LONG-TERM EFFECTS OF STAND-REPLACING WILDFIRES ON NUTRIENT CYCLING AND DECOMPOSITION IN SOUTHWESTERN PONDEROSA PINE FORESTS

By Valerie J. Kurth

A Dissertation

Submitted in Partial Fulfillment

of the Requirements for the degree of

Doctor of Philosophy

In Forest Science

Northern Arizona University

May 2011

Approved:

Peter Z. Fulé, Ph.D., Co-Chair

Stephen C. Hart, Ph.D., Co-Chair

Catherine A. Gehring, Ph.D.

Karen A. Haubensak, Ph.D.

ABSTRACT

LONG-TERM EFFECTS OF STAND-REPLACING WILDFIRES ON NUTRIENT CYCLING AND DECOMPOSITION IN SOUTHWESTERN PONDEROSA PINE FORESTS

Valerie J. Kurth

The frequency and size of stand-replacing wildfires in the western U.S. has increased in recent years as a result of complex interacting factors, including increased temperatures, drought frequency, and the build-up of forest fuels from a century of fire suppression. Stand-replacing fires are a relatively novel disturbance in southwestern ponderosa pine forests, so the long-term ecosystem consequences of this type of fire are uncertain. The objective of the research presented here is to improve our understanding of post-fire ecosystem functioning by examining controls on carbon (C) accumulation and release patterns from several different perspectives. The first study addresses post-fire nitrogen (N) cycling dynamics because N availability is a limiting factor to net primary productivity in these forests, so it may be critical to post-fire carbon accumulation. I quantified rates of net and gross nitrogen (N) mineralization and nitrification in burned and paired unburned forests representing a range of fire ages (2-34 years). The results demonstrate that fire effects on N cycling can persist for decades, and support the hypothesis that post-fire vegetation drives ecosystem processes such as N cycling. The second study examines communities of wood-decay fungi as a potential control on post-fire C release as CO_2 from decomposing wood. I identified the community structure of wood-decay fungi in burned and paired unburned forest representing a

range of burn years (2005, 2000, 1996, 1984, and 1977). The results suggest that fire reduces species richness for up to 4 years, and it alters species composition for up to 32 years. However, composition was accompanied by substantial heterogeneity in both burned and unburned sites. A companion experiment using a subset of the fungal species demonstrated that fungi differ in their abilities to decompose wood, which may have implications for fungal succession and wood decomposition rates. The third study addresses the effect of wildfire and two common management treatments (thinning and thinning/burning) on the decomposition of fine roots over a 27-month period. Although I did not observe a significant effect of forest treatment, the overall decomposition rates of fine roots were faster than those of comparable leaf litter. This observation, coupled with the relatively high nutrient content of fine roots, suggests that fine root turnover is an integral part of maintaining soil fertility in these ecosystems. The overall results of these three studies have dual implications for forest management in this region. First, to maintain forested landscapes, managers should implement treatments (e.g., thinning) that reduce the risk of catastrophic wildfire. Second, managers should prepare for this type of wildfire by prioritizing resources (e.g., watersheds, habitat, recreation) and anticipating post-wildfire ecosystem recovery.

ACKNOWLEDGEMENTS

There are many, many people who provided some form of assistance in the development, execution, and completion of the studies presented in this dissertation. Special thanks to my committee co-chairs, Steve Hart and Pete Fulé, for providing guidance, support, and expertise throughout my graduate research. I am especially grateful that they encouraged me to explore my own dissertation research journey, despite the circuitousness of my path. Thank you also to my other committee members, Kitty Gehring and Karen Haubensak. Kitty has been an incredibly generous and supportive mentor to me over the last four years, and Karen has been a great person to talk about ideas with and has provided me with excellent editorial help.

I received an enormous amount of support from various field and lab technicians throughout this work. I could not have done this work without field assistance from many of the students at the Ecological Research Institute. I received countless hours of lab assistance from Stephanie Jackson, and critical technical help and support from Morgan Luce and Tamara Max. I would also like to thank all former and current members of the Hart, Gehring and Fulé lab groups for the providing thoughtful support on all aspects of this work.

Finally, I would like to thank my amazing support network of family and friends, in Flagstaff and beyond. Thank you to Tom DeLuca, who first had the idea that I could get my doctorate. Thank you to my parents, who never doubted my ability to find my way. And thank you to my two most beloved people, Ellie Kurth and Sam Harworth, for everything.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

Chapter One is the overall introduction to the dissertation. Chapter Two is a literature review summarizing information relevant to Chapter Four. Chapters Three, Four and Five are formatted as individual papers for submission to scientific journals. These three chapters may include some redundancy and each has a separate literature cited section. Chapter Six is the overall summary and management implications.

CHAPTER 1

INTRODUCTION

Recent alterations in global climate patterns, including increasing mean temperatures and drought severity, have the potential to alter natural disturbance regimes, as well as the direction of the post-disturbance ecosystem response (Savage and Mast 2005, Marshall et al. 2008). Severe drought conditions can trigger sudden and large-scale mortality events (Breshears et al. 2005), and are correlated with the rising frequency and amount of area burned by wildfires in the western U.S. (Westerling et al. 2003, Westerling et al. 2006). In particular, stand-replacing wildfires have long-term effects on ecosystem functioning, but specific post-fire recovery patterns are difficult to predict given the innate complexities within climate change and land management practices (Beschta et al. 2004, Marshall et al. 2008, Williams et al. 2010). However, given that drought frequency and intensity are predicted to rise in the future (Seager et al. 2007; Easterling et al. 2003; Hoerling and Kumar 2003), we may expect to continue on the trajectory of more frequent stand-replacing wildfires. Therefore, it has become increasingly important to understand forest ecosystem response to these large-scale disturbances in order to provide appropriate information to land managers (Beschta et al. 2004).

Ponderosa pine forests of the southwestern U.S. are sensitive to drought conditions and increasing mean temperatures (Williams et al. 2010). This sensitivity means that they are more likely to suffer from tree mortality due to water stress, and have higher susceptibility to mortality from more frequent bark beetle outbreaks and wildfires. Wildfires are of particular interest because of their ability

to rapidly alter ecosystem properties; specifically, fires combust large amounts of organic matter stored in living and dead plant biomass and release it to the atmosphere rapidly (Hurteau and Brooks 2011). Fires also lead to changes in microclimate, the deposition of nutrients in ash, a prolonged pulse of inorganic nitrogen (N), increased soil pH, and the potential to alter both plant and microbial community structure (Raison 1979, Neary et al. 1999, Hart et al. 2005). Furthermore, natural regeneration is often limited in southwestern ponderosa pine forests (Heidmann 2008), which gives rise to potential shifts in the overall vegetation community following stand-replacing wildfires, including changes to alternative stable states such as grassland or shrubland (Savage and Mast 2005).

Southwestern ponderosa pine forests are uniquely suited to studying post-wildfire ecosystem functioning for several reasons. First, these forests are widespread in the Southwest, comprising almost half of the commercial forested land in Arizona, Utah, New Mexico and Colorado (Schubert 1974). The land-use history of this region strongly influenced the wildfire regime, specifically a shift from low-intensity surface fires to stand-replacing wildfires since Euro-American settlement circa 1880 (Covington and Moore 1994). The historic fire regime was frequent, low intensity surface fires that maintained forests in an open structure with prolific understory vegetation. The introduction of land management practices, such as livestock grazing, timber harvest, and fire suppression by Euro-American settlers beginning in the late 1800s has led to a build-up of small diameter trees and an associated fire regime shift to infrequent, stand-replacing wildfires (Cooper 1960, Covington et al. 1997). Finally, research in this ecosystem is relevant to other

forests in the western U.S. because these land management practices, especially active fire suppression, are similarly applied across the region.

The research in this dissertation uses southwestern ponderosa pine forests as a model ecosystem to examine post-wildfire ecosystem response from several different angles, all under the overarching them of carbon (C) and N cycling. In doing so, I hoped to gain a broad, but compelling perspective on post-wildfire ecosystem functioning.

The research in Chapter Three explores changes in N cycling dynamics over time since stand-replacing wildfire using *in situ* measurements of net N transformations and laboratory measurements of gross N production. Nitrogen, as well as water, is limiting to net primary productivity in southwestern ponderosa pine forests (Wagle and Kitchen 1972, Heidmann et al. 1979); thus, its availability to plant growth may constrain post-fire net primary production (i.e., C accumulation). Nitrogen transformations are strongly affected by wildfire, but the effects of stand-replacing wildfire on N cycling in these forests have rarely been assessed (Grady and Hart 2006). Severe fires combust substantial amounts of the organically bound N, but fires also create a pulse of plant-available N, in the form of pyrogenically produced NH_4^+(White 1986, Covington and Sackett 1992, Wan et al. 2001, Smithwick et al. 2005). The fates of this NH_4^+ include: plant uptake, microbial assimilation (immobilization), fixation within clays, loss due to surface erosion, and conversion into NO_3^- (Johnson 1992), another form of plant-available inorganic N. Nitrification rates are typically enhanced for 1-2 years after fire because there is abundant NH_4^+ substrate, plant uptake of NH_4^+ is typically low, and microclimatic

conditions, especially elevated pH and soil temperatures, are favorable for nitrifying bacteria (Raison 1979, Covington and Sackett 1992, Prieto-Fernández et al. 2004, Turner et al. 2007). As burned areas become re-vegetated, demand for N from plants and microbes increases, resulting in an observed decline in nitrification rates.

The temporal aspect of these processes is important because the majority of our knowledge concerning fire effects in southwestern ponderosa pine forests comes from prescribed burning studies. This body of research suggests a correlation between burning intensity and the magnitude of the inorganic N pulse (Kovacic et al. 1986). Here we might predict that a high-intensity, stand-replacing wildfire in these forests would have a much more pronounced effect on N cycling than a lower-intensity prescribed fire. Indeed, evidence from Grady and Hart (2006) supports such a relationship. Based on their findings, I hypothesized that inorganic N availability would remain high after a stand-replacing wildfire, but would gradually decline over time as regeneration proceeds, eventually resembling that of unburned forests.

The research described in Chapter Four centers on communities of wood-decay fungi as a potential control on post-fire wood decomposition (i.e., C release) in southwestern ponderosa pine forests. Stand-replacing wildfires in these forests leave large amounts of C in the form of dead wood on the landscape. As this wood is decomposed by heterotrophic microbes, C is released to the atmosphere as CO_2. Research by Dore et al. (2008) demonstrates that burned forests in northern Arizona are net sources of atmospheric C for at least 10 years because rates of

decomposition exceed C fixation by plants; thus, the fate of wood on these landscapes may be an important component to C cycles (Hurteau and Brooks 2011).

In general, wood decomposition is controlled by abiotic factors (temperature and moisture), substrate (quality and quantity), and colonization by saprotrophic organisms (Harmon et al. 1986). We know comparatively little about the organisms, primarily basdiomycete and ascomycete fungi, that are responsible for much of wood decay. The literature review in Chapter Two summarizes our current knowledge about wood-decay fungi community patterns and emphasizes how little is known about these patterns. The research in Chapter Four explores the effect of stand-replacing wildfire disturbance on communities of wood-decay fungi in southwestern ponderosa pine forests, and examines the decomposition capabilities of a subset of the fungal species found in the observational portion of the research. This work differs from previous research in that it is the first instance where a combination of mycelial isolation and molecular techniques has been used for wood-decay fungi identification in these forests. This research is one of the few works to examine the effects of a high-severity wildfire, as opposed to a prescribed burn, on wood-decay fungal communities.

The research in Chapter Five explores both management and wildfire effects on the decomposition of fine roots in southwestern ponderosa pine forests. The decomposition of fine roots is an important source of nutrients and organic matter for soils of many undisturbed ecosystems (McClaugherty et al. 1984, Silver and Vogt 1993, Berg et al. 1998, Chen et al. 2002), but it may be especially important following a disturbance, such as management activities or stand-replacing wildfire.

This type of disturbance may cause an abrupt cessation in aboveground litter inputs, but the subsequent decay of fine root organic material belowground may serve as a buffer against these sudden declines in aboveground litter inputs (Fahey et al. 1988). There is a relative dearth of fine root decomposition studies compared to those of leaf decomposition for any ecosystem, and fine root decomposition has rarely been measured in ponderosa pine forests. However, if fine root decomposition rates exceed those of their comparable leaf litter, they may constitute a dynamic belowground pool of nutrients and soil organic matter in these ecosystems.

This suite of studies integrates knowledge of two key ecosystem cycles (C and N) with a novel disturbance regime in southwestern ponderosa pine forests. Nitrogen cycles are examined over time since fire, while both above- and below-ground C cycles are studied within communities of wood-decay fungi and fine root decomposition, respectively. The results fill important gaps in our knowledge of how stand-replacing wildfires influence ecosystem functioning in southwestern ponderosa pine forests, and they are especially important given the increasing frequency of this disturbance type in forests of the western U.S.

LITERATURE CITED

Berg, B., M.-B. Johansson, V. Meentemeyer, and W. Kratz. 1998. Decomposition of tree root litter in a climatic transect of coniferous forests in northern Europe: A synthesis. Scandinavian Journal of Forest Research **13**:402-412.

Beschta, R., J. Rhodes, J. Kauffman, R. Gresswell, G. Minshall, J. Karr, D. Perry, F. Hauer, and C. Frissell. 2004. Postfire management on forested public lands of the western United States. Conservation Biology **18**:957-967.

Breshears, D. D., N. S. Cobb, P. M. Rich, K. P. Price, C. D. Allen, R. G. Balice, W. H. Romme, J. H. Kastens, M. L. Floyde, J. Belnap, J. J. Anderson, O. B. Myers, and C. W. Meyer. 2005. Regional vegetation die-off in response to global-change-type drought. Proceedings of the National Academy of Sciences **102**:15144-15148.

Chen, H., M. E. Harmon, J. Sexton, and B. Fasth. 2002. Fine-root decomposition and N dynamics in coniferous forests of the Pacific Northwest, U.S.A. Canadian Journal of Forest Research **32**:320-331.

Cooper, C. 1960. Changes in vegetation, structure, and growth of southwestern pine forests since white settlement. Ecological Monographs **30**:129-164.

Covington, W., P. Fulé, M. Moore, S. Hart, T. Kolb, J. Mast, S. Sackett, and M. Wagner. 1997. Restoring ecosystem health in ponderosa pine forests of the Southwest. Journal of Forestry **95**:23-29.

Covington, W. and M. Moore. 1994. Southwestern ponderosa pine forest structure: Changes since Euro-American settlement. Journal of Forestry **92**:39-47.

Covington, W. and S. Sackett. 1992. Soil mineral nitrogen changes following prescribed burning in ponderosa pine. Forest Ecology and Management **54**:175-191.

Dore, S., T. E. Kolb, M. Montes-Helu, B. W. Sullivan, W. D. Winslow, S. C. Hart, J. P. Kaye, G. W. Koch, and B. A. Hungate. 2008. Long-term impact of a stand-replacing fire on ecosystem CO_2 exchange of a ponderosa pine forest. Global Change Biology **14**:1801-1820.

Fahey, T. J., J. W. Hughes, M. Pu, and M. A. Arthur. 1988. Root decomposition and nutrient flux following whole-tree harvest of northern hardwood forest. Forest Science **34**:744-768.

Grady, K. and S. Hart. 2006. Influences of thinning, prescribed burning, and wildfire on soil processes and properties in southwestern ponderosa pine forests: a retrospective study. Forest Ecology and Management **234**:123-135.

Harmon, M. E., J. F. Franklin, F. Swanson, P. Sollins, S. Gregory, J. Lattin, N. Anderson, S. Cline, N. Aumen, J. Sedell, G. Lienkaemper, K. Cromack, and K. Cummins. 1986. Ecology of coarse woody debris in temperate ecosystems. Advances in Ecological Research **15**:133-302.

Hart, S., T. DeLuca, G. Newman, M. MacKenzie, and S. Boyle. 2005. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. Forest Ecology and Management **220**:166-184.

Heidmann, L. 2008. Forest regeneration research at Fort Valley. In: Fort Valley Experimental Forest - A Century of Research 1908-2008. S. D. Olberding and M. M. Moore, tech. coords. Proceeding RMRS-P-55. U.S. Department of

Agriculture - Forest Service, Rocky Mountain Research Station, Fort Collins, CO.

Heidmann, L., W. J. Rietveld, and D. P. Trujillo. 1979. Fertilization increases cone production and diameter growth of a 55-year-old ponderosa pine stand in Arizona. Pages 197-205 *in* Proc. symp. on flowering and seed development in trees.

Hurteau, M. D. and M. L. Brooks. 2011. Short- and Long-term Effects of Fire on Carbon in US Dry Temperate Forest Systems. BioScience **61**:139-146.

Johnson, D. 1992. Nitrogen retention in forest soils. Journal of Environmental Quality **21**:1-12.

Kovacic, D., D. Swift, J. Ellis, and T. Hakonson. 1986. Immediate effects of prescribed burning on mineral soil nitrogen in ponderosa pine of New Mexico. Soil Science **141**:71-76.

Marshall, J., J. Blair, D. Peters, G. Okin, A. Rango, and M. Williams. 2008. Predicting and understanding ecosystem responses to climate change at continental scales. Frontiers in Ecology and the Environment **6**:273-280.

McClaugherty, C. A., J. D. Aber, and J. Melillo. 1984. Decomposition dynamics of fine roots in forested ecosystems. Oikos **42**:378-386.

Neary, D., C. Klopatek, L. DeBano, and P. Ffolliott. 1999. Fire effects on belowground sustainability: a review and synthesis. Forest Ecology and Management **122**:51-71.

Prieto-Fernández, Á., M. Carballas, and T. Carballas. 2004. Inorganic and organic N pools in soils burned or heated: immediate alterations and evolutions after forest wildfires. Geoderma **121**:291-306.

Raison, R. 1979. Modification of the soil environment by vegetation fires, with particular reference to nitrogen transformations: A review. Plant and Soil **51**:73-108.

Savage, M. and J. Mast. 2005. How resilient are southwestern ponderosa pine forests after crown fires? Canadian Journal of Forest Research **35**:967-977.

Schubert, G. H. 1974. Silviculture of southwestern ponderosa pine: The status-of-our-knowledge. USDA Forest Service Res. Paper RM-123. U.S. Department of Agriculutre, Rocky Mountain Forest and Range Experiment Station, editor., Fort Collins, CO.

Silver, W. L. and K. A. Vogt. 1993. Fine root dynamics following single and multiple disturbances in a subtropical wet forest ecosystem. Journal of Ecology **81**:729-738.

Smithwick, E., M. Turner, M. Mack, and F. Chapin. 2005. Postfire soil N cycling in northern conifer forests affected by severe, stand-replacing wildfires. Ecosystems **8**:163-181.

Turner, M., E. Smithwick, K. Metzger, D. Tinker, and W. Romme. 2007. Inorganic nitrogen availability after severe stand-replacing fire in the Greater Yellowstone ecosystem. Proceedings of the National Academy of Sciences **104**:4782.

Wagle, R. and J. Kitchen. 1972. Influence of fire on soil nutrients in a ponderosa pine type. Ecology **53**:118-125.

Wan, S., D. Hui, and Y. Luo. 2001. Fire effects on nitrogen pools and dynamics in terrestrial ecosystems: a meta-analysis. Ecological Applications **11**:1349-1365.

Westerling, A. L., A. Gershunov, T. J. Brown, D. R. Cayan, and M. D. Dettinger. 2003. Climate and Wildfire in the Western United States. Bull Amer Met Soc **84**:595.

Westerling, A. L., H. G. Hildago, D. R. Cayan, and T. W. Swetnam. 2006. Warming and Earlier Spring Increase Western U.S. Forest Wildfire Activity. Science **313**:940-943.

White, C. 1986. Effects of prescribed fire on rates of decomposition and nitrogen mineralization in a ponderosa pine ecosystem. Biology and Fertility of Soils **2**:87-95.

Williams, A., C. Allen, C. Millar, T. W. Swetnam, J. Michaelsen, C. Still, and S. Leavitt. 2010. Forest responses to increasing aridity and warmth in the southwestern United States. Proceedings of the National Academy of Science **107**:21289-21294.

CHAPTER 2

LITERATURE REVIEW: CURRENT RESEARCH IN WOOD-DECAY FUNGI COMMUNITY STRUCTURES

Introduction

Until recently, testing the hypothesis that microbial diversity was linked to ecosystem processes was alluring, but nearly impossible to rigorously examine because of the lack of appropriate tools. However, the recent introduction of DNA-based molecular techniques has made tests of this hypothesis within reach of our scientific capabilities (Zak et al. 2006). For example, we now know that microbes exhibit biogeographical patterns (Fierer et al. 2007), and we know that differences in microbial communities can equate to functional differences (Strickland et al. 2009). Transitioning from microbial community structure to ecosystem processes is a unique challenge, primarily because of difficulties in manipulating microbial communities (Mcguire and Treseder 2009). Despite this challenge, there is an increasing amount of attention drawn to this area of ecology. As such, it is important to reflect on the status of our current knowledge and synthesize what we have learned because this process may influence future research directions.

I use wood-decaying fungi as a model for our understanding of microbial community patterns in this review for several reasons. First, relative to other microorganisms (e.g., bacteria and archaea), there is substantial information about fungal taxonomy, making it simpler to identify their community structure. Also, wood-decay fungi exhibit a vast diversity in their abilities to process wood (Boddy and Watkinson 1995, Worrall et al. 1997), implying that species compositional

patterns influence associated rates of decomposition. Furthermore, assembly history patterns have already been shown to influence anteceding community structure, as well as affect decomposition rates of the wood substrate (Fukami et al. 2010). Finally, a fundamental understanding of the decomposer community and how it influences rates of decay may be a critical, but currently lacking, component of global carbon (C) models (Cornwell et al. 2009, Mcguire and Treseder 2009). In this review, I begin by discussing some of the major factors that control fungal communities in wood. Then, I describe how molecular techniques can offer alternative information about these communities and suggest future directions for research.

Controls on fungal diversity patterns

The factors controlling wood-decay fungi community patterns are thought to be numerous. Specific information about a given species, such as its colonization strategy, substrate utilization, and both inter- and intra-species interactions are important considerations for community structure. Also, information about the wood environment, chemistry, state of decay, and landscape arrangement can all influence the abundance of a particular species and the community in which it is found (Rayner and Boddy 1988, Jönsson et al. 2008). Because of the plethora of potential factors and interactions, I have limited the scope of this review to a specific set of factors that have been demonstrated to affect fungal community structure patterns (Renvall 1995). I have purposely not included extensive details about such factors as climate (temperature and moisture) and substrate (quality and quantity) because there are comparatively large amounts of information on their role in wood

decomposition (Harmon et al. 1986). Instead, I focused on factors that appear to be important in the literature, but have not been included in a previous literature review. These factors include tree species, wood size, state of decay, and disturbance.

Tree species vary widely in their wood chemistry and physical properties (Rayner and Boddy 1988, Cornwell et al. 2009), so it is not surprising that tree species exert strong influence on their fungal communities. For example, Lumley et al. (2001) incorporated an array of factors potentially driving fungal communities in aspen and white spruce forest, including climate and log variables (size, moisture content, species) into a multivariate analysis. Using mycelial isolation techniques, the authors found that tree species was the most important factor controlling fungal species composition. Kulhánková et al. (2006) reached similar conclusions using a molecular technique (temperature gradient gel electrophoresis; TGGE) in a hardwood forest. Also, Lindner et al. (2006), using sporocarp surveys in mixed hardwood stands, found that tree species was a strong predictor for the five most abundance fungal species encountered. Altogether, this research suggests that fungi exhibit a large degree of tree species preference.

The size of decomposing wood is also a key factor in fungal community structure. In general, fungal species richness tends to increase with log diameter or volume, but smaller woody debris can support more species per unit volume because of its higher surface area to volume ratio (Kruys and Jonsson 1999, Allen et al. 2000, Heilmann-Clausen and Christensen 2004). Therefore, retaining a range of log diameters is usually recommended when managing for fungal species

conservation purposes. Indeed, this was shown in a sporocarp survey in Norway spruce forest where Kruys et al. (1999) observed that uncommon species of fungi showed a preference for larger coarse woody debris (CWD). A related study also observed that certain species were confined to large diameter wood (Jönsson et al. 2008). Lindner et al. (2006) found log diameter to be an important predictor of the five most abundant fungal species in a mixed hardwood forest. Other research suggests that fungal community composition on fine and coarse woody debris (FWD and CWD, respectively) is driven by different factors. Results from Bassler et al. (2010), using sporocarp surveys in a mixed hardwood forest revealed that, although species abundance was best predicted by surface area in both wood size classes, species composition was predominantly controlled by surface area in CWD but by microclimate in FWD. Collectively, these results demonstrate that wood size clearly influences the fungal community structure, but the interactions between size and other environmental variables may be difficult to tease apart.

The state of wood decay is a consistent predictor of fungal community structure, but there is a lot of complexity in these patterns. Several studies have observed that the state of wood decay is the strongest predictor of fungal species composition using both mycelial isolation (Lumley et al. 2001) and sporocarp surveys (Fukasawa et al. 2009a, Pouska et al. 2011). However, other work suggests that state of decay may be less important to some fungal species. For example, Jönsson et al. (2008), using sporocarp surveys in Norway spruce (*Picea abies*), revealed that early fungal colonizers were more affected by the state of decay while secondary colonizers were more likely to be influenced by log characteristics and

arrangement. This suggests that predicting community composition in the later stages of decay may be constrained by environmental factors.

An important consideration for the aforementioned research is the method used for species identification. For example, Rajala et al. (2010) observed that species richness peaked at intermediate stages of decay using sporocarps surveys, but at later stages of decay using denaturing gel gradient electrophoresis (DGGE). Indeed, it is unclear how much general conclusions in the literature would change because of methodological differences (see discussion below). However, research using mycelial isolation and microscopic identification found that fungal species richness increased with the state of decay (Fukasawa et al. 2009b), suggesting that mycecial isolation and molecular techniques may yield comparable results.

Intermediate stages of wood decay may facilitate the most distinct species composition, as well as the most species coexistence because of fungal succession patterns over time. Species with good dispersal capabilities that are able to quickly use the simpler forms of C in the wood would be expected to dominate initially, but as the simpler forms of C are used up, species composition would reflect substrate limitation in that those fungi capable of breaking down complex C (e.g., lignin) would dominate. Hence, species richness might be highest at intermediate stages of decay because the diversity of C substrates would be maximized and thus, open up more niche opportunities. Support for this pattern was revealed by Jonsson et al. (2008); using sporocap surveys, they observed the highest species coexistence at intermediate stages of wood decay. Also, Fukasawa et al. (2009b) observed that

species composition was the most distinct at intermediate stages of decay using mycelial isolation in a beech forest.

McGuire and Treseder (2009) suggest that species identity within microbial communities is most important in the later stages of decomposition when only the very recalcitrant forms of C remain. They point out that only certain fungal species with specific enzymatic capacities can process complex C structures, and, therefore, the species present on a given substrate is rate limiting to decomposition. It is also likely that, given the narrow range of these fungi, that wood in the late stages of decay may harbor more similar communities. Indeed, Rajala et al. (2010) observed this pattern and suggested it was a result of the community becoming more stable after airborne and other forms of colonization by spores was maximized.

Forest disturbance, such as timber harvest, prescribed burning or wildfire, may have a range of consequences for fungal community structure, but few studies have addressed their impacts. Clear-cut logging in Fennoscandinavia had strong effects on fungal species composition, as determined by sporocarp surveys 1 to 4 years following treatment (Junninen et al. 2008). However, the long-term (25 years) effects of slash removal following clear-cut in northern Sweden are presumed to be almost non-existent, since no differences in species richness or abundance were observed (Allmér et al. 2009). Controlled burning in northern coniferous forests favored certain species of fungi, causing short-term (≤ 5 years) alterations in community composition (Olsson and Jonsson 2010, Berglund et al. 2011). A decline in species richness was observed in the first year following burning (Olsson and Jonsson 2010) in one study, but other studies showed that the number of species

recovered within 1 to 5 years following a burn (Berglund et al. 2011). Fungal species richness was also affected by wildfire and timber harvest in aspen and white spruce forest of northern Alberta, Canada, as measured by mycelial isolation. Both types of disturbance led to lower species richness in the recently disturbed forests (one year), while the highest species richness was observed in undisturbed forest (Lumley et al. 2001).

Methodological approaches and future directions

To date, methodologies for assessing communities of wood-decay fungi vary considerably. In climates where moisture is not limiting to sporocarp production, such as northern Europe, sporocarp surveys are predominantly performed as a rapid means of assessment (e.g., Bässler et al. 2010, Ovaskainen et al. 2010a, Berglund et al. 2011). However, sporocarp surveys only identify those fungi that are sporulating at the time of the survey, and, therefore, they do not necessarily represent the entire species richness. Also, in ecosystems that are moisture limited, sporocarp production is confined to wetter periods of the year, such as spring snowmelt or summer monsoon in the southwestern U.S. Because of these limitations, researchers may employ both mycelial isolation and molecular techniques. A relatively large number of wood-decay fungi can be cultured on media using mycelial isolation, wherein emerging fungi are placed in pure culture. Subsequent species identification depends on fungal morphology, which can be difficult because cultures typically only produce vegetative reproductive structures (conidia). An alternative is to use molecular techniques to identify the species in culture (Allmér et al. 2006).

Much of research on the molecular identification of fungi began with work on ectomycorrhizal fungi (Gardes et al. 1991), but is now applied to broader groups of fungi. Ectomycorrhizal DNA can be extracted from root tips, which avoids many of the complications that accompany extracts from soil and wood (e.g., inhibition by polyphenolic compounds); however, methods for extracting fungal DNA from soil and wood continue to improve. For fungi in general, the internal transcribed spacer region (ITS) of the ribosomal DNA is targeted for amplification by polymerase chain reaction (PCR). The ITS region is relatively small in size (600 – 800 base pairs), but high degree of variability, which enables one to distinguish fungal species by the nucleotide sequence (Gardes and Bruns 1993). Its location, between two highly conserved gene regions (5.8S and 28S), facilitated the development of suitable primers for DNA amplification using PCR (Gardes and Bruns 1993). The primer pairs ITS1-F (specific for higher fungi), and ITS4-B (specific for basidiomycetes) or ITS4 (universal primer; Gardes and Bruns 1993, Jasalavich et al. 2000) are commonly used to amplify fungal DNA.

Several DNA fingerprinting techniques, such as restriction fragment length polymorphism (RFLP), terminal restriction fragment length polymorphism (T-RFLP), denaturing gel gradient electrophoresis (DGGE), and temperature gradient gel electrophoresis (TGGE), have been used as a culture-free method of assessing fungal communities in wood (see Zak et al. 2006 for further description). These techniques provide a profile of the community composition without the added effort of cloning (for DNA extracts that contain multiple species). However, DNA fingerprinting techniques tend to detect only the dominant taxa in a given

community, and they do not permit the identification of unique fungal species (Zak et al. 2006). Furthermore, there is no national database of community profiles, which makes it difficult to draw comparisons across studies (Fierer et al. 2009). Recent work has sequenced the DNA bands from TGGE and DGGE analyses, which enhances the community information by identifying the fungal species, but these methods still only detect the dominant species (Kulhánková et al. 2006, Rajala et al. 2010). T-RFLP techniques have made similar advances, and have the same disadvantage (Allmér et al. 2006, Allmér et al. 2009). Allmér et al. (2006) conducted a comparision of T-RFLP, mycelial isolation followed by DNA sequencing, and sporocarp surveys, and assessed the limitations of each technique. Mycelial isolation detected the most species of the three methods, but it is impossible to know if other species were present but not culturable. T-RFLP detected only five species in total, and only one of these was unique for that method.

The use of high-throughput sequencing (also known as pyrosequencing or 454 sequencing) to identify microbial community structure from environmental samples is increasing in popularity because the cost is no longer prohibitive. The advantage of this type of sequencing is that one can submit a DNA extract containing multiple microbial taxa (e.g., from soil) and obtain thousands of sequences at a time. Therefore, one can be relatively certain of identifying all the species in the community while also obtaining a measurement of the relative abundance for each. The disadvantages of high-throughput sequencing are the possibility of sequencing errors and subsequent overestimations of diversity.

Ovaskainen et al. (2010b) recently used 454 sequencing for the identification of wood decay fungal communities in Finnish spruce forests, and compared the results with two other methods (sporocarp surveys and DGGE). The 454 sequencing detected the greatest number of species (30), and 19 of these were not detected by either of the other methods. DGGE profiling detected the lowest number of species (9), and only one of these was unique to that method. Sporocarp surveys detected 10 unique species not found using the other methods. The results show that high-throughput sequencing is a promising technique and would be especially useful in arid climates, where sporocarp production is often moisture limited.

In general, all the molecular techniques are limited by the strength of the database used to match the sequences observed. Zak et al. (2006) reviewed some of the databases used in microbial ecology; of these, the largest is GenBank, hosted by the National Center for Biotechnology Information (NCBI; http://www.ncbi.nih.gov/). This annotated database is comprehensive, but not curated, and, therefore, it has the potential to contain a high amount of errors. Also, despite containing over one hundred million sequences, researchers using the Basic Local Alignment Search Tool (BLAST) to match their sequences often encounter instances where a sequence does not align to any of the sequences in the database (Ovaskainen et al. 2010b, Rajala et al. 2010). Despite these issues, GenBank is extremely advantageous, both for its convenience and wide accessibility, and it provides some of the most up-to-date sequence information available for microbial ecologists. The continued submission of quality, annotated sequences by researchers will undoubtedly improve the resolution of the database in the future.

Clearly, there is no single best way to identify wood-decay fungal community structure. However, the combination of traditional (sporocarp surveys and mycelial isolation) and molecular techniques appears to yield the most comprehensive assessments. High-throughput sequencing can provide the most information with the least amount of effort, but in situations where the cost is prohibitive, cloning and direct sequencing may provide a comparative level of resolution with a considerable amount of effort. Community profiling techniques are advantageous when coarse measurements of community composition are required, but do little to advance our understanding of fungal diversity unless combined with another method, such as sporocarop surveys.

Future work on wood-decay fungal community structure is promising because these communities are conveniently used as model communities to test ecological theory (e.g., Fukami et al. 2010, Ovaskainen et al. 2010a). Also, because not all communities are equal in their ability to process C (Fukami et al. 2010), a comprehensive understanding of wood-decay fungal communities could better inform global C models (Mcguire and Treseder 2009). In turn, this could lead to more realistic and potentially mechanistic global C models. Therefore, we can expect this field to evolve rapidly in the coming years because of the advantages in using wood-decay fungi as a model community, the importance for biodiversity assessments, and the relevance to global C cycles.

LITERATURE CITED

Allen, R. B., P. K. Buchanan, P. W. Clinton, and A. J. Cone. 2000. Composition and diversity of fungi on decaying logs in a New Zealand temperate beech (*Nothofagus*) forest. Canadian Journal of Forest Research **30**:1025-1033.

Allmér, J., J. Stenlid, and A. Dahlberg. 2009. Logging-residue extraction does not reduce the diversity of litter-layer saprotrophic fungi in three Swedish coniferous stands after 25 years. Canadian Journal of Forest Research **39**:1737-1748.

Allmér, J., R. Vasiliauskas, K. Ihrmark, and A. Dahlberg. 2006. Wood-inhabiting fungal communities in woody debris of Norway spruce (*Picea abies* (L.) Karst.), as reflected by sporocarps, mycelial isolations, and T-RFLP identification. FEMS Microbiology Ecology **55**:57-67.

Bässler, C., J. Müller, F. Dziock, and R. Brandl. 2010. Effects of resource availability and climate on the diversity of wood-decaying fungi. Journal of Ecology **98**:822-832.

Berglund, H., M. T. Jönsson, R. Penttilä, and I. Vanha-Majamaa. 2011. The effects of burning and dead-wood creation on the diversity of pioneer wood-inhabiting fungi in managed boreal spruce forests. Forest Ecology and Management **261**:1-13.

Boddy, L. and S. C. Watkinson. 1995. Wood decomposition, higher fungi, and their role in nutrient redistribution. Canadian Journal of Botany **73(Suppl. 1)**:S1377-S1383.

Cornwell, W. K., J. H. C. Cornelissen, S. D. Allison, J. Bauhus, P. Eggleton, C. M. Preston, F. Scarff, J. T. Weedon, C. Wirth, and A. E. Zanne. 2009. Plant traits and wood fates across the globe: rotted, burned, or consumed? Global Change Biology **15**:2431-2449.

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

Fierer, N., J. Morse, S. Berthrong, E. Bernhardt, and R. Jackson. 2007. Environmental controls on the landscape-scale biogeography of stream bacterial communities. Ecology **88**:2162-2173.

Fukami, T., I. Dickie, J. Paula Wilkie, B. Paulus, D. Park, A. Roberts, P. Buchanan, and R. Allen. 2010. Assembly history dictates ecosystem functioning: evidence from wood decomposer communities. Ecology Letters **13**:675-684.

Fukasawa, Y., T. Osono, and H. Takeda. 2009a. Dynamics of physicochemical properties and occurrence of fungal fruit bodies during decomposition of coarse woody debris of Fagus crenata. Journal of Forest Research **14**:20-29.

Fukasawa, Y., T. Osono, and H. Takeda. 2009b. Microfungus communities of Japanese beech logs at different stages of decay in a cool temperate deciduous forest. Canadian Journal of Forest Research **39**:1606-1614.

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

Gardes, M., T. J. White, J. A. Fortin, T. D. Bruns, and J. W. Taylor. 1991. Identification of indigenous and introducted symbiotic fungi in ectomycorrhizae by

amplification of nuclear and mitochondrial ribosomal DNA. Canadian Journal of Botany **69**:180-190.

Harmon, M. E., J. F. Franklin, F. Swanson, P. Sollins, S. Gregory, J. Lattin, N. Anderson, S. Cline, N. Aumen, J. Sedell, G. Lienkaemper, K. Cromack, and K. Cummins. 1986. Ecology of coarse woody debris in temperate ecosystems. Advances in Ecological Research **15**:133-302.

Heilmann-Clausen, J. and M. Christensen. 2004. Does size matter? On the importance of various dead wood fractions for fungal diversity in Danish beech forests. Forest Ecology and Management **201**:105-117.

Jasalavich, C., A. Ostrofsky, and J. Jellison. 2000. Detection and identification of decay fungi in spruce wood by restriction fragment length polymorphism analysis of amplified genes encoding rRNA. Applied and Environmental Microbiology **66**:4725-4734.

Jönsson, M., M. Edman, and B. G. Jonsson. 2008. Colonization and extinction patterns of wood-decaying fungi in a boreal old-growth *Picea abies* forest. Journal of Ecology **96**:1065-1075.

Junninen, K., J. Kouki, and P. Renvall. 2008. Restoration of natural legacies of fire in European boreal forests: an experimental approach to the effects on wood-decaying fungi. Canadian Journal of Forest Research **38**:202-215.

Kruys, N., C. Fries, B. G. Jonsson, T. Lämås, and G. Ståhl. 1999. Wood-inhabiting cryptogams on dead Norway spruce (Picea abies) trees in managed Swedish boreal forests. Pages 178-186 Canadian Journal of Forest Research.

Kruys, N. and B. G. Jonsson. 1999. Fine woody debris is important for species richness on logs in managed boreal spruce forests of northern Sweden. Pages 1295-1299 Canadian Journal of Forest Research.

Kulhánková, A., T. Béguiristain, J. Moukoumi, J. Berthelin, and J. Ranger. 2006. Spatial and temporal diversity of wood decomposer communities in different forest stands, determined by ITS rDNA targeted TGGE. Pages 547-556 Annals of Forest Science.

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

Lumley, T. C., D. L. Dennis Gignac, and R. S. Currah. 2001. Microfungus communities of white spruce and trembling aspen logs at different stages of decay in disturbed and undisturbed sites in the boreal mixedwood region of Alberta. Canadian Journal of Botany **79**:76-92.

Mcguire, K. L. and K. K. Treseder. 2009. Microbial communities and their relevance for ecosystem models: Decomposition as a case study. Soil Biology and Biochemistry:1-7.

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

Ovaskainen, O., J. Hottola, and J. Siitonen. 2010a. Modeling species co-occurrence by multivariate logistic regression generates new hypotheses on fungal interactions. Ecology **91**:2514-2521.

Ovaskainen, O., J. Nokso-Koivisto, J. Hottola, T. Rajala, T. Pennanen, H. Ali-Kovero, O. Miettinen, P. Oinonen, P. Auvinen, and L. Paulin. 2010b. Identifying wood-inhabiting fungi with 454 sequencing – what is the probability that BLAST gives the correct species? Fungal Ecology **3**:274-283.

Pouska, V., J. Lepš, M. Svoboda, and A. Lepšová. 2011. How do log characteristics influence the occurrence of wood fungi in a mountain spruce forest? Forest Ecology **4**:201-209.

Rajala, T., M. Peltoniemi, T. Pennanen, and R. Mäkipää. 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.

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

Renvall, P. 1995. Community structure and dynamics of wood-rotting Basidiomycetes on decomposing conifer trunks in northern Finland. Karstenia **31**:1-51.

Strickland, M., C. Lauber, N. Fierer, and M. Bradford. 2009. Testing the functional significance of microbial community composition. Ecology **90**:441-451.

Worrall, J. J., S. E. Anagnost, and R. A. Zabel. 1997. Comparison of wood decay among diverse lignicolous fungi. Pages 199-219 Mycologia.

Zak, D., C. Blackwood, and M. Waldrop. 2006. A molecular dawn for biogeochemistry. Trends in Ecology & Evolution **21**:288-295.

CHAPTER 3

LONG-TERM ALTERATIONS OF NITROGEN CYCLING FOLLOWING SEVERE WILDFIRE IN SOUTHWESTERN PONDEROSA PINE FORESTS

ABSTRACT

Stand-replacing wildfires are a novel disturbance within southwestern ponderosa pine forests and, as a result, there are many uncertainties regarding their influence on post-fire ecosystem functioning. In particular, N cycling patterns may be altered to a greater extent under this fire regime relative to the historic, low-intensity fire regime. However, few studies have examined the direct effects of this type of fire on N cycling, nor the long-term consequences. We used a stand-replacing wildfire chronosequence to examine the long-term dynamics of net and gross nitrogen (N) transformations following severe burning. To control for environmental variation among the burned sites, we established paired, adjacent unburned sites for each burn year. We tested direct effects of fire by comparing burned and unburned sites for each year, and we assessed long-term changes over time using the differences between each burned and unburned pair. We expected N transformations and pool sizes in burned areas to become more similar to comparable unburned forest as these areas become re-vegetated. Net N transformations continued to be affected by fire after 30+ years. Burned sites had consistently higher rates of net nitrification than unburned sites. Overall rates of net N mineralization were lower in burned than unburned sites, but net nitrification was a greater proportion of net mineralization in the burned sites. Burned sites had

higher NO_3^- pools than unburned sites, but NH_4^+ pools were similar between burned and unburned sites. Gross rates of N mineralization were not different between burned and unburned paired sites, but burned sites had generally higher rates of gross nitrification than unburned sites. Overall, our findings indicate that the effects of high-severity wildfires on N cycling in these ecosystems persist for several decades, and we hypothesize that this is the result of altered vegetation dynamics in burned sites. These results may have long-term implications for the recovery of ecosystem N and associated rates of net primary productivity in these ecosystems.

INTRODUCTION

The complex interactions between land management and climate change have led to pronounced changes in the wildfire disturbance regimes of coniferous forests in the western U.S. (McKenzie et al. 2004, Westerling et al. 2006, Williams et al. 2010). Wildfire is the most pervasive natural disturbance in these ecosystems (Agee 1998), and a century of fire exclusion, coupled with a warmer and drier climate, have culminated in widespread increases in the frequency and size of stand-replacing wildfires throughout this region (Covington and Moore 1994a, Westerling et al. 2003, Westerling et al. 2006). Severe fires can alter a suite of community and ecosystem properties, including plant community structure (Hebel et al. 2009, Ojeda et al. 2010), primary productivity (Sabo et al. 2008), microbial communities (Smithwick et al. 2005), and the biogeochemical cycling of carbon (Wardle et al. 2003, Rothstein et al. 2004, Kashian et al. 2006) and nitrogen (DeLuca et al. 2002, Grady and Hart 2006, Turner et al. 2007). Some coniferous forests are adapted to high-severity wildfires (e.g., jack pine, *Pinus banksiana* Lamb.; lodgepole pine, *Pinus contorta* Douglas ex Louden var. *latifolia* Engelm. ex S. Watson), but others, such as southwestern ponderosa pine (*Pinus ponderosa* var. *scopulorum* Engelm), are adapted to low-intensity surface fire regimes (Cooper 1960). For these forests, the relatively common occurrence of high-severity fires over the last ~40 years (Covington et al. 1997) constitutes a major shift in their natural disturbance regime. Climate predictions suggest that stand-replacing wildfires will occur with increased in frequency in the future (Westerling et al. 2006, Marshall et al. 2008, Williams et

al. 2010); however, the long-term implications of this disturbance type on ecosystem functioning are uncertain.

The effects of stand-replacing wildfires on nitrogen (N) dynamics are of particular interest because N, along with water, is limiting to cone production and diameter growth in southwestern ponderosa pine forests (Heidmann et al. 1979). As a result, the post-fire N availability may be critical to pine regeneration and net primary productivity (NPP). Historically, the relatively rapid turnover of N was the result of a frequent, low-intensity surface fire regime, which maintained forests in open stands with abundant herbaceous openings (Cooper 1960, Covington and Moore 1994a). Hart et al. (2005) hypothesized that this vegetation structure influenced the belowground microbial community, leading to the regulation of rapid N cycling. However, historic fire regimes in this region were virtually eliminated by practices introduced by Euro-American settlers: livestock grazing reduced herbaceous fuels that would carry a surface fire, and active fire suppression led to extended periods of fire exclusion (Covington et al. 1997). Current forests are characterized by high densities of small diameter trees (White 1985, Covington and Moore 1994b), and associated rates of net N transformations are less than half that of the historic regime, as suggested by forest restoration treatments (Kaye and Hart 1998a).

Although N transformations in southwestern ponderosa pine forests have been substantially altered due to a century of fire exclusion, the current regime of infrequent, stand-replacing wildfires has the potential to induce alternative trajectories in N cycling. Fires oxidize much of the N stored organically in biomass

(Johnson et al. 2005), but they also cause a temporary pulse in available N in the soil, which may facilitate post-fire regeneration (White 1986, Covington and Sackett 1992, Wan et al. 2001). This pulse is initially comprised of pyrogenically produced ammonium (NH_4^+), which remains elevated for approximately 1 to 2 years following fire (Raison 1979, Covington and Sackett 1992, Prieto-Fernández et al. 2004, Turner et al. 2007, Boerner et al. 2009). Associated with this is the enhancement of nitrification arising from the increased substrate availability (NH_4^+) and higher soil pH (Christensen 1973, Bauhus et al. 1993). Elevated levels of nitrate (NO_3^-) are typically found within a few days of the fire and reach their maximum approximately 1 year after prescribed burning (Covington and Sackett 1992, Wan et al. 2001). Importantly, stand-replacing wildfires may have greater effects on N mineralization patterns than prescribed burning because they tend to burn more intensely (Kovacic et al. 1986, Covington and Sackett 1992). Indeed, Grady and Hart (2006) observed longer-term pulses in net N mineralization, seven years after a stand-replacing wildfire in a southwestern ponderosa pine forest.

Given the multitude of studies that have been conducted on the short-term responses of wildfire, there is comparatively little known about the long-term effects on N cycling. Long-term studies employing a chronosequence approach showed declines in inorganic N availability over time since stand-replacing wildfire in northern boreal forests (DeLuca et al. 2002) and lodgepole pine forests (Turner et al. 2007). In contrast, patterns of initial decline and subsequent increase over time in net N mineralization and NH_4^+ concentrations following stand-replacing wildfire have been observed in jack pine forests (Yermakov and Rothstein 2006), and

southern boreal forests (Brais et al. 1995). Regardless of the inconsistent patterns observed, these long-term studies all occur in forests that are adapted to stand-replacing fire regimes. It is difficult to draw comparisons to southwestern ponderosa pine forest because stand-replacing wildfires are a novel disturbance to these ecosystems, and, therefore, N cycling response patterns may be altogether different.

To assess long-term N cycling dynamics following stand-replacing wildfires in southwestern ponderosa pine forests, we measured net and gross N transformation rates along a wildfire chronosequence. Each burned site was paired with an adjacent site in unburned forest to better isolate the effect of time since fire. We hypothesized (1) that burned and unburned sites would have different rates of net N transformations because of inherent microclimate differences caused by the fire, and that these differences would be more pronounced during the summer monsoon season (mid-July to mid-September) when plant growth rates are maximized. Next, we hypothesized (2) that rates of net N mineralization and associated (NH_4^+) pool sizes would decline with time since fire as vegetation demand for N increases. To test this, we assessed the size of the differences between burned and paired unburned sites over time since fire. We expected the differences to become smaller over time as the burned sites began to resemble unburned forests. We also hypothesized (3) that rates of net nitrification and associated (NO_3^-) pool sizes would decline with time since fire as a result of increase plant-microbe competition for NH_4^+ (Kaye and Hart 1997). Similar to hypothesis (2), we examined the differences over time since fire between burned and unburned paired sites. In

addition, we calculated the relative proportion of net nitrification to N mineralization at each site and examined how the difference in the ratio at burned and unburned paired sites changed over time. We expected the differences in this ratio to decline over time and eventually resemble that of the paired unburned sites. Finally, we hypothesized (4) that rates of gross N mineralization would be low initially following fire because of C limitations resulting from combustion (Koyama et al. 2010), but these rates would increase over time since fire as C inputs from plant litter increase (Turner et al. 2007). Concurrently, we expected that post-fire rates of gross nitrification would be high initially following fire because of warmer soil temperatures and higher pH (Raison 1979, Hobbs and Schimel 1984), but would similarly decline with time since fire, eventually resembling rates in comparable undisturbed forest (Davidson et al. 1992). For both gross N transformations we expected these temporal trends to be reflected as a decline over time since fire, in the difference between burned and unburned forests.

METHODS

Study sites and experimental design

We conducted this study across seven post-wildfire sites in ponderosa pine forests of northern Arizona, U.S. According to the Western Regional Climate Center (www.wrcc.dri.edu), the Fort Valley weather station (within 23 km and at a similar elevation to all sites) received 65.4 cm of precipitation during the 12-month study period (01-July-2007 to 01-July-2008). The mean daily maximum air temperature for the same period was 17.2°C, and the mean daily minimum air temperature was -3.7°C. The 30-year averages (1971-2000) for the station were 56.3 cm/y of

precipitation, with mean daily maximum and minimum air temperatures of 16.3 °C and -3.6°C, respectively. Study sites were located on basaltic parent material and had each experienced one stand-replacing crown fire (> 95% tree mortality) within the last 60 years according to records at Coconino and Kaibab National Forests.

We employed a chronosequence (space-for-time substitution) approach to examine the long-term effects of stand-replacing wildfires on these ecosystems. We chose to sample a broad temporal extent (seven sites over a 32-year period) rather than having replicated sites over a smaller number of years because this design was better suited to illustrating changes in N cycling dynamics over time. Two of the older wildfire sites (23 and 34 years) were salvage logged and replanted after the fire, which was the typical management practice at the time. The other sites did not receive active management after the fire. We acknowledge that the management difference compromises our study design to some extent. However, we considered the need for a clearer understanding of long-term post-wildfire ecosystem functioning, coupled with the limited number of suitable wildfire sites in this region to be the most important factors for conducting this research. Furthermore, a companion study performed at this chronosequence indicates that both live plant biomass C and woody debris C remained relatively consistent with time since fire (Ross et al., In review), suggesting that these management activities did not substantially compromise the chronosequence.

Seven wildfire sites were selected encompassing a 32-year period between 1973 and 2005 (Table 1). Each site was paired with an adjacent unburned patch of forest to better isolate the effects of fire and reduce other sources of variation

(Johnson and Miyanishi 2008). Unburned sites were located in the nearest accessible unburned forest. Vegetation in the unburned sites consisted of ponderosa pine with some Gambel oak (*Quercus gambelii* Nutt.) at lower elevations or mixed conifers (*Pseudotsuga menziesii* (Mirb.) Franco and *Pinus strobiformis* Engelm.) at higher elevations.

At each wildfire site, we established three, 200-m transects within the perimeter of the burn. Burn area heterogeneity prevented identical transect orientation and layout across all the sites, but we attempted to sample in a similar manner across all sites as much as possible; this included covering a similar areal extent at all the burned and unburned sites. Three, 10-m radius plots were located 100 m apart along each of the three transects for a total of nine plots at each site (n = 9). Plots were at least 100 m from roads and 20 m from trails or old logging roads. At most of the sites, transects were at least 100 m from unburned forest; however, at the two most recent burns (time since fire: two and six years), the size and perimeter of the burn meant that plots were approximately 25 m from unburned forest. We collected above- and below-ground C data at each plot, including tree biomass C, herbaceous C, organic horizon C, woody debris C, and mineral soil total C, total N, pH, gravimetric water content (GWC), and bulk density, as described by Ross et al. (In review).

Quantification of net N mineralization rates

We measured *in situ* net N transformations (i.e., net N mineralization and net nitrification) rates over two incubation periods spanning 12-months. The first incubation went from early July through October, 2007 (monsoonal period; growing

season), and the second incubation went from November to early July, 2008 (dormant season). We used the resin core method (DiStefano and Gholz 1986), as modified by Kaye and Hart (1998). This modification allows for the concurrent measurement of net N transformations in the forest floor (O horizon) and mineral soil (0-15 cm) using the same resin core. The cores were incubated at a fixed distance (7.5 m) and angle from the plot center. We estimated net N mineralization by calculating the difference between the initial and final soil (NH_4^+ + NO_3^-)-N pools plus the (NH_4^+ + NO_3^-)-N adsorbed onto the mixed-bed, ion exchange resin (IER) bag fixed beneath the mineral soil core. We estimated net nitrification in a similar manner using the initial and final soil NO_3^--N pools and the IER adsorbed NO_3^--N. Forest floor net N mineralization and nitrification were estimated by placing a second IER bag on top of the mineral soil core and putting forest floor material on top of this bag. Rates were calculated using the difference between the initial and final forest floor N (NH_4^+ + NO_3^- for mineralization; NO_3^- for nitrification) pools, plus that component adsorbed onto the IER bag beneath the forest floor. There was not sufficient forest floor material at the wildfire sites to estimate N transformations, but we placed an IER bag on top of the mineral soil to ensure that inputs from throughfall were not included in the measurements.

The soils were stored at 4°C (≤ 5 days) until sieved field-moist (<4 mm) and extracted. Thirty grams of mineral soil were extracted in 100 ml of 2 M KCl and the IER was extracted in 100 ml of 2 M KCl. The forest floor material was processed by removing items >1cm in diameter, cutting up the remainder with scissors and homogenizing before extracting 5 g in 25 ml of 2 M KCl. Extracts were mechanically

shaken for one hour, filtered through preleached (with deionized water) Whatman #1 filters, and frozen until inorganic N concentrations could be analyzed. Extracts were analyzed for NH_4^+, and NO_3^- on a Lachat AE Flow-Injection Analyzer (Lachat Instruments Inc., Loveland, CO, USA), using indophenol blue (Lachat Instruments Inc., 1990) and cadmium reduction-diazotization (Lachat Instruments Inc., 1992) methods, respectively. The mean bulk density for all four mineral soil cores taken during the study period (2 incubation periods, 1 initial and 1 final for each) was used to convert the mineral soil mass-based transformation rates to an areal basis. For the forest floor transformations, the mean bulk density computed from two 30 x 30 cm quadrats taken at each plot was used to convert mass-based forest floor rates to an areal basis.

Quantification of gross N transformation rates

We used the ^{15}N isotope dilution method (Hart et al. 1994) to measure gross N transformation rates in a 24-hour laboratory incubation. For logistical reasons, we selected a subset of the burned and unburned chronosequence sites (5 of the 7 original: 2, 7, 11, 30, and 34 years since fire) and plots (6 plots of the original 9 at each site) for these measurements. In doing so, we reduced the number of analyses, which are both time consuming and expensive, while retaining enough sites to compare older to more recent fires. We conducted this measurement in mid-August because the soil was moist and warm from the summer monsoons, and we expected N transformations to be maximized during this period (Kaye and Hart 1998a). We also measured soil temperatures at these sites using data logger per site (Hobo Pendant Data Loggers; Onset Computer Corporation, Bourne, MA, USA) at a depth of

5 cm in the mineral soil; values were recorded every 10 seconds for 3.5 weeks. All values were stored and mean soil temperatures were calculated for each site.

We collected mineral soil (0-10 cm) for the gross N transformation incubation on 11-13 August 2008 by taking cores with an Oakfield sampler (Oakfield Apparatus, Inc.; Oakfield, WI, USA). The cores were 1.9-cm diameter and were taken from a 0.5 m^2 area at a fixed (7.5 m) distance from the plot center and in the opposite direction from where the net transformation cores were incubated. We chose this location because we wanted to avoid soil disturbance created by companion research at the plots. Soils were kept on ice while in transport and refrigerated in the laboratory for 24-48 hours. All soils were sieved at field-moisture content (<4 mm) on 13 August. On 14-August-2008, we weighed approximately 25 g of sieved soil into each of five specimen containers. The first sample was immediately extracted in 100 ml 2 M KCl, and the concentrations of NH_4^+ and NO_3^- were used to estimate initial pool sizes. The other four samples were divided into nitrification or mineralization groups at time 0 hours (t0) or time 24 hours (t24). We injected 0.35 ml of a labeled solution, $^{15}NO_3^-$ as $K^{15}NO_3^-$ or $^{15}NH_4^+$ as $(^{15}NH_4^+)_2SO_4$ (200 mg N/L; 99 atom % ^{15}N enrichment) into the appropriate soil using a syringe and needle. Each 0.35 ml aliquot was divided into four equal proportions and injected into the soil at four evenly spaced locations. Each injection was made approximately halfway in between the soil surface and the bottom of the specimen container to ensure even distribution of the label. The solution injections added approximately 3.5 µg N/kg dry soil for both of the N species and increased the GWC by approximately 14%. The t0 soils were immediately extracted in 100 ml

of 2 M KCl. The t24 soils were incubated inside sealed glass Mason jars (~1 L) with distilled water at the bottom to maintain the moisture content. The jars were incubated for 24 h in the dark at 20°C before the soils were extracted in 100 ml 2M KCl. All extractions were performed as described above for net N transformations and frozen until subsequent analysis on the Lachat (initial pool sizes) or preparation for ^{15}N analysis.

We performed the ^{15}N analyses using the diffusion procedure described by Stark and Hart (1996). The ^{15}N contents were determined on the diffusion extracts using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (University of California, Davis, Stable Isotope Facility; PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer; Sercon Ltd., Cheshire, UK). We used the NH_4^+ and NO_3^- pool sizes at t0 and t24, the atom % ^{15}N excess at t0 and t24, and the equations of Kirkham and Bartholomew (1954) to calculate rates of gross mineralization and nitrification.

Statistical Analyses

All data were checked for adherence of the assumptions of regression and ANOVA using the Shapiro-Wilk's test for normality and the O'Brien test of equality of variance. Outliers in the net N transformation data were identified as points with studentized residuals outside the 99% confidence interval of the standard normal distribution, and were excluded from the analyses. This resulted in the removal of one or two outlying data points for each site and process. An *a priori* alpha level was set at 0.05. All statistical analyses were conducted using JMP statistical software (SAS Institute; version 5.0.1.2).

We examined the mineral soil characteristics (total C, total N, C:N, pH, and GWC) using a two-way analysis of variance (ANOVA) with time since fire (years) and treatment (burned or unburned) as the main effects. All of these analyses contained significant year X treatment interactions; thus, treatment differences were assessed for each year separately.

We assessed the effect of fire on net N transformations in the mineral soil using a two-way ANOVA with time since fire (years), treatment (burned or unburned) as the main effects, and means were separated using Tukey's HSD. When a significant year X treatment interaction was encountered (net nitrification), we assessed treatment differences for each year separately using a one-way ANOVA. We examined the effect of season (growing or dormant) on net N transformations using a three-way ANOVA with time since fire, treatment, and season as the main effects; however, there were significant interactions between season and time since fire ($P = 0.02$), and between season and treatment ($P < 0.0001$). Thus, we proceeded by analyzing season and treatment for each burn year separately using a two-way ANOVA. We used Tukey's HSD to separate means for season and treatment; however, when significant season X treatment interactions occurred for a given time since fire year, we analyzed season and treatment separately for that year.

We assessed changes in net N mineralization rates and NH_4^+ pools over time since fire by calculating the differences between the burned and unburned paired sites (burned – unburned). We then explored temporal patterns in the differences using linear regression. We examined patterns in net nitrification and NO_3^- pool sizes in a similar manner. In addition, we calculated the ratio of net nitrification to

net N mineralization at each site; we then examined the differences in the ratios between burned and unburned paired sites over time using linear regression. Finally, we investigated the effect of fire on the NH_4^+ and NO_3^- pool sizes using two-way ANOVA, with time since fire and treatment as the main effects.

We assessed the effect of fire on gross N transformations in the mineral soil using a two-way analysis of variance (ANOVA), with time since fire (years) and treatment (burned or unburned) as the main effects, and means were separated using Tukey's HSD. We analyzed changes in the gross N transformation rates over time by computing the difference between the burned and unburned paired sites (burn – unburned) and performing a linear regression with time since fire. In addition, we calculated the ratio of gross nitrification to gross N mineralization at each site; we then examined the differences in the ratios between burned and unburned paired sites over time using linear regression

RESULTS

The mineral soil characteristics varied between the burned and unburned treatments across the burn years (Table 3.1). The most consistent differences were observed for pH, wherein the burned soils had higher pH values than the unburned soils at all the sites except for year 23. Also, C:N ratios were lower in the burned soils than the unburned soils at years 6, 7, 11, and 30.

Both time since fire and treatment (burned or unburned) had a significant effect on net N mineralization rates ($P = 0.03$ and $P = 0.0002$, respectively), and there was no significant interaction term ($P = 0.44$). Tukey's HSD did not reveal statistical differences between burned and unburned pairs for any of the burn years

(Figure 3.1A). Pools of NH_4^+ were affected by treatment ($P = 0.04$), but not time since fire ($P = 0.33$; Figure 3.2A), nor was there a significant interaction ($P = 0.14$). A significant time since fire X treatment interaction was observed for net nitrification rates ($P = 0.008$); thus, we compared the treatments separately for each year. Net nitrification rates were significantly higher in the burned than the unburned sites for all years ($P < 0.05$; Figure 3.1B). Pools of NO_3^- were affected by treatment ($P < 0.0001$) but not time since fire ($P = 0.11$), nor was there a significant interaction ($P = 0.19$). Nitrate pools were statistically higher in the burned sites than unburned at years 2, 23, and 30. We did not include the net N transformation rates in the organic horizon at the unburned sites in the net N transformation rate site estimate because adding them did not alter the overall patterns between treatments and time since fire; however, net N mineralization rates in the organic horizon ranged from 0.36 – 1.0 g N m^{-2} y^{-1}, and net nitrification rates ranged from 0.09 – 0.27 g N m^{-2} y^{-1}.

Net N transformation rates were generally higher during the growing season (July – October) than the dormant season (November – June; Figures 3.3, 3.4). Differences between burned and unburned sites were evident for net nitrification during both growing and dormant season, except for year 23, when no seasonal differences were observed, and year 34, when there were no differences in the dormant season rates. Distinctions between burned and unburned sites were also more apparent during the growing season for net N mineralization (Figures 3.3A, 3.4A). During the growing season, net N mineralization was higher at the unburned sites for all years except 6 and 30, while this pattern was only observed at year 7 during the dormant season.

The differences in net N mineralization rates (burned – unburned) were not significantly correlated with time since fire (R^2 = 0.29, P = 0.21; Figure 3.5A), and neither were the differences in NH_4^+ pool sizes (R^2 = 0.05, P = 0.64; data not shown). Similarly, net nitrification rate differences and NO_3^- pool size differences were not correlated with time since fire (R^2 = 0.12, P = 0.45 and R^2 = 0.26, P = 0.24, respectively; Figure 3.5B). The differences in the net nitrification: net N mineralization ratios were also not correlated with time since fire (R^2 = 0.05, P = 0.62; Figure 3.5).

Rates of gross N mineralization varied with year (P = 0.01), but not with treatment (burned or unburned; P = 0.49; Figure 3.6A). In contrast, rates of gross nitrification varied by both year (P = 0.01) and treatment (P < 0.0001). Tukey's HSD revealed that burned and unburned pairs were significantly different from each other at years 7 and 30 (Figure 3.6B). The ratios of net nitrification to gross N mineralization were generally higher in the burned than the unburned soils (Figure 3.6C) with the exception of year 34, when rates of gross N mineralization were very close to zero. The differences between burned and unburned rates of gross N mineralization and nitrification were not correlated with time since fire (R^2 = 0.22; P = 0.43; R^2 = 0.004; P = 0.92, respectively; data not shown), nor were the differences in the ratio of gross nitrification rates to gross N mineralization (R^2 = 0.003; P = 0.93; data not shown). The average daily temperatures were approximately 4 °C warmer in burned than unburned soils (14.4 °C and 18.7 °C, respectively), though lack of replication prevented us from confirming this statistically.

DISCUSSION

This research provides new insights on the long-term N cycling patterns in southwestern ponderosa pine forests following a stand-replacing wildfire, which is a novel disturbance type in this ecosystem. Our results suggest that the effects of stand-replacing wildfire on both net and gross N transformation rates and inorganic N pool sizes persist for several decades in this ecosystem. Since soil N transformations closely parallel vegetation recovery following wildfire (Hobbs and Schimel 1984, Hart et al. 2005, Koyama et al. 2010), the patterns we observed are potentially a reflection of an ecosystem state change driven by post-fire establishment.

Our net N transformation results suggest that the effects of stand-replacing wildfires on N cycling dynamics in these ecosystems last longer than the length of our 34-year chronosequence. In support of our first hypothesis, annual rates of net nitrification were consistently higher in the burned sites than the paired unburned sites, and there was a strong burn effect on annual net N mineralization rates. This pattern was also observed for the NH_4^+ and NO_3^- pool sizes, wherein levels of NO_3^- were elevated at three of the burned sites, and NH_4^+ pools were generally affected by burning. The patterns we observed for net nitrification and NO_3^- pool sizes may be explained by environmental conditions often observed in recently burned sites that facilitate nitrification, including higher pH and soil temperatures (Raison 1979, Hart et al. 2005). Our soil temperature data suggest that daily mineral soil temperatures in the burned sites are approximately 4° C higher than the unburned sites. Also, the mineral soils in the unburned sites were generally more acidic than the burned sites (Table 3.1), which may be inhibiting autotrophic nitrifiers (Johnson

1992), and therefore, may also explain the patterns of elevated net nitrification rates.

Net N transformation rates were two to three times as high during the growing season than the dormant season, confirming that N availability is highest when soil temperatures are elevated and soil moisture contents are sufficient, and coinciding with maximum plant growth rates. Also, differences between the burned and unburned pairs were more apparent during the growing season, which further supports our hypothesis (1). This was especially true for net N mineralization, where 5 of the sites had higher rates in the unburned than the burned sites during the growing season, and suggests that distinctions among the net N transformations may have been diluted when expressed at the annual scale. In general, the dormant season rates were more variable than the growing season rates, which may have made treatment differences harder to detect. Approximately half of the annual precipitation falls during the summer monsoon season (mid-July to mid-September) in this water-limited region (Schubert 1974); this, coupled with higher summer soil temperatures, would support higher soil microbial activity during this period (Kaye and Hart 1998b), .

We did not observe a statistically significant time since fire association among the differences for either net N mineralization or net nitrification rates, nor for NH_4^+ or NO_3^- pool sizes. In addition, the differences in the relative proportion of net N mineralization resulting from net nitrification (net nitrification: net mineralization ratio) remained relatively flat over time, demonstrating that net nitrification made a substantial contribution to net N mineralization rates in the

burned sites throughout the length of our chronosequence. The lack of temporal trends in our data is contrary to our hypotheses (2 and 3) because we expected rates of N cycling and pools in burned forests to become similar to unburned forests over time since fire, suggesting a relatively slow post-fire recovery of N cycling in southwestern ponderosa pine forests.

We observed a similar lack of temporal trend with time since fire in the gross N production rate measurements. Again, the differences over time remained relatively constant for both gross N mineralization and gross nitrification rates, suggesting persistent differences in mineral N production rates, and providing no support for our hypothesis (4). However, the gross N transformations are consistent with the net N transformations, suggesting that rates of NH_4^+ and NO_3^- production are driving the observed patterns between treatments and over time since fire.

It is notable that the ratios of nitrification:N mineralization are generally much higher for the gross than the net N transformations. This may have resulted from using field incubated, intact cores for the net N transformations and lab incubated, sieved soils for the gross N transformations. Nitrogen transformation rates measured in mixed soils are not necessarily representative of those in undisturbed soils (Booth et al. 2006). In our study, sieving may have increased substrate (NH_4^+) availability to nitrifiers, and thus, stimulating nitrification rates to a higher degree for the gross N transformation measurements.

One potential explanation for the sustained effects of stand-replacing wildfire on net and gross nitrification rates is the effect of fire-deposited charcoal. Although the exact mechanism has not been identified, charcoal may affect soil processes

through the sorption of phenolic compounds (Zackrisson et al. 1996) or by enhancing microbial activity (Pietikäinen et al. 2000). DeLuca and Sala (2006) demonstrated experimentally that recently deposited charcoal can increase the nitrification potentials in unburned forest soils in northwestern ponderosa pine forests. However, this effect has not been demonstrated under field conditions.

We speculate that limited tree regeneration following large, stand-replacing wildfires in this region has led to prolonged impacts on N dynamics, and this is primarily visible in elevated rates of nitrification. Ponderosa pine forests are known to take on various vegetation trajectories following stand-replacing wildfires, including grassland, shrubland, or dense forest (Savage and Mast 2005). Natural regeneration can be limited in these forests (Heidmann 2008) because it requires a specific combination of seed production, weather conditions, and a favorable seed bed (Gaines and Shaw 1958). Although cone production in ponderosa pine is enhanced by N fertilization (Heidmann et al. 1982), and, thus, may be facilitated after wildfire, the widespread fire-induced pine mortality would impede natural regeneration. Additioanlly, seedling establishment may depend on a host of other factors, including moisture conditions (Gaines and Shaw 1958) and specific mycorrhizal linkages (Booth and Hoeksema 2010).

The post-wildfire vegetation trajectory is thought to drive microbially mediated ecosystem processes (Hart et al. 2005), and the shift in vegetation at the burned sites may have ecosystem consequences for N retention and cycling. The wildfire sites in the current study have little-to-no natural pine regeneration, and the resultant shift to an herbaceous-dominated plant community coincides with the

prolonged changes in N transformation rates we observed. As burned sites become re-vegetated, we expect plants, especially trees, to be more effective competitors for N than heterotrophic microbes over the long term (Johnson 1992). However, a lack of tree regeneration at the burned sites in the current study may reduce overall ecosystem N demand. When coupled with the sustained increases in N transformations rates that we observed, the implication is that considerable N could be lost from the site via leaching of NO_3^- or denitrification (Johnson 1992). This may be especially critical in ponderosa pine ecosystems because both of these processes are enhanced when soils are wet, and the wet growing (monsoon) season coincides with greater rates of N transformations in these soils. A major implication of progressive ecosystem N loss is an overall reduction in long-term NPP (Vitousek and Howarth 1991).

Our findings contrast with some long-term studies of stand-replacing wildfires in the literature, where temporal trends in N availability and net N mineralization patterns were observed (DeLuca et al 2002; Yermakov and Rothstein 2006; Turner et al 2007). It is worth noting, however, that the aforementioned studies all occurred in northern coniferous forests that are adapted to stand-replacing wildfire disturbance. Our results are more consistent with those of Durán et al. (2009) who observed long-term (> 17 years) effects of stand-replacing wildfires on net N transformations in *Pinus canariensis* forests of the Canary Islands. The authors attribute this pattern to changes in soil organic C quality and quantity, as well as a lack of understorey vegetation recovery leading to higher soil erosion rates.

We observed that elevated rates of net nitrification, and, in some instances, gross nitrification, after wildfire are sustained over a long time period in ponderosa pine forests. This result is somewhat unexpected given the abundant literature showing that prescribed burning effects on N transformations are relatively short term (~1 year) in these forests (Kovacic et al. 1986, Covington and Sackett 1992, Monleon et al. 1997). However, our results are consistent with those by Grady and Hart (2006) wherein they observed elevated rates of net nitrification seven years following stand-replacing wildfire in southwestern ponderosa pine forests. Furthermore, our results demonstrate that this is a multi-decadal effect. Thus, this novel disturbance regime can lead to long-term effects on N cycling dynamics in this ecosystem, which may have broader implications for ecosystem N retention and associated net primary productivity.

ACKOWLEDGEMENTS

This work was supported by the USDA NRICG program (Award # 2005-35101-16179). We thank the students of the Ecological Research Institute at Northern Arizona University for assistance in the field and the lab, as well as Doug Manning and Sara Eckert for help establishing the field sites. We also thank Morgan Luce, Paul Selmants, Stephanie Jackson, and Brittany Widner for assistance with the gross N production measurements.

LITERATURE CITED

Agee, J. K. 1998. The landscape ecology of western forest fire regimes. Northwest Science **72**:24-34.

Bauhus, J., P. Khanna, and R. Raison. 1993. The effect of fire on carbon and nitrogen mineralization and nitrification in an Australian forest soil. Soil Biology and Biochemistry **31**:621-639.

Boerner, R. E. J., J. Huang, and S. C. Hart. 2009. Impacts of Fire and Fire Surrogate treatments on forest soil properties: a meta-analytical approach. Ecological Applications **19**:338-358.

Booth, M. G. and J. D. Hoeksema. 2010. Mycorrhizal networks counteract competitive effects of canopy trees on seedling survival. Ecology **91**:2294-2302.

Booth, M. S., J. M. Stark, and S.C. Hart. 2006. Soil-mixing effects on inorganic nitrogen production and consumption in forest and shrubland soils. Plant Soil **289**:5-15.

Brais, S., C. Camiré, Y. Bergeron, and D. Paré. 1995. Changes in nutrient availability and forest floor characteristics in relation to stand age and forest composition in the southern part of the boreal forest of northwestern Quebec. Forest Ecology and Management **76**:181-189.

Christensen, N. L. 1973. Fire and the nitrogen cycle in California chaparral. Science **181**:66.

Cooper, C. 1960. Changes in vegetation, structure, and growth of southwestern pine forests since white settlement. Ecological Monographs **30**:129-164.

Covington, W., P. Fulé, M. Moore, S. Hart, T. Kolb, J. Mast, S. Sackett, and M. Wagner. 1997. Restoring ecosystem health in ponderosa pine forests of the Southwest. Journal of Forestry **95**:23-29.

Covington, W. and M. Moore. 1994a. Postsettlement changes in natural fire regimes and forest structure: ecological restoration restoration of old-growth ponderosa pine forests. Journal of Sustainable Forestry **2**:153-181.

Covington, W. and M. Moore. 1994b. Southwestern ponderosa forest structure: Changes since Euro-American settlement. Journal of Forestry **92**:39-47.

Covington, W. and S. Sackett. 1992. Soil mineral nitrogen changes following prescribed burning in ponderosa pine. Forest Ecology and Management **54**:175-191.

Davidson, E., S. C. Hart, and M. Firestone. 1992. Internal cycling of nitrate in soils of a mature coniferous forest. Ecology **73**:1148-1156.

DeLuca, T. H., M.-C. Nilsson, and O. Zackrisson. 2002. Nitrogen mineralization and phenol accumulation along a fire chronosequence in northern Sweden. Oecologia **133**:206-214.

DeLuca, T. H. and A. Sala. 2006. Frequent fire alters nitrogen transformations in ponderosa pine stands of the Inland Northwest. Ecology **87**:2511-2522.

DiStefano, J. and H. Gholz. 1986. A proposed use of ion exchange resins to measure nitrogen mineralization and nitrification in intact soil cores. Communications in Soil Science and Plant Analysis **17**:989-998.

Durán, J., A. Rodríguez, J. M. Fernández-Palacios, and A. Gallardo. 2009. Changes in net N mineralization rates and soil N and P pools in a pine forest wildfire chronosequence. Biol Fertil Soils **45**:781-788.

Gaines, E. M. and E. W. Shaw. 1958. Half a century of research—Fort Valley Experimental Forest 1908-1958. Station Paper No. 38, Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. 16 p.

Grady, K. and S. Hart. 2006. Influences of thinning, prescribed burning, and wildfire on soil processes and properties in southwestern ponderosa pine forests: a retrospective study. Forest Ecology and Management **234**:123-135.

Hart, S., T. DeLuca, G. Newman, M. MacKenzie, and S. Boyle. 2005. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. Forest Ecology and Management **220**:166-184.

Hart, S. C., J. Stark, E. Davidson, and M. Firestone. 1994. Nitrogen mineralization, immobilization, and nitrification. Methods of Soil Analysis, Part 2: Microbiological and Biochemical Properties. Soil Science Society of America, Madison, WI.

Hebel, C., J. Smith, and K. Cromack. 2009. Invasive plant species and soil microbial response to wildfire burn severity in the Cascade Range of Oregon. Applied Soil Ecology **42**:150-159.

Heidmann, L. 2008. Forest regeneration research at Fort Valley. In: Fort Valley Experimental Forest - A Century of Research 1908-2008. S. D. Olberding and M. M. Moore, tech. coords. Proceeding RMRS-P-55. U.S. Department of

Agriculture - Forest Service, Rocky Mountain Research Station, Fort Collins, CO.

Heidmann, L., T. Johnsen, Q. Cole, and G. Cullum. 1982. Establishing natural regeneration of ponderosa pine in central Arizona. Journal of Forestry **80**:77-79.

Heidmann, L., W. J. Rietveld, and D. P. Trujillo. 1979. Fertilization increases cone production and diameter growth of a 55-year-old ponderosa pine stand in Arizona. Pages 197-205 *in* Proc. symp. on flowering and seed development in trees.

Hobbs, N. T. and D. S. Schimel. 1984. Fire effects on nitrogen mineralization and fixation in mountain shrub grassland communities. Journal of Range Management **37**:402-405.

Johnson, D. 1992. Nitrogen retention in forest soils. Journal of Environmental Quality **21**:1-12.

Johnson, D., J. Murphy, R. Susfalk, T. Caldwell, W. Miller, R. Walker, and R. Powers. 2005. The effects of wildfire, salvage logging, and post-fire N-fixation on the nutrient budgets of a Sierran forest. Forest Ecology and Management **220**:155-165.

Johnson, E. and K. Miyanishi. 2008. Testing the assumptions of chronosequences in succession. Ecology letters **11**:419-431.

Kashian, D., W. Romme, D. Tinker, M. Turner, and M. Ryan. 2006. Carbon storage on landscapes with stand-replacing fires. BioScience **56**:598-606.

Kaye, J. and S. Hart. 1998a. Ecological restoration alters nitrogen transformations in a ponderosa pine-bunchgrass ecosystem. Ecological Applications **8**:1052-1060.

Kaye, J. and S. Hart. 1998b. Restoration and canopy-type effects on soil respiration in a ponderosa pine-bunchgrass ecosystem. Soil Science Society of America Journal **62**:1062-1072.

Kaye, J. P. and S. C. Hart. 1997. Competition for nitrogen between plants and soil microorganisms. TREE **12**:139-143.

Kirkham, D. and W. Bartholomew. 1954. Equations for following nutrient transformations in soil, utilizing tracer data. Soil Science Society of America Journal **18**:33-34.

Kovacic, D., D. Swift, J. Ellis, and T. Hakonson. 1986. Immediate effects of prescribed burning on mineral soil nitrogen in ponderosa pine of New Mexico. Soil Science **141**:71-76.

Koyama, A., K. Kavanagh, and K. Stephan. 2010. Wildfire effects on soil gross nitrogen transformation rates in coniferous forests of central Idaho, USA. Ecosystems **13**:112-1126.

Marshall, J., J. Blair, D. Peters, G. Okin, A. Rango, and M. Williams. 2008. Predicting and understanding ecosystem responses to climate change at continental scales. Frontiers in Ecology and the Environment **6**:273-280.

McKenzie, D., Z. Gedalov, D. Peterson, and P. Mote. 2004. Climatic change, wildfire, and conservation. Conservation Biology **18**:890-902.

Monleon, V., K. Cromack, and J. Landsberg. 1997. Short- and long-term effects of prescribed underburning on nitrogen availability in ponderosa pine stands in central Oregon. Canadian Journal of Forest Research **27**:369-378.

Ojeda, F., J. G. Pausas, and M. Verdú. 2010. Soil shapes community structure through fire. Oecologia **163**:729-735.

Pietikäinen, J., O. Kiikkilä, and H. Fritze. 2000. Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. Oikos **89**:231-242.

Prieto-Fernández, Á., M. Carballas, and T. Carballas. 2004. Inorganic and organic N pools in soils burned or heated: immediate alterations and evolutions after forest wildfires. Geoderma **121**:291-306.

Raison, R. 1979. Modification of the soil environment by vegetation fires, with particular reference to nitrogen transformations: A review. Plant and Soil **51**:73-108.

Ross, C. S., J. P. Kaye, M. W. Kaye, V. J. Kurth, R. J. Brimmer, S. C. Hart, and P. Z. Fulé. In Review. Labile carbon constrains soil respiration in ponderosa pine forests following crown fires and precipitation manipulations. Ecosystems.

Rothstein, D., Z. Yermakov, and A. Buell. 2004. Loss and recovery of ecosystem carbon pools following stand-replacing wildfire in Michigan jack pine forests. Canadian Journal of Forest Research **34**:1908-1918.

Sabo, K., S. Hart, C. Sieg, and J. Bailey. 2008. Tradeoffs in overstory and understory aboveground net primary productivity in southwestern ponderosa pine stands. Forest Science **54**:408-416.

Savage, M. and J. Mast. 2005. How resilient are southwestern ponderosa pine forests after crown fires? Canadian Journal of Forest Research **35**:967-977.

Schubert, G. H. 1974. Silviculture of southwestern ponderosa pine: The status-of-our-knowledge. USDA Forest Service Res. Paper RM-123. U.S. Department of Agriculture, Rocky Mountain Forest and Range Experiment Station, editor., Fort Collins, CO.

Smithwick, E., M. Turner, M. Mack, and F. Chapin. 2005. Postfire soil N cycling in northern conifer forests affected by severe, stand-replacing wildfires. Ecosystems **8**:163-181.

Stark, J. and S. C. Hart. 1996. Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. Soil Science Society of America Journal **60**:1846-1855.

Turner, M., E. Smithwick, K. Metzger, D. Tinker, and W. Romme. 2007. Inorganic nitrogen availability after severe stand-replacing fire in the Greater Yellowstone ecosystem. Proceedings of the National Academy of Sciences **104**:4782.

Vitousek, P. M. and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea: How can it occur? Biogeochemistry **13**:87-115.

Wan, S., D. Hui, and Y. Luo. 2001. Fire effects on nitrogen pools and dynamics in terrestrial ecosystems: a meta-analysis. Ecological Applications **11**:1349-1365.

Wardle, D. A., G. Hörnberg, O. Zackrisson, M. Kalela-Brundin, and D. A. Coomes. 2003. Long-term effects of wildfire on ecosystem properties across an island area gradient. Science **300**:972-975.

Westerling, A. L., A. Gershunov, T. J. Brown, D. R. Cayan, and M. D. Dettinger. 2003. Climate and wildfire in the western United States. Bull Amer Met Soc **84**:595.

Westerling, A. L., H. G. Hildago, D. R. Cayan, and T. W. Swetnam. 2006. Warming and earlier spring increase western U.S. forest wildfire activity. Science **313**:940-943.

White, A. 1985. Presettlement regeneration patterns in a southwestern ponderosa pine stand. Ecology **66**:589-594.

White, C. 1986. Effects of prescribed fire on rates of decomposition and nitrogen mineralization in a ponderosa pine ecosystem. Biology and Fertility of Soils **2**:87-95.

Williams, A., C. Allen, C. Millar, T. W. Swetnam, J. Michaelsen, C. Still, and S. Leavitt. 2010. Forest responses to increasing aridity and warmth in the southwestern United States. Proceedings of the National Academy of Science **107**:21289-21294.

Yermakov, Z. and D. E. Rothstein. 2006. Changes in soil carbon and nitrogen cycling along a 72-year wildfire chronosequence in Michigan jack pine forests. Oecologia **149**:690-700.

Zackrisson, O., M.-C. Nilsson, and D. A. Wardle. 1996. Key ecological function of charcoal from wildfire in the Boreal forest. Oikos **77**:10-19.

Table 3.1 Site and mineral soil (0-15 cm) characteristics for the southwestern ponderosa pine wildfire chronosequence. Data are means with one standard error in parentheses. Bold values indicate significant differences between burned and unburned pairs for a given fire year.

Characteristic	Years since fire (from 2007) and burn treatment													
	2		6		7		11		23		30		34	
	B†	U†	B	U	B	U	B	U	B	U	B	U	B	U
Area burned (ha)	28	-	450	-	268	-	3495	-	1258	-	1858	-	3162	-
Total C ($g\ m^{-2}$)	1898.5 (114)	2404.4 (132)	2894.8 (117)	2973.5 (196)	2785.2 (62)	2466.1 (168)	1946.5 (223)	1950.3 (222)	**2804.6 (178)**	**2178.7 (151)**	**1942.2 (73)**	**2846.7 (226)**	1851.6 (141)	2156. (136)
Total N ($g\ m^{-2}$)	80.07 (5.6)	91.4 (7.5)	**156.9 (6.8)**	**128.9 (11.5)**	**127.6 (5.7)**	**100.4 (9.5)**	96.9 (11.5)	83.8 (8.7)	139.8 (10.9)	113.0 (7.7)	85.4 (5.4)	102.4 (10.5)	80.6 (6.8)	97.3 (9.2)
C:N	24.1 (1.2)	27.0 (1.5)	**18.5 (0.5)**	**23.9 (1.5)**	**22.0 (0.7)**	**25.1 (1.2)**	**20.2 (1.0)**	**23.0 (0.6)**	20.3 (0.6)	19.4 (0.8)	23.2 (1.0)	28.3 (1.0)	23.3 (0.7)	22.8 (1.2)
pH	**5.56 (0.06)**	**5.36 (0.06)**	**5.73 (0.09)**	**5.12 (0.09)**	**5.55 (0.04)**	**5.04 (0.08)**	**5.58 (0.05)**	**4.97 (0.8)**	5.38 (0.05)	5.22 (0.09)	**5.71 (0.06)**	**5.16 (0.1)**	**5.81 (0.04)**	**5.43 (0.06**

GWC*	0.08	0.06	**0.12**	**0.09**	0.10	0.11	0.09	0.08	**0.08**	**0.10**	0.05	0.09	0.08	0.05
	(0.03)	(0.005)	**(0.01)**	**(0.01)**	(0.01)	(0.01)	(0.004)	(0.01)	**(0.01)**	**(0.01)**	(0.001)	(0.01)	(0.01)	(0.002

*GWC = Gravimetric water content; value is the mean of the July and October 2007 measurements.
† B = Burned; U = Unburned

FIGURE LEGENDS

Figure 3.1 Mean net N mineralization (A) and net nitrification (B) annual rates in mineral soil (0–15 cm) measured *in situ* at seven burned (closed bars) and adjacent unburned (open bars) wildfire chronosequence sites. Error bars are ±1 standard error; note different scales in y-axis. Significant differences ($P \leq 0.05$) between burned and unburned rates for each year are marked with an asterisk and results from the two-way ANOVA are shown. Panel (C) shows the ratio of net nitrification to net N mineralization for burned and unburned sites at each year.

Figure 3.2 Mean inorganic N pools (NH_4^+, A; NO_3^-, B) in the mineral soil (0-10 cm) at each of the burned (closed bars) and unburned (open bars) wildfire chronosequence sites. Bars are the mean of nine plots, taken at the two initial time periods for the net N transformation measurements (n = 18; July and October, 2007). Error bars are ±1 standard error, and significant differences ($P \leq 0.05$) between burned and unburned sites at each fire year are marked with an asterisk. Results from the two-way ANOVA are shown. Pools under the detection limit are marked "UDL."

Figure 3.3 Mean net N mineralization (A) and net nitrification (B) rates in the mineral soil (0-15 cm) measured during the growing season (July to October) converted to a daily basis. Measurements were performed in paired burned (closed bars) and unburned (open bars) sites along a wildfire chronosequence. Error bars are ±1 standard error, and significant differences ($P \leq 0.05$) between burned and unburned sites at each fire year are marked with an asterisk.

Figure 3.4 Mean net N mineralization (A) and net nitrification (B) rates in the mineral soil (0-15 cm) measured during the dormant season (November to June) converted to a daily basis. Measurements were performed in paired burned (closed bars) and unburned (open bars) sites along a wildfire chronosequence. Error bars are ±1 standard error, and significant differences ($P \leq 0.05$) between burned and unburned sites at each fire year are marked with an asterisk.

Figure 3.5 Differences in annual net N transformation rates (net N mineralization, A; net nitrification, B) between burned and adjacent unburned sites at seven wildfire chronosequence sites. Panel (C) shows the differences in the ratios of net N mineralization to net nitrification. Each point represents the difference between the mean of each fire site and its paired unburned site for each fire year.

Figure 3.6 Mean gross rates of N mineralization (A), nitrification (B) measured by isotope dilution technique in a 24-hour laboratory incubation. Measurements were performed on soils from six plots originating from paired burned (black filled) and unburned (gray filled) sites at five wildfire chronosequence sites (n = 6). Error bars are ±1 standard error, and significant differences ($p \leq 0.05$) between burned and unburned sites at each fire year are marked with an asterisk. Panel (C) shows the proportion ratio of gross nitrification to gross N mineralization for burned and unburned sites at each year.

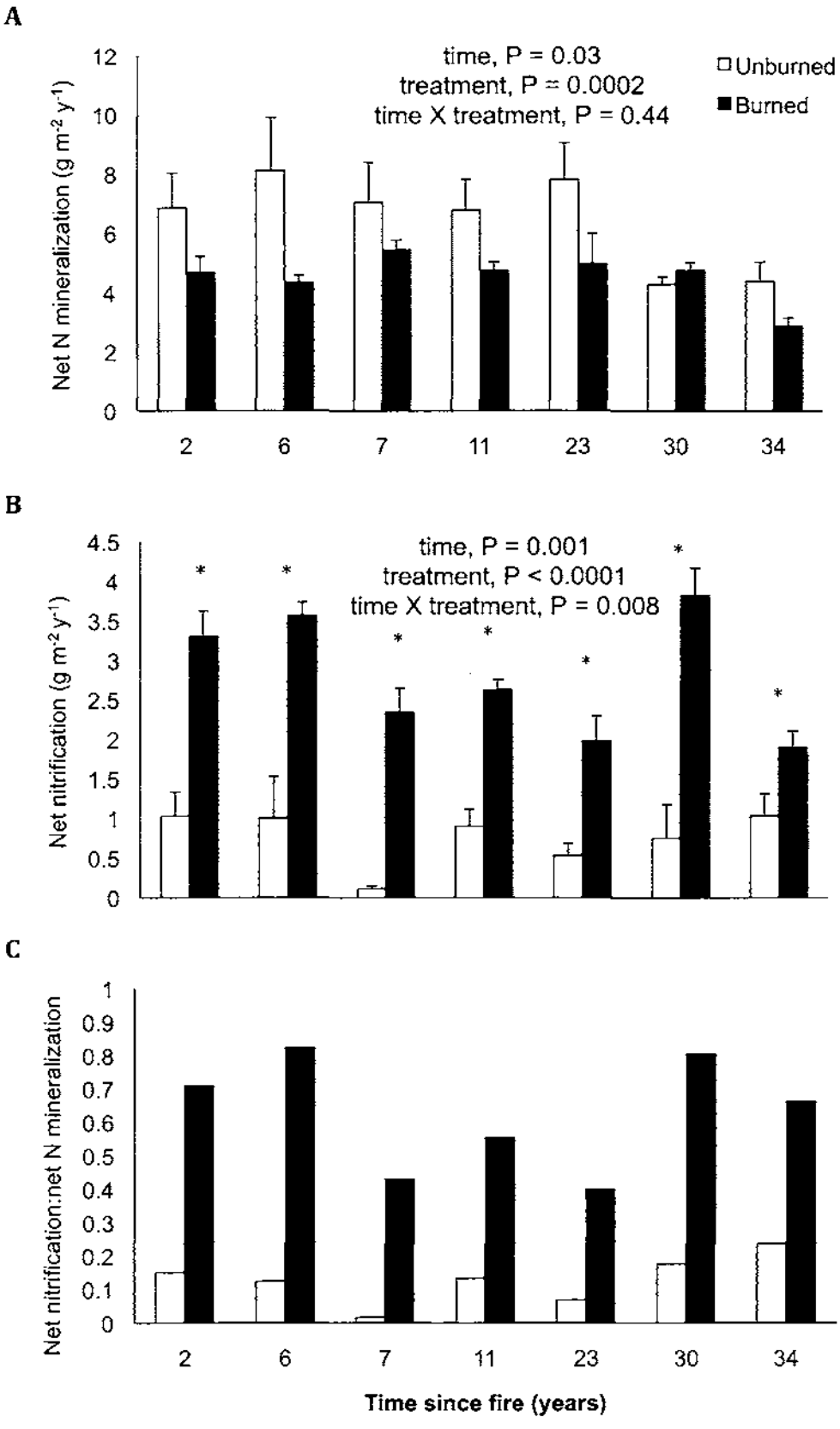


Figure 3.1

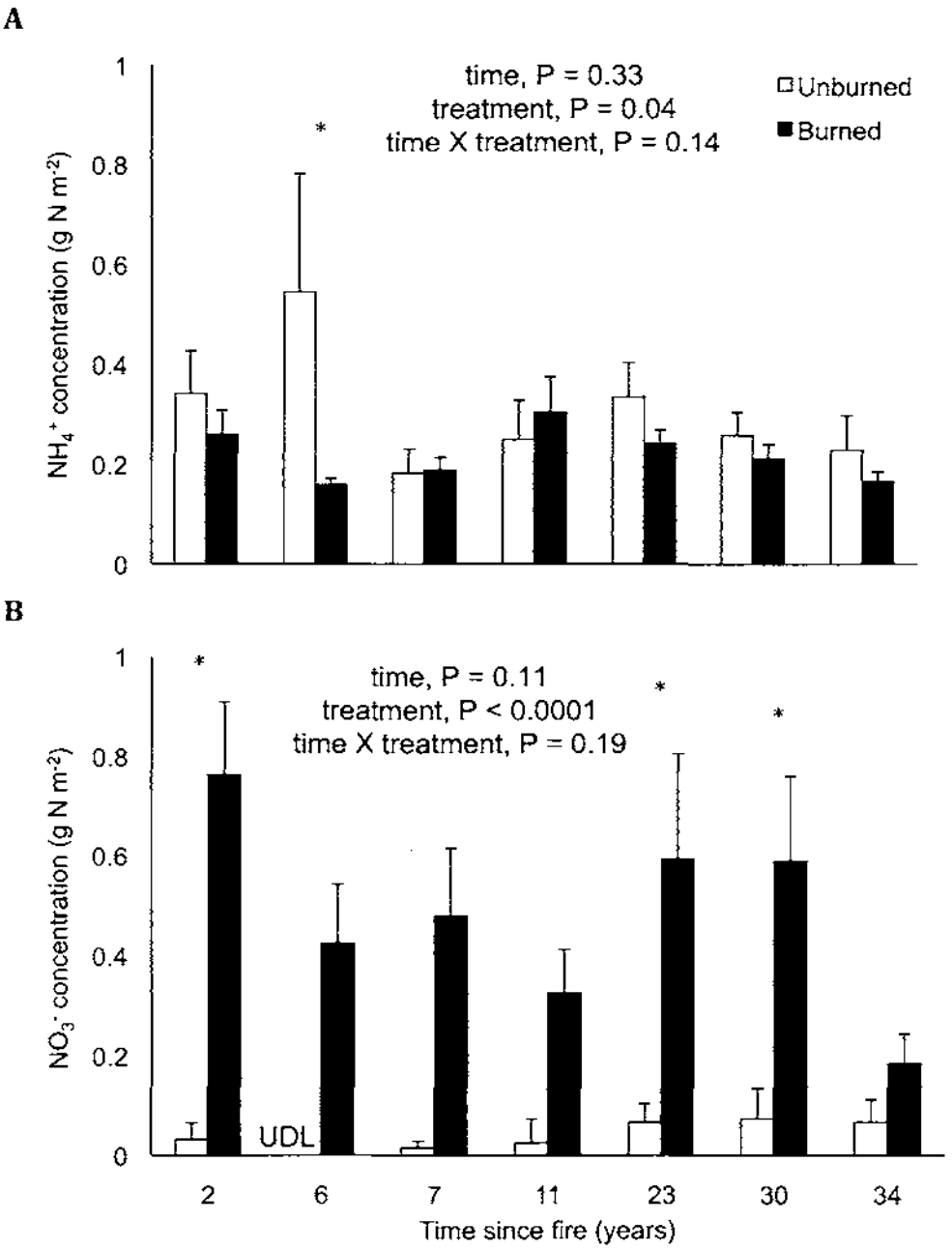


Figure 3.2

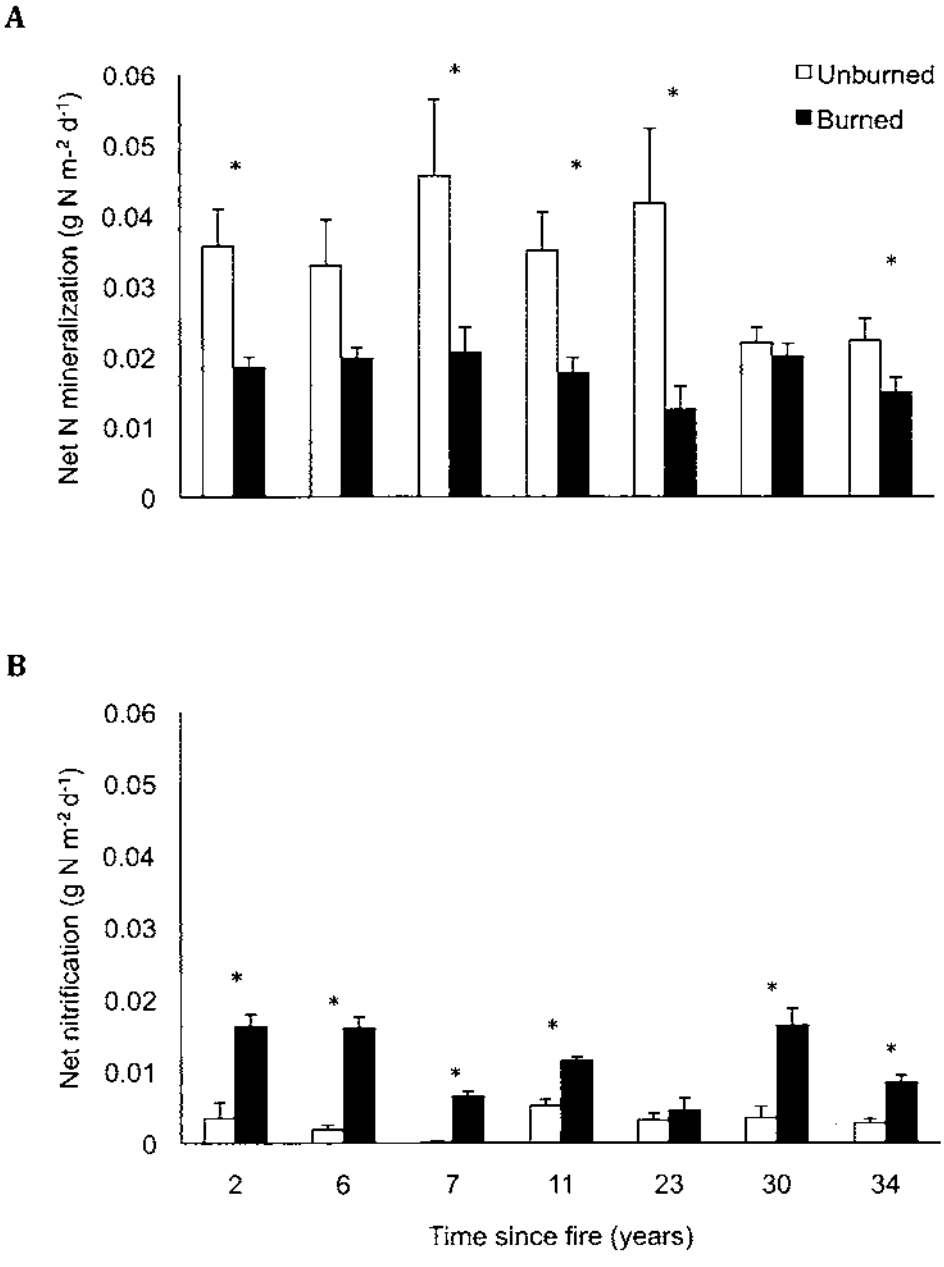
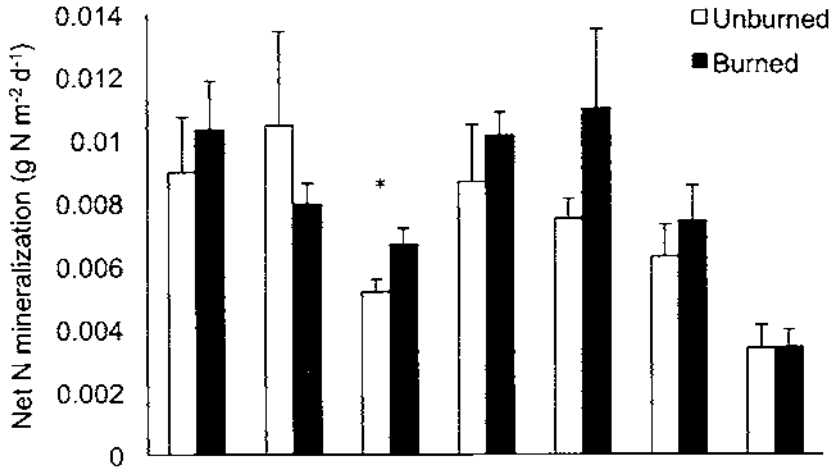


Figure 3.3

A



B

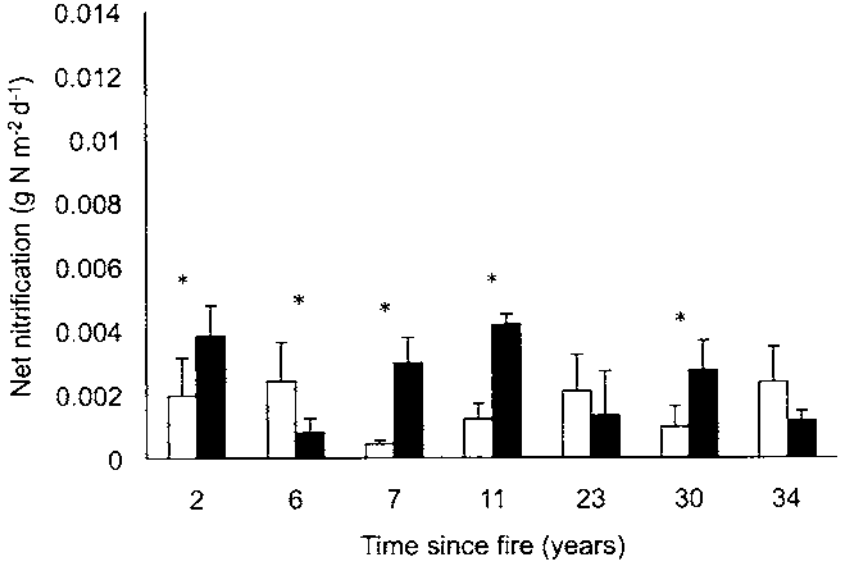


Figure 3.4

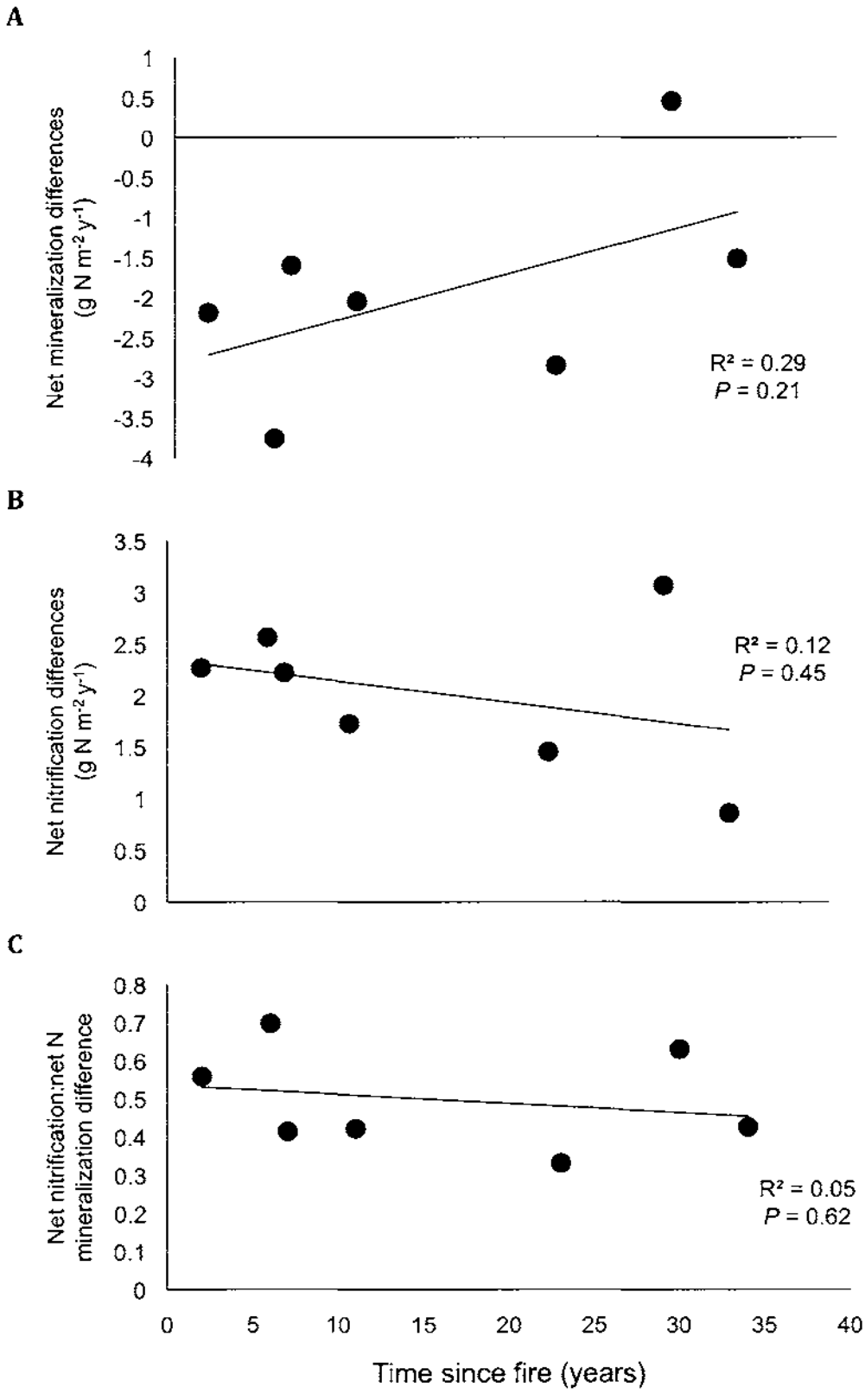


Figure 3.5

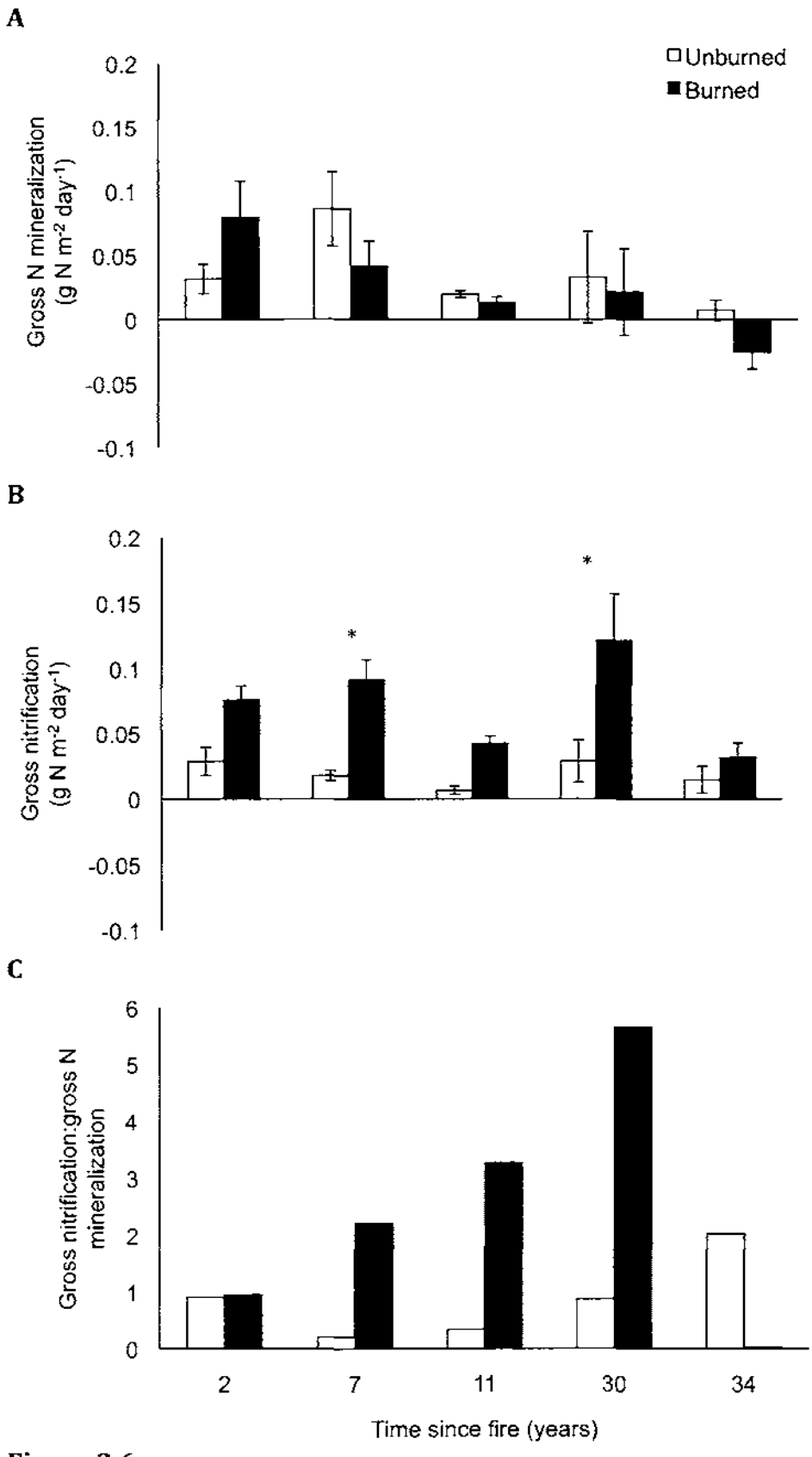


Figure 3.6

CHAPTER 4

STAND-REPLACING WILDFIRES ALTER THE COMMUNITY STRUCTURE OF WOOD-DECAY FUNGI IN SOUTHWESTERN PONDEROSA PINE FORESTS

ABSTRACT

Increasing tree mortality patterns in the western U.S. due to drought, wildfire, and insect outbreaks have widespread implications for ecosystem carbon (C) cycling. In particular, the activity of heterotrophic microbes in dead, decomposing trees can represent a relatively long-term source of CO_2 to the atmosphere. We used southwestern ponderosa pine forests as a model system to investigate the effects of stand-replacing wildfires on the community structure of wood-decay fungi. We established a wildfire chronosequence to assess these effects, using a combination of culture-based and molecular approaches. Our assessment of the fungal decay communities revealed dominance by ascomycete fungi, which contrasts with previous studies that used sporocarps. Fire suppressed species richness for up to 4 years and altered species composition for the entire length of the chronosequence (32 years). An experimental incubation with a subset of the fungal isolates demonstrated that species varied in their capacity to decompose wood. Our results suggest that stand-replacing wildfires have long-term effects on fungal community composition, which may have associated consequences for wood decomposition and C cycling.

INTRODUCTION

Tree mortality in the western U.S. has increased in recent years due to a combination of drought, insect outbreaks, and wildfire (Westerling et al. 2006, Van Mantgem et al. 2009), and these patterns may have profound implications for carbon (C) storage and cycling. Severe drought conditions can lead to sudden and massive tree species die-off (Breshears et al. 2005), and the climate factors that are associated with drought conditions (e.g., warmer spring and summer temperatures, lower precipitation) are also correlated with the increasing frequency and size of wildfires in the western U.S. (Westerling et al. 2003; 2006). Large, high-severity wildfires can have long-term consequences for ecosystem C cycles because they often kill trees but do not consume all the tree biomass; the C flux from decomposition of these dead trees often exceeds ecosystem photosynthesis rates for years to decades (Kashian et al. 2006). The quantity of CO_2 released by post-fire decomposition can be substantial, equaling three times the amount of emissions by the fire itself in some forests (Auclair and Carter 1993). The length of time that recently burned forests function as net CO_2 sources is uncertain; high-severity burns in mixed conifer and ponderosa pine forests of the Inland Northwest remain net atmospheric sources for 4-5 years (Meigs et al. 2009), whereas similar burns in southwestern ponderosa pine forests are likely to remain a net source for decades. (Dore et al. 2008, Hurteau and Brooks 2011). Clearly, a strong mechanistic knowledge of the controls on wood decomposition would enhance our understanding of ecosystem C dynamics following high severity wildfires.

Many of the factors that limit the decomposition of wood, including colonization by saprotrophic organisms, substrate quality and quantity, and abiotic environmental characteristics (Harmon et al. 1986), are likely altered by wildfire. Wildfire reduces fungal inoculum sources due to heat-induced mortality (Raison 1979, Choromanska and DeLuca 2002, Korb et al. 2004); indeed, the maximum ground temperatures reached in forest fires range from 200° C to 300° C (Neary et al. 1999), while fungal mortality occurs at temperature <100° C (Dunn et al. 1985). Reductions of soil-based fungal inoculum by fire make dispersal abilities, either via airborne spores, animal transport, or migration of mycelium in the soil, critical to fungal establishment on wood. The distance and arrangement of the inoculum source are key factors to fungal colonization. For example, in spruce stands of central Sweden, the probability of colonization of stumps by basidiomycete fungi of the same genet decreased with distance from the point of establishment (Kirby et al. 1990). Fungi that overcome dispersal limitations in a recently burned area are likely to encounter abundant wood substrate, but it will probably be at least partially charred. Charring decreases decomposition rates (Cornwell et al. 2009), but may provide greater habitat opportunities for microbes because of increased surface area (Pietikäinen et al. 2000). Finally, post-fire changes in abiotic variables, primarily microclimate and nutrient availability, may also alter the responses of wood-decaying fungi. Wildfires generally cause an increase in land surface temperatures, and they alter chemical characteristics, notably by increasing the pH and nutrient availability (Hart et al. 2005); the interactions of all these variables may affect the establishment of fungi.

Although the environmental changes resulting from fire are likely to affect the diversity and community composition of decay fungi, few studies have examined the short- and long-term effects of fire on fungi (Cairney and Basitas 2007). Controlled burning in northern coniferous forests favored certain species of fungi, causing moderate to strong alterations in community composition (Olsson and Jonsson 2010, Berglund et al. 2011). A decline in species richness was observed in the first year following burning (Olsson and Jonsson 2010) in one study, but another study showed that the number of species recovered within 1 to 5 years following a burn (Berglund et al. 2011). The only study we know of that investigated the effects of wildfire on wood-decay fungi communities did not observe substantial community differences, only that species richness was lower one year after fire compared to undisturbed forest (Lumley et al. 2001).

Fungal community composition of the early succession species may also have cascading effects on the composition of later succession fungal communities, and this may be reflected in rates of wood decay. Several experimental laboratory experiments have confirmed that complex species interactions, including a legacy of exudates in the wood, promote the growth of certain secondary fungal species and inhibit others (Niemelä et al. 1995, Holmer et al. 1997, Heilmann-Clausen and Boddy 2005). In addition, Fukami et al. (2010) demonstrated that the colonization history of fungi led to direct effects on the proportion of wood mass lost during the incubation period, suggesting that wildfire effects on fungal community composition may have functional consequences. Fungal community structure is thought to be more important in the later stages of decay, when the majority of the C remaining is

in the form of lignin (McGuire and Treseder 2009). However, it is clear that early successional decomposer communities are instrumental in determining subsequent species succession and may have large-scale implications for wood decomposition and C cycles (Fukami et al. 2010).

Southwestern ponderosa pine (*Pinus ponderosa* var. *scopulorum* Engelm.) forests are an excellent model ecosystem to examine wildfire effects on wood decomposition and C cycling for numerous reasons. First, ponderosa pine forests are widespread in the Southwest, comprising almost half of the commercial forest land in Arizona, Utah, New Mexico and Colorado (Schubert 1974). Also, the Southwest is predicted to suffer large-scale tree mortality due its high sensitivity to drought, predicted increases in ambient temperatures, and the related increases in wildfire frequency and bark beetle outbreaks (Williams et al. 2010). The land-use history in these forests also has strongly influenced the risk of wildfire and associated tree mortality. Historic forests underwent frequent, low-intensity surface fires, but the introduction of land management practices (cattle grazing, timber harvest, and active fire suppression) by Euro-American settlers led to a shift to infrequent, stand-replacing wildfires (Baker 1994, Fleischner 1994, Beschta et al. 2004). Research on southwestern ponderosa pine ecosystems is relevant to other forests in the western U.S. because these land management practices are widely applied across the region, and climate models predict widespread drought conditions and warmer temperatures (Easterling et al. 2000, Hoerling and Kumar 2003). Finally, the relatively common incidence of stand-replacing wildfires in the last ~40 years (Stephens 2005, Littell et al. 2009) has provided the opportunity to

construct a chronosequence of burned sites in which to examine the post-fire legacy of decaying wood.

In this study, we compared the diversity and species composition of wood decay fungi associated with wildfires of varying ages to that of nearby unburned stands of ponderosa pine in northern Arizona. Relatively little is known about the fungi responsible for wood decay in these forests beyond sporocarp surveys (Gilbertson 1974), and evidence suggests that sporocarps may not be representative of the entire fungal community in wood (Allmér et al. 2006). Therefore, we used a combination of mycelial isolation and molecular techniques to identify wood-decay fungi community structure. We also measured the decomposition potentials of a subset of the species observed. We hypothesized (1) that fires would cause a decrease in fungal species diversity due to heat-induced mortality and dispersal limitations. Concurrently, we expected fire to cause alterations in species composition as a result of changes in environmental conditions. We also hypothesized (2) that fungal community structure would become more similar with increased time since fire, as the effects of fire on the environmental conditions diminish, and substrate quality becomes more limiting to fungi than dispersal abilities. Finally (3), we hypothesized that the species of fungi we isolated would vary in their ability to decompose the same substrate.

METHODS

Study sites

This study was conducted at five locations in ponderosa pine forest covering a region approximately 615 km^2 within the Coconino and Kaibab National Forests of

northern Arizona, U.S.A. (Table 4.1). Each site had experienced only one stand-replacing crown fire (> 95% tree mortality) within the last 60 years according to forest records (range of burn years: 1977 – 2005). The burned sites were each paired with an adjacent unburned site to better isolate the effects of fire and reduce other sources of environmental and temporal variation (Johnson and Miyanishi 2008). All sites were located on basaltic parent material. Woody vegetation in the unburned sites consisted of ponderosa pine with some Gambel oak (*Quercus gambelii* Nutt.) at lower elevations and Douglas-fir and southwestern white pine (*Pseudotsuga menziesii* (Mirb.) Franco and *Pinus strobiformis* Engelm.) at higher elevations. Woody vegetation was limited to small shrubs at all the burned sites except at the 1983 burn site, where there were some small ponderosa pine trees. Only one of the five sites was subjected to salvage logging following the wildfire, the1983 fire. At this site, we sampled from an area on a slope that we are confident was not logged based on the lack of stumps and the prevalence of charred logs. Therefore, we are confident that the only major disturbance that the sites experienced was their respective stand-replacing wildfire. More detailed information on site characteristics can be found in Ross et al. (In review). Although Ross et al.'s measurements were taken at different locations within the sites than those sampled in our current study, all of our samples were taken within 1 km of their plots.

The month we sampled (April, 2009) was drier than average, but temperatures were similar to 30-year averages (Western Regional Climate Center, Fort Valley Station; www.wrcc.dri.edu). The region received 1.7 cm of precipitation

in April of 2009, and the cumulative total for that spring (January – April) was 8.3 cm compared to the 30-year mean (1971 – 2000) for April of 3.2 cm and 21.4 cm for the spring. The mean temperature during April 2009 was 4.2° C, while the 30-year mean April temperature was 4.5° C. The mean spring temperature for the months of January – April, 2009, was 0.9° C, and the 30-year spring mean temperature was 0.5° C.

Field sampling and laboratory analyses

At each site, we selected areas to sample where the fire perimeter could be visually delineated. We then established a sampling swath inside the burned area between 100 and 300 m from the fire perimeter. Within this swath, we selected 6 dead and downed tree boles (hereafter referred to as "logs") to sample. Logs were chosen based on several criteria: they were representative of those in the burned area in terms of size (length and diameter), state of decay (Maser et al. 1979), and they were spatially distinct (logs were sampled in groups of 2; each log pair was approximately 100 m from the others). Four cubic samples, each approximately 6 x 8 x 1.5 cm, were removed from each log using a chainsaw or bow saw and a hatchet. One sample was taken at each end of the log, and the other two samples were evenly spaced across the length of the log. All samples were removed from the highest point along the side of the log. We recorded log length, diameter (both ends and middle), aspect, and state of decay. We calculated log volume using the formula for the volume of a frustum ($V = \pi * length/3 (r_1^2 + r_2^2 + r_1 r_2)$, wherein "r" equaled the radius at either end.

We followed a similar procedure in each of the adjacent unburned areas in that we established a swath between 100 and 300 m from the burn perimeter. To reduce variation in fungal community composition that might be associated with log size and age, we sampled logs that were similar in size and state of decay as those we sampled in the burned area. All wood pieces were placed in polyethylene bags and transported on ice back to the lab and stored at 4° C until processing (< 10 days).

To isolate fungi from within the wood samples, a small piece (5 x 5 x 10 mm) was removed from each wood sample using a coping saw. The wood sample was surface sterilized by briefly dipping in 70% ethanol and then burning off the ethanol with a flame. The wood segments were immediately placed on a sterile petri dish containing Hagem agar (Allmér et al. 2006) and monitored for hyphal growth. Emerging hyphae were placed in pure culture on Hagem agar and grouped by their morphology. A total of 240 wood pieces from 60 logs were plated (6 logs per site).

Multiple representatives from each culture morphotype were selected for DNA extraction. A small portion of hyphae was scraped off the culture using a sterile knife (approximately 0.5 g) and placed in a 96-well DNA extraction plate. The DNA was then extracted using Qiagen DNEasy (Qiagen, Valencia, CA, USA) plant kits according to the manufacturer's instructions, using the modification recommended for fungal tissue. The internal transcribed spacer region of the ribosomal DNA was amplified using polymerase chain reaction (PCR) with the forward ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and reverse ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primer pair (Gardes and Bruns 1993). Restriction

fragment length polymorphism (RFLP) data were obtained following the methods of Gehring et al. (1998) using restriction enzyme digestion with *Hinf*I and *Mbo*I, and morphological groupings were confirmed using the distinctive RFLP band patterns (RFLP types).

The ITS region from no fewer than two representatives of each RFLP type was sequenced. Forward and reverse sequencing was performed on an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) at the Environmental Genetics and Genomics Laboratory (Northern Arizona University, Flagstaff, AZ, USA). Sequences were aligned and edited using Geneious Pro software (Drummond et al. 2010). BLAST searches were also performed using Geneious and the GenBank database (http://www.ncbi.nlm.nih.gov). Sequences with similar BLAST results were aligned using Geneious Consensus alignment to verify their similarity (>97.0% pairwise identity). Final morphological groupings were determined using the sequence results, and samples that did not amplify or did not yield a high quality sequence were grouped based on RFLP pattern and culture morphology.

Fungal community composition was analyzed using PC-ORD software (McCune and Mefford 2006). Each log was considered a plot and species were collapsed into genera because it reduced the number of singletons. Collapsing samples into genera had no effect on the general conclusions drawn in other studies of wood-decay fungi (Crawford et al. 1990). We used a 2-way factorial PerMANOVA (Anderson 2001) with treatment (burned or unburned control) and year of fire as the main effects. We encountered a significant treatment X year interaction ($p = 0.0002$), so we subsequently analyzed the effects of treatment and year separately

using a multi-response permutation procedure (MRPP). An indicator species analysis (Dufrene and Legendre 1997) was conducted based on the year of burn and burned or unburned control. Because of the high diversity in the fungal communities, in addition to the inherent spatial variability, we set the *a priori* alpha level at 0.10 for the community analyses.

Species richness and Shannon's diversity index (H'; computed in PC-ORD) were computed for each log (as a plot) and differences were analyzed using a 2-way analysis of variance (ANOVA) in JMP statistical software (SAS Institute; Version 5.0.1.2). Treatment (burned or unburned control) and year of burn were the main effects. Upon encountering a significant treatment X time interaction ($p = 0.009$ for species richness, $p = 0.021$ for H'), we examined each burn/control pair separately for each fire year. Temporal changes in the burned sites were assessed for both species richness and H' using a one-way ANOVA with burn year as the main effect, and means were separated using Tukey's HSD. Species richness and H' were computed for each site, and these values were used to compare burned and unburned treatments using a paired t-test. Sampling effort was assessed by producing species accumulation curves for the burned and unburned treatments.

The influence of environmental variables (log length, volume, aspect, organic horizon C and woody debris C) on fungal community composition was assessed using a non-metric multidimensional scaling (NMDS) ordination in PC-ORD. The secondary environmental matrix was overlaid on the species matrix and correlations between the environmental variables and species composition were displayed as joint biplots (Murray et al. 2010). Correlations coefficients ($r \geq 0.669$

(burned/unburned separately) or ≥ 0.497 (burned and unburned together) were considered significant at alpha = 0.1 based on a table of critical values (df = 5 or df = 10) Separate ordinations were performed for the burned sites, the unburned sites, and the combined dataset for the burned and unburned sites.

Lab incubation to assess the decomposition capabilities of fungal isolates

To further explore the role of individual species of fungi on wood decomposition, we experimentally measured the decomposition of standard pine dowels following inoculation with isolates of individual species that were frequently found in our field study. We selected 8 species that were dominant in a range of burn ages. We placed five (5 x 5 x 5 mm) cubes of hyphal-rich agar onto nutrient-poor media (Bacto-Agar). We obtained pine dowels (8 mm diameter; www.dowelsondemand.com), cut them into 8 mm lengths, and sterilized them in an autoclave. Five pieces of wood were weighed together and then placed in the media such that each wood piece was situated next to one of the five agar cubes. In total, 10 plates were made for each of the 8 morphotypes (n = 10).

Plates were monitored for fungal colonization of the wood pieces for six months and 15 days before harvesting. The five wood pieces contained in each plate were removed from the petri dish and placed together in a small envelope. The wood pieces were dried at 60° C for 48 hours, and then any fungal hyphae remaining on the wood were scraped off using a sterile knife. The wood pieces were weighed and decomposition was measured as the percentage of initial mass loss. A one-way ANOVA was used to compare the percentage mass loss across the species and Tukey's HSD was used to separate differences.

RESULTS

We isolated fungi from 222 of the 240 wood samples (92.5%), 109 from burned sites and 114 from control sites. Only one species of fungus was isolated from most of the wood samples (62%); of the remaining samples, 27% had two species isolated, 10% had three, and 1% had four.

We sequenced a total of 280 samples, and almost three-quarters (203) of them yielded high quality DNA sequences. We observed 68 distinct fungal taxa; 43 were identified to species, five were identified to genus, one was identified to family, and the remaining taxa (20), mostly singletons, remained unidentified (Table 4.2). Of these, 42 were members of the division Ascomycota, five were members of the division Basidiomycota, and two were members of the Mucormycotina (formerly part of the Zygomycota).

There was no consistent effect of burning on fungal species richness or diversity across all of the sites, but some sites differed significantly from their paired unburned controls. Mean site level species richness did not differ between burned (18.4 species) and unburned (16.4 species) treatments ($p = 0.60$). Similarly, Shannon's diversity did not vary between the burned (1.1444) and unburned (1.2012) treatments ($p = 0.803$). At the log spatial-scale, mean species richness was lower at the burned than the unburned site at the 2005 burn ($p = 0.07$), and it was higher at the burned than the unburned site in the 1984 burn ($p = 0.0006$). For the other three burns, species richness was similar at the burned and unburned sites (Figure 4.1). Shannon's diversity index at the log spatial-scale was higher in the burned sites than unburned sites at years 2000 and 1983 (Table 4.3).

Community composition showed more consistent differences between unburned and burned sites. Species composition differed significantly between four of the five burned and unburned site pairs (Figure 4.2). The indicator species analysis revealed several species as indicators for particular burn and year combinations. *Aspergillus* sp., *Hypocrea* sp., and unknown *Phialophora* were indicators of the 1984, 2005, and 1996 burns, respectively. In the unburned sites, *Biscogniauxia mediterranea* was an indicator of 1996, while both *Byssochlamys nivea* and Pezizomycotina spp. were indicators of 1984 (Table 4.4).

Mean species richness (at the log level) varied by year in the burned sites ($p = 0.002$), as did H' ($p = 0.007$). Richness was significantly lower at the 2005 site than the 2000, 1996, and 1983 sites, but similar to the 1977 site. The 1997 site was similar to all of the other burned sites (Figure 4.2). An identical pattern was revealed for H'. Species richness was similar at all the unburned sites. Shannon's diversity index was higher at the 1996 unburned site than the 1983; the rest of the sites were similar to each other. Community composition at the burned sites varied with time since fire ($A = 0.0985$, $p < 0.0001$; Figure 4.3A); pairwise comparisons showed that community composition differed among all site pairs except for 1984 and 1977. The unburned sites also were distinct from each other in community composition ($A = 0.1178$, $p < 0.0001$; Figure 4.3B) except at the two most recent years, 2005 and 2000. None of the environmental variables (log length, diameter, aspect, organic horizon C and woody debris C) were significantly correlated with an ordination axis (axis 1 or axis 2) for ordinations using data from the burned, unburned, or burned and unburned sites combined. The species accumulation

curves for both the burned and unburned treatments exhibited an asymptotic trend (Figure 4.4).

All of the fungal species tested were able to decompose the wood substrate, but the percent of initial mass that was lost ranged from 1.7% to 11.1% and varied by species. The wood colonized by *Hypocrea lixii* was less decomposed than the other species tested, which were not significantly different from each other (Figure 4.5).

DISCUSSION

Our findings partially support our first hypothesis, that wildfire would cause a decrease in species richness. Average species richness per log was lower at the most recent fire site (2005) compared with its paired unburned site (Figure 4.3). Although our sampling occurred four years following this fire, we were still able to capture limited evidence of a decline in richness caused by fire. Our findings contrast with other studies that documented fungal communities after prescribed burning in boreal forests. For instance, Ohlsson and Jonsson (2011) found that species richness was similar in burned and unburned plots four years post-burn, and Junninen et al. (2008) did not observe differences in species richness one year following fire. However, these studies examined the impact of prescribed burning, a much weaker disturbance than a high-severity wildfire. We also observed that the species richness at the 1984 burned site was higher than the unburned site. We attribute this to the high relative abundance of one species, *Byssochlamys nivea*, in the unburned site, which may indicate that this species is occupying a disproportionate amount of niche space compared to the paired burned site (Figure

2D). *Byssochlamys nivea* is a known inhibitor of some fungal pathogens (Hoff et al. 2004), which may enhance its abilities to outcompete other decomposer fungi.

Fungal community composition differed in burned sites compared to their unburned pairs at all the sites except for the 1996 burn, providing support for our first hypothesis that community composition would differ as result of fire. Other researchers also have found that prescribed burning caused alterations in fungal species composition up to four or five years post-fire (Junninen et al. 2008, Olsson and Jonsson 2010, Berglund et al. 2011), but ours is the first research we know of to demonstrate altered community composition up to 32 years (1977) after a stand-replacing wildfire. The relatively high number of species observed at the 1996 sites suggests a high degree of species co-existence. Similarly, Jonsson et al. (2008) observed higher species co-existence at intermediate stages of wood-decay, suggesting a higher degree of niche availability in these logs. The majority of the logs sampled at the 1996 sites were of the intermediate decay class 3. This could explain the high level of species co-existence at the 1996 sites; however, we are unable to demonstrate this empirically.

We observed a high degree of heterogeneity in the species composition among the unburned sites, which could be a result of spatial or environmental variation across sites. Another source of variation among the unburned sites may be high levels of forest organic material in the unburned sites (as compared to the burned). Tedersoo et al. (2008) demonstrated that the most common ectomycorrhizal fungal species in decaying wood were also common in the forest floor material. Also, the forest floor may be an underestimated source of inoculum of

wood-inhabiting species (Allmér et al. 2009). Together, these studies demonstrate a strong link between fungi in the litter layer and that in decaying wood. In our study, it is possible that organic material is such a rich source of inoculum in the unburned sites, and, as such, it facilitates a much larger degree of community heterogeneity than we expected. But, given this high degree of dissimilarity, we cannot be completely certain that the differences we observed in composition between burned and unburned sites are due to the effect of the fire, and not simply an artifact of high variability in this region.

Post-fire fungal colonization may be limited by the relative scarcity of soil organic material in the forest floor in burned areas compared to unburned forests. Because pine regeneration is extremely slow following stand-replacing wildfires in southwestern ponderosa pine (Heidmann 2008), it is likely that levels of organic material will remain low for an extended period following this type of fire. Low levels of organic material were observed at our burned study sites by Ross et al. (In Review, Table 1), which may explain the long-term alterations in fungal species composition that we observed.

We observed distinctive fungal species composition at the three recent burns (2005, 2000, and 1996), but similar compositions at the two older burns (1984 and 1977; Figure 4.1), which provides support for our second hypothesis, that fungal communities would become more similar with time since fire. These results are consistent with Rajala et al. (2010) who observed a succession of fungal species over the course of log decay in spruce forests. The more decomposed logs had similar community composition. The authors suggest that airborne colonization by

fungal spores is maximized after a certain point in wood decomposition, resulting in a more stable community structure at later stages of decay (Rajala et al. 2010).

Our finding, that *Hypocrea* sp. decomposed wood about five times slower than the other seven species of fungi we tested, partially supported our third hypothesis (Figure 4.4). Members of the genus *Hypocrea* are cosmopolitan soil-borne fungi also common on decaying wood (Druzhinina and Kubicek 2005). However, members of the order Hypocreales, to which the *Hypocrea* belong, do not possess strong abilities to decompose wood substrates (Worrall et al. 1997). This suggests that members of the genus *Hypocrea* are rapid colonizers and generalists, but may lack the enzymatic capacity to process complex C. We also observed that members of the Hypocrea genus were an indicator for the most recent burn (2005) and had a high relative abundance there (Figure 4.2; Table 4.4). Consistent with our findings, members of the anamorph of this genus, *Trichoderma*, have been observed in high abundance in soil following prescribed burning (Froelich et al. 1978).

The remaining species that we tested experimentally all decomposed the wood to a similar degree, suggesting some functional redundancy among the species we tested. Similar studies have found more variability in decomposition abilities among fungal species. In a microcosm experiment, (Clinton et al. 2009) found that fungal species varied widely in their abilities to decay *Nothofagus* wood. Worrall et al (1997) tested a variety of fungi from different taxonomic orders and also observed varying degrees of ability to decay pine wood. Notably, they found an intermediate ability in members of the order Sordariales, of which three of the species we tested are members (*Chaetomium* sp., *Coniochaeta ligniaria*, and

Neurospora terricola). In particular, we expected *Coniochaeta ligniaria* to demonstrate strong decomposition abilities since it is an ascomycete that produces some of the enzymes required for lignin degradation (Lopez et al. 2007). We also expected *Penicillium corylophilum* to exhibit high decomposition based on results by Allison et al. (2009),where they observed that *Penicillium* sp. exhibited strong abilities to decay spruce wood. However, our experiment was short-term and it only assessed the initial stages of decay, and, therefore, may not be indicative of longer-term wood decay.

The inferences we can draw from our single species incubations are also limited because we could not include interactions among species of decay fungi, which are complex and usually antagonistic (Boddy 2000). Although the precise mechnanism is not entirely understood, it is thought that the production of secondary metabolites by earlier colonizing fungi can have inhibitive or stimulative effects on the growth of successive colonizers in wood (Heilmann-Clausen and Boddy 2005). This predecessor legacy effect is substantial; indeed, it has been shown to influence fungal community structure and associated decomposition rates in wood (Fukami et al. 2010). Clearly, interspecific interactions are an important consideration for assessing a species decay capacity, and this is a factor that should be incorporated into future research.

Our study is limited in that we were only able to identify those fungal species that grew in culture; therefore, our analysis missed any species that were not able to exploit the media. Extraction of fungal DNA directly from wood pieces followed by one of several advanced molecular techniques (cloning and sequencing, Terminal

RFLP, DGGE, high throughput sequencing) would potentially be able to identify more species. However, when some of these methods were compared (sporocarp collection, culturing, and T-RFLP), the combination of sporocarps and culturing identified more species than T-RFLP; in fact, T-RFLP only revealed one taxa that had not been identified by either of the other two methods (Allmér et al. 2006). Further, the asymptotic trend in our species accumulation curves suggests that our culture work was able to adequately capture the fungal species present. This evidence, coupled with the high diversity we observed in the present study, makes us confident that we have identified real patterns in fungal community composition.

This research is the first we know of to assess the community structure of wood-decay fungi in southwestern ponderosa pine forests using mycelial isolation and molecular techniques. Indeed, the only other assessment for this region was limited to sporocarop surveys and only included basidiomycetes (Gilbertson 1974). Our results show a dominance of ascomycetes compared to basidiomycetes in these logs; indeed, only two species (*Dichomitus squalens* and *Gloeophyllum saepiarium*) and one genera (*Coniophora*) overlap between the two studies. This comparison emphasizes the importance of utilizing both traditional and molecular techniques in characterizing wood-decay fungal communities, especially in semi-arid climates.

The findings presented here are early reports for a field of ecology that is being transformed by the capability of molecular techniques to rapidly and accurately identify fungal communities. As such, our results will be useful for future hypothesis generating because they can be widely applied to wood decomposition, C cycling, and stand-replacing wildfire disturbance. Our results suggest stand-

replacing wildfire disturbance may have substantial, long-term impacts on these communities. Species richness recovered relatively slowly, as compared to studies of prescribed burning in boreal forests. Also, the species composition in the burned areas was persistently different from comparable unburned areas, up to 32 years after a wildfire. These findings may have wide implications for post-wildfire C cycling because differences in species composition may lead to alterations in wood decomposition rates (Fukami et al. 2010). In particular, the increasing frequency and size of stand-replacing wildfires in the western U.S. suggests that there will be larger amounts of C stored in the decaying wood in the future. Our research suggests a deficiency in current global C models that could be ameliorated with more information on the community structure of wood-decaying fungi (McGuire and Treseder 2010).

ACKNOWLEDGEMENTS

This work was supported by funding from National Science Foundation Doctoral Dissertation Improvement Grant (Award number DEB-1011415). We thank Sam Harworth for field assistance, Todd Wojtowicz for suggesting the incubation experiment and Karen Haubensak for comments on the manuscript.

LITERATURE CITED

Allison, S., D. LeBauer, M. Ofrecio, R. Reyes, A. Ta, and T. Tran. 2009. Low levels of nitrogen addition stimulate decomposition by boreal forest fungi. Soil Biology and Biochemistry **41**:293-302.

Allmér, J., J. Stenlid, and A. Dahlberg. 2009. Logging-residue extraction does not reduce the diversity of litter-layer saprotrophic fungi in three Swedish coniferous stands after 25 years. Canadian Journal of Forest Research **39**:1737-1748.

Allmér, J., R. Vasiliauskas, K. Ihrmark, and A. Dahlberg. 2006. Wood-inhabiting fungal communities in woody debris of Norway spruce (*Picea abies* (L.) Karst.), as reflected by sporocarps, mycelial isolations, and T-RFLP identification. FEMS Microbiology Ecology **55**:57-67.

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

Auclair, A. and T. Carter. 1993. Forest wildfires as a recent source of CO_2 at northern latittudes. Canadian Journal of Forest Research **23**:1528-1536.

Baker, W. 1994. Restoration of landscape structure altered by fire suppression. Conservation Biology **8**:763-769.

Berglund, H., M. T. Jönsson, R. Penttilä, and I. Vanha-Majamaa. 2011. The effects of burning and dead-wood creation on the diversity of pioneer wood-inhabiting fungi in managed boreal spruce forests. Forest Ecology and Management **261**:1-13.

Beschta, R., J. Rhodes, J. Kauffman, R. Gresswell, G. Minshall, J. Karr, D. Perry, F. Hauer, and C. Frissell. 2004. Postfire management on forested public lands of the western United States. Conservation Biology **18**:957-967.

Boddy, L. 2000. Interspecific combative interactions between wood-decaying basidiomycetes. FEMS Microbiology Ecology **31**:185-194.

Breshears, D. D., N. S. Cobb, P. M. Rich, K. P. Price, C. D. Allen, R. G. Balice, W. H. Romme, J. H. Kastens, M. L. Floyde, J. Belnap, J. J. Anderson, O. B. Myers, and C. W. Meyer. 2005. Regional vegetation die-off in response to global-change-type drought. Proceedings of the National Academy of Sciences **102**:15144-15148.

Cairney, J. W. G. and B. Basitas. 2007. Influences of fire on forest soil fungal communities. Canadian Journal of Forest Research **37**:207-215.

Choromanska, U. and T. DeLuca. 2002. Microbial activity and nitrogen mineralization in forest mineral soils following heating: evaluation of post-fire effects. Soil Biology and Biochemistry **34**:263-271.

Clinton, P., P. Buchanan, J. Wilkie, S. Smaill, and M. Kimberley. 2009. Decomposition of Nothofagus wood in vitro and nutrient mobilization by fungi. Canadian Journal of Forest Research **39**:2193-2202.

Cornwell, W. K., J. H. C. Cornelissen, S. D. Allison, J. Bauhus, P. Eggleton, C. M. Preston, F. Scarff, J. T. Weedon, C. Wirth, and A. E. Zanne. 2009. Plant traits and wood fates across the globe: rotted, burned, or consumed? Global Change Biology **15**:2431-2449.

Crawford, R. H., S. E. Carpenter, and M. E. Harmon. 1990. Communities of filamentous fungi and yeasts in decomposing logs of *Pseudotsuga menziesii*. Mycologia **82**:759-765.

Dore, S., T. E. Kolb, M. Montes-Helu, B. W. Sullivan, W. D. Winslow, S. C. Hart, J. P. Kaye, G. W. Koch, and B. A. Hungate. 2008. Long-term impact of a stand-replacing fire on ecosystem CO_2 exchange of a ponderosa pine forest. Global Change Biology **14**:1801-1820.

Drummond, A. J., B. Ashton, M. Cheung, J. Heled, M. Kearse, R. Moir, S. Stones-Havas, T. Thierer, and A. Wilson. 2010. Geneious v5.0. Available from www.geneious.com.

Druzhinina, I. and C. P. Kubicek. 2005. Species concepts and biodiversity in Trichodermaand Hypocrea: from aggregate species to species clusters? J. Zhejiang Univ. Sci. **6B**:100-112.

Dufrene, M. and P. Legendre. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs **67**:345-366.

Dunn, P. H., S. C. Barro, M. Poth. 1985. Soil moisture affects survival of micro-organisms in heated chaparral fire. Soil Biology and Biochemistry **17**:143-148.

Easterling, D. R., G. A. Meehl, C. Parmesan, S. A. Changnon, T. R. Karl, and L. O. Mearns. 2000. Climate Extremes: Observations, Modeling, and Impacts. Science **289**:2068-2074.

Fleischner, T. 1994. Ecological costs of livestock grazing in western North America. Conservation Biology **8**:629-644.

Froelich, R. C., C. S. J. Hodges, and S. Sackett. 1978. Prescribed burning reduces severity of annosus root rot in the South. Forest Science **24**:93-100.

Fukami, T., I. Dickie, J. Paula Wilkie, B. Paulus, D. Park, A. Roberts, P. Buchanan, and R. Allen. 2010. Assembly history dictates ecosystem functioning: evidence from wood decomposer communities. Ecology Letters **13**:675-684.

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

Gehring, C., T. Theimer, T. Whitham, and P. Keim. 1998. Ectomycorrhizal fungal community structure of pinyon pines growing in two environmental extremes. Ecology **79**:1562-1572.

Gilbertson, R. L. 1974. Fungi That Decay Ponderosa Pine. The University of Arizona Press, Tucson, AZ.

Harmon, M. E., J. F. Franklin, F. Swanson, P. Sollins, S. Gregory, J. Lattin, N. Anderson, S. Cline, N. Aumen, J. Sedell, G. Lienkaemper, K. Cromack, and K. Cummins. 1986. Ecology of coarse woody debris in temperate ecosystems. Advances in Ecological Research **15**:133-302.

Hart, S., T. DeLuca, G. Newman, M. MacKenzie, and S. Boyle. 2005. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. Forest Ecology and Management **220**:166-184.

Heidmann, L. 2008. Forest regeneration research at Fort Valley. In: Fort Valley Experimental Forest - A Century of Research 1908-2008. S. D. Olberding and M. M. Moore, tech. coords. Proceeding RMRS-P-55. U.S. Department of Agriculture - Forest Service, Rocky Mountain Research Station, Fort Collins, CO.

Heilmann-Clausen, J. and L. Boddy. 2005. Inhibition and stimulation effects in communities of wood decay fungi: exudates from colonized wood influence growth by other species. Microbial ecology **49**:399-406.

Hoerling, M. and A. Kumar. 2003. The perfect ocean for drought. Science **299**:691-694.

Hoff, J., N. B. Klopfenstein, G. I. McDonald, J. R. Tonn, M. S. Kim, P. J. Zambino, P. F. Hessburg, J. Rogers, T. Peever, and L. Carris. 2004. Fungal endophytes in woody roots of Douglas-fir (Pseudotsuga menziesii) and ponderosa pine (Pinus ponderosa). Forest Pathology **34**:255-271.

Holmer, L., P. Renvall, and J. Stenlid. 1997. Selective replacement between species of wood-rotting basidiomycetes, a laboratory study. Mycological Research **101**:714-720.

Hurteau, M. D. and M. L. Brooks. 2011. Short- and Long-term Effects of Fire on Carbon in US Dry Temperate Forest Systems. BioScience **61**:139-146.

Johnson, E. and K. Miyanishi. 2008. Testing the assumptions of chronosequences in succession. Ecology letters **11**:419-431.

Jönsson, M., M. Edman, and B. G. Jonsson. 2008. Colonization and extinction patterns of wood-decaying fungi in a boreal old-growth *Picea abies* forest. Journal of Ecology **96**:1065-1075.

Junninen, K., J. Kouki, and P. Renvall. 2008. Restoration of natural legacies of fire in European boreal forests: an experimental approach to the effects on wood-decaying fungi. Canadian Journal of Forest Research **38**:202-215.

Kashian, D., W. Romme, D. Tinker, M. Turner, and M. Ryan. 2006. Carbon storage on landscapes with stand-replacing fires. BioScience **56**:598-606.

Kirby, J., J. Stenlid, and O. Holdenrieder. 1990. Population structure and responses to disturbance of the basidiomycete Resinicium bicolor. Oecologia **85**:178-184.

Korb, J., N. Johnson, and W. Covington. 2004. Slash pile burning effects on soil biotic and chemical properties and plant establishment: recommendations for amelioration. Restoration Ecology **12**:52-62.

Littell, J. S., D. McKenzie, D. Peterson, and A. L. Westerling. 2009. Climate and wildfire area burned in western U.S. ecoprovinces, 1916–2003. Ecological Applications **19**:1003-1021.

Lopez, M. J., M. C. Vargas-García, F. Suarez-Estrella, N. N. Nichols, B. S. Dien, and J. Moreno. 2007. Lignocellulose-degrading enzymes produced by the ascomycete Coniochaeta ligniaria and related species: Application for a lignocellulosic substrate treatment. Enzyme and Microbial Technology **40**:794-800.

Lumley, T. C., D. L. Dennis Gignac, and R. S. Currah. 2001. Microfungus communities of white spruce and trembling aspen logs at different stages of decay in

disturbed and undisturbed sites in the boreal mixedwood region of Alberta. Canadian Journal of Botany **79**:76-92.

Maser, C., R. Anderson, J. Cromack, K, J. Williams, and R. Martin. 1979. Dead and downed woody material.*in* J. Thomas, editor. Wildlife Habitats in Managed Forests: the Blue Mountains of Oregon and Washington. Agriculture Handbook No. 553.

McCune, B. and M. J. Mefford. 2006. PC-ORD Version 5.10, Multivariate Analysis of Ecological Data. MjM Software, Gleneden Beach, Oregon, U.S.Q.

McGuire, K. L. and K. K. Treseder. 2009. Microbial communities and their relevance for ecosystem models: Decomposition as a case study. Soil Biology and Biochemistry **42**:529-535.

Meigs, G. W., D. C. Donato, J. L. Campbell, J. G. Martin, and B. E. Law. 2009. Forest fire impacts on carbon uptake, storage, and emission: the role of burn severity in the eastern Cascades, Oregon. Ecosystems **12**:1246-1267.

Murray, T., D. Frank, and C. Gehring. 2010. Ungulate and topographic control of arbuscular mycorrhizal fungal spore community composition in a temperate grassland. Ecology **91**:815-827.

Neary, D., C. Klopatek, L. DeBano, and P. Ffolliott. 1999. Fire effects on belowground sustainability: a review and synthesis. Forest Ecology and Management **122**:51-71.

Niemelä, T., P. Renvall, and R. Penttilä. 1995. Interactions of fungi at late stages of wood decomposition. Annales Botanici Fennici **32**:141-152.

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

Pietikäinen, J., O. Kiikkilä, and H. Fritze. 2000. Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. Oikos **89**:231-242.

Raison, R. 1979. Modification of the soil environment by vegetation fires, with particular reference to nitrogen transformations: A review. Plant and Soil **51**:73-108.

Rajala, T., M. Peltoniemi, T. Pennanen, and R. Mäkipää. 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.

Schubert, G. H. 1974. Silviculture of southwestern ponderosa pine: The status-of-our-knowledge. USDA Forest Service Res. Paper RM-123. U.S. Department of Agriculutre, Rocky Mountain Forest and Range Experiment Station, editor., Fort Collins, CO.

Stephens, S. 2005. Climate and wildfire area burned in western U.S. ecoprovinces, 1916–2003. International Journal of Wildland Fire **14**:213-222.

Tedersoo, L., T. Suvi, T. Jairus, and U. Köljalg. 2008. Forest microsite effects on community composition of ectomycorrhizal fungi on seedlings of *Picea abies* and *Betula pendula*. Environmental Microbiology **10**:1189-1201.

Van Mantgem, P. J., N. L. Stephenson, J. C. Byrne, L. D. Daniels, J. F. Franklin, P. Z. Fulé, M. E. Harmon, A. J. Larson, J. M. Smith, A. H. Taylor, and T. T. Veblen. 2009. Widespread Increase of Tree Mortality Rates in the Western United States. Science **323**:521-524.

Westerling, A. L., A. Gershunov, T. J. Brown, D. R. Cayan, and M. D. Dettinger. 2003. Climate and Wildfire in the Western United States. Bull Amer Met Soc **84**:595.

Westerling, A. L., H. G. Hildago, D. R. Cayan, and T. W. Swetnam. 2006. Warming and Earlier Spring Increase Western U.S. Forest Wildfire Activity. Science **313**:940-943.

Williams, A., C. Allen, C. Millar, T. W. Swetnam, J. Michaelsen, C. Still, and S. Leavitt. 2010. Forest responses to increasing aridity and warmth in the southwestern United States. Proceedings of the National Academy of Science **107**:21289-21294.

Worrall, J. J., S. E. Anagnost, and R. A. Zabel. 1997. Comparison of wood decay among diverse lignicolous fungi. Mycologia **89**:199-219.

Table 4.1 Characteristics for the burned and unburned sites. Organic horizon and woody debris C data are from Ross et al. (In review), and are the mean of 9 plots (± 1 standard error).

Site*	Treatment	Area burned (ha)	Latitude†	Longitude†	Organic horizon C ($g\ C\ m^{-2}$)§	Woody debris C ($g\ C\ m^{-2}$)§
2005	Burned	28	35.30190	111.59126	405.1 (151.8)	1672.3 (445.30)
	Unburned	-	35.29930	111.59090	3851.4 (756.7)	78.1 (21.49)
2000	Burned	268	35.27448	111.81026	558.5 (118.2)	4889.0 (818.3)
	Unburned	-	35.27353	111.80709	3819.9 (493.59)	372.3 (98.31)
1996	Burned	3495	35.44024	111.78300	153.1 (38.8)	584.0 (144.48)
	Unburned	-	35.43988	111.78709	2906.1 (376.8)	495.2 (137.40)
1984	Burned	1258	35.35471	111.92883	410.7 (147.1)	762.4 (261.97)
	Unburned	-	35.35312	111.92653	4199.8 (1111)	446.7 (153.16)
1977	Burned	1858	35.24710	111.60103	598.6 (142.3)	382.9 (157.97)
	Unburned	-	35.24693	111.60328	2192.5 (625.0)	837.9 (406.32)

*Site refers to the year the wildfire occurred.

[†]Latitude and longitude reported in NAD27 decimal degrees.

[§]Organic horizon was sampled from two 30 x 30 cm quadrats per plot and did not include woody debris on the surface (classified as fine wood debris; Ross et al. In Review). Coarse woody debris was classified as those wood pieces >7.62 cm in diameter. Both organic horizon and coarse woody debris were converted to g C m^{-2} (Ross et al. In review).

Table 4.2 The wood-inhabiting fungi observed in this study and their identification based on the best BLAST match to the ITS sequences.

Species	GenBank Accession #	Sequence length	Percentage*	Bit Score†
Ascomycota				
Arhrographis cuboidea	AB213444	455	99.3	825
Aspergillus sp.	FJ770067	647	83.8	564
Aureobasidium pullulans	AF121282	509	99.8	934
Biscogniauxia mediterranea	EF026134	570	99.6	1,043
Byssochlamys nivea	AY265223	928	94.6	1,454
Chaetomium sp.	HM222951	566	99.1	1,015
Chromelosporium carneum	FJ872075	596	99.3	1,078
Coniochaeta ligniaria	AY198390	577	99.7	1,054
Cytospora pruinosa	EU552121	627	96.0	1013
Cytospora austromontana	EU552118	642	99.1	1151

Didymella fabae	GQ305306	544	100.0	1005
Fimetariella rabenhorstii	HM036593	524	99.6	955
Geopyxis carbonaria	Z96986	553	99.8	1,014
Gyromitra infula	AJ698480	773	90.2	961
Hypocrea lixii	AF443917	602	99.8	1,105
Hypocrea lutea	AB027384	596	100.0	1,101
Hypocrea schweinitzii (Anamorph: *Trichoderma citrinoviride)*	EU280098	638	100	1,179
Nemania serpens	EF155504	596	99.5	1,083
Neosartorya hiratsukae	GQ461906	629	98.7	1,116
Neurospora terricola	AY681176	583	99.8	1,072
Ophiodendron griseum	AF062796	514	99.8	945
Ophiostoma sapniodorum	HM031507	544	90.6	701
Ophiostoma deltoideosporum	EU879121	545	88.4	623
Penicillium spinulosum	GU566247	597	99.6	1,088

Penicillium canescens	FJ439586	579	100.0	1,070
Penicillium citreonigrum	EU497959	586	100.0	1,083
Penicillium corylophilum	GU566277	599	100.0	1,107
Penicillium decumbens	AY373909	568	99.6	1,037
Penicillium fellutanum	AY373913	559	99.1	1,002
Penicillium janithellum	AB293968	627	99.2	1,127
Penicillium purpurogenum	GU566210	594	99.5	1,079
Phaeomoniella sp.	GQ153128	540	99.4	981
Phialophora alba	HM116755	608	100.0	1,123
Podospora miniglutinans	AY515362	528	100.0	976
Preussia sp.	FJ210518	537	99.5	976
Pyronema domesticum	HQ115722	592	98.8	1,053
Rhinocladiella atrovirens	AB091215	606	99.0	1,085
Rhinocladiella sp.	GU067765	536	99.4	972
Sydowia polyspora	GQ412722	578	99.8	1,062

Thielavia arenaria	GU966511	555	99.6	1,015
Myxotrichaceae sp.	FJ475803	563	99.6	1,029
Basidiomycota				
Cerinosterus luteoalbus	AY618667	450	98.7	799
Coniophora prasinoides	GU187519	677	98.5	1,198
Dichomitus squalens	AM988622	638	98.7'	1,129
Gloeophyllum sepiarium	AY089732	592	99.8	1,088
Rhodoturula lamellibrachiae	AB263122	605	95.9	967
Zygomycetes				
Umbelopsis sp.	GQ241270	603	99.5	1,096
Umbelopsis ramanniana	EU715662	629	98.7	1,111

*Percent similarity of query to published reference sequence
†Bit score is an evaluation of the query and reference sequence alignment based on their lengths and the number of gaps and substitutions between the two. Bit scores are normalized, thus they can be compared across search results.

Table 4.3 Mean Shannon's diversity index (H') for wood-decay fungi found in logs at burned and unburned sites. Means represent the mean of six logs (plots) ±1 standard error, and p-values are the results of a one-way ANOVA at each site with treatment (burned or unburned) as the main effect. Significant differences between burned and unburned sites for each burn year are denoted by a boldface p-value ($p < 0.1$).

Burn year	Shannon's diversity index		
	Burn	Unburned	p-value
2005	0.6847 (0.24)	1.045 (0.26)	0.330
2000	1.5053 (0.10)	1.1645 (0.13)	**0.069**
1996	1.5060 (0.23)	1.6218 (0.15)	0.676
1984	1.6598 (0.07)	0.847 (0.13)	**0.003**
1977	1.234 (0.21)	1.2298 (0.18)	0.988

Table 4.4 Results of indicator species analysis of wood-decay fungi communities in burned and unburned sites of varying ages.

Species	Indicator value	*P*-value	Indicator year	Indicator treatment
Aspergillus sp.	26.7	0.018	1984	Burned
Chromelosporium carneum	20.5	0.057	1984	Burned
Hypocrea sp.	32.4	0.003	2005	Burned
Phialophora sp.	33.3	0.082	1996	Burned
Biscogniauxia mediterranea	33.3	0.080	1996	Unburned
Byssochlamys nivea	34.8	0.001	1984	Unburned
Coniochaeta ligniaria.	21.3	0.057	1996	Unburned
Penicillium sp.	24.1	0.035	2000	Unburned
Pezizomycotina spp.	27.5	0.022	1984	Unburned

FIGURE LEGENDS

Figure 4.1 Species richness of wood-decay fungi at each burned (closed bars) and unburned (open bars) at each of the five chronosequence sites. Asterisk indicates differences between the burn and unburned treatment at each year. Each bar represents the mean of six logs sampled per site, and error bars represent ± 1 standard error.

Figure 4.2 Percent relative abundance of all the fungal species at burned (closed bars) and unburned sites (open bars) at each of the five chronosequence sites (A-E; 2005, 2000, 1996, 1984, 1977, respectively). Relative abundance was computed for each unburned and burned site separately. Species are arranged in order of decreasing relative abundance beginning with the unburned sites. Statistics shown (A and P values) are the results of MRPP analysis comparing the community structure at each burned and unburned area.

Figure 4.3 Non-metric multidimensional scaling of wood-decay fungi community composition in burned (A) and unburned (B) sites. The final stress after 250 runs with real data was 25.5 (burned) and 25.6 (unburned) for a 2-dimensional solution. Each centroid is the mean of a site in ordination space, and bars represent ± 1 standard error. Six logs were sampled per site and four wood pieces were taken from each log (n = 6). Results of MRPP analysis confirmed that the 2005, 2000, and 1996 burns differed compositionally from each other, and the 1984 and 1977 burns were similar in composition. MRPP analysis on the unburned sites confirmed that the 2000 and 2005 sites were similar compositionally, while the 1996, 1984, and 1977 were distinct.

Figure 4.4 Species accumulation curves for the unburned and burned treatments. Sample units are equivalent to one log, wherein four wood samples were taken for mycelial isolation. The curve is the cumulative number of species for a given number of sample units.

Figure 4.5 Percent mass loss of the pine dowel pieces when incubated with one of nine wood-decay fungal species from culture. Each incubation was replicated ten times (n = 10) and bars represent ± 1 standard error. *Hypocrea lixii* had a lower decomposition rate than the other species, which were all similar. Unique letters represent differences among the fungal species.

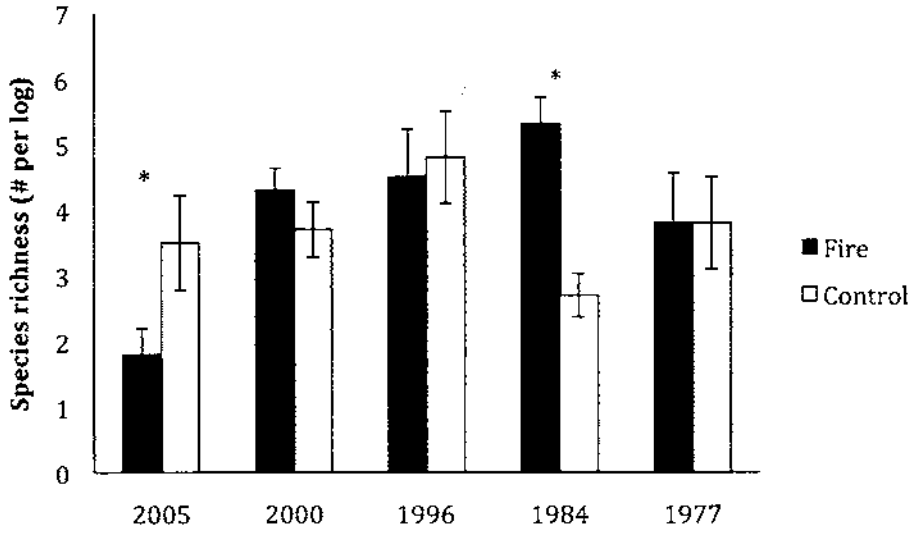
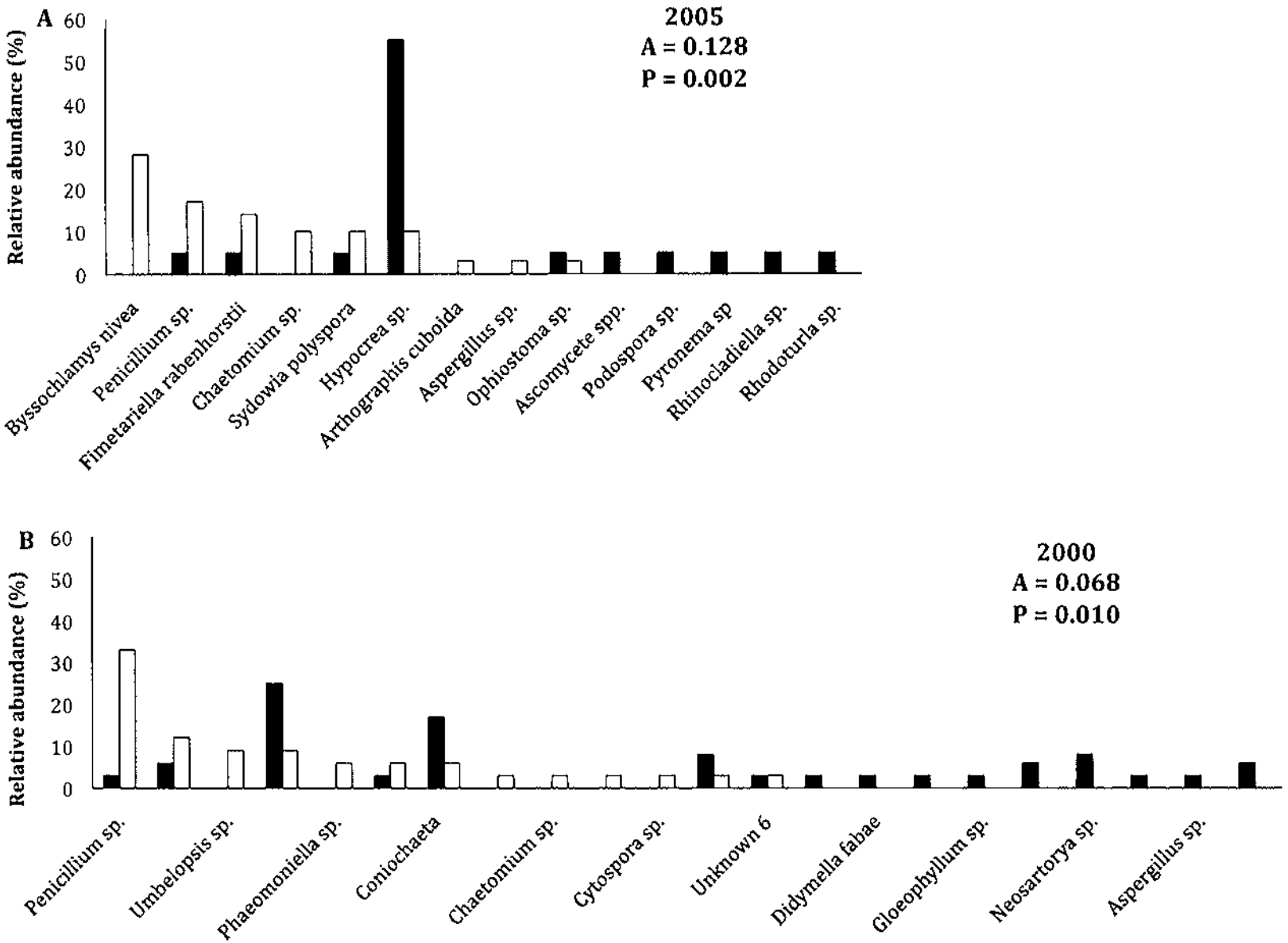
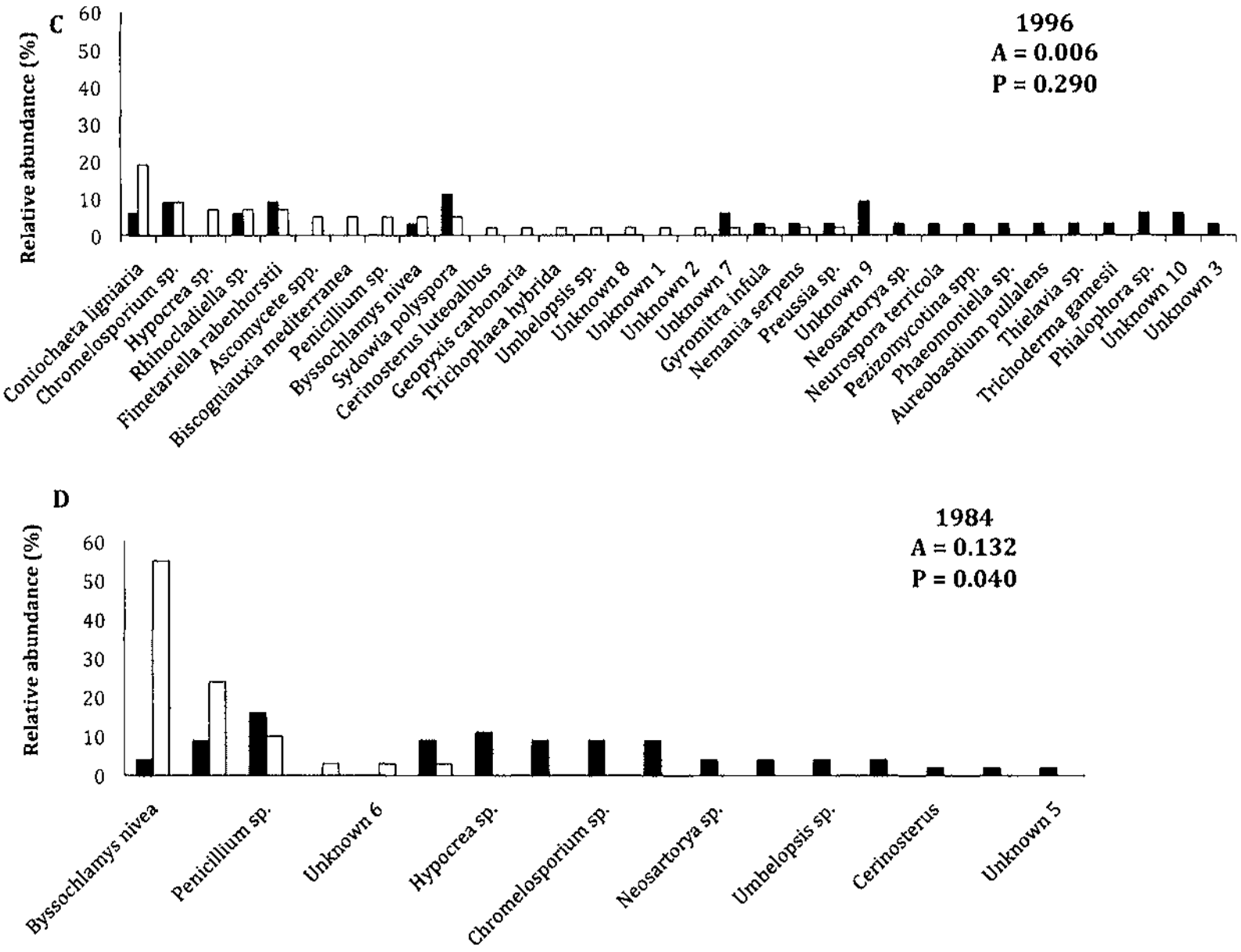


Figure 4.1





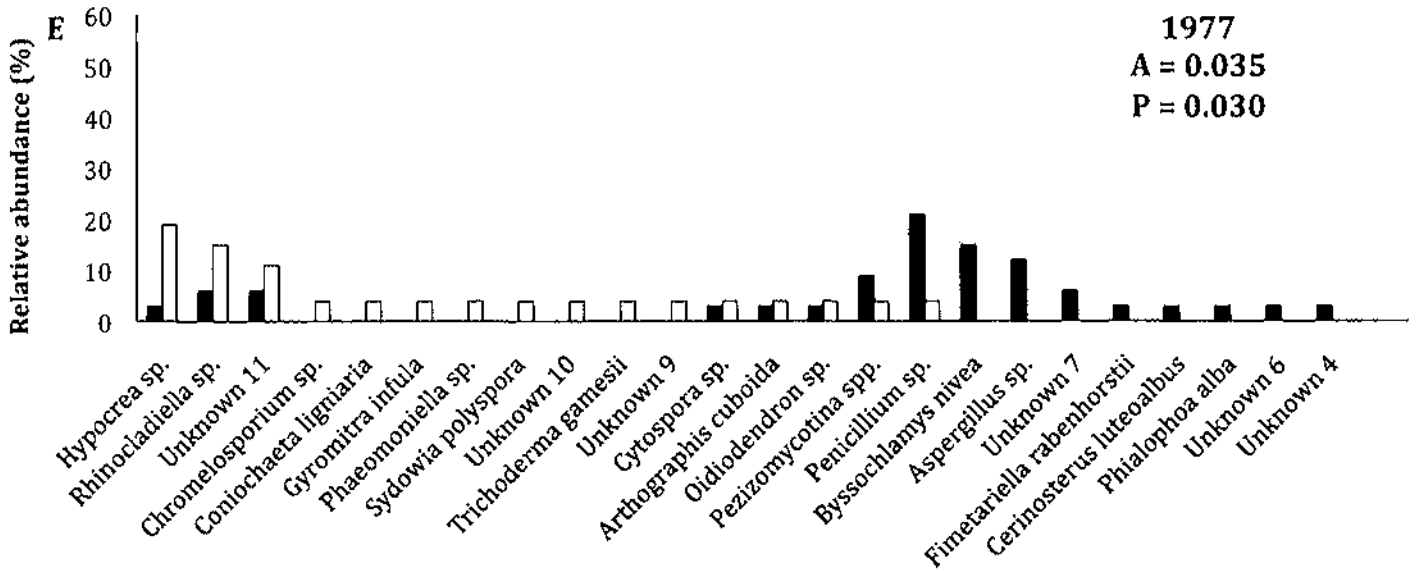
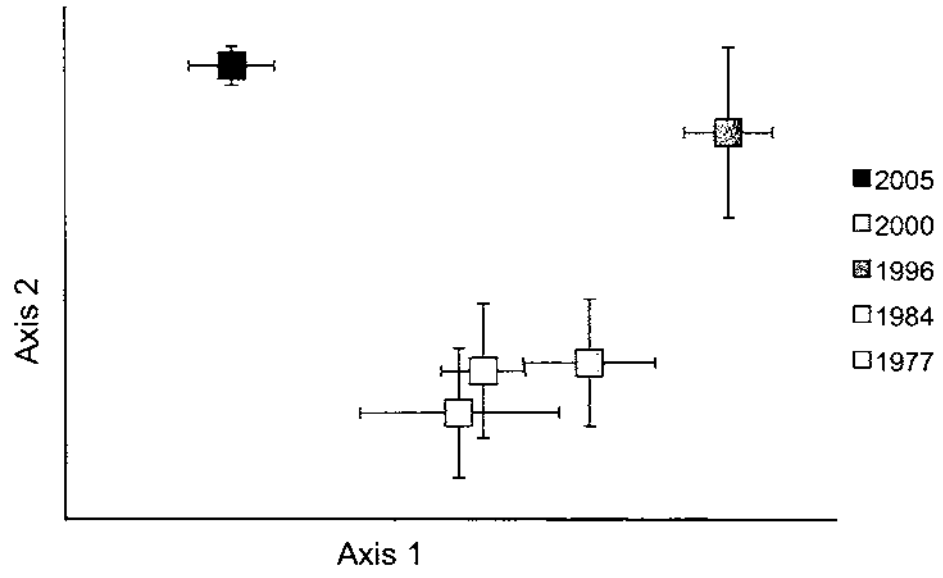


Figure 4.2

A



B

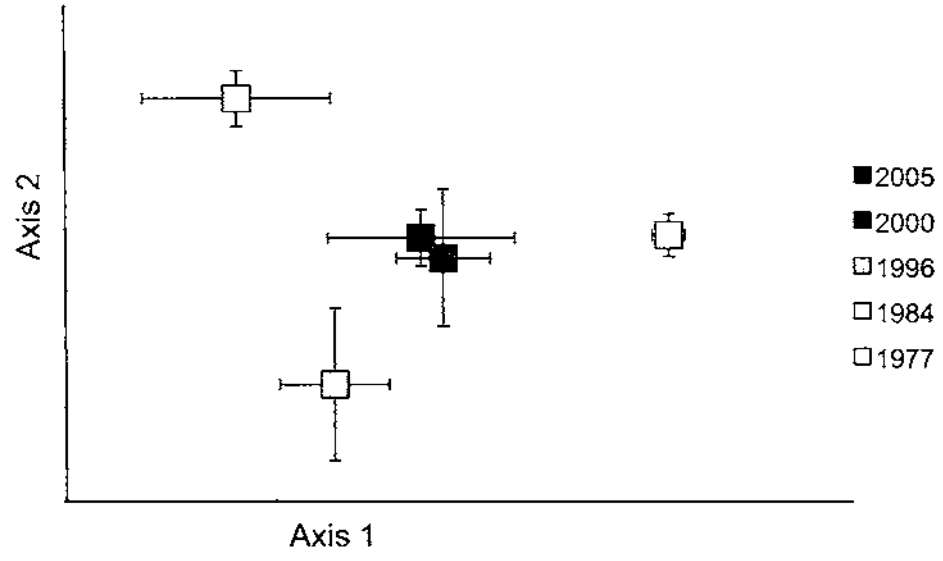


Figure 4.3

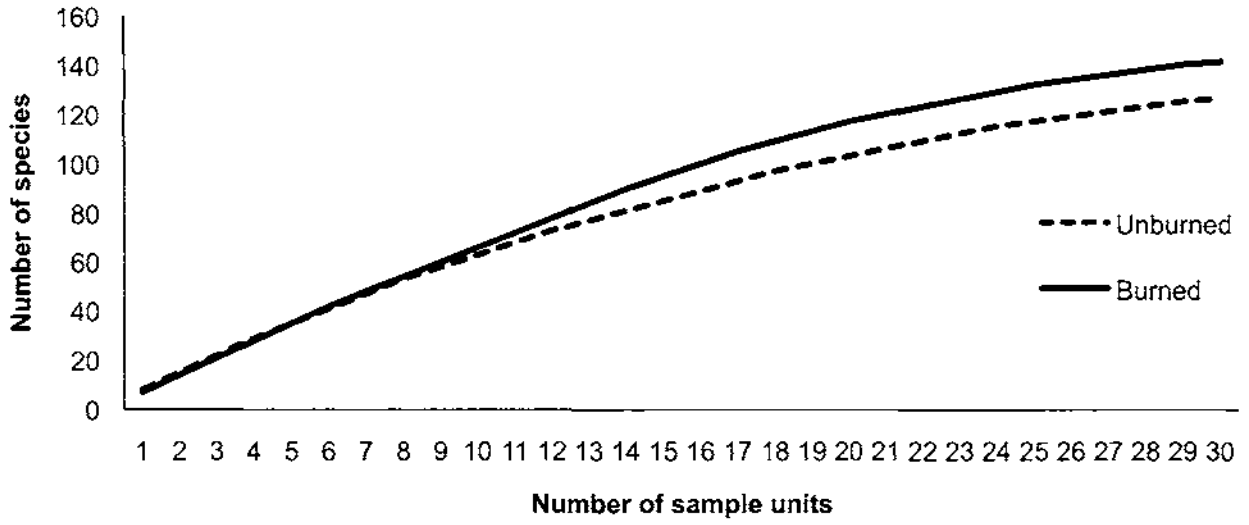


Figure 4.4

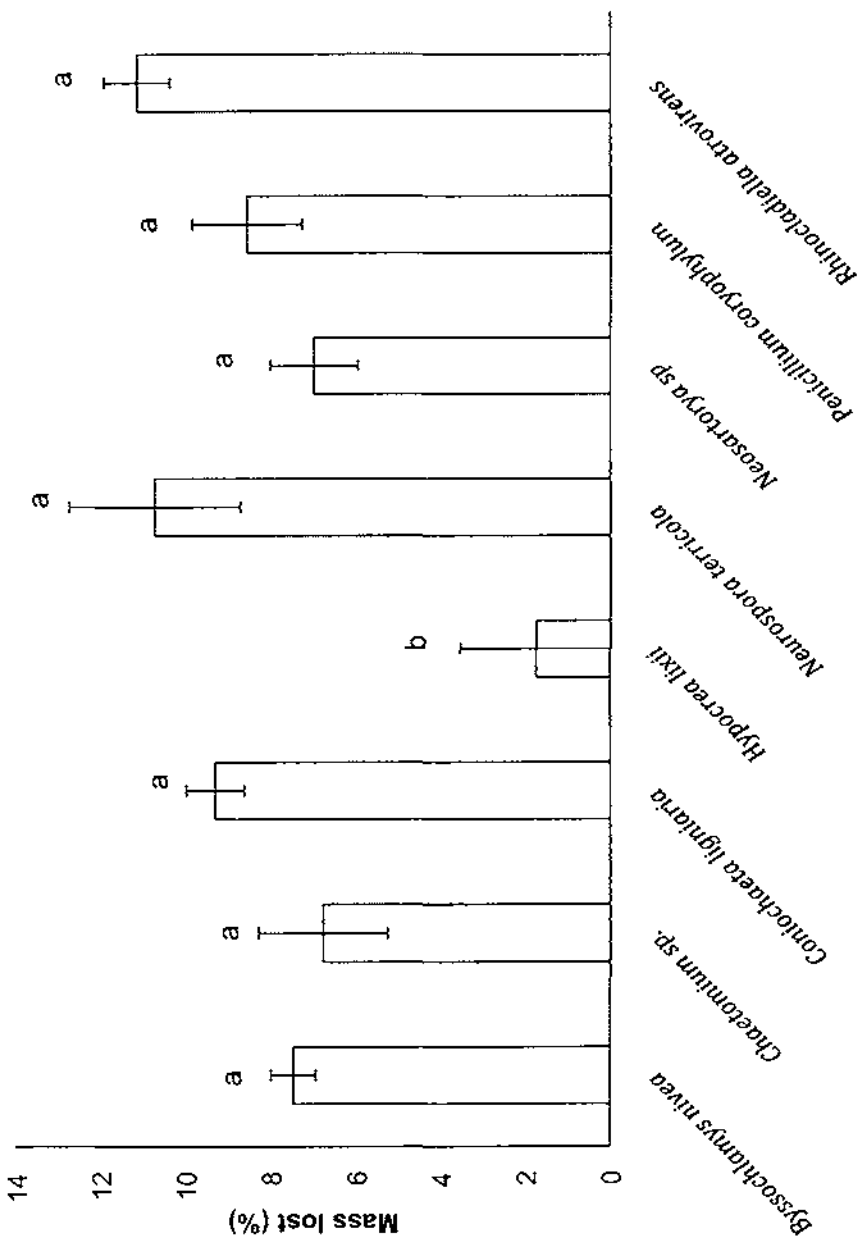


Figure 4.5

CHAPTER 5

WILDFIRE AND FOREST MANAGEMENT INFLUENCES ON FINE ROOT DECOMPOSITION AND SOIL MICROBIAL BIOMASS POOLS IN SOUTHWESTERN PONDEROSA PINE FORESTS

ABSTRACT

The decomposition of fine roots is an essential component of nutrient cycling in terrestrial ecosystems, but the rate of this flux and the factors that regulate it are poorly understood compared to our knowledge of aboveground leaf litter decay. We evaluated the effects of different forest management regimes on rates of fine root decomposition and associated soil microbial carbon (C) pools in southwestern ponderosa pine forests. We selected stands that had previously undergone various management treatments (unmanaged, thinned, thinned/prescribed burned, and wildfire) and installed steel-walled cylinders (trenches) into the ground to sever fine roots. We hypothesized that rates of fine root decomposition would increase with the degree of disturbance across the treated stands: highest at the wildfire site and lowest at the unmanaged site. Additionally, we hypothesized that microbial C would be lowest where the fine root decomposition rate was the highest because the trenches would prevent new belowground C inputs as a substrate for microbial maintenance and growth. To evaluate these hypotheses, we sampled soil inside the trenches at 5 and 27 months following trench installation, and measured fine root biomass and microbial biomass C pools. We compared these measurements to soil samples taken outside the trenches at the 5 month sample date and calculated fine root decomposition rates. Over the 27-month period, rates of fine root

decomposition did not differ among management treatments, but herbaceous roots decomposed significantly faster than pine roots. Microbial C pools were lower inside the trenches at the wildfire sites than the unmanaged and thinned sites at 5 months, but pools were similar across all management treatments at 27 months. We observed a significant positive correlation between fine root decay constants and microbial C ($r = 0.35$; $p = 0.01$) across all the management treatments, suggesting that soil microbes are limited by root-derived labile C. The higher nutrient concentrations and rates of decomposition of fine roots than leaf litter in this region, coupled with the relatively comparable inputs of these two types of detritus to the soil, suggest that fine root turnover is integral to the maintenance of soil fertility in these forest ecosystems.

INTRODUCTION

Fine root decomposition is an important source of nutrients and organic matter for soils in many forest ecosystems (McClaugherty et al., 1984; Silver and Vogt, 1993; Berg et al., 1998; Chen et al., 2002; Hart et al., 2005a), but it may be of particular importance following disturbance. Natural or anthropogenic disturbances, such as wildfires, hurricanes, or tree harvest cause a reduction in aboveground litter inputs to the soil because of a reduction in stand growth; however, the belowground decay of fine root material following the disturbance may buffer the ecosystem against these sudden declines in aboveground nutrient return (Fahey et al., 1988). Decaying fine roots provide a flush of labile carbon (C) for maintaining microbial activity and soil organic matter pools. However, the pattern of fine root decay may depend on the type, severity, and spatial scale of the disturbance (Silver and Vogt, 1993). For example, landscape-level disturbances from high-severity wildfire may cause greater reductions in fine root biomass than smaller, more localized disturbances from tree thinning or prescribed burning (Hart et al., 2005a; Dore et al., 2008; Selmants et al., 2008; Sullivan et al., 2008).

Extended fire exclusion in southwestern ponderosa pine forests has led to significant changes in stand structure, which contribute to the increase of uncharacteristically large and severe wildfires in this region (Covington and Moore, 1994). The goal of many recent forest management efforts in southwestern ponderosa pine is to reduce the likelihood of stand-replacing, catastrophic wildfires and to restore these forests to their historic stand structure, composition, and function (Covington et al., 1997; Allen et al., 2002). These management treatments,

primarily a combination of thinning and burning, promote higher quality litter inputs by increasing herbaceous productivity (Kaye et al., 2005; Sabo et al., 2008), however, comparatively little is known regarding how these treatments influence the turnover of fine roots. For instance, Hart et al. (2005a) did not observe differences in fine root decay between control and repeatedly burned plots, but their study was limited to one growing season. In similar forests, Selmants et al. (2008) found that fine root biomass decreased as a result of forest restoration treatments, but their study did not quantify the rate of fine root production or decay.

Although fine root dynamics may significantly influence nutrient cycling following forest management activities, such as tree thinning and prescribed burning (Hart et al., 2005a; Kaye et al., 2005), few studies have evaluated fine root decomposition across a range of disturbances within a similar habitat (Fahey et al., 1988; Silver and Vogt 1993; Hart et al., 2005). Fewer still have linked fine root decay to microbial C, despite evidence suggesting that the exclusion of fine roots can alter microbial community composition (Brant et al., 2006) and reduce CO_2 efflux and microbial biomass (Li et al., 2005).

We assessed the linkages between fine root decay and microbial biomass C under the premise that if fine roots are a significant C source for microbes, then changes in fine root biomass should be closely coupled to changes in microbial biomass C. We employed several different forest management treatments in southwestern ponderosa pine as a framework for examining this link because the treatments (unmanaged, thinned, thinned and burned, and wildfire) provide a

gradient of soil C availability, in both C quantity and quality (Grady and Hart, 2006). We hypothesized that rates of fine root decomposition would increase with the degree of disturbance across these stands: highest at the wildfire stand, intermediate in the thinned and thinned and burned, and lowest in the unmanaged stands. The basis for this prediction was twofold: 1) an increase in soil temperature and N availability that occurs in these forests with increasing disturbance (Hart et al., 2005b; Grady and Hart, 2006), and 2) a concomitant increase in the relative cover of herbaceous understory species (Grady and Hart, 2006; Sabo et al., 2008), which have a higher substrate quality for decomposing organisms than pine roots (Kaye and Hart, 1998a). Higher soil temperature, N availability, and root litter quality in more disturbed stands should all contribute to faster rates of fine root decomposition (Gill and Jackson, 2000; Silver and Miya, 2001). We also hypothesized that, because soil microbial C pools are coupled to plant litter inputs (Zak et al., 1994; Grady and Hart, 2006; Li et al., 2010), changes in microbial C pools would covary with changes in fine root biomass. If soil microorganisms are truly maintained via access to fine root C, then we would expect to find a correlation between fine root biomass and microbial biomass C. We predicted that levels of microbial biomass C would be lowest in stands where decomposition is the fastest (wildfire), intermediate where decomposition is intermediate (thinned and thinned and burned) and highest where decomposition is the slowest (unmanaged).

METHODS

Study site and forest treatments description

This study was conducted in ponderosa pine (*Pinus ponderosa* var. *scopulorum* Engelm.) forests in the Coconino National Forest of northern Arizona, USA, near the city of Flagstaff (35° 8' N, 111° 39' W). Historically (1909-2000), annual precipitation in this region averaged 56.6 cm and annual air temperature averaged 6.3°C (Fort Valley Arizona Meteorological Data (FVAMD), 2006). Precipitation during our study period (2004, 2005 and 2006) was 43.7 cm, 54.6 cm, and 38.6 cm respectively, and mean annual air temperature was 5.9°C, 6.5°C, and 6.8°C respectively (FVAMD, 2006). Elevations across stands ranged from 2160 to 2440 m. Monsoonal rains are common in the southwestern USA between approximately mid-July and September, with approximately half the yearly precipitation occurring during this season.

Ponderosa pine is the dominant overstory vegetation in all stands except in the wildfire stands where the overstory is absent (but was previously ponderosa pine). The understory community is dominated by Arizona fescue (*Festuca arizonica* Vasey) and mountain muhly (*Muhlenbergia montana* Hitch.) in unmanaged, thinned, and thinned and burned stands, and by squirreltail (*Elymus elymoides* (Raf.) Swezey), foxtail (*Hordeum jubatum* L.) and cheatgrass (*Bromus tectorum* L.) in wildfire stands (Sabo et al., 2009). Soils are Typic Argiborolls and Mollic Eutroboralfs, with flow basalt and basaltic cinders as the parent materials (Miller et al., 1995).

We used the experimental sites of Grady and Hart (2006), where plots were established across replicated stands with different management histories (e.g., thinning, thinning/burning) or wildfire). The forest treatments were part of the Stand Treatment Impacts on Forest Health (STIFH) study, designed to evaluate the effects of thinning, prescribed burning, and wildfire on ecosystem structure and function (Bailey et al., 2001). A retrospective approach was employed to quantify the long-term effects of silvicultural treatments (as surrogates for forest restoration) and wildfire.

We randomly selected four stands from each of the following four forest treatment groups (≥20 ha each within 750 km^2 landscape area, and out of a potential pool of 10 of each treatment group).

- *Unmanaged stands* (UN) – stands dominated by dense patches of even-aged, small diameter ponderosa pine trees (< 40 cm diameter at breast height [1.37 m]) that had not received a density altering treatment within the last 30 years, such that the stands had > 90% crown closure. Mean basal area in UN stands was 35 m^2/ha and tree density averaged 815 trees/ha.
- *Thinned stands* (TH) – stands of even-aged ponderosa pine trees as above, with greater than 30% basal area removed between 1988 and 1995, of which 50% or more came from diameter classes < 30 cm. The percent of basal area removed in TH stands ranged from 32 to 59% with a mean of 40%.
- *Thinned and burned stands* (TB) – stands like those from TH above that also received a broadcast burn treatment within three to four years following

thinning (1989 to 1997). The percent of basal area removed in TB stands ranged from 33 to 70% and averaged 57%.

- *Wildfire stands* (WF) – similar to unmanaged stands prior to being consumed by the Hochderffer stand-replacing wildfire in 1996. Ponderosa pine tree mortality was greater than 90%. This wildfire site was selected because it occurred at about the same time as the other treatments.

Sampling design

Within each replicate stand of each treatment, we sampled from three plots, spaced between 150 to 1000 m apart. These plot data were averaged to calculate the responses for each of the four replicate stands in each treatment (n = 4).

We used root trenches to assess fine root (≤ 2-mm diameter) decomposition and associated changes in microbial C. These trenches were installed in May of 2004. Their original intent was to sever all fine roots, and thus allow the partitioning of heterotrophic and autotrophic soil respiration rates (Hanson et al., 2000). The trenches consisted of thin-walled (~1.6 mm) steel cylinders, 20.3 cm in diameter. The cylinders were driven into the soil to a depth of 35 cm using a steel driving cap and a sledgehammer. Because of the methodological similarity between the cylinders and traditional trenches (e. g., Silver and Vogt, 1993), we will refer hereafter to the cylinders as "trenches."

We made several assumptions to estimate fine root decomposition rates using the trenched plots. First, we assumed that the trench installation killed all fine roots, which allowed us to calculate decomposition as the difference between the root mass in the soil outside the cylinder (untrenched) and that inside the cylinder

(trenched). We also assumed that, by randomly assigning the trench locations, the fine root biomass was the same among the treatments before the trenches were installed. Finally, we assumed that the fine root biomass did not change outside the trenches (untrenched) from the time the trenches were installed until the first sampling date (after 5 months). Previous work in ponderosa pine forests in this region suggests that fine root biomass pools are not very dynamic over one growing season (Hart et al., 2005a). Furthermore, we tested the statistical similarity of fine root biomass outside the trenches at the two sampling dates (5- and 27-months) using a 2-way analysis of variance with sample date and forest treatment as effects. The fine root biomasses of the untrenched soils were statistically similar between these two time periods ($p = 0.741$), and there was no significant sample date x forest treatment interaction. Therefore, we are confident that fine root biomass outside of the trenches (untrenched) did not change substantially during the first five months following trench installation, and we proceeded to use the untrenched biomass at 5 months as our initial fine root biomass pool size for estimating fine root decomposition.

Fine root and microbial biomass

We took two mineral soil cores (4.8 cm dia. x 15 cm depth) from inside and in the immediate vicinity (within 0.5 m) outside the trenches in October 2004 and August 2006 (5 and 27 months after trenching, respectively). One core from each trenched and untrenched pair was used for estimating fine root biomass. Two plots had herbaceous plants growing inside the trench at the 27 month sample date, and, thus, were excluded from all subsequent analyses.

We separated fine roots from the mineral soil using a hydro-pneumatic elutriator (Scienceware Bel-Art products, Pequannock, N.J., USA; Hart et al., 2005a). At the 5-month sampling, all roots, live and dead, were separated from other organic material using a shallow pan containing deionized water. At the 27-month sampling, the roots were further separated by vegetation type: pine and herbaceous. This separation was guided by root morphological differences between the two types of vegetation (e.g., the dichotomous branching structure of the pine roots versus the irregular branching patterns of the herbaceous roots; Kaye and Hart, 1998b). All fine roots were oven dried at 70 °C for 48 h and then weighed. A decomposition constant (k, year^{-1}) was calculated for each forest treatment assuming that mass loss fit a single exponential decomposition model (Olson, 1963). The decomposition constant was calculated for the total (live + dead) fine root biomass based on the difference between the untrenched fine root biomass at the 5-month reference time and the trenched fine root biomass at the two sampling times. The decomposition constants for the herbaceous and pine fine root biomasses were calculated using the difference between the trenched and untrenched biomass values at 27 months because roots were not separated by type at the earlier sample date.

Soil samples taken from the second core from each trenched and untrenched pair were immediately extracted for microbial C using the chloroform ($CHCl_3$) fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987; Haubensak et al., 2002). Soil samples were sieved (< 4 mm), and approximately 30 g of field-moist soil was extracted with 100 mL of 0.5 M K_2SO_4. A second 30-g subsample of mineral soil was also placed inside a desiccator with a beaker containing 30 mL of

ethanol-free $CHCl_3$. The desiccator was repeatedly evacuated to boil the $CHCl_3$ and then left under vacuum for 5 days (Haubensak et al., 2002). After 5 days, the $CHCl_3$ was removed from the soil by repeated evacuations and then soil subsamples were immediately extracted with 100 mL of 0.5 M K_2SO_4. Extracts were shaken on a reciprocating shaker for one h, filtered with Whatman #1 filters (pre-leached with deionized water), and frozen until analysis.

Organic C concentrations in unfumigated and fumigated extracts were determined by ultraviolet-enhanced persulfate oxidation using a Dohrmann DC-80 Carbon Analyzer with infrared detection (Tekmar-Dohrmann, Cincinnati, OH, USA). Soil microbial C was computed by the difference between the unfumigated and fumigated extracts. We used a k_{EC} of 0.39 (Sparling et al., 1980) to convert fumigation C flushes to microbial C.

Statistical analysis

We analyzed fine root biomass, microbial biomass, and gravimetric water content (GWC) by forest management type across the three sample dates (untrenched, 5 months after trenching, and 27 months after trenching). For these variables, we used repeated measures analysis of variance (RMANOVA) and Greenhouse-Geissner adjustments to account for autocorrelation over time, and tested for forest management treatment and time main effects and their interactions. Significant forest management treatment effects were followed by additional one-way ANOVAs for each sample date and Tukey's Honestly Significant Difference (HSD) multiple comparisons to separate treatment means. We also calculated Pearson's correlation coefficients to examine the relationship between

fine root biomass and microbial biomass C pools across all forest management treatments.

We analyzed the effects of forest treatment on the 27-month decomposition rate constants, *k*, using two approaches. For the total fine root decomposition rate constant, we used a one-way ANOVA with management treatment as the main effect. For the pine and herbaceous *k* values, we used a two-way ANOVA with root type and management treatment as the main effects, and their interaction. When the overall model was significant, Tukey's HSD multiple comparisons were used to separate means among treatments. All statistical analyses were performed using JMP 5.0.1.2 (SAS Institute Inc., Cary, NC, USA), and an alpha level of 0.05 was used to determine statistical significance.

RESULTS AND DISCUSSION

Fine root decomposition

Substrate quality is an important driver of fine root decomposition (Gholz et al., 2000; Silver and Miya, 2001; Parton, et al., 2007), and we expected the previously observed differences in dominant vegetation across the treatment gradient (Sabo et al., 2009) to be reflected in the rates of fine root decomposition. Specifically, we expected the highly disturbed wildfire site to exhibit the highest rate of fine root decomposition because of its increased amounts of herbaceous vegetation, higher soil N availability and higher soil temperatures compared to the other three sites. However, our analysis demonstrated that forest management treatment did not affect fine root decomposition in this study. We subsequently analyzed the rates of pine and herbaceous decomposition separately by pooling

each root type across all forest treatments at the 27-month sample date. In this analysis, we found that the herbaceous fine roots decayed faster than the pine ($p < 0.0001$). Thus, the ostensibly low fine root biomass at the wildfire site (Figure 5.1) compared to the other treatments suggests that perhaps the low statistical power of our study ($n = 4$) limited our ability to detect management differences.

The range of fine root decomposition rates we observed for ponderosa pine (wildfire = 0.10 y^{-1}; unmanaged = 0.35 y^{-1}; Table 5.1) are consistent with rates found for conifers at regional and global scales, as well as across methodologies. Decomposition rates of between 0.48 y^{-1} (control) and 0.73 y^{-1} (repeatedly burned) have been reported for southwestern ponderosa pine forests (Hart et al., 2005a). In addition, the percent of fine root biomass remaining (data not shown) is also similar to values reported for ponderosa pine in the Pacific Northwest over a similar time period (Chen et al., 2002). At larger scales, the decomposition rates we observed are comparable to those for conifer species in other temperate ecosystems (0.18 – 0.29 y^{-1}; Hobbie et al., 2010), and the overall global mean for conifers (0.17 y^{-1}; Silver and Miya, 2001). Since nearly all of the above-mentioned studies used the litterbag method, it is useful to note that the trench method we employed yielded comparable results to litterbags.

Our research demonstrates that fine root turnover is a more important source of nutrients and soil organic matter in this ecosystem than comparable leaf litter. Although inputs from fine roots and foliage are similar in unmanaged and thinned southwestern ponderosa pine forests (Kaye et al., 2005), the rates of pine fine root decomposition we observed are generally faster than those reported for

ponderosa pine needle litter (Klemmedson, et al., 1985; Hart et al., 2005b). Furthermore, the net release of N from fine roots is higher than that of comparable needle litter during decomposition in these forests (Wright, 1996; Hart et al., 2005b). Taken together, this evidence suggests that fine root inputs to nutrient cycling processes and soil organic matter may be greater than those from needle litter in these ecosystems.

Coupling of fine root decomposition and microbial biomass C pools within forest management treatments

Patterns in microbial C pools provide limited support for our hypothesis that microbial biomass C levels would be lowest at the wildfire stands, highest at the unmanaged stands and intermediate at the thinned and thinned/burned. Unlike fine root decomposition, microbial biomass C differed among management treatments ($p = 0.02$; Figure 5.2). The untrenched samples had the greatest treatment differences, wherein microbial biomass C was significantly lower at the wildfire site than the unmanaged and thinned sites. This pattern is consistent with previous findings by Grady and Hart (2006) for untrenched soils at the same study plots. Treatment differences in the current study were also evident at the 5-month sample date, where the wildfire site had lower microbial biomass C than the thinned site.

Microbial biomass C decreased over time across all the management treatments (untrenched > trenched 5 months > trenched 27 months; $p < 0.0001$; Figure 2). This decline suggests a progressive constraint of labile C on soil microbial activity over the course of our study (Zak et al., 1994). Because fine root turnover is thought to cycle soil C more rapidly than associated aboveground plant litter (Ruess

et al., 2003), it is likely that fine root material is an important source of this belowground labile C for soil microbes (Hart and Sollins, 1998; Li et al., 2010). The trenches limited new belowground C inputs from fine roots, and may have increased nutrient availability and soil moisture content (Fisher and Gosz, 1986), all of which are artifacts of the study design. However, we found a decrease in GWC over time inside the trenches ($p < 0.001$), but no effect of management treatment (Table 5.1). This suggests the lack of active roots inside the trenches did not increase the soil moisture content, which could have led to higher microbial biomass C and accelerated fine root decomposition. Furthermore, because both fine root biomass and microbial biomass C declined over time inside the trenches, we argue that soil microbes in these forests are highly constrained by belowground labile C inputs from fine roots (either from root exudation or decomposition), regardless of the management treatment imposed.

To further explore the link between belowground microorganisms and C inputs from fine roots, we analyzed correlations between fine root biomass and microbial biomass C. There was a significant correlation between fine root biomass and microbial biomass C when all treatments were pooled together across all sample dates (Figure 5.3; $r = 0.35$, $p = 0.01$, $n = 48$); however, a substantial portion of the variation (~88%) was still unexplained. When the two variables were analyzed separately for each management treatment, only the unmanaged treatment had a significant correlation ($r = 0.62$; $p = 0.03$, $n = 12$). The thinned treatment was marginally significant ($r = 0.46$; $p = 0.13$, $n = 12$), while the thinned/burned and wildfire treatments were not significantly correlated. This

pattern suggests that the larger management disturbances (wildfire and thinning/burning) uncoupled the C supply as a controller of microbial biomass C, and this may explain some of the variation in the correlation analysis. We also think that the relationship between fine root biomass and microbial biomass C became weaker at the wildfire site because the same conditions we expected to enhance decomposition (higher N availability, surface temperatures and litter quality) were offset by the lower abundance of decomposers. Finally, the two variables may be controlled by different factors; our research shows that microbial biomass C is linked to fine root-derived labile C availability while fine root decomposition may be a function of changes in microclimate or substrate quality (Silver and Miya, 2001), both of which were not measured in our study.

CONCLUSIONS

This is one of few existing studies to quantify fine root decomposition and associated microbial biomass C pools under common forest management treatments, and our findings suggest that fine root turnover is an important component of nutrient cycling in these forests. Fine root decomposition may represent a substantial portion of terrestrial C fluxes and source of mineral nutrients (Silver and Miya, 2001; Fornara et al., 2009), and our research emphasizes the importance of considering belowground organic matter inputs and turnover when making management decisions. Despite the lack of statistical difference in fine root decomposition among the forest treatments, we found that herbaceous roots decomposed faster than pine; therefore, we recommend implementing silvicultural treatments that open forest canopies and encourage the development of herbaceous

understory to promote dynamic nutrient cycling. Furthermore, these treatments reduce the risks of catastrophic wildfires, which, in turn, reduce soil microbial biomass C and may have long-term implications for microbial community dynamics.

ACKNOWLEDGMENTS

We thank Morgan Luce and Greg Newman for laboratory assistance and Karen Haubensak, Catherine Gehring, and Pete Fulé for helpful comments on this manuscript.

LITERATURE CITED

Allen, C.D., Savage, M., Falk, D.A., Suckling, K.F., Swetnam, T.W., Schulke, T., Stacey, P.B., Morgan, P., Hoffman, M., and Klingel, J.T. 2002. Ecological restoration of southwestern ponderosa pine ecosystems: A broad perspective. Ecol. Appl. **12**(5): 1418-1433. doi: 10.1890/1051-0761(2002)012[1418:EROSPP]2.0.CO;2.

Bailey, J.D., Wagner, M.R., Smith, J.J., 2001. Stand treatment impacts on forest health (STIFH): structural responses associated with silvicultural treatments. In: Van Riper III, C., Thomas, K.A., Stuart, M. (Eds.), Fifth Biennial Conference of Research on the Colorado Plateau. U.S. Geological Survey/FRESC Report Series USGSFRESC/COPL/2001/24.

Berg, B., Johansson, M.-B., Meentemeyer, V., and Kratz, W. 1998. Decomposition of tree root litter in a climatic transect of coniferous forests in northern Europe: A synthesis. Scandinavian Journal of Forest Research, **13**(4): 402-412. doi: 10.1080/02827589809383000.

Brant, J.B., Myrold, D.D., and Sulzman, E.W. 2006. Root controls on soil microbial community structure in forest soils. Oecologia. **148**(4): 650-659. doi: 10.1007/s00442-006-0402-7.

Brookes, P.C., Landman, A., Pruden, G., and Jenkinson, D.S. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. **17**(6): 837-842. doi: 10.1016/0038-0717(85)90144-0.

Chen, H., Harmon, M.E., Sexton, J., and Fasth, B. 2002. Fine-root decomposition and N

dynamics in coniferous forests of the Pacific Northwest, U.S.A. Can. J. For. Res. **32**(2): 320-331. doi: 10.1139/X01-202.

Covington, W.W., and Moore, M.M. 1994a. Postsettlement changes in natural fire regimes and forest structure: ecological restoration of old-growth ponderosa pine forests. J. Sustain. For. **2**: 153-181. doi: 10.1300/J091v02n01_07.

Covington, W.W., Fulé, P.Z., Moore, M.M., Hart, S.C., Kolb, T.E., Mast, J.N., Sackett, S.S., and Wagner, M.R. 1997. Restoring ecosystem health in ponderosa pine forests of the Southwest. J. For. **95**(4): 23-29.

Dore, S., Kolb, T.E., Montes-Helu, M., Sullivan, B.W., Winslow, W.D., Hart, S.C., Kaye, J.P., Koch, G.W., and Hungate, B.A. 2008. Long-term impact of a stand-replacing fire on ecosystem CO_2 exchange of a ponderosa pine forest. Glob. Change Biol. 14(8): 1801-1820. doi:10.1111/j.1365-2486.2008.01613.

Fahey, T.J., Hughes, J.W., Pu, M., and Arthur, M.A. 1988. Root decomposition and nutrient flux following whole-tree harvest of northern hardwood forest. For. Sci. **34**(3): 744-768.

Fisher, F.M., and Gosz, J.R., 1986. Effects of trenching on soil processes and properties in a New Mexico mixed-conifer forest. Biol. Fert. Soils. **2**(1): 35-42. doi: 10.1007/BF00638959.

Gholz, H.L., Wedin, D.A., Smitherman, S.M., Harmon, M.E., Parton, W.J., 2000. Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. Glob. Change Biol. **6**(7): 751-765. doi:10.1046/j.1365-2486.2000.00349.

Gill, R.A., and Jackson, R.B. 2000. Global patterns of root turnover for terrestrial

ecosystems. New Phytol. **147**(1): 13-31. doi:10.1046/j.1469-8137.2000.00681.

Grady, K.C., and Hart, S.C. 2006. Influences of thinning, prescribed burning, and wildfire on soil processes and properties in southwestern ponderosa pine forests: A retrospective study. For. Ecol. Manag. **234**(1-3): 123-135. doi:10.1016/j.foreco.2006.06.031.

Hanson, P.J., Edwards, N.T., Garten, C.T., and Andrews, J.A. 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. Biogeochemistry, **48**(1): 115-146. doi: 10.1023/A:1006244819642.

Hart, S.C., and Sollins, P. 1998. Soil carbon and nitrogen pools and processes in an old-growth conifer forest 13 years after trenching. Can. J. For. Res. **28**(8): 1261-1265. doi: 10.1139/cjfr-28-8-1261.

Hart, S.C., Classen, A.T., and Wright, R.J., 2005a. Long-term interval burning alters fine root and mycorrhizal dynamics in a ponderosa pine forest. J. Appl. Ecol. **42**: 752-761. doi:10.1111/j.1365-2664.2005.01055.

Hart, S.C., DeLuca, T.H., Newman, G.S., MacKenzie, D.M., and Boyle, S.I. 2005b. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. For. Ecol. Manag. **220**(1-3) 166-184. doi: 10.1016/j.foreco.2005.08.012.

Haubensak, K.A., Hart, S.C., and Stark, J.M. 2002. Influences of chloroform exposure time and soil water content on C and N release in forest soils. Soil Biol. Biochem. **34**(11): 1549-1562. doi: 10.1016/S0038-0717(02)00124-4.

Hobbie, S.E., Oleksyn, J., Eissenstat, D.M., and Reich, P.B. 2010. Fine root decomposition rates do not mirror those of leaf litter among temperate tree species. Oecologia, **162**(2): 505-513. doi: 10.1007/s00442-009-1479-6.

Kaye, J.P., and Hart, S.C. 1998a. Ecological restoration alters nitrogen transformations in a ponderosa pine-bunchgrass ecosystem. Ecol. Appl. **8**(4): 1052-1060. doi: 10.2307/2640960.

Kaye, J.P., and Hart, S.C. 1998b. Restoration and canopy-type effects on soil respiration in a ponderosa pine-bunchgrass ecosystem. Soil Sci. Soc. Am. J. **62**(4): 1062-1072. doi: 10.2136/sssaj1998.03615995006200040030x.

Kaye, J.P., Hart, S.C., Fulé, P.Z., Covington, W.W., Moore, M.M., and Kaye, M.W. 2005. Initial carbon, nitrogen, and phosphorus fluxes following ponderosa pine restoration treatments. Ecol. Appl. **15**(5): 1581-1593. doi: 10.1890/04-0868.

Klemmedson, J.O., Meier, C.E., and Campbell, R.E. 1985. Needle decomposition and nutrient release in ponderosa pine ecosystems. For. Sci. **31**(3) : 647-660.

Li, Y., Xu, M., Zou, X., and Xia, Y. 2005. Soil CO_2 efflux and fungal and bacterial biomass in a plantation and a secondary forest in wet tropics in Puerto Rico. Plant Soil, **268**(1): 151-160. doi:10.1007/s11104-004-0234-3.

Li, X., Han, S., Guo, Z., Shao, D., and Xin, L. 2010. Changes in soil microbial biomass carbon and enzyme activities under elevated CO_2 affect fine root decomposition processes in a Mongolian oak ecosystem. Soil Biol. Biochem. **42**: 1101-1107. doi:10.1016/j.soilbio.2010.03.007.

McClaugherty, C.A., Aber, J.D., and Melillo, J. 1984. Decomposition dynamics of fine roots in forested ecosystems. Oikos, **42**(3): 378-386. doi: 10.2307/3544408.

Miller, G., Ambos, N., Boness, P., Reyer, D., Robertson, G.P., Scalzone, K., Steinke, R., and Surbirge, T. 1995. Terrestrial Ecosystem Survey of the Coconino National Forest. USDA For. Serv., Southwestern Region, 405 pp.

Olson, J.S. 1963. Energy storage and the balance of producers and decomposers in ecological systems. Ecology, **44**(2): 322-331. doi: 10.2307/1932179.

Parton, W., Silver, W.L., Burke, I.C., Grassens, L., Harmon, M.E., Currie, W.S., King, J.Y., Adair, E.C., Brandt, L.A., Hart, S.C., and Fasth, B. 2007. Global-scale similarities in nitrogen release patterns during long-term decomposition. Science, **315**(5810): 361-364. doi:10.1126/science.1134853.

Sabo, K.E., Hart, S.C., Sieg, C.H., and Bailey, J.D. 2008. Tradeoffs in overstory and understory aboveground net primary productivity in southwestern ponderosa pine stands. For. Sci. **54**(4): 408-416.

Sabo, K.E., Sieg, C.H., Hart, S.C., and Bailey, J.D. 2009. The role of disturbance severity and canopy closure on standing crop of understory plant species in ponderosa pine stands in northern Arizona, USA. For. Ecol. Manag. **257**(8): 1656-1662. doi:10.1016/j.foreco.2009.01.006.

Selmants, P.C., Hart, S.C., Boyle, S.I., Gehring, C.A., and Hungate, B.A. 2008. Restoration of a ponderosa pine forest increases soil CO_2 efflux more than either water or nitrogen additions. J. Appl. Ecol. **45**(3): 913-920. doi:10.1111/j.1365-2664.2008.01460.

Silver, W.L., and Miya, R.K. 2001. Global patterns in root decomposition: comparisons of climate and litter quality effects. Oecologia, **129**(3): 407-419. doi: 10.1007/S004420100740.

Silver, W.L., and Vogt, K.A. 1993. Fine root dynamics following single and multiple disturbances in a subtropical wet forest ecosystem. J. Ecol. **81**(4): 729-738. doi: 10.2307/2261670.

Sparling, G.P., Feltham, C.W., Reynolds, J., West, A.W., and Singleton, P. 1980. Estimation of soil microbial C by a fumigation-extraction method: use of soils of high organic matter content, and a reassessment of the k_{EC} factor. Soil Biol. Biochem. **22**(3): 301-307. doi: 10.1016/0038-0717(90)90104-8.

Sullivan, B.W., Kolb, T.E., Hart, S.C., Kaye, J.P., Dore, S., and Montes-Helu, M. 2008. Thinning reduces soil carbon dioxide but not methane flux from southwestern ponderosa pine forests. For. Ecol. Manag. **255**: 4047-4055. doi:10.1016/j.foreco.2008.03.051.

Vance, E.D., Brookes, P.C., and Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. **19**(6): 703-707. doi: 10.1016/0038-0717(87)90052-6.

Wright, R. J. 1996. Fire effects on fine roots, mycorrhizae, and nutrient dynamics in a southwestern ponderosa pine forest. M.S. thesis, School of Forestry, Northern Arizona University, Flagstaff, AZ.

Zak, D.R., Tilman, D., Parmenter, R.R., Rice, C.W., Fisher, F.M., Vose, J., Milchunas, D., and Martin, C.W. 1995. Plant production and soil microorganisms in late-successional ecosystems: a continental-scale study. Ecology, **75**(8): 2333-2347. doi: 10.2307/194088.

Table 5.1 Mean gravimetric water content (GWC), fine root biomass and associated decomposition rate constants (k, y^{-1}) for each forest treatment. The untrenched category under GWC (± one standard error) denotes values taken from areas outside of the trenches 5 months after the trenches were installed. The 5- and 27-month categories denote values taken from inside the trenched areas at the corresponding time after the trenches were installed. Fine root biomass (± one standard error) and associated k values are shown for the 27-month sampling date. GWC decreased over time ($p < 0.001$), but there was no effect of forest management treatment. Forest treatment did not affect the fine root biomass (pine, herbaceous or total) or the associated rates of decomposition; however, when all treatments were pooled together, herbaceous roots decomposed significantly faster than pine roots ($p < 0.0001$; $n = 16$)

	Gravimetric water content (GWC)			Fine root biomass ($g\ m^{-2}$)		Decomposition constant (k, y^{-1})		
	Untrenched	5 months	27 months	Pine	Herbaceous	Pine	Herbaceous	Total root
Unmanaged	0.368 (0.02)	0.363 (0.01)	0.306 (0.01)	164.5 (33.9)	3.1 (0.6)	0.35	0.34	0.15
Thinned	0.337 (0.01)	0.348 (0.01)	0.266 (0.01)	98.8 (17.1)	7.7 (3.0)	0.19	0.55	0.36
Thin/Burn	0.369 (0.02)	0.345 (0.03)	0.300 (0.03)	120.0 (25.8)	6.5 (3.2)	0.24	0.92	0.34
Wildfire	0.327 (0.02)	0.335 (0.02)	0.243 (0.02)	34.3 (8.4)	1.7 (7.1)	0.10	0.76	0.48

FIGURE LEGENDS

Figure 5.1 Mean fine root biomass in southwestern ponderosa pine forests subjected to different forest management treatments or a stand-replacing wildfire. On the x-axis, the untrenched category denotes values taken from areas outside of the trenches 5 months after the trenches were installed. The 5- and 27-month categories denote values taken from inside the trenched areas at the corresponding time after the trenches were installed. Fine root biomass was not affected by forest management treatment. Vertical bars denote ± one standard error.

Figure 5.2 Mean microbial biomass C at southwestern ponderosa pine forest stands subjected to different management treatments or stand-replacing wildfire. On the x-axis, the untrenched category denotes values taken from areas outside of the trenches 5 months after the trenches were installed. The 5- and 27-month categories denote values taken from inside the trenched areas at the corresponding time after the trenches were installed. Different letters denote significant differences among the forest treatments within sample date categories at $\alpha = 0.05$. Vertical bars denote ± one standard error.

Figure 5.3 Correlation between fine root biomass and microbial biomass C at southwestern ponderosa pine forest stands subjected to different management treatments or stand-replacing wildfire. Each symbol represents a stand average of three subplots. Soils were sampled outside and inside trenches 5 months after the trenches were installed, as well as at 27-months post-installation. All samples were pooled under each management treatment for correlation analysis. Fine root

biomass and microbial biomass C were significantly correlated ($r = 0.35$, $p = 0.01$, $n = 48$)

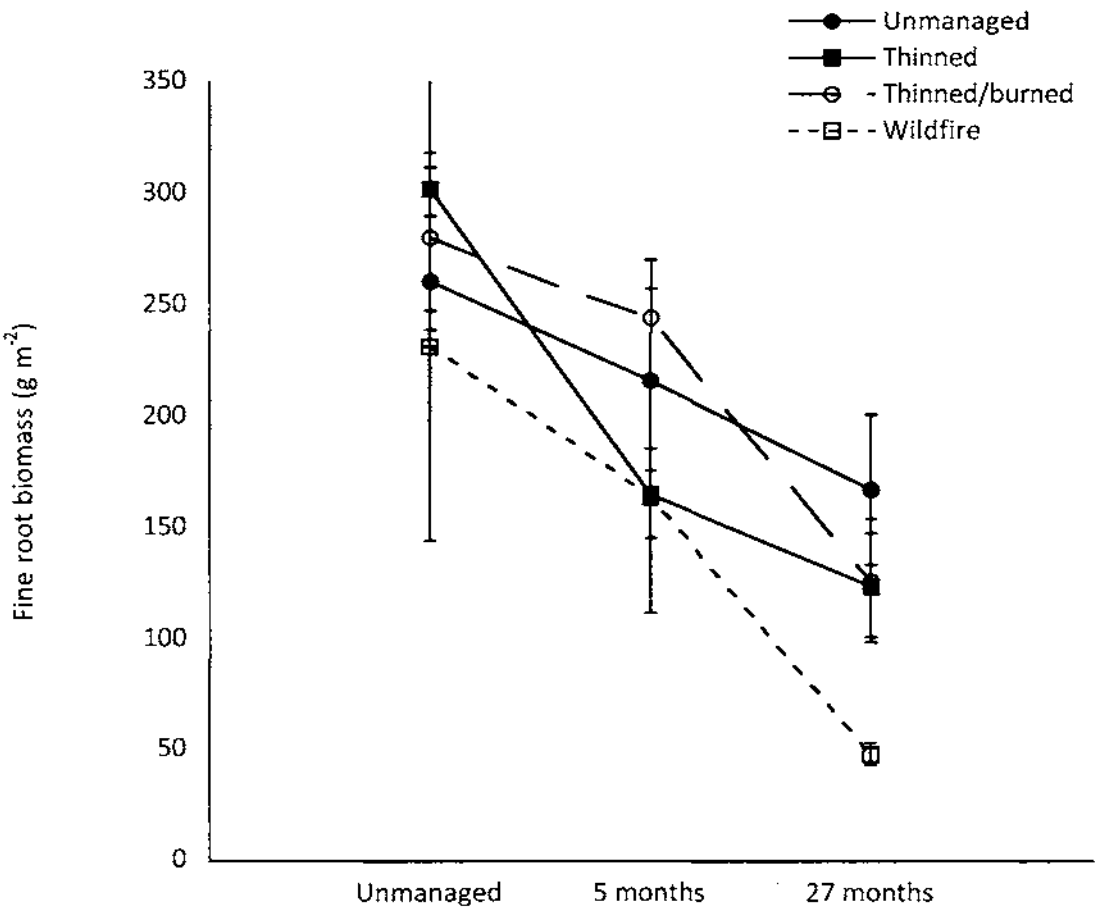


Figure 5.1

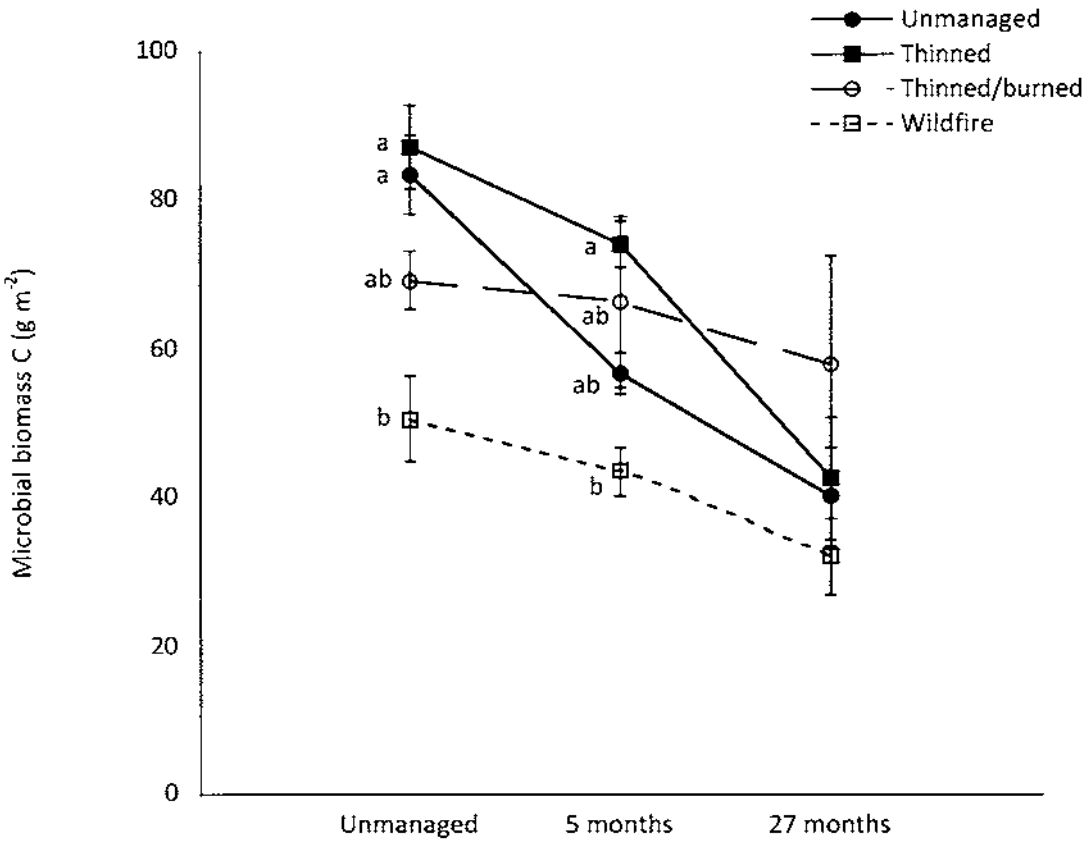


Figure 5.2

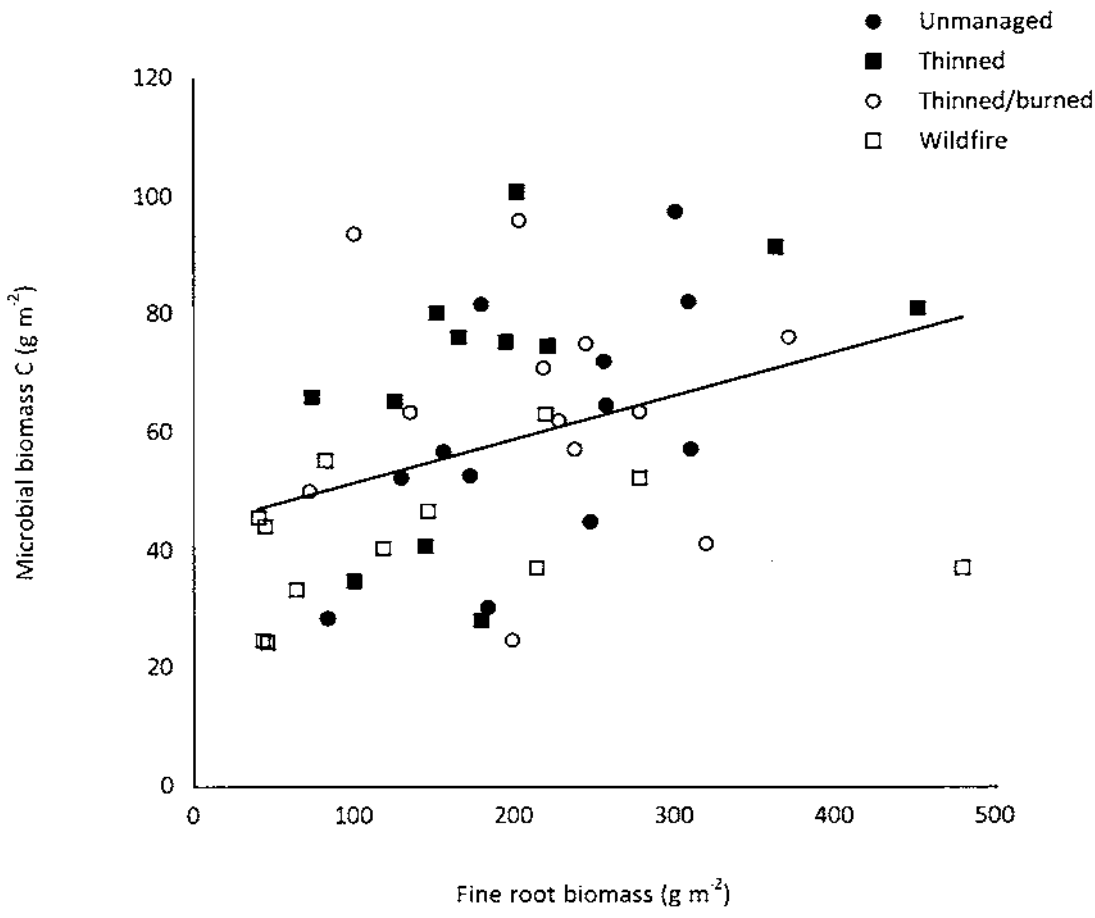


Figure 5.3

CHAPTER 6

OVERALL SUMMARY AND MANAGEMENT IMPLICATIONS

The results of my dissertation studies underscore the broad effects of stand-replacing wildfire in southwestern ponderosa pine forests from several different ecosystem and community level perspectives. In particular, they demonstrate that severe wildfire influences nitrogen (N) cycling and wood-decay fungi communities for multiple decades, and may alter fine root decomposition rates via a shift in vegetation type from pines to herbaceous plants. Importantly, they suggest the effects of stand-replacing wildfires on these ecosystems are persistent, perhaps multi-decadal. The broad implications for management include both minimizing the risks of this type of wildfire (an uncharacteristic disturbance in these ecosystems), as well as adapting management practices for alternative stable states when type conversions result from stand-replacing wildfires.

As suggested in Chapter Three, tree regeneration following stand-replacing wildfires in southwestern ponderosa pine forest is limited. In the past, burned areas were usually salvage logged and replanted, but planting resulted in varying degrees of success. Even the burned sites in this dissertation research that were successfully replanted have had limited recovery in their soil organic horizon. The paucity of tree regeneration and associated lower levels of soil organic material appear to be important controllers of the ecosystem alterations we observed.

The results in Chapter Three suggest that elevated levels of nitrification at burned sites can be explained by these post-fire shifts in vegetation, which result in a dominance by herbaceous biomass. This may have implications for wider

ecosystem functioning because of an overall decrease in plant demand for N compared to undisturbed forest. This decrease in plant-demand, coupled with elevated rates of nitrification, may result in long-term losses of N from the ecosystem via leaching of NO_3^-. The consequence of this may be lower rates of net primary productivity and associated reductions in atmospheric CO_2 fixed by plants. Overall, this suggests that these wildfires lead to long-term effects on carbon (C) cycles, and this may be at least partially due to elevated rates of N cycling.

In Chapter Four, I observed different fungal community structures in burned sites compared to unburned sites, which may be partially due to reductions in tree regeneration and associated soil organic material. Soil organic material is a rich source of fungal propagules and energy for heterotrophs in undisturbed forest, and the relative lack of it in burned areas may result in altered fungal community structure. Although the specific mechanisms are not well-understood, it is possible, given the complex interactions of fungi during succession and associated implications for wood decomposition rates, that the community-level differences we observed may result in ecosystem level differences in decomposition rates. This is an area of research that begs for more work because of the implications for global C cycles.

The results in Chapter Five demonstrate the importance of wildfire-related vegetation shifts to organic matter turnover belowground. In one of few existing studies to quantify fine root decomposition rates, I observed that herbaceous roots decompose faster than pine roots. Although this was not reflected in a statistically significant treatment effect, it suggests that organic matter turnover and subsequent

nutrient release may be more rapid in burned areas because of the higher substrate quality of herbaceous fine roots. Again, these results may have implications for global C cycles because of faster belowground organic matter turnover.

The implications of this dissertation for management are two-fold. First, my research suggests that avoidance of catastrophic wildfires in this region may be the single best way to maintain forested landscapes. However, extended fire exclusion in this region has led alterations in stand structure, with associated consequences for ecosystem functioning (Kaye et al. 2005). Forest management practices in semi-arid climates that utilize a combination of thinning and burning to treat high-density forest stands are likely to suffer less tree mortality during a wildfire than untreated forests (North and Hurteau 2011), which may mitigate some of the long-term effects of stand-replacing wildfires. Furthermore, these types of treatments restore some of the ecosystem functions of the historic fire regime. I suggest that the areas most vulnerable to wildfire be prioritized for treatments to be most effective at preventing catastrophic wildfires at the landscape level.

Secondly, these results suggest that land managers need to be prepared for what to expect following stand-replacing wildfire. Our research supports the work of others in suggesting that many of these forests do not necessarily recover along a predictable successional trajectory back to a forested state. Instead, some burned areas appear to transition to alternative stable states, such as grassland or shrubland (Savage and Mast 2005), and this is reflected in both their N cycling and C storage. Therefore, managers should practice adaptive management in burned regions to maintain vital ecosystem functions (e.g., watershed and soil health and

native diversity of flora and fauna) following a stand-replacing wildfire (Williams et al. 2010).

LITERATURE CITED

Kaye, J., S. Hart, P. Fulé, W. Covington, M. Moore, and M. Kaye. 2005. Initial carbon, nitrogen, and phosphorus fluxes following ponderosa pine restoration treatments. Ecological Applications **15**:1581-1593.

North, M. P. and M. D. Hurteau. 2011. High-severity wildfire effects on carbon stocks and emissions in fuels treated and untreated forest. Forest Ecology and Management **261**:1115-1120.

Savage, M. and J. Mast. 2005. How resilient are southwestern ponderosa pine forests after crown fires? Canadian Journal of Forest Research **35**:967-977.

Williams, A., C. Allen, C. Millar, T. W. Swetnam, J. Michaelsen, C. Still, and S. Leavitt. 2010. Forest responses to increasing aridity and warmth in the southwestern United States. Proceedings of the National Academy of Science **107**:21289-21294.