retained, non-thinned control), and soil amendments (biochar, fertilizer+biochar, fertilizer, non-amended control).

	Fruiting Bodies				Drilled Pine Stakes				
	DF	F.Model	R^2	*p*		*DF*	F.Model	R^2	*p*
2014					*2015*				
Site	1,24	2.37	0.06	0.014*		1,24	27.02	0.211	0.002
Biomass Treatment	3,24	2.35	0.19	0.003*		3,24	11.01	0.259	0.003
Soil Amendment	3,24	1.18	0.09	0.301		3,24	14.58	0.342	0.003
2015					*2016*				
Site	1,24	3.98	0.11	0.067		1,24	3.69	0.075	0.028
Biomass Treatment	3,24	1.32	0.11	0.213		3,24	4.32	0.261	0.035
Soil Amendment	3,24	1.68	0.13	0.064		3,24	2.94	0.018	0.094
2016					*2017*				
Site	1,24	3.97	0.11	0.212		1,24	3.96	0.22	0.025
Biomass Treatment	3,24	2.12	0.17	0.059		3,24	5.60	0.31	0.007
Soil Amendment	3,24	0.71	0.06	0.675		3,24	3.13	0.06	0.034

Table 3.3. Fungal operational taxonomic units (OTUs) that were identified within both the fruiting body surveys and wood stake analyses conducted from the University of Idaho Experimental Forest over three years of data collection (fall 2014 - spring 2017). Of the 2,333 OTU sequences found within the wood stake data, only 23 or 1% were found within the fruiting body sequences.

Wood Stake OTUs	Fruiting Body Sequences
OTU559	*Athelia_bombacina*_DK15_10
OTU497	*Botryobasidium_sp.*1_DK16_17
OTU2217	*Botryobasidium_subcoronatum*_DK14_125
OTU529	*Botryobasidium_subcoronatum*_DK14_160
OTU951	*Botyryobasidium_tubulicystidium*_DK14_139
OTU139	*Ceratobasidium_sp.*1_DK14_105
OTU1945	*Ceratobasidium_sp.*1_DK16_233
OTU296	*Ceratobasidium_sp.*1_DK16_404
OTU1019	*Chaetodermella_luna*_DK15_19
OTU27	*Coniophora_arida*_DK14_102
OTU1778	*Dichomitus_squalens*_DK16_304
OTU1272	*Hyphoderma_setigerum*_DK15_32
OTU62	*Hypochnicium_albostramineum*_DK16_339
OTU193	*Hypochnicium_sphaerosporum*_DK16_256B
OTU2189	*Kneifiella_sp.*1_DK16_226
OTU151	*Leptosporomyces_sp.*2_DK14_157
OTU729	*Peniophorella_praetermissa*_DK14_90
OTU1766	*Peniophorella_pubra*_DK16_286
OTU100	*Phanerochaete_sordida*_DK16_198
OTU998	*Pluteus_brunneidiscus*_DK16_242
OTU11	*Scytinostroma_sp.*1_DK14_195
OTU1157	*Scytinostroma_sp.*1_DK14_195
OTU833	*Stereum_sanguinolentum*_DK14_205

Fig. 3.1. Box plots showing operational taxonomic unit (OTU) richness differences between sampling methods per plot (fruiting body surveys vs. sequencing fungal DNA extracted from drilled pine wood stakes) from plots on the University of Idaho Experimental Forest over three years of data collection (fall 2014 - spring 2017). ANOVA of OTU richness found a significant difference between sampling methodologies ($F=999.68$, $p < 0.001$). Sequencing of drilled wood stakes found an average of 86 more OTUs than fruiting body surveys (Tukey HSD, $p < 0.001$).

Concluding Remarks

Without frequent forest fires, forest management that includes selective thinning is an important source of disturbance in the intermountain western United States. The sustainability of biomass harvesting for biofuels will depend on cooperative studies that assess effects on the forest ecosystem as a whole. Because many types of fungi (whether saprophytic, mutualistic, or somewhere in between) depend on wood substrates for growth and reproduction, it is important to consider whether those species will be affected by management practices that may alter the quantity and quality of wood substrates. Fungal sampling for sporocarps yielded information relative to colonization of all woody debris types on the plots, in various stages of decomposition, as opposed to high-throughput sequencing of drilled wood stakes which yielded information relative to the fungal colonization of one type of woody debris.

Analysis of fungal DNA obtained from drilled wood stakes resulted in greater operational taxonomic unit (OTU) richness to assess treatment effects. The future of high-throughput sequencing of mixed communities remains strong because of availability of sequencing technologies and the potential to observe statistically significant differences. Simply, DNA assessment of drilled wood stakes provided an enormous amount of sequence data from the uniform, wood stake substrates. At the time of first retrieval of wood stakes, the fungal community composition reflected effects of both biomass treatment and soil amendment. And because the use of wood stakes has been used primarily to assess decomposition over time is standardized methodology, this data can be used comparatively against other future studies. However, those effects were difficult to discern because the majority of identified OTUs were Ascomycete fungi, for which there is little taxonomic information beyond the level of order.

Further, because the wood stakes represent small diameter, bark-less, freshly killed wood substrates, fungi that would colonize large diameter and well-decomposed substrates would not be expected to be present.

Sporocarp surveys provided the most information about wood substrates on the plots, because the whole plots were sampled. All wood substrates, from small diameter twigs to large diameter logs, were assessed for fungal sporocarps, providing a dataset that encompassed mycorrhizal species through plant pathogens. Because some wood substrates tended to be rarer on the plots, the fungal species that were present on those substrates also tended to be rare within this study. The rare species identified on well decomposed wood substrates included saprophytic fungi such as *Trechispora farinacea* (Pers.) Liberta and *Phlebia centrifuga* P. Karst. Additionally, *Phaeolus schweinitzii* (Fr.) Pat., an important forest pathogen, and *Antrodia carbonica* (Overh.) Ryvarden, a brown rot fungus, were only found on freshly killed large diameter logs, of which there were few on the plots. The sampling methods were designed primarily to measure diversity of wood-inhabiting fungi at the stand level, and future studies would be necessary to address sampling for individual species that occur very infrequently at the landscape level. Sampling efforts that assess a specific number of large diameter logs at specific levels of decomposition would greatly reduce data variability and provide a more thorough assessment, though that sampling design would be more difficult if that range of substrates were not readily available. Future sampling would further establish whether certain species are in fact rare, or just under-sampled. Because fungal assessments overall are rare in this location, this research provides a baseline of data.

This research has highlighted the benefit of fungal diversity assays as a tool to assess effects of forest management practices and make predictions about the health of future forests.

Many of the most important fungal pathogens can be easily identified due to the visibility and distinct morphologies of their sporocarps. For example, fruiting bodies of important root disease fungi in *Armillaria* are mushrooms; those in *Heterobasidion* are conks. These specific fungi represent diseases of site that are essentially permanent; therefore, forest managers having familiarity with fungi and their diverse roles will have more predictive insight into the next-generation forests than those who do not.

Using the University of Idaho Experimental Forest study site as an example, forest managers could make a number of inferences simply from the presence of *Phaeolus schweinitzii*. First, the easily-recognizable and characteristically brown-lobed polyporous fruiting body indicates the presence of a brown-rotting, heart- and butt- rotting fungus, common in old-growth timber (Sinclair et al., 1987). GPS tagging of the tree/stump/log where the fruiting body was found would allow researchers to track the progress of decay within the wood, as well as inform artisans where to collect sources of natural dyes (Aurora, 1991). Brown-rotted wood contains high concentrations of recalcitrant lignins (Rayner and Boddy, 1988) and has important implications for long term carbon sequestration, similar to biochar. Therefore, tagging of the wood substrates would allow researchers to assess how wood decomposes into soil and specifically test whether brown rotted wood leads to greater carbon sequestration. Best management practices include the retention of coarse woody debris in forest landscapes, and selection of the wood materials for removal should not be random. By selecting a diseased tree as a legacy tree, based on observations of fruiting bodies of heart/butt-rot pathogenic fungi, a forest manager could promote biodiversity with managerial decisions that take into account centuries of long term impacts (e.g. carbon sequestration, wildlife habitat) and a range of ecological diversity (Mazurek and Zielinski, 2004).

References

Aurora, D., 1991. All That the Rain Promises and More: A Hip Pocket Guide to Western Mushrooms. Ten Speed Press, Berkeley, California.

Mazurek, M.J., Zielinski, W.J., 2004. Individual legacy trees influence vertebrate wildlife diversity in commercial forests. Forest Ecology and Management 193: 321-334.

Rayner, A.D.M., Boddy, L., 1988. Fungal Decomposition of Wood: Its Biology and Ecology. John Wiley & Sons Ltd., New York.

Sinclair, W.A., Lyon, H.H., Johnson, W.T., 1987. Diseases of Trees and Shrubs. Cornell University Press, Ithaca, New York.

Appendices

Appendix 1a. Fungal observations during the fall sampling periods of 2014, 2015, and 2016 by biomass treatments 0X, 1X, 2X, non-thinned, and combined. An observation was defined as the occurrence of a fruiting body, greater than 1 cm^2, on a substrate. Totals are reported on the bottom of the table for all treatments separately and for treatments combined.

	Biomass Treatment				
Fungal Name	0X	1X	2X	Non-thinned	Combined
Acanthophysellum lividocoeruleum	22	10	4	16	52
Amphinema byssoides	4	0	1	8	13
Amylocorticiellum sp.1	1	0	0	0	1
Anomoporia bombycina	3	0	0	0	3
Antrodia carbonica	0	0	0	1	1
Antrodia serialis	2	0	0	0	2
Antrodia sordida	0	0	1	1	2
Antrodia sp.1	0	0	1	0	1
Antrodia xantha	0	0	0	1	1
Antrodiella pallasii	1	0	1	0	2
Aphanobasidium pseudotsugae	2	0	0	0	2
Armillaria ostoyae	1	0	0	1	2
Athelia bombacina	22	27	19	5	73
Athelia epiphylla	0	0	2	2	4
Athelia fimbulata	1	0	0	0	1
Athelia psychrophila	3	2	7	2	14
Atheliachaete sanguinea	1	1	0	0	2
Auricularia americana	0	0	1	1	2
Auricularia sp.1	0	4	0	0	4
Basidiodendron caesiocinereum	0	1	0	0	1
Botryobasidium botryosum	1	0	0	0	1
Botryobasidium candicans	0	0	0	1	1
Botryobasidium obtusisporum	0	1	0	1	2
Botryobasidium sp.1	1	1	1	3	6
Botryobasidium subcoronatum	8	0	3	5	16
Botryobasidium tubulicystidium	1	0	2	0	3
Ceratobasidium cornigerum	0	0	3	0	3
Ceratobasidium sp. 1	1	0	1	0	2
Chaetodermella luna	2	0	2	5	9
Cinereomyces lindbladii	4	4	0	11	19

Coniophora arida	31	15	43	17	106
Coniophora olivaceum	2	0	0	1	3
Corticium confine	0	1	0	0	1
Crucibulum laeve	1	0	0	0	1
Crustoderma dryinum	1	0	0	0	1
Cryptoporus volvatus	7	1	9	3	20
Dacrymyces chrysospermus	5	5	3	7	20
Dacryobolus sp.1	3	0	0	1	4
Dichomitus squalens	6	1	5	0	12
Fibulomyces fusoideus	0	0	7	0	7
Fibulomyces mutabilis	5	0	0	0	5
Fomitopsis ochracea	0	3	0	3	6
Gloeophyllum abietinum	1	0	0	0	1
Gloeophyllum carbonarium	1	0	0	0	1
Gloeophyllum protractum	2	0	0	0	2
Gloeophyllum sepiarium	1	0	1	0	2
Gloeophyllum sp.1	12	12	12	10	46
Gloeoporus dichrous	0	0	0	1	1
Gloeoporus sp.1	0	1	0	0	1
Guepiniopsis alpina	4	2	2	3	11
Hastodontia hastata	1	0	0	0	1
Heterobasidion occidentale	0	0	1	1	2
Hydnomerulius pinastri	2	0	0	1	3
Hyphoderma amoenum	0	0	0	1	1
Hyphoderma roseocremeum	1	0	0	0	1
Hyphoderma setigerum	0	26	27	0	53
Hyphoderma sp.1	1	0	0	0	1
Hyphodontia breviseta	0	0	0	1	1
Hyphodontia floccosa	1	0	0	2	3
Hyphodontia hastata	2	0	0	0	2
Hyphodontia pallidula	3	0	0	2	5
Hyphodontia sibiricum	1	0	0	0	1
Hyphodontia sp.1	1	0	2	1	4
Hyphodontia sp.2	0	1	0	0	1
Hypholoma fasciculare	0	0	1	2	3
Hypochnicium albostramineum	0	4	13	3	20
Hypochnicium sp.1	0	1	2	0	3
Irpex sp.1	0	1	0	0	1
Kneiffiella sp.1	0	0	1	1	2
Kneifiella subalutacea	3	0	2	2	7

Leptosporomyces fuscostratus	0	0	1	0	1
Leptosporomyces raunkiaeri	1	0	0	0	1
Leptosporomyces septentrionalis	0	0	0	1	1
Leptosporomyces sp.1	0	2	0	1	3
Leucogyrophana mollusca	0	3	1	0	4
Mycena maculata	0	0	0	1	1
Mycena metata	0	1	0	1	2
Oxyporus corticola	0	2	0	0	2
Panellus serotinus	0	0	1	1	2
Panellus sp.1	0	1	0	0	1
Peniophorella praetermissa	1	0	0	0	1
Peniophorella pubera	0	3	0	0	3
Phaeolus schweinitzii	3	0	0	1	4
Phanerochaete burtii	1	0	0	0	1
Phanerochaete krikophora	1	0	2	0	3
Phanerochaete sordida	3	1	5	0	9
Phlebia centrifuga	2	2	0	2	6
Phlebia radiata	0	5	1	0	6
Phlebia segregata	1	0	0	0	1
Phlebia subserialis	1	1	2	1	5
Phlebia tremellosa	0	2	0	0	2
Phlebiopsis gigantea	0	1	3	3	7
Piloderma byssinum	0	0	0	1	1
Piloderma fallax	2	0	0	1	3
Piloderma sphaerosporum	0	0	0	1	1
Pluteus brunneidiscus	0	0	1	0	1
Pluteus posaranus	0	0	0	1	1
Porodaedalea niemelaei	0	0	0	1	1
Pseudomerulius aureus	0	1	0	0	1
Radulomyces sp. 1	0	0	1	0	1
Resinicium bicolor	0	0	0	2	2
Resinicium sp.1	1	1	1	0	3
Rhizochaete sulphurina	1	2	0	1	4
Scytinostroma galactinum	1	0	0	2	3
Serpula himantioides	2	0	1	0	3
Sistotrema brinkmanii	1	0	1	0	2
Sistotrema sp.1	0	0	2	0	2
Stereum sanguinolentum	6	14	19	16	55
Tapinella atrotomentosa	0	1	1	1	3
Trametes sp. 1	1	0	0	0	1

Trametes versicolor	4	0	2	2	8
Trechispora farinacea	0	0	1	2	3
Trechispora sp.1	1	0	2	0	3
Trichaptum abietinum	27	49	51	24	151
Tubulicrinis borealis	0	0	1	0	1
Tubulicrinis calothrix	2	1	0	0	3
Tubulicrinis glebulosus	12	3	4	8	27
Tubulicrinis sp.1	3	1	1	0	5
Tubulicrinis sp.2	1	0	1	0	2
Tubulicrinis subulatus	3	0	0	8	11
Tylospora sp. 1	1	0	0	0	1
Veluticeps fimbriata	0	0	0	1	1
Xenasmatella vaga	0	1	3	1	5
Xylodon nespori	1	3	6	0	10
Xylodon sambuci	2	0	4	0	6
Total number of observations	265	226	299	212	1002
Total species richness	72	46	58	62	126

Appendix 1b. Fungal observations during the fall sampling periods of 2014, 2015, and 2016 by decay classes 1, 2, 3, 4, 5, and combined. An observation was defined as the occurrence of a fruiting body, greater than 1 cm^2, on a substrate. Totals are reported on the bottom of the table for each decay class separately and for decay classes combined.

	Decay Class					
Fungal Name	1	2	3	4	5	Combined
Acanthophysellum lividocoeruleum	1	7	28	14	2	52
Amphinema byssoides	0	0	7	6	0	13
Amylocorticiellum sp.1	0	0	1	0	0	1
Anomoporia bombycina	0	0	3	0	0	3
Antrodia carbonica	0	0	1	0	0	1
Antrodia serialis	0	2	0	0	0	2
Antrodia sordida	0	1	1	0	0	2
Antrodia sp.1	0	0	1	0	0	1
Antrodia xantha	0	0	0	1	0	1
Antrodiella pallasii	0	0	2	0	0	2
Aphanobasidium pseudotsugae	0	1	1	0	0	2
Armillaria ostoyae	0	0	2	0	0	2
Athelia bombacina	0	38	22	13	0	73
Athelia epiphylla	0	3	1	0	0	4
Athelia fimbulata	0	0	1	0	0	1
Athelia psychrophila	0	12	1	1	0	14
Atheliachaete sanguinea	0	1	1	0	0	2
Auricularia americana	0	1	1	0	0	2
Auricularia sp.1	0	0	4	0	0	4
Basidiodendron caesiocinereum	0	0	0	1	0	1
Botryobasidium botryosum	0	0	1	0	0	1
Botryobasidium candicans	0	0	1	0	0	1
Botryobasidium obtusisporum	0	2	0	0	0	2
Botryobasidium sp.1	0	1	4	1	0	6
Botryobasidium subcoronatum	0	3	11	2	0	16
Botryobasidium tubulicystidium	0	1	2	0	0	3
Ceratobasidium cornigerum	0	3	0	0	0	3
Ceratobasidium sp. 1	0	1	1	0	0	2
Chaetodermella luna	0	0	5	4	0	9
Cinereomyces lindbladii	0	4	14	1	0	19
Coniophora arida	0	49	41	15	1	106
Coniophora olivaceum	0	1	2	0	0	3

Corticium confine	0	0	1	0	0	1
Crucibulum laeve	0	0	0	1	0	1
Crustoderma dryinum	0	0	1	0	0	1
Cryptoporus volvatus	6	14	0	0	0	20
Dacrymyces chrysospermus	0	12	5	3	0	20
Dacryobolus sp.1	0	1	1	2	0	4
Dichomitus squalens	0	10	2	0	0	12
Fibulomyces fusoideus	0	7	0	0	0	7
Fibulomyces mutabilis	0	5	0	0	0	5
Fomitopsis ochracea	0	3	3	0	0	6
Gloeophyllum abietinum	0	0	1	0	0	1
Gloeophyllum carbonarium	0	0	1	0	0	1
Gloeophyllum protractum	0	1	1	0	0	2
Gloeophyllum sepiarium	0	1	1	0	0	2
Gloeophyllum sp.1	0	30	16	0	0	46
Gloeoporus dichrous	0	0	1	0	0	1
Gloeoporus sp.1	0	1	0	0	0	1
Guepiniopsis alpina	0	9	2	0	0	11
Hastodontia hastata	0	0	1	0	0	1
Heterobasidion occidentale	0	2	0	0	0	2
Hydnomerulius pinastri	0	1	1	1	0	3
Hyphoderma amoenum	0	1	0	0	0	1
Hyphoderma roseocremeum	0	0	1	0	0	1
Hyphoderma setigerum	0	38	15	0	0	53
Hyphoderma sp.1	0	1	0	0	0	1
Hyphodontia breviseta	0	0	1	0	0	1
Hyphodontia floccosa	1	1	1	0	0	3
Hyphodontia hastata	0	1	1	0	0	2
Hyphodontia pallidula	0	1	3	1	0	5
Hyphodontia sibiricum	0	0	1	0	0	1
Hyphodontia sp.1	0	1	2	1	0	4
Hyphodontia sp.2	0	0	0	1	0	1
Hypholoma fasciculare	0	1	2	0	0	3
Hypochnicium albostramineum	0	8	10	2	0	20
Hypochnicium sp.1	0	2	1	0	0	3
Irpex sp.1	0	0	1	0	0	1
Kneiffiella sp.1	0	1	0	1	0	2
Kneifiella subalutacea	0	0	5	2	0	7
Leptosporomyces fuscostratus	0	0	1	0	0	1
Leptosporomyces raunkiaeri	0	0	1	0	0	1

Leptosporomyces septentrionalis	0	1	0	0	0	1
Leptosporomyces sp.1	0	2	1	0	0	3
Leucogyrophana mollusca	0	1	2	1	0	4
Mycena maculata	0	0	0	1	0	1
Mycena metata	0	1	1	0	0	2
Oxyporus corticola	0	0	1	1	0	2
Panellus serotinus	0	1	1	0	0	2
Panellus sp.1	0	1	0	0	0	1
Peniophorella praetermissa	0	1	0	0	0	1
Peniophorella pubera	0	0	2	1	0	3
Phaeolus schweinitzii	1	3	0	0	0	4
Phanerochaete burtii	0	1	0	0	0	1
Phanerochaete krikophora	0	2	1	0	0	3
Phanerochaete sordida	0	5	4	0	0	9
Phlebia centrifuga	0	0	5	1	0	6
Phlebia radiata	0	6	0	0	0	6
Phlebia segregata	0	0	1	0	0	1
Phlebia subserialis	0	4	1	0	0	5
Phlebia tremellosa	0	2	0	0	0	2
Phlebiopsis gigantea	0	6	1	0	0	7
Piloderma byssinum	0	0	1	0	0	1
Piloderma fallax	0	0	3	0	0	3
Piloderma sphaerosporum	1	0	0	0	0	1
Pluteus brunneidiscus	0	0	1	0	0	1
Pluteus posaranus	0	0	1	0	0	1
Porodaedalea niemelaei	0	1	0	0	0	1
Pseudomerulius aureus	0	0	1	0	0	1
Radulomyces sp. 1	0	1	0	0	0	1
Resinicium bicolor	1	0	1	0	0	2
Resinicium sp.1	0	0	2	1	0	3
Rhizochaete sulphurina	0	0	3	1	0	4
Scytinostroma galactinum	0	0	3	0	0	3
Serpula himantioides	0	0	3	0	0	3
Sistotrema brinkmanii	0	2	0	0	0	2
Sistotrema sp.1	0	0	2	0	0	2
Stereum sanguinolentum	1	47	7	0	0	55
Tapinella atrotomentosa	0	1	2	0	0	3
Trametes sp. 1	0	1	0	0	0	1
Trametes versicolor	0	8	0	0	0	8
Trechispora farinacea	0	0	0	3	0	3

Trechispora sp.1	0	0	2	1	0	3
Trichaptum abietinum	0	124	21	6	0	151
Tubulicrinis borealis	0	1	0	0	0	1
Tubulicrinis calothrix	0	1	1	1	0	3
Tubulicrinis glebulosus	0	11	12	4	0	27
Tubulicrinis sp.1	0	1	2	2	0	5
Tubulicrinis sp.2	0	0	2	0	0	2
Tubulicrinis subulatus	0	0	4	7	0	11
Tylospora sp. 1	0	1	0	0	0	1
Veluticeps fimbriata	0	0	1	0	0	1
Xenasmatella vaga	0	1	2	2	0	5
Xylodon nespori	0	5	3	2	0	10
Xylodon sambuci	0	4	1	1	0	6
Total number of observations	12	531	346	110	3	1002
Total species richness	7	74	93	38	2	126

Appendix 2 – Responses of wood-colonizing fungal community structure to thinning in *Pinus tabuliformis* plantations in northern China: the assessment of drilled wood stake shavings from wood stakes in the same plots

1. Introduction

Because of known biases in DNA extraction and PCR amplification (Wintzingerode et al. 1997), this study aimed to assess which steps, if any, significantly affected the final fungal community analyses results from drilled wood stakes. This test aimed to assess whether there was a significant effect of pooling DNA samples, and if timing of DNA pooling was a factor affecting the fungal community analyses results.

2. Methods

2.1. *Site and treatments*

Organic matter decomposition was evaluated using a standardized wood stake decomposition study at the University of Idaho's Experimental Forest (46.849512, -116.845068). The Experimental Forest has silt-loam soils with naturally regenerated mixed conifer species dominating the landscape. Four soil amendment treatment plots: non-amended control, fertilizer (224 kg N ha^{-1} as urea), biochar (2 Mg ha^{-1}), and fertilizer plus biochar were tested for efficacy at this site.

2.2. *Wood stakes and extraction*

Using protocols described by Jurgensen et al. (2006), loblolly pine (*Pinus taeda* L.) and aspen (*Populus tremuloides* Michx.) stakes (2.5 x 2.5 x 15 cm) were fixed on the surface of the soil-litter layer with large metal staples in the spring of 2014. After 1 year, five pine and five aspen stakes were removed from each of the four plots for sampling, yielding 20 pine and 20 aspen stakes in total.

Each individually labeled stake was processed at the Forest Service's Rocky Mountain Research Station, Moscow, ID within 24 hours of extraction from the soil. Soil debris was scraped from the surface of each stake before drilling 1 cm deep using sterile 4 mm bits. Surface stakes were drilled at two points on opposite ends, and the drill shavings were combined and collected in 2 mL strip tubes in 96-well format. The shavings were covered with filter-sterilized cell lysis solution (CLS) and frozen at 20 degrees C until DNA was extracted.

Fungi inhabiting the wood stakes were identified using DNA-based methods described by Lindner et al. (2011). The DNA was cleaned using GeneClean III kits (Qbiogene), that were previously found to be reliable and cost effective for the recovery of high quality genomic fungal DNA from wood samples (Lindner et al., 2011).

2.3. *Experimental design of wood stake pooling*

Because the five aspen and five pine stake replicates within each treatment plot represent sub-sampling, this study was conducted to determine if the same fungal communities could be observed when cell lysis solution (CLS) containing wood shavings from the aspen stakes (and

pine stakes, respectively) was pooled together prior to extraction. Aspen and pine wood stakes were processed from each soil amendment treatment plot. See table 1 for details.

Separate extractions and separate PCR products

For each individual wood stake, the DNA was extracted from the wood shavings separately, and the PCR reactions were conducted separately. With 40 individual stakes (five aspen + five pine stakes/plot), 40 PCR products were uniquely barcoded prior to high through-put sequencing with Ion Torrent PGM^{TM}.

Separate extractions combined before PCR reaction

Within each plot, the DNA from the five separately extracted aspen stakes was combined equally before the PCR reaction. The combined PCR product was uniquely barcoded prior to sequencing. This procedure was repeated for processing the pine stakes.

Separate extractions combined after PCR reaction

Within each plot, the DNA from the five separately extracted aspen stakes produced five separate PCR products. Those five separate PCR products were combined equally and uniquely barcoded prior to sequencing. This procedure was repeated for processing the pine stakes.

Combining prior to both extraction and PCR reaction

Within each plot, the CLS containing wood shavings from the five aspen stakes was combined equally before the DNA extraction. CLS was heated to 65 C for 2 hours, and then the CLS from the five stakes was combined. The pooled CLS solution was extracted, and the

extracted DNA was processed to yield a PCR product that was uniquely barcoded prior to sequencing. This procedure was repeated for processing the pine stakes.

2.4. *Sequencing*

The procedures for high-throughput sequencing follow the Ion PGM manufacturer's instructions and Lindahl (2013). DNA was extracted from the drilled wood stake shavings using the tagged and barcoded primers fITS7 (Ihrmark et al., 2012) and ITS4 for the PCR reaction. Following DNA amplification, Zymo Research's Select-A-Size DNA Clean & Concentrator spin columns were used to clean the resulting PCR product. Equimolar proportions of DNA were templated in an emulsion PCR step where the product was attached and amplified onto the Ion Sphere Particles (ISPs). Sequencing of the cleaned, template positive ISPs was performed using the Ion PGM Hi-Q sequencing kit according to manufacturer's recommendations. Sequence reads obtained from the PGM were trimmed, filtered, clustered, and assigned operational taxonomic units (OTUs) with USEARCH8, facilitated by the uFITS program (Palmer, 2016).

2.5. *Data Analyses*

Sequenced fungal community composition data was analyzed to determine if combination of DNA (i.) prior to extraction, (ii.) prior to PCR, and/or (iii.) after PCR reaction significantly affected the fungal community analyses compared with DNA that was (iv.) extracted separately with separate PCR reactions. The final OTU data from the five separately extracted and separately sequenced pine stakes within each plot was combined for analyses and

represents the “non-pooled” community. This procedure was repeated for the aspen stakes. Community analyses of the wood stake data was conducted using R Statistical Software (R Core Team, 2015) and the Vegan package (Oksanen et al., 2018). To assess the effects of pooling on the fungal community composition, a nonparametric permutational multivariate ANOVA (PERMANOVA) test (Anderson, 2001) was performed by the adonis function in the Vegan package of R.

3. Results

PERMANOVA analysis concluded that there was no significant difference between fungal communities if DNA was combined: (i.) prior to extraction, (ii.) prior to PCR, (iii.) after PCR reaction, or (iv.) not combined [adonis: Pseudo-F (5,26) = 0.763, p-value = 0.6087].

Methodically, it is simplest to initially pool the CLS containing wood shavings prior to extraction, as opposed to performing separate extractions and PCR reactions. Therefore, this research also assessed whether there was a difference between the fungal communities analyzed from combing the CLS prior to extraction, as opposed to the non-pooled, control, community. PERMANOVA analysis concluded that pooling the CLS containing wood shavings from the aspen stakes (and pine stakes, respectively) prior to extraction did not produce significantly different fungal communities from DNA that was extracted and processed separately [adonis: Pseudo-F (3,12) = 0.296, p-value = 0.9697].

4. Conclusions

The five aspen and five pine stake replicates within each treatment plot represents sub-sampling. Therefore, because there was no significant difference between the fungal community compositions generated through pooling the CLS containing wood shavings samples or not, this experiment will benefit future wood stake research by reducing the processing time and high-throughput sequencing costs.

Pooling the aspen stakes (and pine stakes, respectively) will result in a 1/5 reduction in time and cost. The Ion Torrent located at the Forest Products Laboratory (Madison, Wisconsin) can process about 100 samples per run, at a cost of ~ $1,000; therefore, the sequencing and labor savings are significant.

5. References

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46.

Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal ITS2 region--evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol. Ecol. 82, 666–77.

Jurgensen, M., Reed, D., Page-Dumroese, D., Laks, P., Collins, A., Mroz, G., Degórski, M., 2006. Wood strength loss as a measure of decomposition in northern forest mineral soil. Eur. J. Soil Biol. 42, 23–31.

Lindahl, B, D., Nilsson, R.H., Tedersoo, L., Abarenkov, K., Carlsen, T., Pennanen, T., Stenlid, J., Kauserud, H., 2013. Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. New Phytol. 199, 288–299.

Lindner, D.L., Vasaitis, R., Kubartová, A., Allmér, J., Johannesson, H., Banik, M.T., Stenlid, J., 2011. Initial fungal colonizer affects mass loss and fungal community development in *Picea abies* logs 6yr after inoculation. Fungal Ecol. 4, 449–460.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2018. Package 'vegan. https://cran.r-project.org/web/packages/vegan/vegan.pdf (accessed 7 March 2018).

Palmer, J., 2017., AMPtk: Amplicon Toolkit for NGS data. https://github.com/nextgenusfs/amptk (accessed 7 March 2018).

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna Austria, https://www.R-project.org/ (accessed 7 March 2018).

Wintzingerode, F.v., Gobel, U.B., Stackebrandt, E., 1997. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiology Reviews 21: 213-229.

Table 1. Experimental design of DNA extraction and PCR reaction combinations per plot, treatment, and stake type. Stakes include aspen and pine. Treatment include non-amended control, biochar, fertilizer, and biochar + fertilizer (B+F).

Plot #	Stake Type	Treatment	DNA Extraction	PCR Reaction	# of Barcodes
1	Aspen	Control	5 stakes, 5 separate DNA extractions	5 stakes, 5 separate PCR reactions	5
1	Aspen	Control	5 separate DNA extractions	DNA combined, pre-PCR reaction	1
1	Aspen	Control	5 separate DNA extractions	DNA combined, post-PCR reaction	1
1	Aspen	Control	5 stakes' shavings combined pre-extraction	Combined PCR reaction	1
1	Pine	Control	5 stakes, 5 separate DNA extractions	5 stakes, 5 separate PCR reactions	5
1	Pine	Control	5 separate extractions	DNA combined, pre-PCR reaction	1
1	Pine	Control	5 separate extractions	DNA combined, post-PCR reaction	1
1	Pine	Control	5 stakes' shavings combined pre-extraction	Combined PCR reaction	1
2	Aspen	Biochar	5 stakes, 5 separate DNA extractions	5 stakes, 5 separate PCR reactions	5
2	Aspen	Biochar	5 separate extractions	DNA combined, pre-PCR reaction	1
2	Aspen	Biochar	5 separate extractions	DNA combined, post-PCR reaction	1
2	Aspen	Biochar	5 stakes' shavings combined pre-extraction	Combined PCR reaction	1
2	Pine	Biochar	5 stakes, 5 separate DNA extractions	5 stakes, 5 separate PCR reactions	5
2	Pine	Biochar	5 separate extractions	DNA combined, pre-PCR reaction	1
2	Pine	Biochar	5 separate extractions	DNA combined, post-PCR reaction	1
2	Pine	Biochar	5 stakes' shavings combined pre-extraction	Combined PCR reaction	1
3	Aspen	Fertilizer	5 stakes, 5 separate DNA extractions	5 stakes, 5 separate PCR reactions	5
3	Aspen	Fertilizer	5 separate extractions	DNA combined, pre-PCR reaction	1
3	Aspen	Fertilizer	5 separate extractions	DNA combined, post-PCR reaction	1
3	Aspen	Fertilizer	5 stakes' shavings combined pre-extraction	Combined PCR reaction	1
3	Pine	Fertilizer	5 stakes, 5 separate DNA extractions	5 stakes, 5 separate PCR reactions	5
3	Pine	Fertilizer	5 separate extractions	DNA combined, pre-PCR reaction	1
3	Pine	Fertilizer	5 separate extractions	DNA combined, post-PCR reaction	1
3	Pine	Fertilizer	5 stakes' shavings combined pre-extraction	Combined PCR reaction	1
4	Aspen	B + F	5 stakes, 5 separate DNA extractions	5 stakes, 5 separate PCR reactions	5
4	Aspen	B + F	5 separate extractions	DNA combined, pre-PCR reaction	1
4	Aspen	B + F	5 separate extractions	DNA combined, post-PCR reaction	1
4	Aspen	B + F	5 stakes' shavings combined pre-extraction	Combined PCR reaction	1
4	Pine	B + F	5 stakes, 5 separate DNA extractions	5 stakes, 5 separate PCR reactions	5
4	Pine	B + F	5 separate extractions	DNA combined, pre-PCR reaction	1
4	Pine	B + F	5 separate extractions	DNA combined, post-PCR reaction	1
4	Pine	B + F	5 stakes' shavings combined pre-extraction	Combined PCR reaction	1