FIRE-SEVERITY EFFECTS ON PLANT-FUNGAL INTERACTIONS:

IMPLICATIONS FOR ALASKAN TREELINE DYNAMICS IN A WARMING CLIMATE

By

Rebecca. E. Hewitt

RECOMMENDED:

Dr. T. Scott Rupp
Advisory Committee Member

Dr. D. Lee Taylor
Advisory Committee Member

Dr. Teresa N. Hollingsworth
Advisory Committee Co-Chair

Dr. F. Stuart Chapin III
Advisory Committee Co-Chair

Dr. Diane Wagner
Chair, Department of Biology and Wildlife

APPROVED:

Dr. Paul W. Layer
Dean, College of Natural Science and Mathematics

Dr. John C. Eichelberger
Dean of the Graduate School

14 August 2014
Date

FIRE-SEVERITY EFFECTS ON PLANT-FUNGAL INTERACTIONS: IMPLICATIONS FOR ALASKAN TREELINE DYNAMICS IN A WARMING CLIMATE

A

DISSERTATION

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

By

Rebecca E. Hewitt, B.A.

Fairbanks, Alaska

August 2014

Abstract

Understanding the complex mechanisms controlling treeline advance or retreat in the arctic and subarctic has important implications for projecting ecosystem response to climate change. Changes in landcover due to a treeline biome shift could alter climate feedbacks and ecosystem services such as wildlife and berry habitat. Major sources of uncertainty in predicting treeline advance or retreat are the controls over seedling establishment at treeline and in tundra. One often-overlooked yet physiologically important factor to seedling establishment is the symbiosis with ectomycorrhizal fungi (EMF), the obligate mycobionts of all boreal tree species. EMF provide soil nutrients and water to seedlings and protect against pathogens, enhancing their growth and reducing drought stress. The availability of these critical mycobionts may be limited across the forest-tundra ecotone and by disturbance events such as wildfire. Wildfires are the primary large-scale disturbance in Alaskan boreal forests and are increasingly prevalent in tundra and at treeline. Fire is the major driver of boreal tree seedling recruitment; however, fire also alters the community structure and reduces biomass of EMF, especially after high-severity fires. To investigate the potentially critical role of EMF in seedling establishment at and beyond current treeline in Alaska, I conducted two observational studies and one experimental study that address how fire-severity influences EMF community structure and plant-fungal interactions.

These studies indicated that shrubs that survived and resprouted after fires at treeline and in tundra were a source of resilience for EMF diversity and function. Shrubs maintained late-successional stage EMF taxa, and the EMF taxa associated with shrubs at treeline were compatible with tree seedlings that naturally established after fire. Many of the EMF taxa that were shared by seedlings and shrubs were present across the low Arctic, suggesting that EMF compatible with boreal tree species are not limited within the predicted geographic range of

treeline expansion. Additionally, I found that seedling growth was correlated with post-fire fungal inoculum. Seedling growth was promoted by EMF inoculum provided by resprouting shrubs after fire. However, when fungal inoculum lacked EMF in post-fire tundra soils, seedling biomass was related to the negative effect of soil pathogens and the positive influence of dark septate endophytes. Together these results illustrate the important role of resprouting tundra shrubs as fungal nurse plants for establishment of boreal tree species at and potentially beyond current treeline, and that biotic factors such as EMF-tree interactions are important to seedling performance. My results suggest that the inclusion of biotic effects, like plant-fungal interactions, in simulation models of treeline dynamics will improve the accuracy of predictions of forestation and associated landscape flammability with future warming in Alaska.

Table of Contents

Page

Page

List of Figures

Page

Page

Page

List of Tables

Page

List of Appendices

Page

Acknowledgements

This research was supported by funding from the Joint Fire Science Graduate Research Innovation Award, several grants and a fellowship from the Alaska Experimental Program to Stimulate Competitive Research (National Science Foundation Award 0701898) and the state of Alaska, a National Science Foundation Graduate Research Fellowship (Division of Graduate Education-0639280 and 1242789), a graduate research grant from Alaska IDeA Networks of Biomedical Research Excellence, a grant-in-aide from the Arctic Institute of North America, and a student research grant from the Center for Global Change, the Arctic Long-Term Ecological Research Program (National Science Foundation - Office of Polar Programs 0856853 and National Science Foundation -Division of Environmental Biology 1026853), and in-kind support from the Bonanza Creek Long-Term Ecological Research site, U.S. Forest Service Pacific Northwest Research Station, and Bureau of Land Management Alaska Fire Service. A Lowell Thomas Jr. Scholarship, Dissertation Completion Fellowship, John B. Henderson Scholarship, and Howenstein Field Research scholarship from the University of Alaska Fairbanks and the University of Alaska Foundation also supported this dissertation. Additional support was provided by the Department of Biology and Wildlife through Teaching Assistantships and tuition waivers and Dr. F. S. Chapin III and Dr. T. N. Hollingsworth for summer and supplemental financial support.

I am deeply grateful for the guidance and intellectual mentorship of my co-advisors Dr. F. S. Chapin III and T. N. Hollingsworth and my two committee members Dr. D. L. Taylor and Dr. T. S. Rupp. Each of my committee members has contributed substantially to my intellectual development and the ideas and execution of my

dissertation chapters. I would like to thank the Scenarios Network for Alaska and Arctic Planning and especially the Treeline and Vegetation Dynamics working group with the Alaska Integrated Ecosystem Model for their insights regarding the integration of my dissertation research into a landscape-modeling framework. I thank Randi Jandt, Eric Miller, David Yokel, Dana Nossov, Gus Shaver, Amy Breen, Cody Johnson, and Adrian Rocha for field and logistical support. I thank Jamie Hollingsworth for creating maps for each of my manuscripts and presentations. I thank John Alden for his generosity in sharing his knowledge of the boreal forest and his archived seed collections for my experiments. I thank the Institute of Arctic Biology Greenhouses and especially Mark Wright and Heather McIntire for their help with my greenhouse experiments. Most importantly I thank my parents, Janet and Geoff Hewitt, and my brother, Gregory Hewitt, for their unconditional support.

Chapter 1

General Introduction

Changes in the distribution of arctic tundra and boreal forest have important effects on the climate system resulting from altered carbon storage (McGuire et al. 2001) and regional albedo (Chapin et al. 2000). The expansion of treeline in Alaska will have positive feedbacks to climate warming that arise with conversion of tundra to forest (Chapin et al. 2005). There is evidence that in the boreal forest climate-induced changes in the fire regime may be a more critical driver of landscape processes and species migrations than the direct effects of warming (Dale et al. 2001). Consequently, fire may play a critical role in determining the successful establishment of tree seedlings in previously unforested tundra and therefore vegetation feedbacks to the climate system.

Soil microbes are key drivers of ecosystem processes, yet their role in regulating landscape-scale vegetation change is not known. Ectomycorrhizal fungi (EMF) are obligate symbionts of all boreal tree species (Molina et al. 1992). The availability of compatible mycobionts directly relates to seedling establishment and growth, which are life history stages considered most important to the advance of boreal forest into previously unforested tundra (Harsch and Bader 2011). Fire frequency and extent has increased in the last half-century in the boreal forest and in tundra in response to warmer weather and lower precipitation. Changes in fire regime have been hypothesized to increase vegetation transitions from tundra to forest (Landhausser and Wein 1993); however, fire can reduce EMF abundance and alter species composition (Dahlberg 2002, Certini 2005, Cairney and Bastias 2007). Therefore, biogeographic or ecological constraints on EMF availability may preclude tree seedling

establishment and migration. The main goal of this dissertation research is to elucidate the role of EMF in tree seedling establishment and growth post-fire, specifically at and beyond current treeline. This will improve the accuracy with which we can forecast forestation under predicted scenarios of future warming.

Historical perspective on landcover change in Alaska

Spruce-dominated latitudinal treeline is currently at its farthest, northern Holocene-extent in Alaska (Bigelow et al. 2003). In both tundra and boreal forest biomes, paleoecological reconstructions of vegetation infer that there have been substantial shifts in dominant vegetation since the Last Glacial Maximum (LGM ~~21-18 thousand years before present (Ka BP)). With warm and dry conditions during the early Holocene (~13-10 Ka BP) the mosaic of shrub, graminoid, and forb tundra in Alaska transitioned to deciduous woodland (Bigelow et al. 2003, Edwards et al. 2005). Development of the modern coniferous boreal forest occurred when conditions shifted to a warmer, moister climate beginning in the mid-Holocene (~ 10-6 Ka BP), which could support coniferous canopy species. White spruce, *Picea glauca*, dominated the landscape from 10-6 Ka BP, followed by a transition to black spruce-dominated, *Picea mariana*, forests in the late-Holocene (~5 Ka BP to present) when conditions cooled. In the past, shifts in vegetation were strongly linked to fire activity on the landscape. For example, in the boreal forest the transition from dominance of white to black spruce due to cooler, moister conditions resulted in a shift from a low- to a high-frequency fire regime. In tundra, however, fires were more prevalent during the early Holocene, when birch-dominated shrub tundra was more abundant than graminoid or forb tundra (Higuera et al. 2008). The paleoecological perspective

on the relatiohsip between dominant vegetation and fire can aid in hypothesis development and predictions of future climate-fire-vegetation dynamics.

The role of tree seedling establishment in understanding and predicting treeline dynamics

Both paleoecological and present-day demographic studies indicate that understanding the controls over seedling establishment is key to predicting treeline advance (Germino et al. 2002, Lloyd 2005). However, the most important factors influencing seedling establishment at treeline and in tundra are not well understood. Controls over seedling growth and mortality are the most important factors linked to seedling establishment and treeline position (Hobbie and Chapin 1998, Danby and Hik 2007, Harsch and Bader 2011). One often-overlooked yet physiologically important control affecting seedling establishment and mortality is the symbiosis with ectomycorrhizal fungi (EMF), the obligate mycobionts of all boreal tree species (Smith and Read 2008). EMF provide soil nutrients and water to seedlings and may sometimes protect against pathogens, they may also enhance tree growth and reduce drought stress (Horton and van der Heijden 2008). The primary sources of seedling mortality at and beyond treeline are related to inadequate carbon gain, desiccation, and exposure to heat or frost, and the detrimental effects of parasitic snow fungi (Harsch and Bader 2011), all of which can be partially mitigated through the positive effects of EMF on the water relations and nutrient status of seedlings. EMF may, therefore, be particularly important to successful seedling establishment and growth under stressful environmental conditions such as at treeline, but EMF may be limited after fire, making for a complex fire-EMF-seedling relationship at the treeline ecotone.

Fire effects on treeline position and vegetation transitions

The boreal forest is North America's largest biome (Johnson *et al.* 1995), and wildfires are the dominant large-scale disturbance in Alaskan boreal forests. In Alaska, changes in climate have directly affected the fire regime in the boreal forest (Rupp *et al.* 2007). From 1959–99 there has been a doubling of both the annual area burned and frequency of large fire years in North American boreal forests (Kasischke and Turetsky 2006). As climate changes, fires at treeline and tundra are expected to become more frequent due to drier fuel loads and more frequent thunderstorms (Dissing and Verbyla 2003).

Studies in tundra and boreal forest indicate that feedbacks between vegetation and fire severity or return interval can result in the transition of one tundra type or forest canopy structure to another (Racine et al. 2004, Johnstone et al. 2010). In the boreal forest, fire is a major driver of tree recruitment as well as tree migration (Johnstone et al. 2004). Post-fire successional trajectories are closely related to fire severity, which affects density and composition of tree seedlings that establish after fire. High-severity fires expose mineral soils, which are favorable to seedling germination, net seedling establishment, and growth of transplanted seedlings (Johnstone and Chapin 2006). The northern migration of lodgepole pine into Alaska also appears to be tightly linked to high-severity fires (Johnstone and Chapin 2003). Because fire disturbance is critical to boreal forest vegetation patterns, it seems likely that a changing fire regime will influence where on the landscape trees will occur in the future.

EMF availability on the landscape and effects on seedlings

Comprehensive studies of factors influencing treeline position have noted that EMF may be an important factor delineating the boundary between forest and tundra (Sveinbjornsson et al.

2002). Fungal inoculum is generally not considered limited in soils. However there are two primary situations where ectomycorrhizal symbionts may be limited in their availability: (1) disturbance events such as severe fire that can result in substantial alterations in mycorrhizal community composition, and (2) vegetation transition zones where one mycorrhizal fungal guild decreases and another increases in dominance as a consequence of host plant cover. Alaska treeline can be characterized by both of these scenarios where fungal inoculum may be limiting. Treeline is the boundary between forest and tundra where there is a transition from dominance of host plants that associate with EMF to ericoid mycorrhizal fungi (ERM), the symbionts of primarily ericaceous plants (Read 1991, Gardes and Dahlberg 1996). Increasingly treeline may be subject to fire activity as both boreal forest and tundra fires become more prevalent (Kasischke and Turetsky 2006, Rocha et al. 2012).

The absence of compatible mycobionts beyond current treeline could inhibit tree seedling establishment. Across vegetation boundaries in other ecosystems, a lack of compatible EMF symbionts reduced seedling establishment (Horton et al. 1999, Haskins and Gehring 2005). In habitats where mycorrhizal type might be limited by the structure of the plant communities such as ecotones, abundance of a mycorrhizal guild (i.e., EMF or ERM) can be limited by spore dispersal capability or vegetative growth of mycelium. Observational studies and experimental manipulations suggest that seedlings may partner with atypical mutualists if the appropriate mycorrhizal type is not abundant (Horton et al. 1998, Chen and Cairney 2002). However, over time seedlings will engage in symbiosis with the typical guild, presumably the most efficient mycorrhizal partner (Jones et al. 1998), if available. When compatible mycobionts are not available seedling establishment and growth is limited (Nunez et al. 2009).

Seedling establishment may be further constrained when disturbances occur in ecosystems where there is already limitation of mutualists due to low densities of host plants (i.e. ectomycorrhizal tundra shrubs or tree islands in ERM-dominated tundra). In boreal and temperate regions, severe wildfire activity resulted in decreased EMF biomass (Stendell *et al.* 1999) and colonization of roots (Treseder *et al.* 2004) and altered EMF community structure (Baar et al. 1999, Grogan et al. 2000), but what that means for seedling establishment is more ambiguous. After fire seedlings can be colonized by spores or sclerotia that survive fire in the resistant propagule community (Baar et al. 1999, Taylor and Bruns 1999); however, integration of seedlings into a CMN through root colonization by mycelium from established vegetation can be more beneficial to seedlings because the high carbon costs of supporting a large foraging network is subsidized by established host plants (Selosse et al. 2006, Horton and van der Heijden 2008). There is variation in EMF species-level effectiveness at providing soil nutrients (Nara 2006). Consequently, differences in mycorrhizal community composition can affect individual plant fitness, competitive dynamics, and plant species coexistence within a community (Booth 2004, Bever et al. 2010). Therefore, fire-altered EMF community structure could influence patterns of vegetation in ecosystems and across landscapes.

The primary goals of this dissertation were to 1) determine how fire severity affects fungal inoculum associated with resprouting vegetation and in soils, 2) assess the effects of post-fire inoculum on tree and shrub seedling performance for plant species expected to expand in tundra over the next century, and 3) integrate our mechanistic understanding of post-fire plant-fungal interactions into a framework useful for predicting landscape patterns of treeline movement with predicted scenarios of warming and fire. To investigate the potentially critical role of EMF in seedling establishment at and beyond current treeline in Alaska, I present two

observational studies and one experimental study that address how fire-severity influences EMF community structure and plant-fungal interactions (Figure 1.1). In chapter two I characterized the mycorrhizal communities associated with dwarf birch (*Betula nana*) shrubs that resprouted after the 2007Anaktuvuk River Fire (ARF), across a fire-severity gradient using molecular tools. In chapter three I experimentally tested fire-severity effects on the fungal inoculum potential of tundra soils and associated effects on plant performance. Finally, in chapter four I investigated whether shrubs that resprout after fire maintain their EMF mycobionts and whether these shrub EMF taxa colonize and enhance the growth of tree seedlings that establish after wildfire at treeline in Alaska.

In the conclusion chapter I present a synthesis of these studies and integrate these results into a landscape model of climate-fire-vegetation dynamics using the model ALFRESCO (Figure 1.1) currently under development. Together the three studies illustrate fire-severity effects on inoculum sources associated with resprouting vegetation (chapters 2 and 4) and soils (chapter 3) at and beyond current treeline and the relationship between post-fire fungal community structure and boreal tree seedling attributes (chapters 3 and 4). This research suggests that the incorporation of EMF-tree seedling interactions into landscape model simulations of post-fire vegetation dynamics will improve the accuracy of predictions of vegetation change and associated landscape flammability under predicted scenarios of climate warming.

REFERENCES

Baar, J., T. R. Horton, A. M. Kretzer, and T. D. Bruns. 1999. Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. New Phytologist 143:409-418.

Bever, J. D., I. A. Dickie, E. Facelli, J. M. Facelli, J. Klironomos, M. Moora, M. C. Rillig, W. D. Stock, M. Tibbett, and M. Zobel. 2010. Rooting theories of plant community ecology in microbial interactions. Trends in Ecology & Evolution 25:468-478.

Bigelow, N. H., L. B. Brubaker, M. E. Edwards, S. P. Harrison, I. C. Prentice, P. M. Anderson, A. A. Andreev, P. J. Bartlein, T. R. Christensen, W. Cramer, J. O. Kaplan, A. V. Lozhkin, N. V. Matveyeva, D. F. Murray, A. D. McGuire, V. Y. Razzhivin, J. C. Ritchie, B. Smith, D. A. Walker, K. Gajewski, V. Wolf, B. H. Holmqvist, Y. Igarashi, K. Kremenetskii, A. Paus, M. F. J. Pisaric, and V. S. Volkova. 2003. Climate change and Arctic ecosystems: 1. Vegetation changes north of 55 degrees N between the last glacial maximum, mid-Holocene, and present. Journal of Geophysical Research-Atmospheres 108.

Booth, M. G. 2004. Mycorrhizal networks mediate overstorey-understorey competition in a temperate forest. Ecology Letters 7:538-546.

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

Certini, G. 2005. Effects of fire on properties of forest soils: a review. Oecologia. 143:1-10.

Chapin, F. S., A. D. McGuire, J. Randerson, R. Pielke, D. Baldocchi, S. E. Hobbie, N. Roulet, W. Eugster, E. Kasischke, E. B. Rastetter, S. A. Zimov, and S. W. Running. 2000. Arctic and boreal ecosystems of western North America as components of the climate system. Global Change Biology 6:211-223.

Chapin, F. S., M. Sturm, M. C. Serreze, J. P. McFadden, J. R. Key, A. H. Lloyd, A. D. McGuire, T. S. Rupp, A. H. Lynch, J. P. Schimel, J. Beringer, W. L. Chapman, H. E. Epstein, E. S. Euskirchen, L. D. Hinzman, G. Jia, C. L. Ping, K. D. Tape, C. D. C. Thompson, D. A. Walker, and J. M. Welker. 2005. Role of land-surface changes in Arctic summer warming. Science 310:657-660.

Chen, D. M., and J. W. G. Cairney. 2002. Investigation of the influence of prescribed burning on ITS profiles of ectomycorrhizal and other soil fungi at three Australian sclerophyll forest sites. Mycological Research 106:532-540.

Dahlberg, A. 2002. Effects of fire on ectomycorrhizal fungi in fennoscandian boreal forests. Silva Fennica 36:69-80.

Dale, V. H., L. A. Joyce, S. McNulty, R. P. Neilson, M. P. Ayres, M. D. Flannigan, P. J. Hanson, L. C. Irland, A. E. Lugo, C. J. Peterson, D. Simberloff, F. J. Swanson, B. J. Stocks, and B. M. Wotton. 2001. Climate change and forest disturbances. Bioscience 51:723-734.

Danby, R. K., and D. S. Hik. 2007. Responses of white spruce (Picea glauca) to experimental warming at a subarctic alpine treeline. Global Change Biology 13:437-451.

Dissing, D., and D. L. Verbyla. 2003. Spatial patterns of lightning strikes in interior Alaska and their relations to elevation and vegetation. Canadian Journal of Forest Research 33:770-782.

Edwards, M. E., L. B. Brubaker, A. V. Lozhkin, and P. M. Anderson. 2005. Structurally novel biomes: A response to past warming in Beringia. Ecology 86:1696-1703.

Gardes, M., and A. Dahlberg. 1996. Mycorrhizal diversity in arctic and alpine tundra: An open question. New Phytologist 133:147-157.

Germino, M., W. Smith, and A. C. Resor. 2002. Conifer seedling distribution and survival in an alpine-treeline ecotone. Plant Ecology 162:157-168.

Grogan, P., J. Baar, and T. D. Bruns. 2000. Below-ground ectomycorrhizal community structure in a recently burned bishop pine forest. Journal of Ecology 88:1051-1062.

Harsch, M. A., and M. Y. Bader. 2011. Treeline form - a potential key to understanding treeline dynamics. Global Ecology and Biogeography 20:582-596.

Haskins, K., and C. Gehring. 2005. Evidence for mutualist limitation: the impacts of conspecific density on the mycorrhizal inoculum potential of woodland soils. Oecologia 145:123-131.

Higuera, P. E., L. B. Brubaker, P. M. Anderson, T. A. Brown, A. T. Kennedy, and F. S. Hu. 2008. Frequent fires in ancient shrub tundra: implications of paleorecords for arctic environmental change. PLoS One 3:Article No.: e0001744.

Hobbie, S. E., and F. S. Chapin. 1998. An experimental test of limits to tree establishment in Arctic tundra. Journal of Ecology 86:449-461.

Horton, T. R., T. D. Bruns, and V. T. Parker. 1999. Ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment. Canadian Journal of Botany-Revue Canadienne De Botanique 77:93-102.

Horton, T. R., E. Cazares, and T. D. Bruns. 1998. Ectomycorrhizal, vesicular-arbuscular and dark septate fungal colonization of bishop pine (Pinus muricata) seedlings in the first 5 months of growth after wildfire. Mycorrhiza 8:11-18.

Horton, T. R., and M. G. A. van der Heijden. 2008. The role of symbioses in seedling establishment and survival. Pages 189-213 *in* M. A. Leck, V. T. Parker, and R. L. Simpson, editors. Seedling Ecology and Evolution. Cambridge University Press, Cambridge.

Johnson, D., L. Kershaw, A. MacKinnon, and J. Pojar. 1995. Plants of the Western Boreal Forest and Aspen Parkland. Lone Pine Publishing, Edmonton.

Johnstone, J., F. S. Chapin, J. Foote, S. Kemmett, K. Price, and L. Viereck. 2004. Decadal observations of tree regeneration following fire in boreal forests. Canadian Journal of Forest Research 34:267-273.

Johnstone, J. F., and F. S. Chapin. 2003. Non-equilibrium succession dynamics indicate continued northern migration of lodgepole pine. Global Change Biology 9:1401.

Johnstone, J. F., and F. S. Chapin. 2006. Effects of soil burn severity on post-fire tree recruitment in boreal forest. Ecosystems 9:14-31.

Johnstone, J. F., F. S. Chapin, T. N. Hollingsworth, M. C. Mack, V. Romanovsky, and M. Turetsky. 2010. Fire, climate change, and forest resilience in interior Alaska. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 40:1302-1312.

Jones, M. D., D. Durall, and P. Tinker. 1998. A comparison of arbuscular and ectomycorrhizal Eucalyptus coccifera: growth response, phosphorus uptake efficiency and external hyphal production. New Phytologist 140:125-134.

Kasischke, E. S., and M. R. Turetsky. 2006. Recent changes in the fire regime across the North American boreal region—Spatial and temporal patterns of burning across Canada and Alaska. Geophysical Research Letters 33:L09703.

Landhausser, S. M., and R. W. Wein. 1993. Postfire vegetation recovery and tree establishment at the arctic treeline: climate-change-vegetation-response hypotheses. Journal of Ecology 81:665-672.

Lloyd, A. H. 2005. Ecological histories from Alaskan tree lines provide insight into future change. Ecology 86:1687-1695.

Lloyd, A. H., M. E. Edwards, B. P. Finney, J. A. Lynch, V. A. Barber, and N. H. Bigelow. 2005. Development of the Boreal Forest. Pages 62-80 *in* F. S. Chapin, M. W. Oswood, K. Van Cleve, L. A. Viereck, and D. L. Verbyla, editors. Alaska's Changing Boreal Forest. Oxford University Press, New York.

McGuire, A. D., S. Sitch, J. S. Clein, R. Dargaville, G. Esser, J. Foley, M. Heimann, F. Joos, J. Kaplan, D. W. Kicklighter, R. A. Meier, J. M. Melillo, B. Moore, I. C. Prentice, N. Ramankutty, T. Reichenau, A. Schloss, H. Tian, L. J. Williams, and U. Wittenberg. 2001. Carbon balance of the terrestrial biosphere in the twentieth century: analyses of CO2, climate and land use effects with four process-based ecosystem models. Global Biogeochemical Cycles 15:183-206.

Molina, R., H. Massicotte, and J. M. Trappe. 1992. Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. Pages 357-423 *in* M. F. Allen, editor. Mycorrhizal functioning: An integrative plant-fungal process. Chapman and Hall, New York, New York, USA.

Nara, K. 2006. Ectomycorrhizal networks and seedling establishment during early primary succession. New Phytologist 169:169-178.

Nunez, M. A., T. R. Horton, and D. Simberloff. 2009. Lack of belowground mutualisms hinders Pinaceae invasions. Ecology 90:2352-2359.

Racine, C., R. Jandt, C. Meyers, and J. Dennis. 2004. Tundra fire and vegetation change along a hillslope on the Seward Peninsula, Alaska, USA. Arctic Antarctic and Alpine Research 36:1-10.

Read, D. J. 1991. Mycorrhizas in ecosystems. Experientia 47:376-391.

Rocha, A. V., M. M. Loranty, P. E. Higuera, M. C. Mack, F. S. Hu, B. M. Jones, A. L. Breen, E. B. Rastetter, S. J. Goetz, and G. R. Shaver. 2012. The footprint of Alaskan tundra fires during the past half-century: implications for surface properties and radiative forcing. Environmental Research Letters 7:044039.

Rupp, T. S., X. Chen, M. Olson, and A. D. McGuire. 2007. Sensitivity of simulated boreal fire dynamics to uncertainties in climate drivers. Earth Interactions 11:1-19.

Selosse, M. A., F. Richard, X. H. He, and S. W. Simard. 2006. Mycorrhizal networks: des liaisons dangereuses? Trends in Ecology & Evolution 21:621-628.

Smith, S. E., and D. J. Read. 2008. Mycorrhizal Symbiosis. third edition. Academic Press, New York, New York, USA.

Stendell, E. R., T. R. Horton, and T. D. Bruns. 1999. Early effects of prescribed fire on the structure of the ectomycorrhizal fungus community in a Sierra Nevada ponderosa pine forest. Mycological Research 103:1353-1359.

Sveinbjornsson, J., A. Hofgaard, and A. Lloyd. 2002. Natural causes of the tundra-taiga boundary. Ambio:23-29.

Taylor, D. L., and T. D. Bruns. 1999. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. Molecular Ecology 8:1837-1850.

Treseder, K., M. Mack, and A. Cross. 2004. Relationships among fires, fungi and soil dynamics in Alaskan boreal forests. Ecological Applications 14:1826-1838.

FIGURES

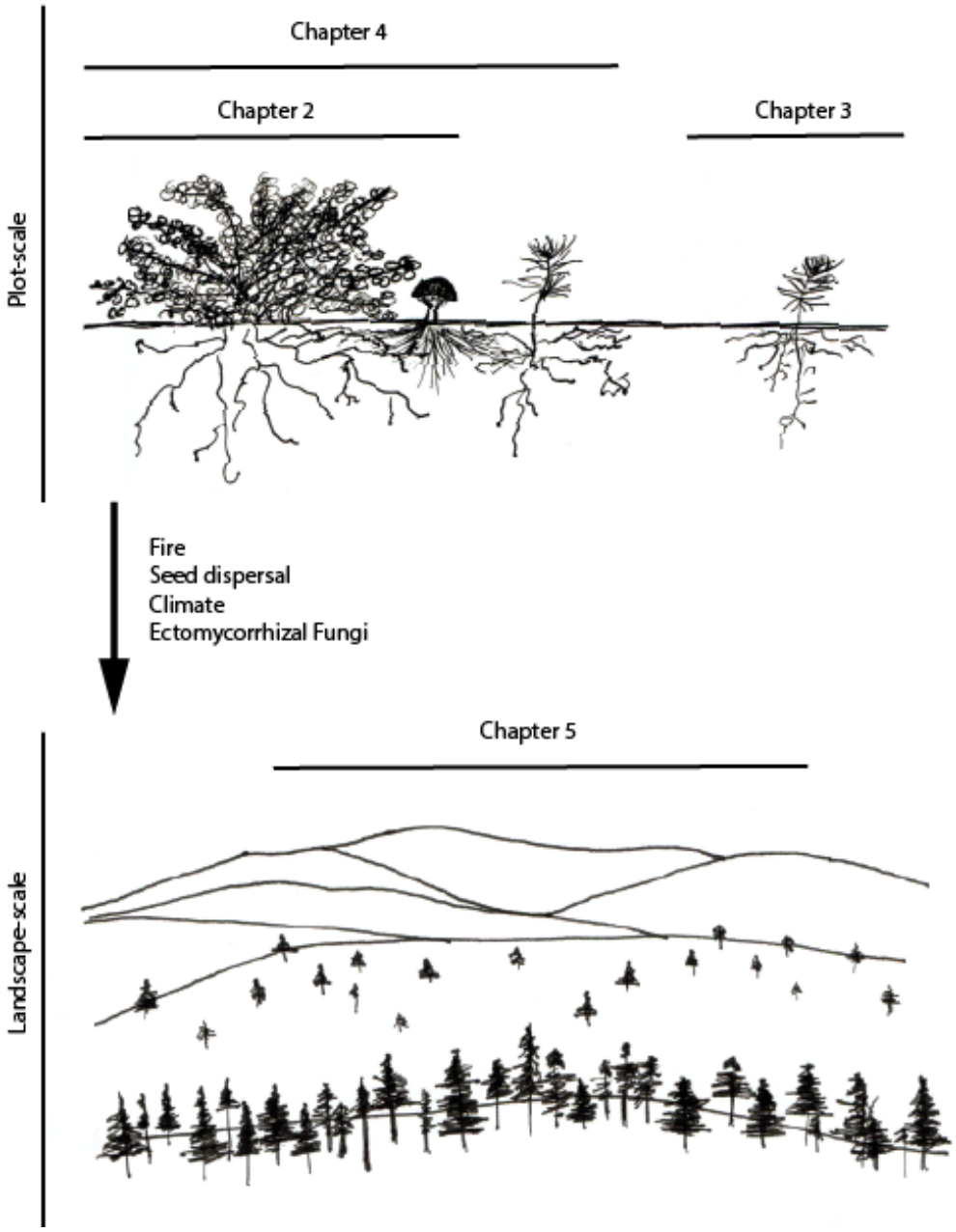


Figure 1.1. A conceptual diagram of how my dissertation chapters address fire-severity effects on ectomycorrhizal fungi associated with resprouting shrubs (chapters 2 and 4), soils (chapter 3), and plant-fungal interactions (chapters 3 and 4) and consequently, how these processes along with other variables inform landscape-modeling of treeline dynamics (chapter 5).

Chapter 2

Resilience of Arctic mycorrhizal fungal communities after wildfire facilitated by resprouting shrubs[1]

ABSTRACT

Climate-induced changes in the tundra fire regime are expected to alter shrub abundance and distribution across the Arctic. However, little is known about how fire may indirectly impact shrub performance by altering mycorrhizal symbionts. We used molecular tools, including ARISA and fungal ITS sequencing, to characterize the mycorrhizal communities on resprouting *Betula nana* shrubs across a fire-severity gradient after the largest tundra fire recorded in the Alaskan Arctic (July-October 2007). Fire effects on the components of fungal composition were dependant on the scale of taxonomic resolution. Variation in fungal community composition was correlated with fire severity. Fungal richness and relative abundance of dominant taxa declined with increased fire severity. Yet, in contrast to temperate and boreal regions with frequent wildfires, mycorrhizal fungi on resprouting shrubs in tundra were not strongly differentiated into fire-specialists and fire-sensitive fungi. Instead, dominant fungi, including taxa characteristic of late successional stages, were present regardless of fire severity. It is likely that the resprouting life history strategy of tundra shrubs confers resilience of dominant mycorrhizal fungi to fire disturbance by maintaining an inoculum source on the landscape after fire. Based on these results, we suggest that resprouting shrubs may facilitate post-fire vegetation regeneration and potentially the expansion of trees and shrubs under predicted scenarios of increased warming and fire disturbance in Arctic tundra.

[1] Hewitt, R. E., E. Bent, T. N. Hollingsworth, F. S. Chapin, and D. L. Taylor. 2013. Resilience of Arctic Mycorrhizal Fungal Communities after Wildfire Facilitated by Resprouting Shrubs. Ecoscience **20**:296-310.

INTRODUCTION

Fire frequency and severity play a strong role in regulating shrub regeneration and distribution in the Arctic (Racine *et al.*, 2004; Lantz *et al.*, 2010), yet little is known about the response to fire of soil fungal communities critical to shrub growth. Shifts in the tundra fire regime could influence vegetation change directly by opening new microsites for colonization and succession (White, 1979) or indirectly by altering the availability of critical fungal symbionts, ectomycorrhizal fungi (EMF), that influence host plant performance (Hoeksema *et al.*, 2010). In general, fire effects on mycobionts are governed by burn severity, which impacts the availability of host plants, and the depth of burning in the soil profile (Neary *et al.*, 1999; Certini, 2005). It is therefore likely that EMF response to fire disturbance may be an important determinant of post-fire vegetation regeneration and expansion in the Alaskan Arctic.

High-latitude climate warming has altered landscape-scale disturbance regimes in the Arctic (Hu *et al.*, 2010). Tundra fires on the North Slope of Alaska were rare occurrences over the last 5000 years (Hu *et al.*, 2010) due to cool, moist conditions and low biomass of dry fuels (Wein, 1976). Increased frequency of warm weather together with low summer precipitation (Shulski & Wendler, 2007), however, has caused a non-linear increase in tundra area burned annually (Hu *et al.*, 2010). In 2007, the Anaktuvuk River Fire (ARF), the largest tundra fire recorded in the circumpolar Arctic, burned 1039 km^2 on the North Slope of Alaska. The ARF was unprecedented in both size and severity in the modern fire record and may be a harbinger of greater landscape flammability in a future warmer climate (Jones *et al.*, 2009). Climate-sensitive changes in the fire regime are predicted to accelerate vegetation change attributed to climate warming across the

Arctic (Landhausser & Wein, 1993), including increased shrub abundance and shrub tundra expansion into graminoid tundra.

Betula nana is one of the four dominant vascular plants in northern Alaskan tundra (Walker *et al*., 2005), exhibits increased productivity in response to warming (Bret-Harte *et al*., 2001), and is the only dominant species that is obligately ectomycorrhizal (Molina *et al*., 1992). While dwarf shrubs such as *B. nana* resprout from belowground stems after fire, recovery to pre-fire productivity levels commonly takes over a decade (Fetcher *et al.*, 1984). If a fire is of high severity, it will not only kill more plant structures and likely lengthen the time required for post-fire shrub recovery (Wein, 1976), but also cause substantial shifts in EMF community composition. The primary regulators of EMF composition are host plant diversity and abundance (Molina *et al*., 1992) and edaphic properties, including soil pH and organic matter content (Kernaghan & Harper, 2001), both of which are impacted by fire severity (Dahlberg, 2002). In forested ecosystems, fire-specialist fungi primarily in the Ascomcota, such as *Wilcoxina* sp. and *Tuber* sp. in the Ascomycota and *Rhizopogon* sp. in the Basidiomycota, respond positively to fire (Baar *et al*, 1999; Taylor & Bruns, 1999). Whereas, fire-sensitive fungi primarily in the Basidiomycota, i.e., species in the Russulaceae, Thelophoraceae, and Amanitaceae, respond negatively to fire, are abundant in unburned stands, and are eliminated or reduced by fire disturbance (Taylor & Bruns, 1999). After fire several decades may be required for EMF composition, richness, and root tip colonization to return to pre-fire levels (Visser, 1995; Treseder *et al.,* 2004).

The potentially long-lived fire effects on EMF composition could result in alterations in host plant performance due to the shift from pre- to post-fire fungal

communities. EMF are well known to undergo successional changes in species composition in tandem with maturation of their hosts. These phenomena led to the categorization of 'early-stage' fungi, which colonize young hosts effectively from spores, and 'late-stage' fungi, which colonize roots of more mature trees and spread by mycelial growth but not by spores (Deacon *et al.*, 1983; Fox, 1986; Newton, 1992). While this classification system is likely over-simplistic, the wide dispersal and rapid colonization of the early colonizers is suggestive of a r-strategy, while the later colonizers display some attributes consistent with a K-strategy. In addition to EMF, host plants that are primarily ectomycorrhizal sometimes associate with dark septate endophytes (DSE) and ericoid mycorrhizal fungi (ERM) (Tedersoo *et al.*, 2009). Mycorrhizal composition has differential effects on host plant performance through nutrient translocation, water uptake, carbon cost, and pathogen resistence, among other factors (Smith & Read, 2008). Thus, it is likely that shifts in mycorrhizal composition due to fire impact mycorrhiza-mediated host plant performance and may influence shrub expansion into non-shrub tundra. Host plants that survive fire, such as resprouting shrubs, however, may maintain their pre-fire mycorrhizal partners and facilitate mycorrhizal resilience to fire.

Here, we conducted the first investigation on the effects of fire on mycorrhizal community structure in Arctic tundra. We sampled roots from resprouting *B. nana* and used molecular tools to characterize fungal community composition across a fire-severity gradient in the ARF. This burn scar is ideal for studying fire-severity effects on mycorrhizal communities because the large area burned allowed us to examine landscape effects of fire on EMF communities, and the wide spectrum of burn severities within the burn scar allowed us to investigate fire effects on a scale that would be logistically

impossible to replicate experimentally. Using this study site, we hypothesized that plant-associated mycorrhizal community structure and composition would change with burn severity (Figure 2.1). In particular, we tested the following hypotheses: 1) fire severity has a greater effect on shrub mycorrhizal community structure than do abiotic factors such as soil pH and landscape position; 2) total richness of shrub mycorrhizal taxa decreases with increasing fire severity; and 3) distribution of shrub mycorrhizal taxa varies along a fire-severity gradient (unburned to severely burned), with late-stage taxa decreasing and fire-specialists increasing across the fire-severity gradient.

MATERIALS AND METHODS

STUDY AREA AND FIELD SAMPLING

Between July and October 2007 the ARF burned 1039 km^2 of upland shrubby tussock tundra underlain by continuous permafrost (Mack *et al.*, 2011). The dominant vegetation before the fire was moist acidic tundra (54%) with moist nonacidic tundra (15%) and shrubland (30%) covering smaller areas (Jandt *et al.*, 2012). We focused our sampling in the moist acidic tundra that is co-dominated by sedges (*Eriophorum vaginatum* L., *Carex bigelowii* Torr. Ex Schwein), evergreen ericoid mycorrhizal shrubs (*Ledum palustre* L. and *Vaccinium vitis-idaea* L.), deciduous ectomycorrhizal shrubs (*Betula nana* L. and *Salix pulchra* Cham.), mosses, and lichens (Walker *et al.*, 2005).

To monitor post-fire vegetation recovery, the Bureau of Land Management (BLM) established sites in the ARF during a 2008 field campaign. In July 2009, two growing seasons after the fire, we visited fourteen burned sites within the ARF burn scar corresponding with different fire severities and one unburned site near the burn scar (15

sites total, Table 2.1). Sites were chosen to represent a continuous fire-severity gradient, based on the composite burn index (CBI), a fire-severity metric, between two endpoints from unburned to severely burned. The ARF burn is accessible only by helicopter in the summer or snow machine in winter. Therefore, we chose three logistically accessible sites to sample intensively: one unburned road-accessible site near the ARF burn scar (site 1), one moderately burned site (site 104), and one high burn-severity site (site 101) both with helicopter access (Table 2.1). At each intensive site we harvested ten *B. nana* resprouts along five parallel 1 m X 30 m transects 10 m apart. Two shrubs were sampled from each transect with the first sampling location randomly determined and then 10 m between each sampled shrub. To increase the spatial extent of our study and potentially better represent the richness of taxa sampled across a fire-severity gradient, we obtained additional *B. nana* root systems from less intensively studied sites, i.e. extensive sites, through a collaborative effort with the BLM during their helicopter-accessed, post-fire vegetation surveys. At these 12 extensive sites, *B. nana* resprouts were sampled when they were present during BLM post-fire vegetation surveys along 50 m transects. Shrubs occurred in low densities along these BLM transects, so 1-3 shrubs were sampled per site (Table 2.1). Harvested shrub root balls were approximately 30 cm diameter with the root crown in the center and 30 cm deep. In total, we sampled 45 root systems with intact rootballs, 15 at the extensive sites and 30 at the intensive sites. Low shrub density on the landscape and limited helicopter time precluded sampling of larger numbers of shrubs. The shrubs were then transported to the University of Alaska Fairbanks, where they were gently washed with distilled water within one week of harvest. Roots were traced back to the root crown and stored for eight months at 4°C in RNAlater (Life Technologies

Corporation, Foster City, CA, USA), which preserves sample morphology and nucleic acids indefinitely until samples can be processed (Lader, 2001; Bent & Taylor 2010). Note that RNAlater halts all metabolic activity and protects extremely labile RNA, as well as DNA (http://www.invitrogen.com). We have recovered equivalent DNA from fresh samples and samples stored for over 2 years in RNAlater at room temperature. Mycorrhizal morphology and anatomy were also preserved (Taylor, D. L., unpubl. data).

To explore the relationship between composition of mycorrhizal fungi, fire severity, and other potentially controlling factors, each site was characterized in terms of latitude, vegetation cover (estimated in the field or by photographs), mineral soil pH (measured in the lab post hoc), active layer (i.e. summer thaw in permafrost soils) depth (measured in the field at 20 points and then averaged across site), and remaining soil organic layer depth (measured at three points and averaged across a site). Fire-severity variables included: CBI (measured in the field in 30m radius plot and calculated as an average rating of consumption for fuel layers in tundra, including burn severity of substrate (litter, duff, and mineral soil exposure) and burn severity of vegetation from low vegetation and tall shrubs) (Jandt *et al.*, 2012) and Normalized Burn Ratio Index (dNBR- calculated as the change in reflectance pre- to post-fire based on Landsat imagery) (Kolden, 2010). Post hoc, we created fire-severity classes across our severity gradient for each site based on CBI, dNBR values, and site descriptions (Table 2.1). Detailed methods for data collection and summarized fire, soil, and environmental site data are presented in Jandt *et al.* (2012). Environmental datasets for the ARF have been archived with the Fire Research and Management Exchange System at www.frames.gov and with the Arctic Long-Term Ecological Research (LTER) data archive http://ecosystems.mbl.edu/arc/datacatalog.html.

FUNGAL SAMPLING

In March 2010, root systems were cut into 4 cm segments and placed in dishes of ultrapure water. Sampling all tips from the root ball was logistically unfeasible, and there was little visible morphological variation, i.e. few morphotypes, on each shrub. Therefore, ten segments of roots were randomly selected from each *B. nana* root system and sampled for ectomycorrhizal root tips. Healthy ectomycorrhizal root tips from *B. nana* roots were identified using a dissecting microscope (up to 40x magnification), and individual root tips were removed with forceps. Criteria for selecting healthy root tips included a lack of root hairs, no sign of necrosis, and turgid, intact tips following the both the workflow for root sampling and diagnostic features of EMF in Brundrett *et al*. (1996) with reference to morphologies in Agerer (1987-2002). Preliminary analyses on six shrubs demonstrated that 18 tips were sufficient to represent the richness of fungal amplicons present on a root system (18 tips vs 36 tips $T_{(6)}$=-1.00, p=0.423; Appendix 2.1). Hence, sampling more tips would not have drastically changed community composition results. For that reason, eighteen tips were randomly chosen from all the ectomycorrhizal tips identified on the root system of an individual *B. nana* shrub and pooled for DNA sequence analysis and Automated Ribosomal Intergenic Spacer Analysis (ARISA) of mycorrhizal fungi community structure. Pooled root tips were then placed in a single 0.6 ml Eppendorf tube, frozen in a small amount of ultrapure water, and lyophilized.

MOLECULAR TECHNIQUES AND BIOINFORMATICS

We conducted a preliminary analysis using ARISA to verify that we had adequately captured the fungal community associated with each individual shrub (Appendix 2.1.). We used DNA sequencing to investigate mycorrhizal community structure of *B. nana* resprouts. DNA was extracted from 45 lyophilized pooled root tip

samples using the Qiagen DNEasy Plant Mini Kit (*QIAGEN* Inc., Valencia, California, USA) according to the manufacturer's instructions and following the protocol of Bent & Taylor (2010).

Clone libraries were created for the pooled fungal DNA from each shrub (i.e., 45 clone libraries). Fungal ITS gene region sequences were obtained by PCR amplification with the PCR primers USER-ITS1F and USER-ITS4 following Bent *et al*. (2011). USER primers have an additional eight bases, which enable them to work with the USER Friendly Cloning Kit (New England Biolabs, Ipswich, MA, USA). The resulting primer sequences are as follows, with the additional bases underlined: USER-ITS1F, *GGAGACAU*CTTGGTCATTTAGAGGAAGTAA (Gardes & Bruns, 1993); USER-ITS4, *GGGAAAGU*TCCTCCGCTTATTGATATGC (White *et al.*, 1990). Each reaction mix (8.5 µl) was electrophoretically separated for 15 minutes on an agarose gel in TBE buffer (0.8% agarose, 100V, 40 minutes) to remove all primers and partially amplified PCR products from the amplicons of interest. One of the 45 samples did not produce amplicons, either in the ARISA experiment or in this one, and was not included in subsequent manipulations.

The 300-1000 bp region containing amplicons of each lane was excised from agarose gels with a scalpel. Amplicons were recovered from the gel slices using a Qiagen Gel Extraction Kit (Qiagen Inc., Valencia, California, USA) following the manufacturer's instructions and ligated into pNEB205A (New England Biolabs, Ipswich, MA, USA). Ligation reactions were then used for the transformation of competent *E. coli* (One Shot MAX Efficiency DH5α-T1R Competent Cells, Invitrogen, Grand Island, NY, USA) following the manufacturer's instructions (USER Friendly Cloning Kit, New England

Biolabs, Ipswich, MA, USA). Growth of colonies on plates was verified, then plates containing at least 13 well-separated white colonies (one per sample) were shipped on wet ice overnight to a facility for plasmid purification and sequencing (Functional Biosciences Inc., Madison, WI, USA). Twelve or 13 clones were sequenced for each shrub.

Cloned sequences were assembled using Codoncode Aligner 3.7 (CodonCode Corporation, Dedham, MA, USA) using PHRED. We used in-house perl scripts to mask low-quality bases (cutoff Q20), orient, and purge sequences containing >3% Ns after end-trimming. We tested for chimeric sequences with an open-source chimera checker (Edgar – Uchime) using an in-house bioinformatics tool (Taylor & Houston, 2011) and manual inspection of aligned sequences using SeAl alignment software (Rambaut, 2002). We eliminated 43 putative chimeric sequences. We grouped sequences into Operational Taxonomic Units (OTUs) using CAP3 (Huang & Madan, 1999) at 97% sequence identity. A representative sequence was selected for each OTU after manual inspection in SeAl alignment software. To provide species identities we ran a BLAST search using the representative sequence for each OTU. The top 10 hits from the BLAST search were assessed for length of overlap between the query and the hit and the % identity. When the top 10 hits did not have high overlap, % identity, or consistency in identification, we built maximum likelihood trees to attempt to resolve the identity of our query sequence using the top vouchered and isolate sequences from GenBank and utilizing the curated specimen fungal ITS search filter (http://biotech.inbre.alaska.edu/fungal-metagenomics/). To construct trees, we aligned sequences in MUSCLE (Edgar, 2004) and used the maximum likelihood method with default settings in Garli v.1.0 (Zwickl, 2006), which uses (GTR+G+I). We manipulated the tree, including midpoint rooting, in FigTree v1.3.1 (Rambaut, 2009). Trees

and alignments are archived with the Bonanza Creek LTER Data Catalog (http://www.lter.uaf.edu/data_b.cfm). OTUs were named and assigned to different levels of taxonomic identification based on sequence similarity following Timling *et al.* (2012). Sequences for each OTU have been archived with GenBank under accession numbers KC455311-KC455362 (Appendix 2.2).

STATISTICAL ANALYSIS

We calculated OTU richness (Mao Tau, Chao1) for the fungi using EstimateS 7.5 (Colwell, 2005). Because we sampled different numbers of shrubs at each site (intensively and extensively sampled sites) we randomly subsampled one shrub to compute a standardized OTU richness. We also computed rarefaction curves for each site using EstimateS 7.5. The number of OTUs was estimated by randomly resampling the observed OTUs 50 times.

We tested for correlations between site-level fire severity and environmental variables using Spearman correlations. Site fire-severity factors (CBI; both components of CBI, burn severity of vegetation and burn severity of substrate; and dNBR pixel value), mineral soil pH, latitude, and elevation were all significantly correlated directly or indirectly with one another (Appendix 2.3a), so we used CBI and organic soil depth to test for relationships between environmental and fire variables and richness of mycorrhizal fungi. We also tested for correlations between response variables for regression analysis (estimated OTU richness, Chaol), observed OTU richness (Mao Tau), proportion of richness in the Basidiomycota, and proportion of abundance represented by Basidiomycota. Observed and estimated richness and Basidiomycota richness and proportion of Basidiomycota abundance were correlated (Appendix 2.3b), so we used

proportion of Basidiomycota richness and estimated OTU richness (Chao1) as response variables for subsequent analyses. The normality of the distribution of these response variables was evaluated for skewness and kurtosis, using the Shapiro-Wilks W test. Square root and log10 transformations were used to normalize these factors. We used regression to test for relationships between CBI or organic soil depth and estimated OTU richness or richness of fungi in the Basidiomycota. We used matched pairs t-tests to test for differences between Ascomycota and Basidiomycota richness and abundance within sites.

We used Nonmetric Multidimensional Scaling (NMDS) (Kruskal, 1964) ordinations and correlations between ordination values and environmental variables to determine the relationship between fire, soil, and vegetation variables and site-level fungal community structure. Ordinations were based on OTU abundance data, which is analogous to biomass or percent cover, routine measures used for ordination of plant communities. We excluded all rare OTUs, i.e. the 22 OTUs that occurred once, from the data set (McCune & Grace, 2002). Shrub fungal community profiles were pooled by sampling site in order to compare fungal community structure to site-level environmental variables. Beal's smoothing transformation was used to relieve the "zero truncation problem" (McCune & Grace, 2002). We conducted an outlier analysis on site-level community profiles and two persistent outliers (sites 60A and 69) were eliminated from further analyses. We used the Sorensen distance measure and a random starting configuration. The final solution of both NMDS ordinations was generated using 500 iterations for each dataset. The primary community composition matrix was correlated with a secondary environmental matrix to relate mycorrhizal community structure to

quantitative site and fire-severity factors, and these correlations are graphically represented in a biplot of the ordinated fungal communities.

The Multiple-Response Permutation Procedure (MRPP) (Berry *et al.*, 1983) was used to investigate whether there were differences between fungal communities in burned sites grouped by low, moderate, and high burn severity categories (low= 3 sites, moderate = 5 sites, high =4 sites). For the MRPP, we used the Euclidean distance measure. We performed an Indicator Species Analysis (Dufrene & Legendre, 1997) to detect OTUs that were over-represented for a particular fire-severity class. Fire-severity class was used as the grouping variable (n= 3 groups: low, moderate, and high severity burn categories). For the Indicator Species Analysis we used 4999 permutations in the Monte Carlo test of significant observed maximum indicator values for OTUs. In addition, OTU relative abundance was visualized using two-way cluster analysis dendrograms at the site level. For computation of the dendrogram we used the Sorensen distance measure and the nearest neighbor group linkage method. All statistical analyses were performed in JMP 9.0.2 (SAS Institute Inc., Cary, North Carolina (USA)) with the exception of the multivariate analysis of mycorrhizal communities in PC-ORD 6.0 MJM Software Design, Gleneden Beach, Oregon (USA)).

RESULTS

MOLECULAR OPERATIONAL TAXONOMIC UNITS SAMPLED

To describe the composition of mycorrhizal fungi on resprouting shrubs after fire we sampled 45 *B. nana* shrubs across a fire-severity gradient (30 shrubs from intensively sampled sites and 15 from extensively sampled sites). We had a 97.3% success rate with

sequencing (31 of 1152 reads [576 clones X forward and reverse sequencing] were eliminated during clean up). We obtained 498 non-chimeric sequences from clone libraries from 44 *B. nana* shrubs across all sites (we could not amplify DNA from one of the original 45 shrubs). These sequences were grouped into 52 OTUs (Appendix 2.2). Thirty of the OTUs occurred more than once, and 22 were singletons. Using BLAST searches and tree building, we were able to describe 16 taxa to the species level (31%), 24 taxa to the genus level (46%), 11 taxa to the family, order or class (21%), and one taxon we could identify only to subphylum (2%). The dominant OTUs represented four different functional groups: EMF, DSE, ERM, and pathogens (Table 2.2). Similar to other mycorrhizal studies, the rarefaction curves suggest that we would have recorded more taxa if we had sampled additional shrubs within each site, especially in the extensively sampled sites. On the other hand, additional sampling was not feasible in many of these sites, as we sampled all available shrubs along the sparsely vegetated transects. Rare fungal species were likely underrepresented, given that 42% of the OTUs were singletons. We, therefore, used the rarefied taxonomic richness values for further analysis of OTU richness.

EFFECTS OF FIRE SEVERITY AND SITE-LEVEL CHARACTERISTICS

Variance in fungal community composition was more strongly correlated with fire severity than with other abiotic factors (Figure 2.2, Table 2.3a). The final solution of the ordination for the OTU dataset yielded two dimensions with a final instability of 0.034. The final stress for the OTU dataset was 6.145, suggesting a good ordination with low risk of drawing false inferences (Clarke, 1993; McCune & Grace, 2002). The two axes represented 93.2% of the variance in fungal community structure, with axis one

contributing 49.9% and axis two 43.4%. Axis one was correlated with variables that reflect fire severity (CBI and dNBR) and latitude (Figure 2.2, Table 2.3a). In contrast, axis two represents a complex acidity gradient as indicated by correlations with elevation and mineral soil pH (Figure 2.2, Table 2.3a). This is likely related to landscape position and the associated relationship between topography and soil pH. Although fire severity was an important correlating factor, we did not observe distinct clustering of sites by fire severity class in ordination space, nor a significant difference in fungal community composition among severity classes (MRPP, A= -0.043, p=0.816). Our subsequent analyses, described below, showed that the effects of fire on the components of community composition depended upon the scale of taxonomic resolution.

Surprisingly, when we examined the effects of fire *at the coarsest taxonomic scale*, we observed no shift to dominance of pyrophilic, fire-specialist fungi in the Ascomycota nor a decline in fire-sensitive, late-stage fungi in the Basidiomycota within study sites or across the burned study area contrasting with our third hypothesis. For example, the proportion of taxa in the Basidiomycota did not decline with increasing fire severity ($F_{(15,1)}$=0.251 P=0.624) nor was it related to residual soil organic depth ($F_{(15,1)}$=0.441 P=0.652). There was no difference between the average abundance of clone sequences for fungi in the Ascomycota and the Basidiomycota observed at each site ($T_{(15)}$=1.164, P=0.264). Average raw abundance of clone sequences for fungi in the Basidiomycota per site was 21.1 ($\pm$ 8.65 SE) and 12.2 for fungi in the Ascomycota ($\pm$ 3.18 SE). There was also no significant difference in the taxon richness of fungi in the Ascomycota and Basidiomycota for a site ($T_{(15)}$=-0.670, P=0.514). In total, across all sites, we observed 28 taxa in the Ascomycota

and 24 in the Basidiomycota. Richness varied from 0 to 9 taxa for Ascomycota and 0 to 12 for Basidiomycota across sites.

At the scale of community richness, we observed a decline in the estimated OTU richness as fire severity (CBI) increased ($F_{(15,1)}$=4.775 P=0.048: R^2=0.269, Figure 2.3). This decrease in OTU richness was independent of the residual organic soil depths post-fire (Chao1 $F_{(15,1)}$ =0.508, P=0.489), which contrasted with our hypotheses developed from post-fire temperate and boreal systems (Neary *et al.*, 1999; Dahlberg, 2002; Certini, 2005) (Figure 2.1).

The dominant mycorrhizal taxa did not show strong affinities with fire severities across the gradient. We observed no increase in presence of fire-specialists or decrease in presence of late-stage fungi across the gradient. The most abundant OTUs and the different fungal groups (ERM, EMF, DSE, and one putative pathogen) occurred in multiple fire-severity classes (Table 2.2), indicating that none were highly specialized for a given fire severity. Similarly, the indicator species analysis produced no significant observed indicator values for OTUs in the burn-severity classes showing that the presence and abundance of the common taxa were not specifically associated with a fire-severity class. Counter to our hypothesis (Figure 2.1), late stage-fungi were present across fire-severity classes (Table 2.2). The most abundant OTUs in the Basidiomycota were in the genera *Russula*, *Lactarius*, *Inocybe*, *Clavulina*, and *Tomentella*, several species of which are known late-successional fungi including *Lactarius glyciosmus* (Fleming, 1984) and *Russula decolorans* (Visser, 1995). The most abundant OTUs in the Ascomycota were in the genus *Meliniomyces*, the subclass Sordariomycetidae, and the order Helotiales, and one putative pathogen in the genus *Chalara* (Table 2.2). Though we

could not identify many of the dominant fungi in the Ascomycota to species or genus level, the coarser taxonomic identities indicate that these fungi are not known pyrophilic ascomycetous fungi.

When we considered both the presence and relative abundance of taxa, however, we observed that OTU abundance declined across the fire-severity gradient for many of the OTUs significantly correlated with axis one in the ordination biplot. Almost all of the dominant OTUs had strong positive correlations with axis one representing sites along the decreasing fire-severity gradient (Table 2.3b). The two–way cluster dendrogram highlights these patterns showing a lack of variation in OTU *presence* across fire severities, but strong variation in *abundance* of OTUs across all the sites on the fire-severity spectrum (Figure 2.4). These results show that, although late-stage fungi are present after fire, suggesting continuity between pre- and post-fire fungal communities, variability in the relative abundance of common OTUs follow hypothesized patterns across the fire-severity gradient. When we examined the number of rare OTUs that were excluded from the ordination analysis we observed an increase in the percentage of singletons in more severely burned sites (unburned= 17.39%, low= 8.69 %, moderate= 30.43%, and high=43.48%), perhaps reflecting less competition from abundant taxa.

DISCUSSION

This is the first study to investigate the effect of fire on variation in mycorrhizal composition of a dominant tundra shrub, *Betula nana*. The ARF was a rare event in terms of fire severity and magnitude, and the exploration of post-fire processes offers an opportunity to infer how species may respond to the projected increases in tundra fire

frequency and severity with high-latitude warming. In our study, fungal composition correlated with a hierarchy of fire-related, edaphic and landscape variables. However, disentangling the effect of each predictor variable is difficult because the ARF was a natural fire. Although fire severity affected several aspects of fungal composition, including total OTU richness and abundance of dominant taxa, our observations contrasted with some of our hypotheses based on studies from boreal and temperate forests (Visser, 1995; Baar *et al*, 1999).

DISTRIBUTION OF FUNGI IN RELATION TO FIRE DISTURBANCE

The two principal findings of our study were the lack of fire-specialist fungi across the burn scar and the persistence of late-stage taxa on shrubs regardless of fire severity. In contrast with studies from more fire-prone biomes, we did not detect a decline in fire-sensitive fungi in the Basidiomycota or the pronounced dominance of fire-specialists in the Ascomycota after fire (Grogan *et al.*, 2000; Cairney & Bastias, 2007) but see (Jonsson *et al.*, 1999a). It is possible that we did not capture the peak colonization of shrubs by post-fire fungi because we sampled during the second growing season. The dominance of pyrophilic ascomycetous fungi, mainly from the order Pezizales (Fujimura *et al.*, 2005), is well-documented for the six months to two years after fire (Cairney & Bastias, 2007). However, other studies observed the prevalence of ascomycete fire-fungi in the second growing season after fire (Grogan *et al.*, 2000) and their persistentce for up to six years after fire (Visser, 1995).

The dominant EMF in our study were all Basidiomycota in the genera *Russula*, *Lactarius*, and *Inocybe,* which are abundant across arctic habitats, well represented across several host species, and have a high species diversity (Timling *et al*., 2012). In concert

with the broad distribution patterns of these fungi across the fire severity gradient (Table 2.2), some of the dominant fungi are known to occur in late successional seres, such as *Russula decolorans* and *Lactarius glyciosmus*. This suggests that these fungi survived fire in a mycelial state. After fire mycorrhizal fungi colonize roots from resident mycelium and infected root tips that survive fire, dispersed spores, or fire-resistant propagules (Baar *et al.*, 1999). In contrast with studies from more fire-prone temperate zone, our results indicate that the dominant fungi observed across the ARF were not part of a resistant propagule community distinctive from the composition in the unburned site (Taylor & Bruns, 1999). Many of the dominant taxa observed across the fire-severity gradient were present at the unburned site, where we would expect mycelial growth, not colonization from resistant propagules, to be the dominant process by which new roots are colonized (Jonsson *et al.*, 1999b). Hence, the ubiquitous distribution of dominant species, especially late-stage taxa, suggests that mycelial inoculum on the roots of surviving, resprouting shrubs was maintained on the landscape after fire. In addition to EMF, we detected DSE, ERM, and one putative pathogen on the root systems of *Betula nana.* Similar to the distribution patterns of EMF taxa, the dominant taxa in these fungal groups were ubiquitous root colonizers across the fire severity gradient and showed no specialized fire-response.

FIRE VERSUS ENVIRONMENTAL EFFECTS

Although we observed the persistence of late-stage fungi and a lack of differentiation of fire-specialist and fire-sensitive fungi, wildfire severity was correlated with variance in fungal community structure on resprouting *B. nana* shrubs. This correlation is consistent with other post-fire studies (Dahlberg *et al.*, 2001; Smith *et al.*,

2005; Hamman *et al.*, 2007). The correlation between fungal composition and latitude in the ordination is also likely related to fire severity given the northward spread of the fire and increase in burn severity throughout the duration of the burn (Jandt *et al.*, 2012). While fire-severity variables were the primary factors correlated with overall fungal community composition in Arctic Alaska, there was also a strong relationship between mineral soil pH, elevation, and fungal communities.

As with many observational studies of fire effects, the effects of fire severity and other environmental variables were confounded because the ARF was a natural fire. We observed collinearity between fire severity, landscape, and soil variables. It is likely that elevation and mineral soil pH greatly influence the structure of mycorrhizal communities pre-fire and that these factors also relate to the severity of site-level burn characteristics. It is possible that the distance between sampling sites may have influenced the observed structure of the mycorrhizal communities. However, we observed similar taxa across all the sites, indicating that distance between sites is not the primary driver of differences in mycorrhizal community structure.

COMPARISONS WITH THE ADJACENT BOREAL FOREST

In the boreal forest, fire severity affects the legacies of mycorrhizal fungi in two main ways: 1) by altering host plant availability, which determines the potential for mycelial growth, and 2) through the combustion and heating of organic soil, which directly causes fungal mortality (Dahlberg, 2002). In the tundra ecosystem, however, residual soil organic depths did not correlate with fire severity in the ARF burn scar (Mack *et al.*, 2011) and did not correlate with fungal richness or composition in our study. These observations contrast with the important role of organic soil depth in explaining

the decline in fungal richness after fire in the boreal forest (Dahlberg et al., 2001). At the scale of the entire ARF, host plant cover decreased with increased fire severity (Miller, E.A., unpubl. data), which could explain the lower OTU richness on severely burned sites through a species-area relationship (Peay *et al.*, 2007) or through competitive interactions among fungi for root tips (Kennedy *et al.*, 2009; Kennedy, 2010). We hypothesize that fungi that survive fire on resprouting roots have a strong priority effect, rapidly colonize new short roots, and may be superior competitors for root tips. Overall, the fire response for mycorrhizal communities associated with *B. nana* is different from the adjacent boreal forest. Two possible explanations for these biome differences are that 1) the rarity of tundra fires for at least the last 5000 years may select against fire-specialist fungi or 2) we sampled resprouting host plants that survived fire, whereas studies in other biomes have sampled soil, macrofungal fruit bodies, mycorrhizas in soil cores, and naturally occurring seedlings in sites where the host species was killed by fire.

ECOLOGICAL SIGNIFICANCE OF FUNGAL LIFE HISTORY TRAITS

The persistence of fungal inoculum on the landscape after an unprecedented wildfire event has strong ecological implications for nutrient cycling and vegetation establishment because of the life-history traits of the fungi that survived fire on the roots of resprouting shrubs. One of the central findings from our study is the presence of late-stage fungi, regardless of fire severity. In general, enzymatic competency can be related to fungal successional stage and associated declines in high-resource-quality substrates (Dighton, 2003). For example, *Russula decolorans,* a late-stage fungus, is preferentially found in low-nutrient, late-successional-stage environments due to its affinity for low-quality nitrogen sources (Toljander *et al.* 2006). Although the role of EMF as the primary

conduits of inorganic and organic forms of soil nutrients for host plants in the Betulaceae (Smith & Read, 2008) is well understood, the ecological significance of associations with ERM and DSE remain uncertain (Deslippe and Simard, 2011; Newsham, 2011). The persistence of mycorrhizal taxa may provide resilience in the ecological function of a system by alleviating post-disturbance lag times in ecological processes, such as proteolytic activity, decomposition, or plant acquisition of soil resources, depending on the availability of mycorrhizal and DSE fungi.

FUNGAL RESILIENCE WITH A DYNAMIC TUNDRA FIRE REGIME

In other ecosystems where mycorrhizal availability is constrained after disturbance, early colonizing shrubs provide mycelial inoculum for establishing trees and shrubs, thus influencing successional trajectories (Nara & Hogetsu, 2004; Nara, 2006a, Nara, 2006b). In tundra, the presence and density of resprouting *B. nana* host plants appears to mediate fire-severity effects on fungal composition and may provide a potentially important source of mycorrhizal resilience, both in maintaining mycorrhizal diversity and in facilitating vegetation establishment under future scenarios of warming and fire. After fire in the tundra, where resprouting shrubs are dominant components of the vegetation and fires are commonly of lower severity, we hypothesize that fire effects on fungal community composition will not likely limit shrub performance and vegetation change. If post-fire host plant densities do affect resilience of fungal communities to fire, it seems likely that fire will have the greatest effect on mycorrhizal shrub performance and vegetation change in ecosystems such as graminoid tundra that have low shrub density or in sites that burn so severely that host plants rarely resprout. In tundra, persistence of mycorrhizal communities after fire disturbance could facilitate shrub

expansion and range expansion of neighboring ectomycorrhizal boreal forest tree species, as warming continues at high latitudes.

ACKNOWLEDGEMENTS

This research was supported by funding from the Alaska Experimental Program to Stimulate Competitive Research (National Science Foundation Award 0701898) and the state of Alaska, a National Science Foundation Graduate Research Fellowship (Division of Graduate Education-0639280 and 1242789) and a Center for Global Change student award to REH, the Arctic Long-Term Ecological Research Program (National Science Foundation - Office of Polar Programs 0856853 and National Science Foundation -Division of Environmental Biology 1026853), and in-kind support from the Bonanza Creek Long-Term Ecological Research site, U.S. Forest Service Pacific Northwest Research Station, and Bureau of Land Management Alaska Fire Service. We thank Eric Miller, Gus Shaver, Cody Johnson, and Adrian Rocha for field and logistical support.

REFERENCES

Agerer, R. (ed.), 1987 – 2002. Color Atlas of Ectomycorrhizae. EInhorn Verlag, Schwäbisch Gmünd.

Annotated Checklist of the Panarctic Flora (PAF) Vascular plants. University of Oslo, Oslo, Norway. Online [URL] http://nhm2.uio.no/paf/ (Accessed on 16 July 2012).

Baar, J., T. R. Horton, A. M. Kretzer & T. D. Bruns, 1999. Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. New Phytologist, 143: 409–418.

Bent E. & D.L. Taylor 2010. Direct amplification of DNA from fresh and preserved ectomycorrhizal root tips. Journal of Microbiological Methods, 80: 206-208.

Bent, E., P. Kiekel, R. Brenton R. & D. L. Taylor, 2011. Root-associated ectomycorrhizal fungi shared by various boreal forest seedlings naturally regenerating after a fire in Interior Alaska and correlation of different fungi with host growth responses. Applied Environmental Microbiology, 77: 3351-3359.

Berry, K. J., K. L. Kvamme & P. W. Mielke, 1983. Improvements in the Permutation Test for the Spatial Analysis of the Distribution of Artifacts into Classes. American Antiquity, 48: 547–553.

Bret-Harte, M. S., G. Shaver & J. Zoerner, 2001. Developmental plasticity allows *Betula nana* to dominate tundra subjected to an altered environment. Ecology, 82: 18-32.

Brundrett M., N. Bougher, B. Dell, T. Grove & N. Malajczuk, 1996. Working with Mycorrhizas in Forestry and Agriculture. Australian Centre for International Agricultural Research Monograph, 32: 1-374.

Cairney, J. W. G. & B. A. Bastias, 2007. Influences of fire on forest soil fungal communities. Canadian Journal of Forest Research, 37: 207–215.

Certini, G., 2005. Effects of fire on properties of forest soils: a review. Oecologia, 143: 1–10.

Clarke, K. R., 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology, 18: 117-143.

Colwell, R. K., 2005. EstimateS: Statistical estimation of species richness and shared species from samples. User's Guide and application online [URL] http://purl.oclc.org/estimates. (Accessed on 13 May 2012)

Dahlberg, A., 2002. Effects of fire on ectomycorrhizal fungi in fennoscandian boreal forests. Silva Fennica, 36: 69–80.

Dahlberg, A., J. Schimmel, A. F. S. Taylor, & H. Johannesson, 2001. Post-fire legacy of ectomycorrhizal fungal communities in the Swedish boreal forest in relation to fire severity and logging intensity. Biological Conservation, 100: 151–161.

Deacon, J., S. Donaldson & F. Last, 1983. Sequences and interactions of mycorrhizal fungi on birch. Plant and Soil, 71: 257-262.

Deslippe, J. & S. Simard, 2011. Below-ground carbon transfer among *Betula nana* may increase with warming in Arctic tundra. New Phytologist: 689–698.

Dighton, J., 2003. Fungi in Ecosystem Processes. Marcel Dekker, Inc., New York.

Dufrene, M. & P. Legendre, 1997. Species Assemblages and Indicator Species: The Need for a Flexible Asymmetrical Approach. Ecological Monographs, 67: 345–366.

Edgar, R. C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32: 1792-1797.

Fetcher, N., T. F. Beatty, B. Mullinax, & D. S. Winkler, 1984. Changes in arctic tussock tundra thirteen years after fire. Ecology, 65: 1332-1333.

Fleming, L. V., 1984. Effects of soil trenching and coring on the formation of ectomycorrhizas on birch seedlings grown around mature trees. New Phytologist, 98: 143–153.

Fox, F.M.,1986. Groupings of ectomycorrhizal fungi of birch and pine, based on establishment of mycorrhizas on seedlings from spores in unsterile soils. Transactions of the British Mycological Society. 87: 371-380.

Fujimura, K. E., J. E. Smith, T. R. Horton, N. S. Weber, & J. W. Spatafora, 2005. Pezizalean mycorrhizas and sporocarps in ponderosa pine (*Pinus ponderosa*) after prescribed fires in eastern Oregon, USA. Mycorrhiza, 15: 79–86.

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

Grogan, P., J. Baar, & T. D. Bruns, 2000. Below-ground ectomycorrhizal community structure in a recently burned bishop pine forest. Journal of Ecology, 88: 1051–1062.

Hamman, S. T., I. C. Burke ,& M. E. Stromberger, 2007. Relationships between microbial community structure and soil environmental conditions in a recently burned system. Soil Biology & Biochemistry, 39: 1703–1711.

Hoeksema, J. D., V. B. Chaudhary, C. A. Gehring, N. C. Johnson, J. Karst, R. T. Koide, A. Pringle, C. Zabinski, J. D. Bever, J. C. Moore, G. W. T. Wilson, J. N. Klironomos, & J. Umbanhowar, 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecology Letters, 13: 394–407.

Hu, F., P. Higuera, J. E. Walsh, W. L. Chapman, P. Duffy, L. B. Brubaker, & M. Chipman, 2010. Tundra burning in Alaska: linkages to climatic change and sea ice retreat. Journal of Geophysical Research-Biogeosciences, 115: 1–8.

Huang, X. & A. Madan, 1999. CAP3: A DNA Sequence Assembly Program. Genome Research, 9 898-877.

Index Fungorum. Landcare Research, Lincoln, New Zealand and RBG Kew: Mycology, Surrey, United Kingdom. Online [URL] http:// www.indexfungorum.org (Accessed 12 July 2012).

Jandt, R.R., E. A. Miller, D.A. Yokel, M. S. Bret-Harte, C. A. Kolden, & M. C. Mack, 2012. Findings of Anaktuvuk River Fire Recover Study. US Bureau of Land Management Technical Report. Fairbanks, Alaska.

Jones, B. M., C. A. Kolden, R. Jandt, J. T. Abatzoglou, F. Urban, & C. D. Arp, 2009. Fire Behavior, Weather, and Burn Severity of the 2007 Anaktuvuk River Tundra Fire, North Slope, Alaska. Arctic Antarctic and Alpine Research, 41: 309–316.

Jonsson, L., A. Dahlberg, M.-C. Nilsson, O. Kårén & O. Zackrisson, 1999a. Continuity of ectomycorrhizal fungi in self-regenerating boreal *Pinus sylvestris* forests studied by comparing mycobiont diversity on seedlings and mature trees. New Phytologist, 142: 151–162.

Jonsson, L. M., A. Dahlberg, M.C. Nilsson, O. Zackrisson & O. Karen, 1999b. Ectomycorrhizal fungal communities in late-successional Swedish boreal forests, and their composition following wildfire. Molecular Ecology, 8: 205–215.

Kennedy, P., 2010. Ectomycorrhizal fungi and interspecific competition: species interactions, community structure, coexistence mechanisms, and future research directions. New Phytologist, 187: 895–910.

Kennedy, P. G., K. G. Peay, & T. D. Bruns, 2009. Root tip competition among ectomycorrhizal fungi: are priority effects a rule or an exception? Ecology, 90: 2098–107.

Kernaghan, G. & K. A. Harper, 2001. Community structure of ectomycorrhizal fungi across an alpine / subalpine ecotone. Ecography: 181–188.

Kolden, C. A., 2010. Characterizing Alaskan wildfire regimes through remotely sensed data: assessment of large area pattern and trend. PhD thesis. Clark University, Worcester, Massachusetts.

Kruskal, J. B., 1964. Nonmetric multidimensional scaling: a numerical method. Psychometrika, 29: 115-129.

Lader, E. S., 2001. Methods and reagents for preserving RNA in cell and tissue samples. Ambion, Inc., Austin, Texas. Online [URL] http://www.freepatentsonline.com/6204375.html (Accessed 9 July 2009).

Landhausser, S. M. & R. W. Wein, 1993. Postfire vegetation recovery and tree establishment at the arctic treeline: climate-change-vegetation-response hypotheses. Journal of Ecology, 81: 665–672.

Lantz, T. C., S. E. Gergel, & G. H. R. Henry, 2010. Response of green alder (*Alnus viridis* subsp. fruticosa) patch dynamics and plant community composition to fire and regional temperature in north-western Canada. Journal of Biogeography, 37: 1597–1610.

Mack, M. C., M. S. Bret-Harte, T. N. Hollingsworth, R. R. Jandt, E. A. G. Schuur, G. R. Shaver, & D. L. Verbyla, 2011. Carbon loss from an unprecedented Arctic tundra wildfire. Nature, 475: 489–492.

McCune, B. & J. B. Grace. 2002. Analysis of Ecological Communities. MjM Software Design, Gleneden Beach, Oregon.

Molina, R., H. Massicotte & J. M.Trappe, 1992. Specificity phenomena in mycorrhizal symbioses: Community-ecological consequences and practical implications. Pages 357-423 in M.F Allen (ed.) Mycorrhizal functioning: An integrative plant-fungal process. Chapman and Hall, New York, New York.

Nara, K., 2006a. Pioneer dwarf willow may facilitate tree succession by providing late colonizers with compatible ectomycorrhizal fungi in a primary successional volcanic desert. The New Phytologist, 171: 187–97.

Nara, K., 2006b. Ectomycorrhizal networks and seedling establishment during early primary succession. The New Phytologist, 169: 169–78.

Nara, K. & T. Hogetsu, 2004. Ectomycorrhizal Fungi on Established Shrubs Facilitate Subsequent Seedling Establishment of Successional Plant Species. Ecology, 85: 1700–1707.

Neary, D. G., C. C. Klopatek, L. F. DeBano, & P. F. Ffolliott, 1999. Fire effects on belowground sustainability: a review and synthesis. Forest Ecology and Mangement, 122: 51–71.

Newsham, K. K., 2011. A meta-analysis of plant responses to dark septate root endophytes. New Phytologist, 190: 783-793.

Newton, A. C., 1992. Towards a functional classification of ectomycorrhizal fungi. Mycorrhiza, 2: 75-79.

Peay, K. G., T. D. Bruns, P. G. Kennedy, S. E. Bergemann & M. Garbelotto, 2007. A strong species-area relationship for eukaryotic soil microbes: island size matters for ectomycorrhizal fungi. Ecology Letters, 10: 470–80.

Racine, C., R. Jandt, C. Meyers, J. Dennis, 2004. Tundra fire and vegetation change along a hillslope on the Seward Peninsula, Alaska, USA. Arctic Antarctic and Alpine Research, 36: 1-10.

Rambaut, A.. 2002. Sequence Alignment Editor. Online [URL] http://tree.bio.ed.ac.uk/software/seal/. (Accessed 24 March 2011).

Rambaut, A., 2009. FigTree: Phylogenetic tree production. Online [URL] http://tree.bio.ed.ac.uk/software/figtree/. (Accessed 2 May 2011).

Shulski, M. & G. Wendler, 2007. The climate of Alaska: Fairbanks*, Alaska*. University of Alaska Press, Fairbanks, Alaska.

Smith, J. E., D. McKay, G. Brenner, J. McIver, & J. W. Spatafora, 2005. Early impacts of forest restoration treatments on the ectomycorrhizal fungal community and fine root biomass in a mixed conifer forest. Journal of Applied Ecology, 42: 526–535.

Smith, S. E. & D. J. Read, 2008. Mycorrhizal Symbiosis, 3rd Edition. Elsevier Ltd, Oxford.

Taylor, D. L. & T. D. Bruns, 1999. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. Molecular Ecology, 8: 1837–1850.

Taylor, D. L. & S. Houston, 2011. A bioinformatics pipeline for sequence-based analyses of fungal biodiversity. Methods in molecular biology, 722: 141–155.

Tedersoo, L., K. Pärtel, T. Jairus, G. Gates, K. Põldmaa, & H. Tamm, 2009. Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the Helotiales. Environmental Microbiology, 11: 3166-3178.

Timling, I., A. Dahlberg, D. A. Walker, M. Gardes, J. Y. Charcosset, J. M. Welker, & D. L. Taylor, 2012. Distribution and drivers of ectomycorrhizal fungal communities across the North American Arctic. Ecosphere, 3: doi:10.1890/ES12-00217.1

Toljander, J. F., U. Eberhardt, Y. K. Toljander, L. R. Paul, & A. F. S. Taylor, 2006. Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. New Phytologist, 170: 873–884.

Treseder, K. K., M. C. Mack, & A. Cross, 2004. Relationships among fires, fungi and soil dynamics in Alaskan boreal forests. Ecological Applications, 14: 1826–1838.

Visser, S., 1995. Ectomycorrhizal fungal succession in jack pine stands following wildfire. New Phytologist, 129: 389–401.

Viereck, L. A., C. T. Dyrness, A. R. Batten, & K. J. Wenzlick, 1992. The Alaska vegetation classification. USDA, Forest Service General Technical Report PNW-GTR-286, Portland, Oregon.

Walker, D. A., M. K. Raynolds, F. J. A Daniels, E. Einarsson, A. Elvebakk, W. A. Gould, A. E. Katenin, S. S. Kholod, C. I. Markon, E. S. Melnikov, N. G. Moskalenko, S. S. Talbot, & B. A Yurtsev, 2005. The Circumpolar Arctic vegetation map. Journal of Vegetation Science 16: 267-282.

Wein, R., 1976. Frequency and characteristics of arctic tundra fires. Arctic, 29: 213-222.

White, P. S., 1979. Pattern, process, and natural disturbance in vegetation. The Botanical Review, 45: 229-299.

White, T., T. Bruns, S. Lee & J. Taylor, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–322 in M. Innis, D. Gelfand, J. Sninsky, & T. White (eds.) PCR Protocols: A Guide to Methods and Applications. Academic Press, New York.

Zwickl, D. J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD thesis. The University of Texas at Austin, Austin, Texas.

FIGURES

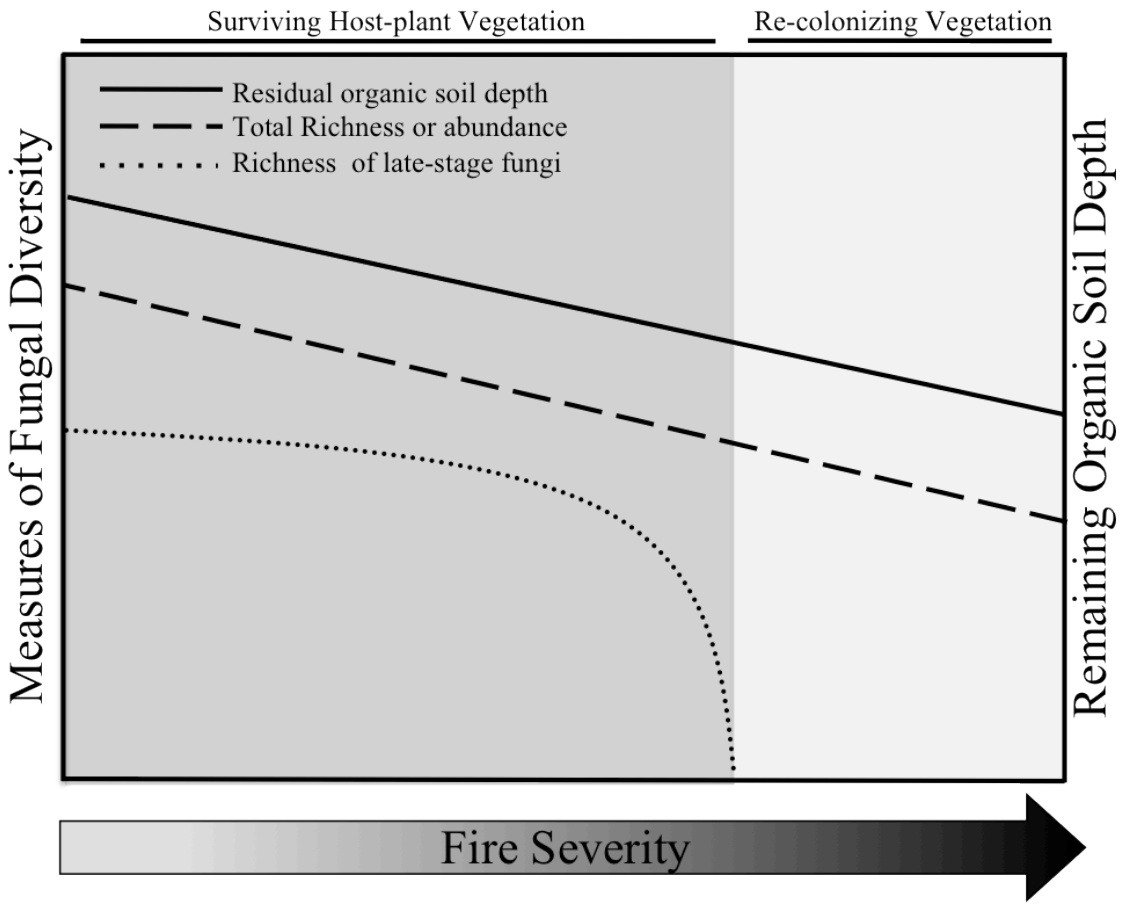


Figure 2.1. Hypothesized changes in measures of fungal community composition across a tundra fire-severity gradient. Late-stage fungi are likely K-strategists and predominantly basidiomycetes.

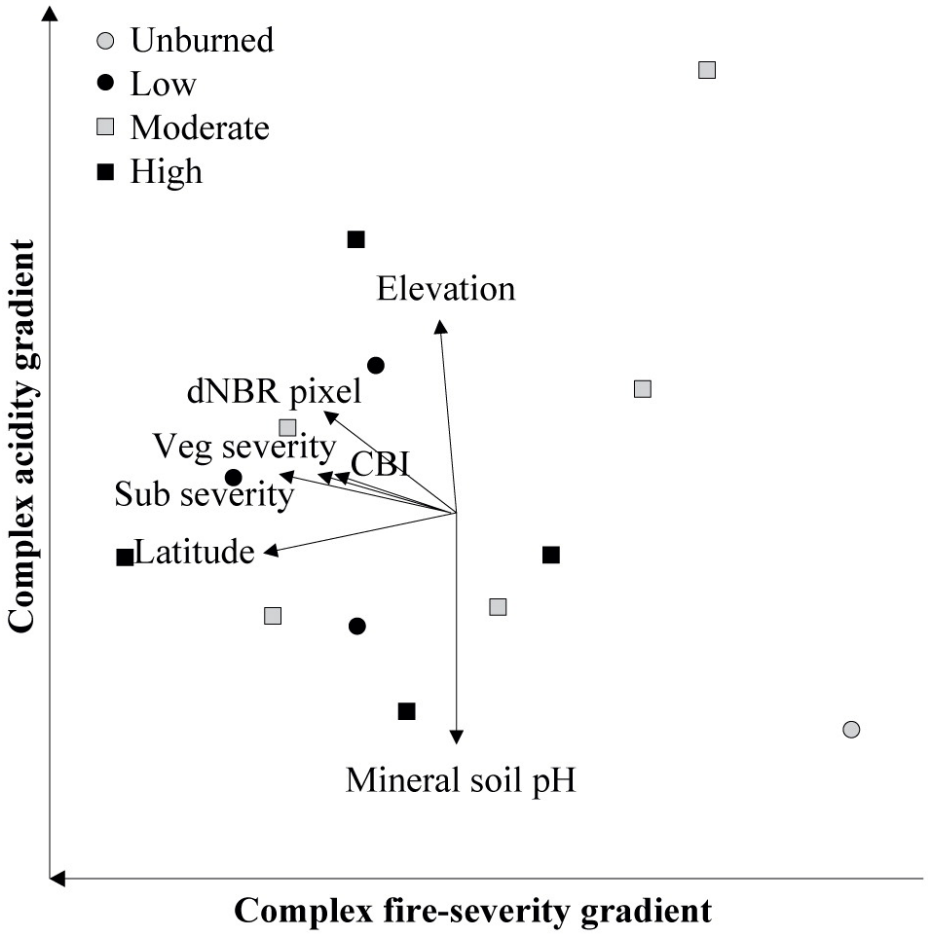


Figure 2.2. NMDS ordinations of mycorrhizal community structure: biplot of important environmental variables significantly correlated ($r^2 \geq 0.200$) with OTU mycorrhizal community structure for unburned, low, moderate, and high severity sites. The vectors show the direction and strength of correlations of environmental variables in relation to each site represented by mycorrhizal community structure (presence and abundance). "Veg severity" is burn severity of vegetation and "Sub severity" is burn severity of substrate from each site.

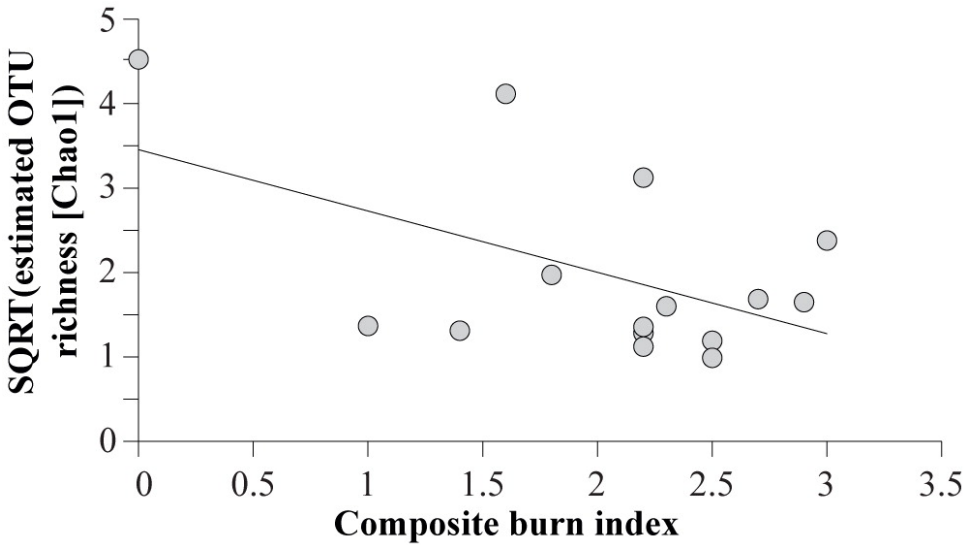


Figure 2.3. Fungal richness decreased with increasing burn severity. Regression of the estimated OTU richness (Chao1) sampled across sites and rarified to an individual shrub ($F_{(15,1)}$ =4.7751 P=0.0478; R^2=0.269). CBI increases with increasing fire severity and is a composite measure of substrate and vegetation burn severity.

Figure 2.4. Two-way cluster analysis dendrogram of OTU abundance across sampled sites in different burn severities. Abundance of OTUs is shown in grey-scale where the white squares show where taxa are absent and the filled, black squares show the highest abundance of the OTU. * denotes taxa that were significantly correlated with axis 1 of the ordination. Relative abundance for each site is based on the pooled number of clones per shrub, which is a common way to infer proportional fungal biomass or relative abundance. OTU data were transformed using the Beal's smoothing function.

TABLES

Table 2.1. Site descriptions for fifteen sampling sites grouped by fire-severity classes within or adjacent to the Anaktuvuk River Fire burn scar.

Fire Severity*	Site†	No. samples	Location	Site Description ‡§
Unburned	**1**	10	69.032° N, 148.833° W	Unburned site with *Eriophorum vaginatum* tussocks and *Betula nana*, *Ledum palustre*, *Rubus chamaemorus*, sphagnum, and feather mosses in the inter-tussock space; 2C2A
Low	5	2	69.341° N, 150.908° W	Moist site with *B. nana*, *R. chamaemorus*, *Eriophorum* sp. tussocks, and some sedges; 2C2H
	27	1	69.055° N, 150.594° W	Wet meadow with *B. nana*, *L. palustre*, *R. chamaemorus*, sphagnum mosses, and non-tussock forming sedges; 3A2I
	51	1	69.256° N, 150.648° W	Moist lowland site with *B. nana* and *Salix* sp. shrubs, *Carex bigelowii*, and *Eriophorum* sp. tussocks; 2C2A
Moderate	**104**	10	68.952° N, 150.210° W	Moist site with resprouting *Eriophorum* sp. tussocks and *B. nana, R. chamaemorus,* and *L. palustre*; 2C2A
	20	1	69.019° N, 150.754° W	Moist site on a north hillslope with *B. nana, R. chamaemorus*, *Eriophorum* sp. tussocks, and *Polygonum bistortoides* (Pursh); 2C2A
	30	1	69.110° N, 150.622° W	Moist site with *B. nana*, *L. palustre*, *R. chamaemorus*, and *Eriophorum* sp. tussocks.; 2C2A
	32	1	69.159° N, 150.691° W	Wet sedge meadow site with *B. nana*, *R. chamaemorus*, and *Carex* sp. in a polygonated valley bottom; 3A3
	41	1	69.336° N, 150.791° W	Moist site with *B. nana*, *L. palustre, Carex* sp. sedges, and *Eriophorum* sp. tussocks; 3A2D with patches of 3A3 polygons
High	**101**	10	68.996° N, 150.281° W	Moist site with exposed mineral soil and resprouting *Eriophorum* sp. tussocks, *R. chamaemorus,* and *B. nana* shrubs*;* 2C2A
	13	1	69.172° N, 150.819° W	Moist site with *Eriophorum* sp. tussocks, *Saussurea angustifolia* (Wild) D.C., *B. nana* shrubs, and combusted sphagnum mosses; 2C2A
	37	1	69.271 ° N, 150.753° W	Moist ridgetop site with exposed mineral soil, *B. nana* and *Salix* sp. shrubs, and *Eriophorum* sp. tussocks ; 3A2D
	60A	1	69.175 ° N, 150.538° W	Moist site with exposed mineral soil, *B. nana* and *Salix* sp. shrubs, *L. palustre, Carex* sp. sedges and *Eriophorum* sp. tussocks; 3A2H
	63	3	69.128° N, 150.445° W	Dry site with *Equisetum* sp. L., graminoids, *Salix* sp., *B. nana* shrubs, and exposed mineral soil; 2C2C
	69	1	69.019° N, 150.412° W	Hill top moist tussock tundra site with *B. nana*, *L. palustre,* and *Eriophorum* sp. tussocks; 2C2A

* Fire-severity classes are based on CBI, dNBR values, and site descriptions.

† Bold site numbers denote intensively sampled sites, while extensively sampled sites are not bold.

‡ We classified vegetation communities using the Viereck (1992) vegetation classification: 2C2A= open low mixed shrub-sedge tussock tundra, 2C2H=open low willow-sedge shrub tundra, 3A21=sedge-birch tundra, 3A2H=sedge-willow tundra, and 3A3=Wet graminoid herbaceous tundra.

§ Environmental site descriptions, mineral soil pH, active layer depth, remaining soil organic layer depth, and CBI data for burned sites have been summarized in Jandt *et al.* (2012) and are available online at www.frames.gov and with the Arctic Long-Term Ecological Research (LTER) data archive http://ecosystems.mbl.edu/arc/datacatalog.html

Table 2.2. Classification and presence of the ten most abundant operational taxonomic units (OTUs) across fire-severity classes.

OTU Abundance	Best match Description	Type*	Unburned	Low	Moderate	High
131	*Russula decolorans*	EMF	x		x	x
50	*Meliniomyces* sp	ERM	x	x	x	x
39	*Phialocephala fortinii*	DSE	x	x	x	
32	Sordariomycetidae sp.	likely DSE		x	x	x
27	*Russula* sp.	EMF	x		x	x
27	Helotiales sp.	likely ERM	x	x	x	x
22	*Chalara* sp.	pathogen			x	
19	*Lactarius glyciosmus*	EMF			x	
13	*Meliniomyces variabilis*	ERM		x	x	x
12	*Inocybe borealis*	EMF	x			

*EMF= ectomycorrhizal fungi, ERM= ericoid mycorrhizal fungi, DSE=dark septate endophyte.

Table 2.3 a, b. Correlations between environmental variables or fungal taxa and NMDS ordination of mycorrhizal communities in the ARF burn: a. Environmental variables correlated with NMDS ordinations. b. Fungal taxa presence and relative abundance correlated with the axes of NMDS ordinations. Note that a positive value on axis 1 indicates low fire severity.

a.	Axis 1*		Axis 2*	
Environmental variables	r	r^2	r	r^2
Latitude	-0.631	**0.398**	-0.309	0.095
Slope	0.029	0.001	0.34	0.116
Elevation	-0.227	0.051	0.553	**0.306**
Burn Severity Substrate	-0.519	**0.27**	0.244	0.06
Burn Severity Vegetation	-0.494	**0.244**	0.258	0.067
CBI	-0.487	**0.237**	0.283	0.08
Mineral soil pH	0.111	0.012	-0.685	**0.47**
Average organic soil depth	0.351	0.123	0.331	0.109
Active layer depth	0.09	0.008	0.286	0.082
dNBR Class	-0.181	0.033	0.433	0.188
dNBR Pixel	-0.466	**0.217**	0.443	0.196

Table 2.3 a, b. continued

b.		Complex Fire-Severity Gradient Axis 1*		Complex Acidity Gradient Axis 2*	
Phylum†	Operational Taxonomic	r	r^2	r	r^2
B	*Russula decolorans*	0.495	**0.245**	-0.218	0.047
A	*Meliniomyces* sp	0.563	**0.317**	-0.600	**0.360**
A	*Phialocephala fortinii*	0.364	0.133	0.295	0.087
A	Sordariomycetidae sp.	-0.738	**0.544**	-0.454	**0.206**
B	*Russula* sp.	0.677	**0.458**	0.382	0.146
A	Helotiales sp.	0.488	**0.238**	-0.684	**0.468**
A	*Chalara* sp.	0.563	**0.317**	0.704	**0.496**
B	*Lactarius glyciosmus*	0.528	**0.279**	0.744	**0.553**
A	*Meliniomyces variabilis*	0.013	0.000	0.699	**0.489**
B	*Inocybe borealis*	0.587	**0.345**	-0.494	**0.244**
B	*Clavulina* sp	-0.246	0.060	-0.524	**0.275**
B	Russulaceae sp.	0.472	**0.223**	-0.648	**0.420**
B	Cantharellales sp.	0.587	**0.345**	-0.494	**0.244**
B	*Lactarius* sp.	0.587	**0.345**	-0.494	**0.244**
B	*Lactarius torminosus*	-0.104	0.011	0.447	0.199
B	*Lactarius* sp.	0.316	0.100	0.006	0.000
B	Agaricomycotina sp.	-0.029	0.001	-0.497	**0.247**
B	*Pseudotomentella tristis*	0.563	**0.317**	0.704	**0.496**
B	*Russula* sp.	-0.547	**0.300**	-0.164	0.027
B	*Tomentella subclavigera*	0.587	**0.345**	-0.494	**0.244**
A	*Cladophialophora*	-0.008	0.000	-0.505	**0.255**
A	*Meliniomyces* sp.	0.553	**0.306**	0.397	0.158
A	*Meliniomyces bicolor*	0.563	**0.317**	0.704	**0.496**
A	Helotiales sp.	0.587	**0.345**	-0.494	**0.244**
B	*Hebeloma* sp.	0.587	**0.345**	-0.494	**0.244**
A	*Phialocephala fortinii*	0.930	**0.864**	0.128	0.017
B	Russulaceae sp.	0.472	**0.223**	-0.648	**0.420**

*Bold r^2 values have significant correlations with NMS axes

†Fungal phyla are as follows A= Ascomycota and B= Basidiomycota.

APPENDICES

Appendix 2.1. Verification of the adequacy of root tip sampling and sequencing effort for each *Betula nana* shrub using ARISA

In our view, sequencing data provide better discrimination of fungal taxa while ARISA provides a sensitive, rapid, and cost-effective method to estimate species richness, although it does not provide phylogenetically explicit information (Liu *et al.*1997). We used ARISA to determine the adequate number of root tips sampled to capture fungal richness for each shrub and to verify that we sufficiently sequenced the taxa for each shrub.

METHODS

For ARISA, 15 ml reaction mixes were prepared using the forward primer FAM-ITS1F, CTTGGTCATTTAGAGGAAGTAA (Gardes & Bruns, 1993), labeled on the 5' end with FAM, a fluorescein amidit (Applied Biosystems, Carlsbad, CA, USA), and the reverse primer ITS4, TCCTCCGCTTATTGATATGC (White *et al.*, 1990), using MapMarker 1000 X-rhodamine size standard (BioVentures, Murfreesboro, TN, USA) and following the protocol of Bent *et al*. (2011). Samples were run on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, NJ, USA; Pop6, 50cm array, T-RFLP_1500 protocol).

BIOINFORMATICS

We used GeneMapper 3.7 (Applied Biosystems, Foster City, NJ, USA) to read each ARISA electropherogram. The peak heights for each fragment were relativized by dividing the fluorescence height for each peak by the total fluorescence for a sample profile (Fisher & Triplett, 1999). ARISA ribotypes were binned at 1 bp bin size (Dunbar

et al., 2001). We used 1 bp ARISA ribotype bins as surrogates for "species" in certain fungal community analyses. Binned ARISA ribotype abundance data has been archived with the Bonanza Creek LTER http://www.lter.uaf.edu/data_b.cfm.

STATISTICS AND RESULTS

To determine the necessary number of root tips that we needed to sample in order to adequately capture the fungal richness associated with each shrub, we used a t-test to compare samples with different numbers of tips from the same shrub. Preliminary analyses on six shrubs demonstrated that 18 tips were sufficient to represent the richness of fungal amplicons present on a root system (18 tips vs 36 tips $T_{(6)}$=-1.00, p=0.423; Appendix 2.I). There was not a significant increase in the richness we observed for each shrub when we sampled more than 18 root tips.

To verify that we adequately sequenced the fungal community we compared our ARISA profiles to the OTU dataset for each shrub. ARISA profiles will represent rare taxa, while sequences from the clone libraries could potentially only capture the dominant OTUs associated with each shrub. The distributions of OTU and ARISA ribotype abundances were examined for normality by visually inspecting normal quantile plots, assessing skewness and kurtosis, and using the Shapiro-Wilks W test. We used a paired t-test to compare the observed ARISA and OTU richness on individual shrubs. We also used the Mantel test (Mantel, 1967) with Monte Carlo permutations to evaluate the null hypothesis that there was no significant relationship between the community structure for site-level community profiles represented in the OTU and ARISA matrices. To format symmetrical matrices for the site-level ARISA and OTU matrices we eliminated two sites (sites 60 and 69) with outlier fungal community profiles. The resulting OTU matrix had 13 sites (rows) with 27 OTUs (columns) and the second matrix had 13 sites (rows) with 25 ARISA ribotypes (columns).

We determined that we sequenced enough clones to adequately represent fungal richness for each shrub because the numbers of OTUs obtained from sequences equaled the numbers of ARISA ribotypes ($T_{(43)}$=1.654, p=0.105). In addition, there was a significant relationship between the structure of ARISA and OTU community datasets (Mantel test site matrices r=0.681, p=0.001). For subsequent analyses we used the OTU dataset because it allowed us to comment on taxon identity in addition to community structure. All statistical analyses were performed in JMP 9.0.2 (SAS Institute Inc, 2010) with the exception of the multivariate analysis of mycorrhizal communities in PC-ORD 6.0.

REFERENCES

Bent, E., P. Kiekel, R. Brenton R. & D. L. Taylor, 2011. Root-associated ectomycorrhizal fungi shared by various boreal forest seedlings naturally regenerating after a fire in Interior Alaska and correlation of different fungi with host growth responses. Applied Environmental Microbiology, 77: 3351-3359.

Dunbar, J., L. Ticknor, & C. Kuske, 2001. Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities. Applied and Environmental Microbiology, 67: 190–197.

Fisher, M. M. & E. W. Triplett, 1999. Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. Applied and Environmental Microbiology, 65: 4630-4636.

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

Liu, W.T., T. L. Marsh, H. Cheng, & L. J. Forney, 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Applied and Environmental Microbiology, 63, 4516-4522.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Research, 27: 209–220.

White, T., T. Bruns, S. Lee & J. Taylor, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–322 in M. Innis, D. Gelfand, J. Sninsky, & T. White (eds.) PCR Protocols: A Guide to Methods and Applications. Academic Press, New York.

Appendix 2.2. Operational taxonomic units (OTUs) from cloned sequences from *Betula nana* shrubs sampled across fire severities in the Anaktuvuk Burn.

Operational Taxonomic Unit	OTU Abundance	No. shrubs	No. sites	Best Match GenBank Accession	Best match Description	Identity (%)*	Overlap†	Bit score‡	GenBank Accession§
OTU8	131	20	4	FJ845432.1	*Russula decolorans*	99.43	696	1348	KC455315
OTU7	50	14	6	EF093175.1	*Meliniomyces* sp.	94.39	532	783	KC455314
OTU17	39	9	5	AY394906.1	*Phialocephala fortinii*	99.47	560	1074	KC455322
OTU12	32	7	7	HM595513.1	Sordariomycetidae sp.	100	531	1053	KC455317
OTU18	27	5	3	AY061719.1	*Russula* sp.	98.29	700	1292	KC455323
OTU34	27	7	6	HM190115.1	Helotiales sp.	97.44	546	926	KC455335
OTU14	22	4	1	HM123472.1	*Chalara* sp.	98.26	518	955	KC455319
OTU1	19	2	2	DQ097872.1	*Lactarius glyciosmus*	99.58	718	1392	KC455311
OTU30	13	6	5	HM190126.1	*Meliniomyces variabilis*	97.17	564	1013	KC455333
OTU22	12	1	1	JN580813.1	*Inocybe borealis*	100	573	1136	KC455326
OTU25	10	1	1	AM882793.2	*Inocybe* sp.	97.98	645	1185	KC455329
OTU26	10	1	1	HQ215809.1	*Tomentella* sp.	99.83	593	1168	KC455330
OTU6	10	1	1	EU862208.1	*Clavulina* sp.	96.53	689	1152	KC455313
OTU13	9	3	2	AM113957.1	Russulaceae sp.	89.63	676	797	KC455318
OTU38	8	1	1	AB369933.1	Cantharellales sp.	88.59	371	361	KC455339
OTU19	7	1	1	DQ421996.1	*Lactarius* sp.	99.55	661	1294	KC455324
OTU28	7	1	1	DQ367908.1	*Lactarius torminosus*	98.91	731	1390	KC455332
OTU36	7	3	3	AB560528.1	*Lactarius* sp.	100	685	1358	KC455337
OTU11	5	1	1	AY963567.1	Agaricomycotina sp.	97.62	168	301	KC455316
OTU15	5	1	1	AJ889968.1	*Pseudotomentella tristis*	99.85	679	1338	KC455320
OTU2	5	1	1	AY061707.1	*Russula sp.*	98.83	680	1275	KC455312
OTU23	3	2	1	HQ215808.1	*Tomentella subclavigera*	98.31	593	1110	KC455327
OTU32	3	1	1	EU035406.1	*Cladophialophora chaetospira*	96.88	607	1031	KC455334
OTU40	3	2	2	HM190126.1	*Meliniomyces* sp.	94.35	564	841	KC455340
OTU16	2	1	1	AY394885.1	*Meliniomyces bicolor*	97.01	869	1509	KC455321
OTU20	2	1	1	JN859274.1	Helotiales sp.	96.17	547	894	KC455325
OTU24	2	1	1	AF430254.1	*Hebeloma* sp.	99.76	416	817	KC455328
OTU27	2	1	1	HQ215809.1	*Tomentella* sp.	95.78	593	961	KC455331
OTU35	2	2	2	AY078133.1	*Phialocephala fortinii*	97.74	837	1487	KC455336
OTU37	2	2	2	DQ367913.1	Russulaceae sp.	91.83	697	866	KC455338
SING13	1	1	1	DQ367913.1	Russulaceae sp.	91.41	697	878	KC455346
SING15	1	1	1	FJ845432.1	Russulaceae sp.	92.68	696	995	KC455347
SING18	1	1	1	AY664502.1	*Phialocephala fortinii*	97.33	375	664	KC455348
SING19	1	1	1	AM292201.1	Herpotrichiellaceae	90.66	359	442	KC455349
SING24	1	1	1	AY762619.1	*Meliniomyces* sp.	94.71	378	613	KC455350
SING25	1	1	1	AB598082.1	Helotiales sp.	98.93	558	1053	KC455351
SING26	1	1	1	JN995644.1	*Articulospora* sp.	95.81	549	890	KC455352
SING3	1	1	1	JF908571.1	*Chalara* sp.	93.33	551	842	KC455341
SING30	1	1	1	AY394907.1	*Rhyzoscyphus ericae*	99.54	863	1671	KC455353
SING35	1	1	1	JN942806.1	*Laccaria* sp.	99.71	683	1338	KC455354
SING37	1	1	1	AB634268.1	*Tomentella* sp.	94.89	372	591	KC455355
SING44	1	1	1	AY699656.1	Helotiales sp.	99.73	369	716	KC455356
SING45	1	1	1	EU998916.1	*Articulospora tetracladia*	98.18	548	999	KC455357
SING46	1	1	1	AY078131.1	*Phialocephala* sp.	94.14	561	839	KC455358
SING47	1	1	1	FJ196296.1	Helotiaceae sp.	90.56	537	620	KC455359
SING48	1	1	1	HQ157840.1	*Meliniomyces* sp.	97.77	537	961	KC455360
SING49	1	1	1	AY880935.1	*Phialocephala* sp. Complex	100	374	741	KC455361
SING5	1	1	1	EF093172.1	*Meliniomyces* sp.	95.18	454	724	KC455342
SING53	1	1	1	EF093177.1	*Meliniomyces variabilis*	97.67	343	611	KC455362
SING6	1	1	1	EF093172.1	*Meliniomyces* sp.	96.92	454	783	KC455343
SING7	1	1	1	HQ157840.1	*Melinomyces* sp.	98.51	537	1001	KC455344
SING9	1	1	1	AJ534914.1	*Tomentella* sp.	99.69	643	1259	KC455345

*Identity (%)= overall fraction of identical positions across high scoring segment pairs

†Overlap= length of the hit participating in alignment without the gaps;

‡Bit cores=higher scores indicate the greater significance of the alignment between the query sequence and the hit sequence

§ GenBank Accession = the assigned GenBank Accession number for the representative OTU sequence.

Appendix 2.3. Correlations between variables: a. environmental and fire variable, b. response variables

a. Independent variables		Spearman's correlation	p
Latitude	Mineral soil pH	0.0679	0.8099
Elevation	Mineral soil pH	-0.613	0.0151
Elevation	Latitude	0.1357	0.6296
Burn severity of substrate	Mineral soil pH	-0.2801	0.312
Burn severity of substrate	Latitude	0.174	0.5351
Burn severity of substrate	Elevation	0.969	0.7313
Burn severity of vegetation	Mineral soil pH	-0.3237	0.2391
Burn severity of vegetation	Latitude	0.2803	0.3115
Burn severity of vegetation	Elevation	0.5301	0.0421
Burn severity of vegetation	Burn severity of substrate	0.6507	0.0086
Composite Burn Index (CBI)	Mineral soil pH	-0.1191	0.6724
Composite Burn Index (CBI)	Latitude	0.2164	0.4385
Composite Burn Index (CBI)	Elevation	0.2705	0.3295
Composite Burn Index (CBI)	Burn severity of substrate	0.7564	0.0011
Composite Burn Index (CBI)	Burn severity of vegetation	0.7087	0.0031
dNBR pixel	Mineral soil pH	-0.538	0.0386
dNBR pixel	Latitude	-0.1393	0.6205
dNBR pixel	Elevation	0.3071	0.2655
dNBR pixel	Burn severity of substrate	0.7605	0.001
dNBR pixel	Burn severity of vegetation	0.7062	0.0033
dNBR pixel	Composite Burn Index (CBI)	0.7016	0.0036

b. Response variables		Spearman's correlation	p
Estimated richness (Chao1)	Observed richness (Mao Tau)	0.975	<0.0001
Estimated richness (Chao1)	Proportion of basidiomycete richness	-0.1536	0.5847
Estimated richness (Chao1)	Proportion of basidiomycete abundance	-0.278	0.3158
Observed richness (Mao Tau)	Proportion of basidiomycete richness	-0.0813	0.7733
Observed richness (Mao Tau)	Proportion of basidiomycete abundance	-0.2074	0.4578
Proportion of basidiomycete richness	Proportion of basidiomycete abundance	0.9452	<0.0001

Chapter 3

Plant-fungal interactions after a novel disturbance in the Arctic: implications for shrub and tree migration[2]

ABSTRACT

Vegetation change in high latitude tundra ecosystems is expected to accelerate due to increased wildfire activity. High-severity fires increase the availability of mineral soil seedbeds, which facilitates recruitment, yet fire also alters soil microbial composition, which could significantly impact seedling establishment. We experimentally investigated the effects of fire severity on soil biota and associated effects on plant performance for two plant species predicted to expand into Arctic tundra. We inoculated seedlings in a growth chamber experiment with soils collected from the largest tundra fire recorded in the Arctic and used molecular tools to characterize fungal communities. Seedling biomass was significantly related to the composition of fungal inoculum. Biomass decreased as fire severity increased and the proportion of pathogenic fungi increased. Our results suggest that effects of fire severity on soil biota, and in particular pathogens, may dampen or even reverse the expected increases in tree and shrub establishment after fire, thus influencing predicted changes in Arctic vegetation.

[2] Hewitt, R. E., T. N. Hollingsworth, F. S. Chapin, and D. L. Taylor. Plant-fungal interactions after a novel disturbance in the Arctic: implications for shrub and tree migration. Prepared for submission to Ecology Letters.

INTRODUCTION

Changing climates and the novel conditions they produce can drive species acclimation or adaptation in the present environment, promote the migration of species into more suitable environments, or allow species to recruit into formerly uninhabitable environments. In the last half century, warming in the Arctic and Subarctic has been correlated with the expansion of tundra shrubs into graminoid tundra (Sturm *et al.* 2001) and the migration of forest into tundra in some locations (Harsch *et al.* 2009). These changes in vegetation could have strong positive feedbacks to the climate system through decreases in albedo, carbon storage, and increases in landscape flammability (Rupp *et al.* 2000; Hinzman *et al.* 2005). Evidence suggests that factors influencing seedling establishment are the most critical determinants of global treeline (Harsch & Bader 2011) and shrubline advances (Myers-Smith *et al.* 2011).

Soil biota may influence the capacity of boreal trees to migrate into tundra and tundra shrubs to expand into non-shrubby tundra. Vegetation establishment is influenced by soil biota, both mutualists and pathogens. Microbial symbionts strongly influence plant performance and community structure (Klironomos 2002), yet their impact on landscape-scale vegetation change is often overlooked. Ectomycorrhizal fungi (EM) are essential to seedling establishment and growth both inside (Horton *et al.* 1999) and outside (Nunez *et al.* 2009) the native range of the host plant. Depending on host plant species and fungal specificity, seedlings that integrate into established EM hyphal networks of mature plants often show increased fitness compared with non-mycorrhizal seedlings due to greater nutrient acquisition, protection from pathogens, and alleviation of drought stress by mycobionts (Booth 2004; Horton & van der Heijden 2008; Smith & Read 2008). On the other hand, the establishment of seedlings can also be limited by the presence of species-specific enemies, including fungal pathogens (Janzen 1970; Connell 1971;

Mangan *et al.* 2010). The net outcome of negative interactions with pathogens and positive interactions with mutualists can influence the relative abundance and migration capacity of a plant species (Horton & van der Heijden 2008; Mangan *et al.* 2010).

The fire regime directly affects seedling recruitment success and migration (Johnstone *et al.* 2010) in the boreal forest, shrub growth and reproduction in the Subarctic (Lantz *et al.* 2010), and shrub expansion in the Arctic (Racine *et al.* 2004) primarily through severity effects on the availability of high-quality, mineral soil seedbeds and time since fire on successional dynamics. Although fire disturbance has been rare in the Arctic tundra for the last 11,000 years (Higuera *et al.* 2008), in the last half- century the extent of tundra fires has increased due to warm and dry weather (Hu *et al.* 2010). Increased fire frequency and severity in Arctic tundra is therefore expected to facilitate both tree migration and shrub expansion.

Despite the expected acceleration of tree migration and shrub expansions associated with warming in the Arctic (Landhausser & Wein 1993), the effects of fire disturbance on fungal symbionts might dampen or reverse this trend. For example, the same severe burns that increase the availability of high-quality establishment sites on mineral soil (Johnstone *et al.* 2010) can also alter the community structure of soil-dwelling fungal symbionts (Neary *et al.* 1999). In temperate and boreal regions, severe fires decrease EM richness (Smith *et al.* 2005) and root colonization (Treseder *et al.* 2004). In addition, burns can induce infection in fire-damaged roots of vegetation that survives fire (Parker *et al.* 2006), thus affecting the prevalence of pathogens that may associate with establishing seedlings. In tundra where fire has been relatively rare, the effect of fire severity on the prevalence of pathogens and availability of critical mycorrhizal partners in tundra is largely unknown.

Currently, predictions of vegetation change in Arctic Alaska are based on assumptions derived from boreal forest research, which suggest that substrate availability is the primary ecological filter that drives seedling establishment (Johnstone *et al.* 2010). This implies that in Arctic Alaska higher fire severity fires will produce a better seedbed and thus facilitate treeline advance and shrub expansion. To incorporate the potentially important biotic effects of soil microbes on vegetation transformations, we experimentally investigated the role of post-fire soil microbes on seedling performance for two plant species predicted to migrate into tundra under future scenarios of warming and fire. Specifically, we tested the hypothesis that increasing fire severity decreases performance in establishing seedlings due to fire effects on fungal mutualists and pathogens. This research develops a new conceptual model for understanding controls over seedling establishment and vegetation change at high latitudes.

MATERIALS AND METHODS

STUDY SPECIES AND FIELD SAMPLING

In Alaska, extensive woody expansion into tundra has been documented for alder shrubs, *Alnus viridis* (Chaix) DC. (Sturm *et al.* 2001), and alder growth and reproduction are greater in burned sites (Lantz *et al.* 2010). Black spruce, *Picea mariana* (Mill.) Britton, Sterns & Poggenb., is the dominant latitudinal treeline species throughout most of North America. In northern Alaska, occurrence of black spruce is determined in part by fire occurrence (Lloyd *et al.* 2007) and is well-suited for cold, moist environments. Both species are obligately ectomycorrhizal (Molina *et al.* 1992). Seeds of both species were collected in Interior Alaska at Washington Creek and Fairbanks and stored at -20 °C until used in our growth chamber experiment.

Between July and October 2007 the Anaktuvuk River Fire (ARF), the largest tundra fire ever recorded on the North Slope of Alaska, burned 1039 km^2 of upland shrubby tussock tundra underlain by continuous permafrost (Mack *et al.* 2011). The dominant vegetation before the fire was moist acidic tundra (54%) with moist nonacidic tundra (15%) and shrubland (30%) covering smaller areas (Jandt *et al.* 2012). We focused our study on moist acidic tundra that is dominated by sedges (*Eriophorum vaginatum* L., *Carex bigelowii* Torr. Ex Schwein), evergreen shrubs (*Ledum palustre* L. and *Vaccinium vitis-idea* L.), deciduous shrubs (*Betula nana* L. and *Salix pulchra* Cham.), mosses, and lichens (Viereck *et al.* 1992) and very similar to the understory of some boreal black spruce forests.

In July 2008, one growing season after the fire, we visited eight burned sites within the ARF burn scar corresponding with different fire severities (Figure 3.1; see Appendix 3.1). Fire severity was measured in the field as the Composite Burn Index (CBI) for each site (Jandt *et al.* 2012). At 20 points along a 50 m transect, we collected 5 ml of organic soil and 5 ml mineral soil from the top 5 cm of the soil horizon. We then pooled and homogenized our 20 samples per site by soil horizon, and stored them at 4 °C at the University of Alaska Fairbanks for three weeks until we inoculated the host plants.

EXPERIMENTAL DESIGN

We conducted a growth chamber experiment using a randomized block design with 18 treatments and 10 replicate blocks and two host plant species. There were 16 treatments with inoculum from field soil (8 sites X 2 soil horizons (organic and mineral)) and two additional treatments to test for unintentional inoculation of seedlings in the growth chamber with sterile inoculum (autoclaved mineral and autoclaved organic soils). In July 2008 we surface-sterilized

black spruce seeds with a solution of 5 % household bleach, 5 % ethanol, and liquinox for five minutes and the smaller alder seeds for one minute followed by ten rinses with ultrapure water. Seeds were placed in sterile Petri dishes on autoclaved filter paper, and RO water was used to keep the seeds moist.

We transplanted seedlings from petri dishes into 150 ml cone-tainers (Stuewe and Sons, Inc., Tangent, Oregon, USA) filled with sterilized silt soil two weeks after germination and inoculated with one of eighteen treatments. Twelve ml of treatment soil was added to the top of each cone-tainer and watered into the autoclaved substrate. Seedlings received one 50 ml application (14 ppm N) of a 9:20:9 NPK fertilizer solution one and half months after inoculation and one 50 ml application (50 ppm N) four months after inoculation. Seedlings were grown in a controlled-environment chamber (Conviron CMP 3246, Winnipeg, Manitoba, Canada) for seven months at 25/10°C day/night with 16-h photoperiod at 300 μmol m^{-2} s^{-1} irradiance at ambient humidity (26-96% RH, mean 73.75 RH ± 0.16 S.E., NOAA National Climate Data Center http://www.ncdc.noaa.gov/). We watered seedlings to excess with RO water as needed. Height and survival were measured monthly. At the time of harvest, roots were rinsed gently with RO water and separated from shoots. We dried shoots and roots after root tip sampling (described below) at 60°C for 48 hours in a drying oven (VWR Scientific Products Forced Air Oven, Radnor, Pennsylvania, USA) and determined the dry weight of roots, stems, and leaves.

CHARACTERIZATION OF FUNGAL COMMUNITIES

Harvested root systems were cut into 4 cm segments and floated in ultrapure water in a petri dish. Using a dissecting microscope (40x magnification) we selected ten live root tips randomly from each root system. For each seedling, root tips were pooled for Automated

Ribosomal Intergenic Spacer Analysis (ARISA) and DNA sequence analysis of root-associated fungal community structure. Pooled root tip samples were placed in a single 0.6 ml Eppendorf tube, frozen in a small amount of ultrapure water, and stored at -80 °C.

In September 2010 we extracted DNA from lyophilized pooled root tip samples of each seedling. From these pooled genomic DNA samples, the fungal ITS gene region was amplified using the primers ITSF and ITS4 following the protocol of Bent and Taylor (2010). We obtained ARISA fungal community profiles and ITS sequences following Bent *et al.* (2011). Fungal taxa were inferred from ITS sequences and matched to dominant ARISA ribotypes based on sequence and ribotype fragment lengths (see Appendix 3.2). Fungal identities were assigned though comparison of our ITS sequences to those from GenBank using a curated fungal-ITS BLAST search (http://www.borealfungi.uaf.edu/) that excludes environmental and uncultured sequences (Taylor & Houston 2011). In addition, we constructed maximum likelihood trees to infer identities for sequences with inconsistent identities resulting from the BLAST search (see Appendix 3.3). Nomenclature for our sequences follows Timling *et al.* (2012). Functional groups were assigned based on Operational Taxonomic Unit (OTU) identities. For example, for species and genera we could categorize OTUs as pathogens, endophytes, dark septate endophytes (DSE), saprotrophs, or ericoid mycorrhizal fungi (ERM). OTUs that were assigned identities at family or higher levels of taxonomic resolution were assigned multiple functional groups (see Appendix 3.4). In a few cases the closest sequence identities matched with a ribotype were from different functional groups, in which case we described ribotypes as either pathogenic or nonpathogenic (i.e. ERM, DSE).

STATISTICAL ANALYSIS

We used Nonmetric Multidimensional Scaling (NMS) (Kruskal 1964) ordinations to assess fungal composition. Ordinations were based on ribotype abundance data and we used Beal's smoothing to relieve the "zero truncation problem" (McCune & Grace 2001). We observed some fungal colonization of roots when we ran ARISA on seedlings that received the sterile inoculum (23 ribotypes on 31 of the 40 seedlings that received the sterile treatment and 5 of the ribotypes occurred more than once). In order to adjust for this contamination we used the most conservative approach, and all ribotypes observed on these seedlings were excluded from multivariate analysis of fungal composition. Following McCune & Grace (2001) we eliminated all rare ribotypes, i.e. those that occurred in less than 5% of the samples. We used the Sorensen distance measure and a random starting configuration with a final solution generated using 500 iterations. First, to determine whether fungal composition was related to seedling biomass, we ordinated the fungal communities associated with all seedlings regardless of treatment or host species. Second, to investigate whether treatment differences in fungal composition were related to seedling biomass, we pooled treatment replicates into one fungal community profile per treatment (i.e., combined soil horizon and burn severity) for each host plant. In both cases, axis scores were produced and then used in regression analysis as a measure of fungal composition. We also used Multiple-Response Permutation Procedures (MRPP) (Berry *et al.* 1983) with the Euclidean distance measure to investigate whether there were differences between fungal communities in burned sites grouped by low, moderate, and high burn-severity categories (low= 2 treatments, moderate = 3 treatments, high =3 treatments) and between soil horizons (organic=

8 treatments, mineral= 8 treatments). Fire-severity categories were defined by CBI and site severity descriptions.

We assessed normality graphically for all response variables (total biomass, shoot biomass, root biomass, shoot:root) and considered skewness, kurtosis, and Shapiro-Wilk's W values before log-transforming data. We evaluated correlations between response variables using Spearman correlations. All response variables (stem weight, leaf weight, life span, shoot:root, and total aboveground biomass) were significantly correlated with total biomass, so we used total biomass as the response variable for subsequent analyses. We used ANCOVA to investigate inoculation effects on seedling biomass with host plant species as the covariate, and ANOVA to investigate the relationship between seedling biomass and treatment for each host species. To evaluate the relationship between fungal composition (NMS axis scores) and seedling biomass, we used the stepwise regression to evaluate the best-fit model comprised of all or a subset of NMS axes. We used regression to test for relationships between the proportion of functional groups of fungi and both fire severity and seedling biomass. Stepwise regression was then used to determine whether the relative abundance of particular taxa within defined functional groups were related to seedling biomass. We used matched pairs T-test to compare the survivorship between host plants species for each moth of the experiment.

Each inoculum treatment reflects both fire severity and soil horizon, so we tested for differences in treatment means of seedling biomass (10 seedlings/inoculum type/host species) across a continuous fire-severity gradient (CBI) and soil horizon using regression and ANOVA, respectively. Initially, we explored models with site as a covariate and found site not to be a significant factor. Because sites were chosen to represent different fire severities we did not include both site and CBI in the final model. All statistical analyses were performed in JMP 9.0.2

(SAS Institute Inc, 2010) with the exception of the multivariate analysis of fungal communities in PC-ORD 6.0.

RESULTS

Over 70% of seedlings survived through the end of the experiment, and percent survivorship was not species-dependent ($T_{(7)=}$=1.50, P=0.184). Compared to controls inoculated with sterilized inoculum, inoculated soils from the ARF reduced seedling biomass for both spruce and alder (Full model $F_{(2)}$ =88.7909, P<0.000; inoculation $F_{(1)}$ =5.642, P=0.018; species $F_{(1)=}$169.752, P<0.000) (Figure 3.2).

TREATMENT EFFECTS ON SEEDLING BIOMASS

The inoculation treatments given to the seedlings reflected both burn severity and soil horizon of a site. In general, fire severity had a stronger effect on seedling biomass than did soil horizon, particularly for alder. For alder we found a decrease in total seedling biomass with increased fire severity ($F_{(1)}$ =4.976, P=0.044) (Figure 3.3a, b). Spruce seedling biomass also decreased with increasing fire severity ($F_{(1)=}$ 4.175, P=0.0636) but only for seedlings given mineral-soil inoculum (($F_{(1)=}$ 17.135, P=0.006 (Figure 3.3a, c). In contrast, soil horizon alone had no significant effect on seedling biomass for either alder or spruce (alder $F_{(16, 1)}$ =0.728, P=0.409; spruce $F_{(1)=}$ 0.704, P=0.418). There was, however, a significant interaction between soil horizon and fire severity on spruce seedling biomass ($F_{(1)=}$ 9.340, P=0.010) (Figure 3.3c).

FUNGAL COMPOSITION AND EFFECTS ON SEEDLING BIOMASS

In an exhaustive search for ectomycorrhizal root tips, we failed to find any. However, we did observe numerous fungal hyphae and indications of some degree of fungal interaction with

plant roots. Microscopic morphological examination (10-40X) of live, turgid root tips that had no root hairs from the root system of each spruce and alder seedling revealed no branched, swollen, or colored root tips with classic EM characteristics. Many of the spruce root tips were smooth with lighter coloration than lateral roots, while alder tips often had white cottony hyphae and dark areas of coloration, some of which appeared necrotic (See Appendix 3.5). We further examined root tips on the compound scope (40-100X) to verify absence of root hairs and a fungal mantle and the presence of dark hyphae or necrosis. These morphological observations were supported by the molecular data. We successfully extracted DNA from 199 seedlings and found no ectomycorrhizal fungi associated with any seedling (see Appendix 3.4). For roots that did not initially amplify, we performed multiple extractions and PCRs to ensure that seedlings indeed had no fungi associated with the root systems. We observed 115 ribotypes across the two host species and obtained sequence identities for 28 ribotypes, including the most abundant ribotypes in our study. The ribotypes that were matched with sequence IDs included a range of functional groups: seven of these are putative endophytes (including dark septate endophytes), thirteen are putative pathogens, two are saprotrophs, and six were identified to a deeper taxonomic level or were associated with multiple functional roles; none were ectomycorrhizal taxa.

Fungal composition associated with spruce and alder seedlings varied significantly with different factors of the treatment regime. For inoculated spruce seedlings, fungal communities differed by fire-severity category (MRPP, A= 0.081, p=0.009, with pairwise comparisons low vs. moderate A= 0.073, P=0.022; moderate vs. high A= 0.056, P=0.042; low vs. high A= 0.067, P=0.065), but not soil horizon (MRPP, A=-0.012, p=0.859). In contrast, inoculated alder seedlings had similar fungal composition across fire severity categories, and instead fungal

composition varied significantly by soil horizon (MRPP for fire-severity category, A= -0.029, P=0.779; MRPP for soil horizon class, A= 0.068, P=0.015).

To determine the effect of fungal inoculum on seedling biomass we used the axes from NMDS to represent fungal composition (see Appendix 3.6). To reiterate, fungal inoculum is the composition of the fungi that colonized bioassay seedlings. Stepwise regression indicated that the model containing fungal composition represented by Axes one and two generated the best-fit model of those considered for seedling biomass, regardless of treatment. We found that seedling biomass was significantly related to the fungal composition of inoculum for seedlings regardless of the burn severity and soil horizon of a site ($F_{(7)}$ =11.051, P<0.000). We further investigated the effect of fungal composition for each treatment (fire severity and soil-horizon) on mean seedling biomass for alder and spruce seedlings. For alder the model of fungal composition represented by Axis one was the best fit, and fungal composition was significantly related to biomass for alder seedlings ($F_{(1)=}$ 6.791, P<0.021). For spruce the models containing Axis one, Axis two, or Axis three provided an equal fit to spruce seedling biomass. However, fungal composition was not significantly related to spruce seedling biomass for each treatment ($F_{(1)=}$ 0.506, P<0.489). Overall, these results illustrate that fungal composition was significantly related to seedling biomass. Alder biomass showed a strong relationship to the variation in fungal composition for individual treatments, while spruce seedling biomass did not.

There was a positive trend between the proportion of fungi identified as pathogens and fire severity across the gradient (Fig. 3.4, Total $F_{(1)=}$ 4.9106, P=0.0686, R^2=0.45); however, this relationship was stronger for fungi associated with spruce than those associated with alder seedlings (alder $F_{(1)=}$ 1.2742, P=0.3021, R^2=0.18; spruce $F_{(1)=}$ 6.7790, P=0.0405, R^2=0.53). The reduction in seedling biomass across the fire-severity gradient (Fig. 3.3a) corresponds with the

shift in composition towards a greater proportion of pathogens (Fig. 3.4). We saw a decrease in seedling biomass related to the relative abundance of pathogens, ($F_{(2)=}$ 5.639, P=0.020), whereas the relationship between seedling biomass and the relative abundance of the DSE functional group was not significantly correlated ($F_{(2)=}$ 3.025, P=0.086). However, three DSE taxa were positively related to seedling biomass: *Phialocephala fortinii* complex (ribotype 25, $F_{(4)=}$ 5.244, P=0.025), *Cadophora finlandica* (ribotype 93, $F_{(4)=}$ 5.029, P=0.024), and *Phialocephala* sp.(ribotype 90, $F_{(4)=}$ 15.743, P<0.000). Together these results show that as fire severity increases fungal composition shifted to a greater proportion of pathogens and seedling biomass declined. Although some DSE taxa were positively correlated with seedling growth, pathogens had a strong negative influence on seedling biomass.

DISCUSSION

We found that soils collected following the Anaktuvuk River tundra fire were not an effective source of EM inoculum for alder or spruce seedlings. Because of the rarity of large-scale fire disturbances for at least 11,000 years (Higuera *et al.* 2008), mycorrhizal inoculum in Arctic Alaska may be sensitive to fire severity and lack fire-specialist taxa that are either resilient to fire or are stimulated by fire. We know that the site of the ARF has not burned for the preceding 5,000 years (Hu *et al.* 2010), which is in stark contrast to the average fire return interval of 150 years in the boreal forest.

EM host plants can facilitate seedling inoculation and establishment by providing a source of mycelium (Selosse *et al.* 2006). Mycelial inoculum sources may be limited in tundra because EM host plants can occur in low densities depending on the tundra vegetation classification, and after fire EM shrub densities can take up to a decade to return to pre-fire

densities (Wein & Bliss 1973). Seedling inoculation and establishment outside of the rooting zone of EM tundra shrubs is restricted by the availability of spores, sclerotia, or other components of the post-fire resistant propagule community (RPC) (Taylor & Bruns 1999). However, the presence of the RPC after fire does not ensure successful inoculation if host plants are not present (Ishida *et al.* 2008) or spores are in low densities (Castellano *et al.* 1985). In another study we observed that resprouting tundra shrubs operate as refugia for late-stage EM fungi regardless of fire severity, thus serving as potential EM nurse plants and an important inoculum source for spruce and alder colonization of post-fire tundra sites (Hewitt *et al.,* 2013). Therefore, mycorrhizal shrubs are a more likely source of inoculum than is the RPC following tundra fires.

The lack of mycorrhizal development in our study could also have been an artifact of the growth chamber conditions. Fertilization, small seedling size, and the storage of inoculum may have inhibited mycorrhizal development. However, other studies have shown that, consistent with our methods, the intermittent application of soluble fertilizer (Castellano *et al.* 1985) and the storage of soil inoculum (Nunez *et al.* 2009) or spore slurries (Castellano *et al.* 1985) for comparable or longer time periods does not inhibit the formation of mycorrhizas on first-year seedlings. Although inoculation with unburned soils in our bioassay study would have allowed us to test this, the focus of this study was the effect of fire severity not fire occurrence on fungal inoculum potential. Recent studies indicate there are fungal taxa compatible with boreal tree and shrub species across the low arctic in unburned sites (Reithmeier & Kernaghan 2013, Timling *et al.* 2014).

In addition to the lack of EM inoculum, seedling performance was likely further limited by the detrimental effects of pathogens observed in the ARF soil inoculum. The significant

colonization of seedlings by pathogens may relate to the ability of pathogens to disperse more widely and more quickly than many EM fungi (Aylor 2003; Peay *et al.* 2012). EM colonization can block or dampen pathogen colonization of roots (Smith & Read 2008); however, the potential role of pseudomycorrhizal taxa, such as DSE, in reducing pathogen colonization is not known. Colonization of roots by DSE is suggested to improve plant nutrition and biomass (Newsham *et al.* 2009), which may indirectly reduce colonization by and effects of pathogenic fungi of seedling growth. Indeed, we found the relative abundance of three DSE taxa to be positively related to seedling biomass. Yet, overall, the relative abundance of pathogenic fungi had the stronger affect on seedling biomass. Thirty-eight percent (5/13) of the sequenced fungi we identified to at least the genus level as pathogens were also observed in soils along a trans-Arctic transect (Timling *et al.*, 2014) This suggests that the pathogens found in our study occur across the Arctic, and that pathogen propagules are robust, surviving for long periods in the soil, or distribute rapidly in tundra. Of the DSE we identified in our bioassay, 100% (11/11) were also observed in soils across the trans-Arctic transect (Timling *et al.* 2014). Though DSE are widespread and can be more frequent at high-latitudes than mycorrhizal fungi (Newsham *et al.* 2009), their proportional decline across the fire-severity gradient suggests that they are sensitive to fire disturbance, similar to mycorrhizal fungi. The proportional shift towards more pathogenic fungal symbionts with an increase in fire severity corresponds with reduced growth for both alder and spruce inoculated with field soils. The capacity of a species to invade a novel environment can depend on resource-use strategy and pathogen release outside the plant's current range. Boreal forest trees migrating into tundra or tundra shrubs expanding into non-shrubby tundra may exhibit low levels of pathogen-release similar to other stress-tolerating species (Blumenthal *et al.* 2009).

In concert with the effect of host plant life history strategy, disturbance severity can alter composition of microbial symbionts, thus impacting plant range expansion. Although several edaphic factors, including nutrients or pheonolics, could lead to changes in seedling biomass, we believe that the effect of fire severity on seedling biomass reflects changes in fungal composition in the soil across the fire-severity gradient (Fig. 3.5a). We dismiss the idea of a nutrient effect because the amount of field soil provided for inoculation was small (12 ml), and all seedlings in the experiment were lightly fertilized. Furthermore, phenolic-effects are not likely because fire consumption of the organic horizon volatilizes inhibitory allelochemicals and phenolics in litter and soil (Neary *et al.* 1999), and charcoal sorbs inhibitory compounds (Zackrisson *et al.* 1996). This suggests that a fire effect on fungal community composition is the most likely explanation for the fire-severity effect on seedling biomass. Along these lines, we observed reduced seedling biomass and changes in the proportion of functional groups of fungi as fire severity increased across the gradient of sites. In addition, seedling biomass was related to fungal composition. Hence, we believe that decreased biomass along the fire-severity gradient is attributable to changes in fungal composition related to fire severity.

The influence of fire severity, however, differed between the two host species. Alder seedling biomass declined as expected with increasing fire severity as the proportion of fungal pathogens increased. There was a strong relationship between fungal composition and mean biomass for each treatment, which appears to drive the effect of fire severity on alder seedling biomass. Spruce seedlings, however, responded to fire severity in a more complex way. Spruce biomass declined with increasing fire severity, though to a lesser degree than alder, and the mean biomass for each treatment did not significantly correlate to fungal composition. Instead of

observing declines in spruce seedling biomass with increasing fire severity for both soil horizons as we expected, we observed sensitivity to fire-severity effects only in mineral soils.

The different response of spruce biomass to inoculation with organic and mineral soils was a surprising result, as we did not find distinct fungal communities between the organic and mineral soil horizons. We hypothesize two potential explanations for this observation: 1) There is another landscape factor that is highly correlated with fire severity influencing spruce biomass, or 2) There is one fungal species that is exerting a strong species effect on spruce growth. However, analysis of the ribotypes that were matched with sequences did not reveal an obvious taxon that could be driving the lack of a fire-severity effect on biomass for spruce seedlings inoculated with organic soils. Overall, our findings illustrate that increases in tundra fire severity reduce seedling growth, likely through growth reduction by fungal pathogens. With respect to plant migration into areas beyond their current range, we anticipate the negative secondary effects on seedling growth resulting from fire severity effects on fungal composition will outweigh the potential positive effects due to pathogen release or the benefits of mycorrhizal or dark septate endophytes. Negative secondary effects thus influence the success of seedling establishment beyond the native range into non-shrubby tundra.

Our results suggest that inoculum effects may be most important to landscape-scale vegetation establishment when fires are high-severity and burn down to mineral soil. In the boreal forest, Johnstone & Chapin (2006) observed decreased tree seedling establishment at extremely high-severity sites. These sites are expected to have open microsites for seedling establishment that can facilitate range expansion; yet, these high-severity microsites may actually exhibit a shift in mycobiont composition that reduces recruitment levels to lower than what has been projected for treeline and tundra (Fig. 3.5b). The post-fire studies from the boreal forest that

show increased coniferous and deciduous tree seedling establishment on exposed mineral soil (Johnstone & Chapin 2006) were conducted in sites where EM nurse plants were nearby and sources of EM fungi were not limited. In parallel with these boreal studies, alder productivity is expected to increase in tundra, especially on previously burned sites (Lantz *et al.* 2010). In contrast, we found a decline in alder seedling biomass with increasing fire severity. Currently, models of post-fire tundra vegetation dynamics are parameterized with abiotic factors such as climate and substrate quality (Rupp, personal communication). Our results suggest that the effects of fire severity on soil biota may dampen or even reverse the expected increases in tree and shrub establishment after fire.

Both spruce migration and alder expansion have large ecosystem impacts due to changes in carbon storage, albedo, ecosystem services, and nutrient cycling. In particular, the expansion of alder has a significant influence on nitrogen and phosphorus cycling because of its role as a nitrogen fixer. On a global scale, Harsch *et al.* (2009) found that 2 of 166 treeline sites receded since 1900 AD and that both of these sites showed evidence of disturbance. These authors infer that disturbance legacies do not likely affect the probability of advance, and instead influence initial recruitment and lag times between warming and treeline advance. However, the effects of warming on future fire regime should not be underestimated, and predictions of vegetation response should account for changes in fire regime, including fire return interval and severity. Currently 2.3% of tundra has been converted to forest in Alaska (Chapin *et al.* 2005). From our study, we would expect the rate of tree migration and shrub expansion to be limited by pathogenic affects of soil biota and mutualist limitation after high-severity fires. However, if boreal EM fungi co-migrate or EM tundra shrubs provide surrogate sources of inoculum on the landscape under low and moderate severity fires, we would expect that boreal forest mycobionts

may then facilitate vegetation change at and beyond current treeline, reducing lag times by facilitating initial recruitment. Thus, mutualistic and pathogenic symbionts may both constrain and facilitate vegetation change in a context-dependent, but powerful manner.

ACKNOWLEDGEMENTS

This research was supported by funding from Alaska EPSCoR (EPS-0701898) and the state of Alaska, a National Science Foundation Graduate Research Fellowship (DGE-0639280 and 1242789), and a Center for Global Change student award to REH. In-kind support was provided by the Bonanza Creek Long-Term Ecological Research site, U.S. Forest Service PNW Research Station, UAF IAB greenhouses, and Bureau of Land Management. We thank Randi Jandt, Eric Miller, and David Yokel for field and logistical support, John Alden for our seed source, and Jamie Hollingsworth for creating Figure 3.1.

REFERENCES

1.

Aylor, D.E. (2003). Spread of plant disease on a continental scale: role of aerial dispersal of pathogens. *Ecology*, 84, 1989-1997.

2.

Bent, E., Kiekel, P., Brenton, R. & Taylor, D.L. (2011). Root-associated ectomycorrhizal fungi shared by various boreal forest seedlings naturally regenerating after a fire in Interior Alaska and correlation of different fungi with host growth responses. *Appl. Environ. Microbiol.*, 77, 3351-3359.

3.

Bent, E. & Taylor, D.L. (2010). Direct amplification of DNA from fresh and preserved ectomycorrhizal root tips. *J. Microbiol. Methods*, 80, 206-208.

4.

Berry, K.J., Kvamme, K.L. & Mielke, P.W. (1983). Improvements in the permutation test for the spatial-analysis of the distribution of artifacts into classes. *American Antiquity*, 48, 547-553.

5.

Blumenthal, D., Mitchell, C.E., Pysek, P. & Jarosik, V. (2009). Synergy between pathogen release and resource availability in plant invasion. *Proc. Natl. Acad. Sci. U.S.A.*, 106, 7899-7904.

6.

Booth, M.G. (2004). Mycorrhizal networks mediate overstorey-understorey competition in a temperate forest. *Ecol. Lett.*, 7, 538-546.

7.

Castellano, M.A., Trappe, J.M. & Molina, R. (1985). Inoculation of container-grown Douglas-fir seedlings with basidiospores of *Rhizopogon vinicolor* and *R. colossus*: effects of fertility and spore application rate. *Can. J. For. Res*., 15, 10-13.

8.

Chapin, F.S., Sturm, M., Serreze, M.C., McFadden, J.P., Key, J.R., Lloyd, A.H., *et al.* (2005). Role of land-surface changes in Arctic summer warming. *Science*, 310, 657-660.

9.

Connell, J.H. (1971). On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In: *Dynamics of Population: Proceedings of the advanced study institute on dynamics of numbers of populations* (eds. Den Boer, P.J. & Gradwell, G.R.). Center for Agricultural Publishing and Documentation. Wageningen, pp. 298-312.

10.

Harsch, M.A. & Bader, M.Y. (2011). Treeline form - a potential key to understanding treeline dynamics. *Global Ecol. and Biogeogr.*, 20, 582-596.

11.

Harsch, M.A., Hulme, P.E., McGlone, M.S. & Duncan, R.P. (2009). Are treelines advancing? A global meta-analysis of treeline response to climate warming. *Ecol. Lett.*, 12, 1040-1049.

12.

Hewitt R.E., Bent E., Hollingsworth T.N., Chapin F.S. & Taylor D.L. (2013). Resilience of Arctic Mycorrhizal Fungal Communities after Wildfire Facilitated by Resprouting Shrubs. *Ecoscience*, 20, 296-310.

13.

Higuera, P.E., Brubaker, L.B., Anderson, P.M., Brown, T.A., Kennedy, A.T. & Hu, F.S. (2008). Frequent fires in ancient shrub tundra: implications of paleorecords for arctic environmental change. *PLoS One*, 3, Article No.: e0001744.

14.

Hinzman, L.D., Bettez, N.D., Bolton, W.R., Chapin, F.S., Dyurgerov, M.B., Fastie, C.L., *et al*. (2005). Evidence and implications of recent climate change in northern Alaska and other arctic regions. *Climatic Change*, 72, 251-298.

15.

Horton, T.R., Bruns, T.D. & Parker, V.T. (1999). Ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment. *Can. J. Bot*., 77, 93-102.

16.

Horton, T.R. & van der Heijden, M.G.A. (2008). The role of symbioses in seedling establishment and survival. In: *Seedling Ecology and Evolution* (eds. Leck, M.A., Parker, V.T. & Simpson, R.L.). Cambridge University Press, Cambridge, UK, pp. 189-213.

17.

Hu, F.S., Higuera, P.E., Walsh, J.E., Chapman, W.L., Duffy, P.A., Brubaker, L.B. *et al*. (2010). Tundra burning in Alaska: Linkages to climatic change and sea ice retreat. *J. Geophys. Res.–Biogeo.*, 115, G04002, doi:10.1029/2009JG001270.

18.

Ishida, T.A., Nara, K., Tanaka, M., Kinoshita, A. & Hogetsu, T. (2008). Germination and infectivity of ectomycorrhizal fungal spores in relation to their ecological traits during primary succession. *New Phytol.*, 180, 491-500.

19.

Jandt, R.R., Miller, A.E., Yokel, D.A., Bret-Harte, M.S., Kolden, C.A. & Mack, M.C. (2012). Findings of Anaktuvuk River Fire Recover Study. In: *BLM Technical Report*. U.S. Department of the Interior, Bureau of Land Management, Alaska Fire Service, Ft. Wainwright, Alaska, USA, pp. 1-39.

20.

Janzen, D.H. (1970). Herbivores and the number of tree species in tropical forests *Am. Nat.*, 104, 501-528.

21.

Johnstone, J.F. & Chapin, F.S. (2006). Effects of soil burn severity on post-fire tree recruitment in boreal forest. *Ecosystems*, 9, 14-31.

22.

Johnstone, J.F., Chapin, F.S., Hollingsworth, T.N., Mack, M.C., Romanovsky, V. & Turetsky, M. (2010). Fire, climate change, and forest resilience in interior Alaska. *Can. J. For. Res.*, 40, 1302-1312.

23.

Klironomos, J. (2002). Feeback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, 417, 67-70.

24.

Kruskal, J.B. (1964). Nonmentric multidimensional scaling: a numerical method. *Psychometrika*, 29, 115-129.

25.

Landhausser, S.M. & Wein, R.W. (1993). Postfire vegetation recovery and tree establishment at the arctic treeline: climate-change-vegetation-response hypotheses. *J. Ecol.*, 81, 665-672.

26.

Lantz, T.C., Gergel, S.E. & Henry, G.H.R. (2010). Response of green alder (*Alnus viridis* subsp *fruticosa*) patch dynamics and plant community composition to fire and regional temperature in north-western Canada. *J. Biogeogr,*, 37, 1597-1610.

27.

Lloyd, A.H., Fastie, C.L. & Eisen, H. (2007). Fire and substrate interact to control the northern range limit of black spruce (*Picea mariana*) in Alaska. *Can. J. For. Res.*, 37, 2480-2493.

28.

Mack, M.C., Bret-Harte, M.S., Hollingsworth, T.N., Jandt, R.R., Schuur, E.A.G., Shaver, G.R. *et al.* (2011). Carbon loss from an unprecedented Arctic tundra wildfire. *Nature*, 475, 489-492.

29.

Mangan, S.A., Schnitzer, S.A., Herre, E.A., Mack, K.M.L., Valencia, M.C., Sanchez, E.I. *et al*.. (2010). Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature*, 466, 752-U10.

30.

McCune, B. & Grace, J.B. (2001). *Analysis of Ecological Communities*. 2nd edn. MjM Software Design, Gleneden Beach, Oregon, USA, pp. 1-300.

31.

Molina, R., Massicotte, H. & Trappe, J.M. (1992). Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In: *Mycorrhizal functioning: An integrative plant-fungal process* (ed. Allen, M.F.). Chapman and Hall, New York, New York, USA, pp. 357-423.

32.

Myers-Smith, I.H., Forbes, B.C., Wilmking, M., Hallinger, M., Lantz, T., Blok, D., *et al*. (2011). Shrub expansion in tundra ecosystems: dynamics, impacts and research priorities. *Environ. Res. Lett*., 6, 045509 doi:10.1088/1748-9326/6/4/045509.

33.

Neary, D.G., Klopatek, C.C., DeBano, L.F. & Ffolliott, P.F. (1999). Fire effects on belowground sustainability: a review and synthesis. *For. Ecol. Manage*., 122, 51-71.

34.

Newsham, K.K., Upson, R. & Read, D.J. (2009). Mycorrhizas and dark septate root endophytes in polar regions. *Fungal Ecol.*, 2, 10-20.

35.

Nunez, M.A., Horton, T.R. & Simberloff, D. (2009). Lack of belowground mutualisms hinders Pinaceae invasions. *Ecology*, 90, 2352-2359.

36.

Parker, T.J., Clancy, K.M. & Mathiasen, R.L. (2006). Interactions among fire, insects and pathogens in coniferous forests of the interior western United States and Canada. *Agric. For. Entomol*., 8, 167-189.

37.

Peay, K.G., Schubert ,M.G., Nguyen, N.H. & Bruns, T.D. (2012). Measuring ectomycorrhizal fungal dispersal: macroecological patterns driven by microscopic propagules. *Mol. Ecol*., 21, 4122-4136.

38.

Racine, C., Jandt, R., Meyers, C. & Dennis, J. (2004). Tundra fire and vegetation change along a hillslope on the Seward Peninsula, Alaska, USA. *Arct., Antarc., Alp. Res*., 36, 1-10.

39.

Reithmeier L. & Kernaghan G. (2013). Availability of Ectomycorrhizal Fungi to Black Spruce above the Present Treeline in Eastern Labrador. *PLoS One*, 8.

40.

Rupp, T.S., Starfield, A.M. & Chapin, F.S. (2000). A frame-based spatially explicit model of subarctic vegetation response to climatic change: comparison with a point model. *Landsc. Ecol.*, 15, 383-400.

41.

Selosse M.A., Richard F., He X.H. & Simard S.W. (2006). Mycorrhizal networks: des liaisons dangereuses? *Trends Ecol. Evol*., 21, 621-628.

42.

Smith, J.E., McKay, D., Brenner, G., McIver, J. & Spatafora, J.W. (2005). Early impacts of forest restoration treatments on the ectomycorrhizal fungal community and fine root biomass in a mixed conifer forest. *J. Appl. Ecol*., 42, 526-535.

43.

Smith S.E. & Read D.J. (2008). *Mycorrhizal Symbiosis*. 3rd edn. Academic Press, New York, New York, USA, pp. 1-787.

44.

Sturm, M., Racine, C. & Tape, K. (2001). Increasing shrub abundance in the Arctic. *Nature*, 411, 546-547.

45.

Taylor, D.L. & Bruns, T.D. (1999). Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. *Mol. Ecol.*, 8, 1837-1850.

46.

Taylor, D.L. & Houston, S. (2011). A Bioinformatics Pipeline for Sequence-Based Analyses of Fungal Biodiversity. In: *Fungal Genomics* (eds. Xu, J-R & Bluhm, B.H.). Humana Press, New York, New York, USA, pp. 141-155.

47.

Timling, I., Dahlberg, A., Walker, D.A., Gardes, M., Charcosset, J.Y., Welker, J.M. *et al.*. (2012). Distribution and drivers of ectomycorrhizal fungal communities across the North American Arctic. *Ecosphere*. 3:art111. http://dx.doi.org/10.1890/ES12-00217.1

48.

Timling I., Walker D.A., Nusbaum C., Lennon N.J. & Taylor D.L. (2014). Rich and cold: Diversity, distribution and drivers of fungal communities in patterned-ground ecosystems of the North American Arctic. *Molecular Ecology*, Accepted.

49.

Treseder, K., Mack, M. & Cross, A. (2004). Relationships among fires, fungi and soil dynamics in Alaskan boreal forests. *Ecol. Appl.*, 14, 1826-1838.

50.

Viereck, L.A., Dyrness, C.T., Batten, A.R. & Wenzlick, K.J. (1992). The Alaska vegetation classification. In: *General Technical Report PNW-GTR-286.* U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, Oregon, USA, p. 278.

51.

Wein, R.W. & Bliss, L.C. (1973). Changes in Arctic *Eriophorum* tussock communities following fire. *Ecology*, 54, 845-852.

52.

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

FIGURES

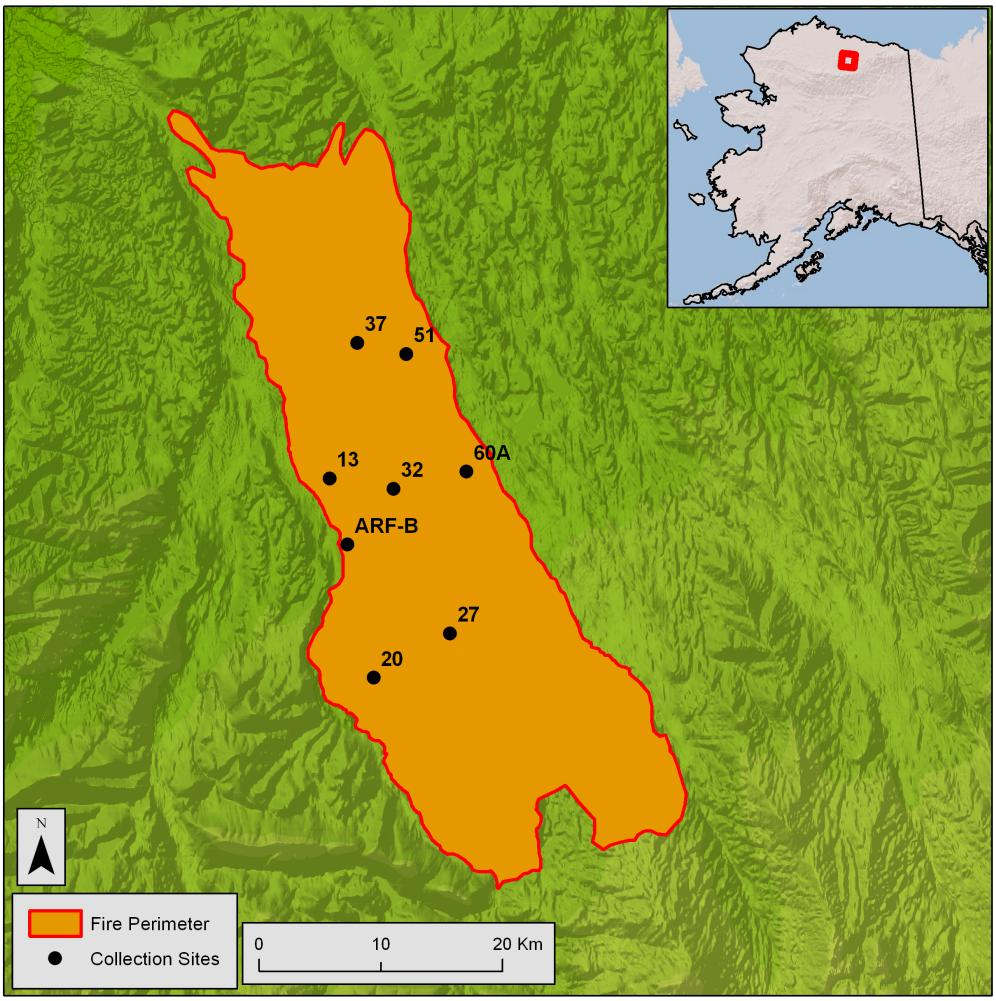


Figure 3.1. Map of soil collection sites that represent a fire-severity gradient within the Anaktuvuk River Fire burn scar: low severity = sites 27 and 51; moderate severity = sites 20, 32, and ARF-B; high severity = sites 13, 37, and 60A.

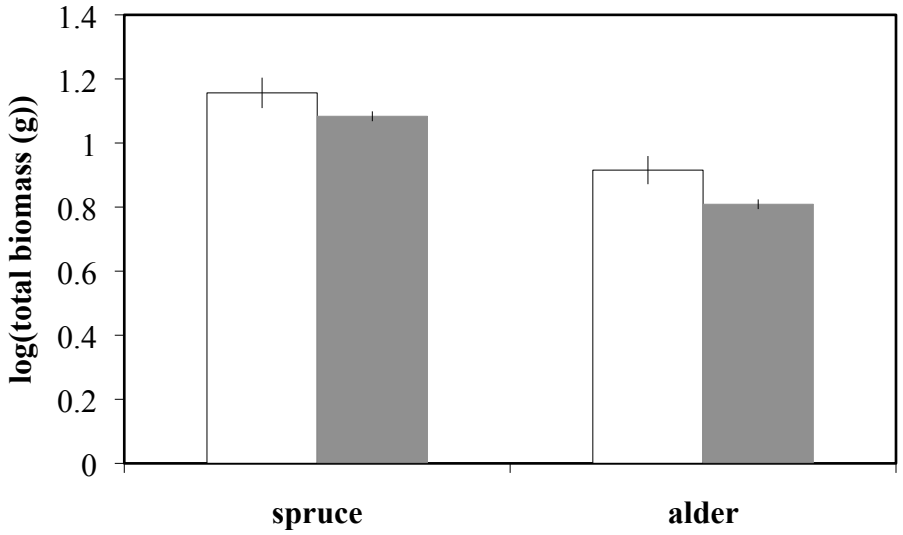


Figure 3.2. Inoculation reduces seedling biomass for spruce and alder seedlings. Open = biomass of all seedlings with sterilized treatment; Filled = all seedlings inoculated with field soils.* indicates significant differences between inoculated and sterile.

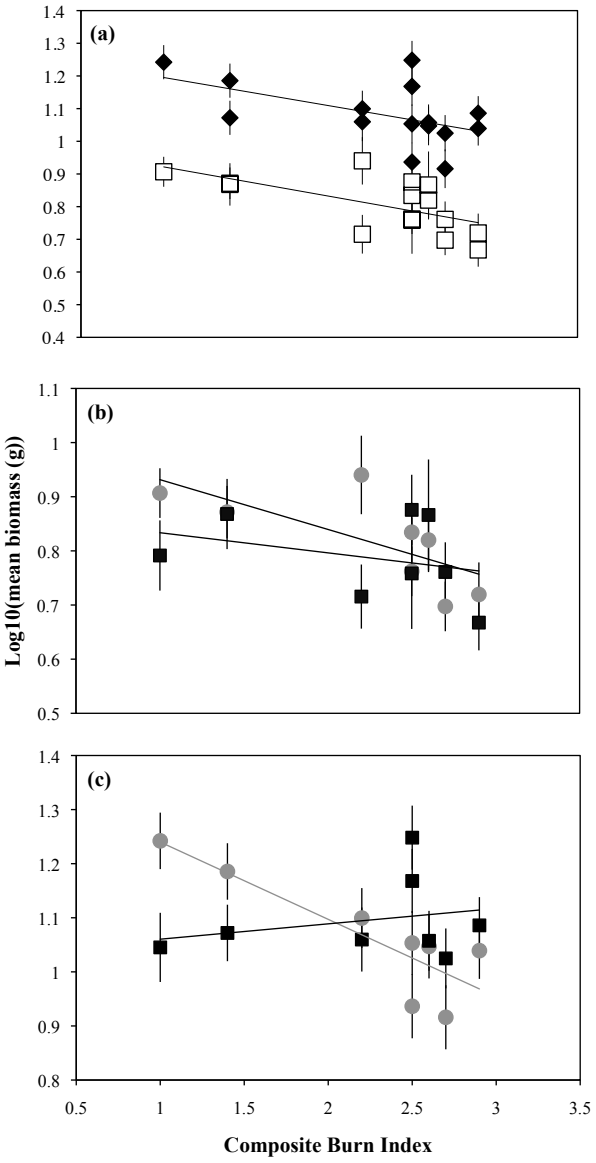


Figure 3.3 a-c. Effects of fire severity on seedling biomass; a: treatment means for spruce and alder biomass decline with increasing fire severity; b. alder biomass declines with increasing fire severity for seedlings grown with inoculum from mineral and organic soil horizons; c. spruce biomass declines with increasing fire severity for seedlings grown with inoculum from the mineral soil horizon. Black diamonds = spruce; open squares = alder; grey circles = mineral soil treatments; black squares = organic soil treatments.

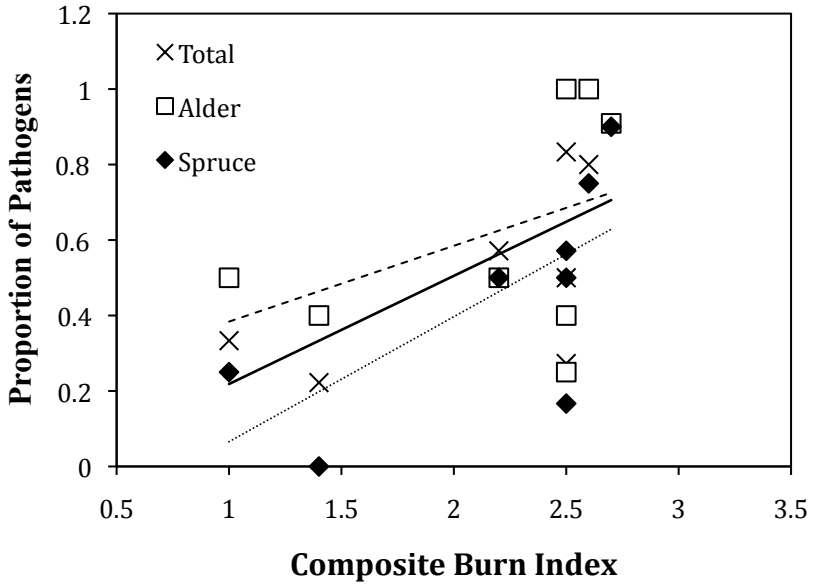


Figure 3.4. Proportion of identified ribotypes that are pathogenic vs. non-pathogenic fungi across the fire-severity gradient. X and solid trend line = total proportion of pathogens occurring with spruce and alder; open squares and dashed trend line = proportion of pathogens occurring with alder; filled diamonds with dotted trend line = proportion of pathogens occurring with spruce. Total $R^2 = 0.44753$; Alder $R^2 = 0.17517$; Spruce $R^2 = 0.47595$.

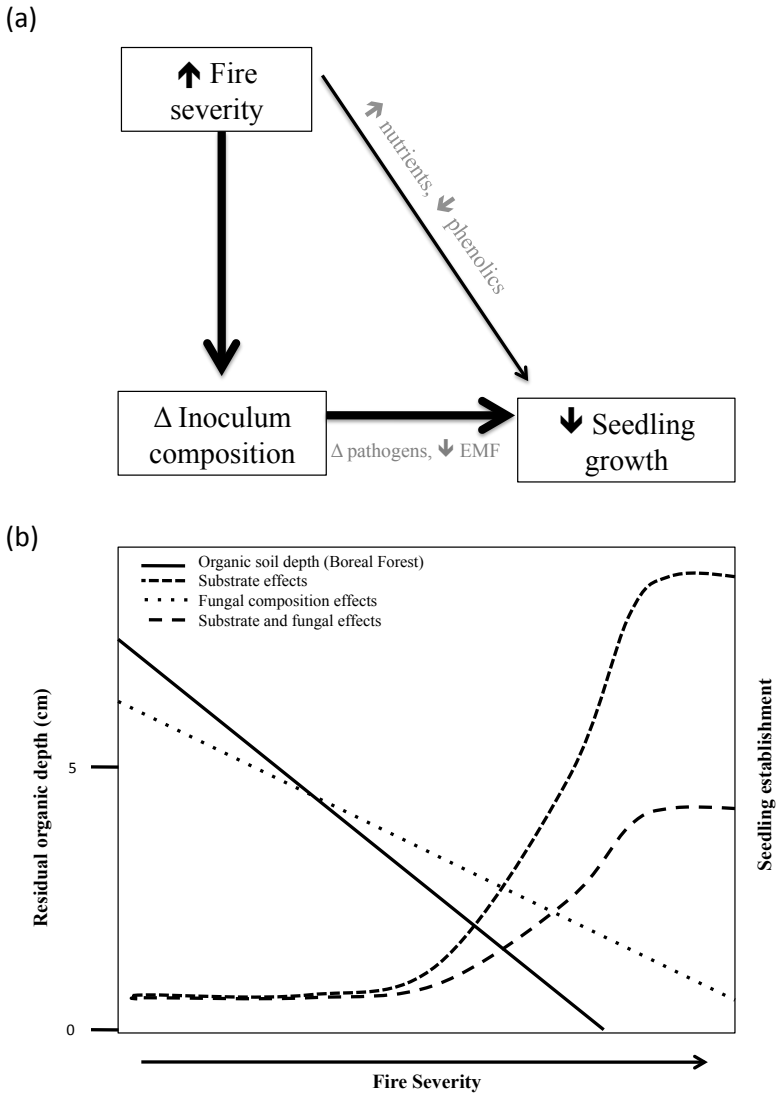


Figure 3.5. Fire-severity effects on vegetation change in the Arctic can be assessed through (a) factors influencing seedling growth and (b) inferences on the net outcome of substrate and microbial community factors on seedling establishment in non-shrubby tundra. Previous research indicates that post-fire substrate availability drives seeding recruitment. Our research suggests that fire-severity effects on soil biota may dampen the positive effects of substrate availability after fire in severely burned sites with no EMF inoculum. Together these finding suggest the influence of post-fire soil biota on seedling biomass is context-dependent.

Appendix 3.1. Site descriptions for eight sampling sites grouped by fire-severity categories within the Anaktuvuk River Fire burn scar. We classified vegetation communities using the Viereck (1992) vegetation classification: 2C2A= open low scrub mixed shrub-sedge tussock tundra, 2C2H=open low scrub willow-sedge shrub tundra, 3A21=mesic graminoid herbaceous sedge-birch tundra, 3A2D=mesic graminoid herbaceous tussock tundra, and 3A3=wet graminoid herbaceous tundra.

Fire Severity	Site	Location	Site Description
Low	27	69.0545 ° N, 150.594 ° W	Wet meadow site with *Betula nana*, *Ledum palustre*, *Rubus chamaemorus*, sphagnum mosses, and non-tussock forming sedges; 3A2I
	51	69.256 ° N, 150.648 ° W	Moist lowland site with *B. nana* and *Salix* sp. shrubs, *Carex bigelowii*, and *Eriophorum* sp. tussocks; 2C2A
Moderate	20	69.019° N, 150.754 ° W	Moist site on a north hillslope with *B. nana, R. chamaemorus*, *Eriophorum* sp. tussocks, and *Polygonum bistortoides* (Pursh); 2C2A
	32	69.159° N, 150.691 ° W	Wet sedge meadow site with *B. nana*, *R. chamaemorus*, and *Carex* sp. in a polygonated valley bottom; 3A3
	ARF-B	69.120° N, 150.790 ° W	Moist site with *Eriophorum vaginatum* tussocks and *Alnus viridis* (Chaix) DC. in adjacent unburned site across a creek; 2C2A
High	13	69.172° N, 150.819° W	Moist site with *Eriophorum* sp. tussocks, *Saussurea angustifolia* (Wild) D.C., *B. nana* shrubs, and combusted sphagnum mosses; 2C2A
	37	69.271 ° N, 150.753° W	Moist ridge top site with exposed mineral soil, *B. nana* and *Salix* sp. shrubs, and *Eriophorum* sp. tussocks; 3A2D
	60A	69.175 ° N, 150.538 ° W	Moist site with exposed mineral soil, *B. nana* and *Salix* sp. shrubs, *L. palustre, Carex* sp. sedges and *Eriophorum* sp. tussocks; 3A2H

Appendix 3.2. Biological key showing ribotype ID, abundance, range of fungal ITS fragment lengths, associated OTU identity, and the best match description as reported in GenBank.

Ribotype ID	Abundance	Range of fragment lengths	OTU assignment	Best match description
11	45	581	OTU 1	*Pyrenochaeta cava*
			OTU12	*Phoma herbarum*
			Singlet15	*Cudoniella* sp.
			Singlet 30	Basidiomycota
31	39	617-618	OTU3	*Pyrenochaeta* sp.
25	18	601-603	OTU 13	*Phialocephala fortinii* complex
41	17	640	OTU 5	*Phoma* sp.
33	12	622-623	OTU 2	*Phialophora lagerbergii*
			Singlet 3	*Phialophora lagerbergii*
			Singlet 27	*Phialophora lagerbergii*
20	10	594		
93	9	928-929	OTU 11	*Cadophora finlandica*
			OTU 15	*Phialophora finlandica*
14	8	585	OTU 7	Helotiaceae sp.
			Singlet 7	Helotiaceae sp.
15	6	587		
87	6	906		
90	6	918-920	OTU 8	*Phialocephala* sp.
12	5	582	Singlet 8	Vibrisseaceae sp.
17	5	590	Singlet 5	*Pezizella* sp.
			Singlet 13	*Rhizoscyphus erica*
18	5	591		
24	5	600	OTU 10	*Coniochaeta* sp.
			Singlet 20	Herpotrichiellaceae sp.
91	5	923		
19	4	593		
29	4	610		
38	4	633	Singlet 10	*Phaeosphaeria* sp.
76	4	872		
94	4	930		
99	4	999	OTU 9	*Pyrenochaeta* cava
5	3	545		
7	3	574	Singlet 17	Nectriaceae
16	3	589		
23	3	598	OTU 6	*Paraphoma* sp.
27	3	605	Singlet 19	*Setomelanomma* sp.
28	3	608	Singlet 9	*Alternaria* sp.
45	3	667		

Appendix 3.2. continued

50	3	685-686	OTU14	Ceratobasidiaceae sp.
68	3	774		
71	3	783		
82	3	882	OTU 4	*Articulospora tetracladia*
4	2	542		
8	2	575		
22	2	596		
32	2	620	Singlet 22	Fungi
34	2	625		
36	2	628		
46	2	670		
58	2	726	Singlet 29	*Candida parapsilosis*
63	2	748		
67	2	765		
69	2	776		
73	2	817		
74	2	823		
78	2	875		
85	2	900	Singlet 6	*Phialocephala* sp.
95	2	935		
100	2	1006		
110	2	1105	Singlet 14	*Phaeosphaeria* sp.
115	2	1175		
1	1	504		
2	1	505		
3	1	520		
6	1	548		
9	1	576		
10	1	579		
13	1	583		
21	1	595	Singlet 11	*Verticillium dahlia*
26	1	604		
30	1	615		
35	1	626		
37	1	629		
39	1	534		
40	1	636		
42	1	643		
43	1	659		
44	1	660		
47	1	671		
48	1	673	Singlet 12	*Exophiala pisciphila*
49	1	682	Singlet 25	*Debaryomyces* sp.

Appendix 3.2. continued

51	1	694		
52	1	697		
54	1	701	Singlet 23	*Pezizella* sp.
55	1	706		
56	1	712		
57	1	724		
59	1	732		
60	1	739		
61	1	742		
62	1	745		
64	1	752		
65	1	755		
66	1	758		
70	1	781		
72	1	813		
75	1	849		
77	1	873		
79	1	877		
80	1	878		
81	1	880		
83	1	885		
84	1	895		
86	1	904		
88	1	907		
89	1	910		
92	1	925		
96	1	951		
97	1	955		
98	1	973		
101	1	1023		
102	1	1037		
103	1	1043	Singlet 1	Orbiliales sp.
104	1	1054		
105	1	1062		
106	1	1086		
107	1	1091	Singlet 16	*Cladosporium* sp.
108	1	1095		
109	1	1103		
111	1	1114		
112	1	1126		
113	1	1144		
114	1	1157		
116	1	1185		

Appendix 3.3. Detailed methods of molecular techniques used to characterize fungal communities.

DNA EXTRACTION

Root tips were suspended in 250 µl lysis buffer and ground in a 0.6 ml microfuge tube with a Kontes pellet pestle (Kimble Chase, Vineland, NJ, USA). We extracted DNA using the Qiagen DNEasy Plant Mini Kit (*QIAGEN* Inc., Valencia, California, USA) according to the manufacturer's instructions. DNA extraction was repeated 2-3 times for samples that showed no ARISA peaks to confirm that no fungal DNA was present.

ARISA

Twenty-five µl PCR reactions were prepared containing 0.65 mM $MgCl_2$, 0.2 mM dNTPs, 0.05 µM forward primer FAM-ITS1F (CTTGGTCATTTAGAGGAAGTAA (Gardes and Bruns 1993) labeled on the 5' end with FAM, a fluorescein amidit (Applied Biosystems, Carlsbad, CA)), 0.05 µM reverse primer ITS4 (TCCTCCGCTTATTGATATGC (White *et al.* 1990), 0.06 mg/ml bovine serum albumin, 0.15 µl JumpStart RED Taq (Sigma-Aldrich, St. Louis, MO, USA), 1X JumpStart RED Taq buffer and 5 µl of DNA extract. Reactions were prepared in 0.2 µl tubes and ran in an MJ Research PTC-225 thermal cycler as follows: 96°C for 3 min, 35 cycles of 94°C for 30s, 52°C for 30s, then 72°C for 3 min, followed by 72°C for 10 min (Bent and Taylor 2010). We diluted the post-PCR DNA extracts (1/10) in ultrapure water based on our own optimization procedure for adjusting PCR product to concentrations appropriate for comparison with the size standard used for ARISA. One microliter of each sample was then mixed with 14.25 µl of formamide and 0.75 µl of size standard (MapMarker 1000 X-rhodamine, BioVentures, Murfreesboro, TN, USA). These reactions were heated to 95ºC for 5 minutes, then immediately placed on ice until run through an ABI 3100 Genetic Analyzer (Applied Biosystems,

Foster City, NJ, USA; Pop6, 50cm array, T-RFLP_1500 protocol). PCR reactions were repeated 2-3 times for samples that showed no ARISA peaks to confirm that no fungal DNA was present.

ITS SEQUENCING

To assign taxonomic identities to the dominant ARISA fragments, fungal ITS gene region sequences were obtained by PCR amplification, as described earlier, except that the PCR primers did not have a fluorescent moiety added to the forward primer. Fungal ITS sequences were obtained directly from pooled root samples in cases where only one dominant ARISA peak was seen. PCR products were shipped on wet ice overnight for sequencing at Functional Biosciences Inc. (Madison, WI, USA).

BIOINFORMATICS

Sequences were assembled in Codoncode Aligner 3.7 (CodonCode Corporation, Dedham, MA, USA) using PHRAP. We used in-house perl scripts to mask low-quality bases based on phred scores (cutoff Q20), orient, and purge sequences containing >3% Ns after end-trimming**.** We grouped sequences into Operational Taxonomic Units (OTUs) using CAP3 (Huang and Madan 1999) at 97% sequence similarity. A representative sequence was selected for each OTU after manual inspection in SeAl alignment software (Rambaut 2002). To assign taxonomic identities we compared the representative sequence for each OTU to ITS sequences from GenBank, utilizing a curated specimen fungal ITS search filter (http://www.borealfungi.uaf.edu/). The top 10 hits from the BLAST search were assessed for the coverage between the query and the hit sequences and the % identity. When the top 10 hits did not have high coverage, % identity, or consistency in identification, we built maximum likelihood trees to resolve the identity of our queried sequence using the top vouchered and isolate sequences from the curated database on the Fungal Metagenomics Project website

(http://www.borealfungi.uaf.edu/). To construct trees, we aligned sequences in MUSCLE (Edgar, 2004) and used the maximum likelihood method with default settings (GTR+G+I) in Garli v.1.0 (Zwickl 2006). We manipulated the tree, including midpoint rooting, in FigTree v1.3.1 (Rambaut 2009). Nomenclature for each OTU follows Timling *et al.* (2012). Sequences for each OTU have been archived with GenBank under accession numbers listed in (see Appendix 3.4.). Sequence lengths were computed using the DNA stats function under the sequence analysis menu at DNA 2.0 Bioinformatics toolbox (https://www.dna20.com/index.php?pageID=216). Direct sequences were matched up with the dominant ribotype in the ARISA profile based on fragment length.

We used GeneMapper 3.7 (Applied Biosystems, Foster City, NJ, USA) to read each ARISA electropherogram. In our study, ARISA was used to estimate community composition by determining the fragment length heterogeneity of the nuclear ribosomal ITS region for fungi present in the pooled community sample. The peak heights for each fragment were relativized by dividing the fluorescence height for each peak by the total fluorescence height for a sample profile (Fisher and Triplett 1999). Fragment length indicates the identity of a fungal taxon (henceforth 'ribotype') and peak height gives a measure of the relative abundance of that taxon. Raw fragment sizes and associated peak heights were exported from GeneMapper. We used raw ARISA ribotypes lengths and analysis of corresponding OTU sequence lengths to bin fragment reads into surrogates for fungal "species" (see Appendix 3.2). In general, ARISA ribotypes were binned at 1 bp bin size where fragment size was rounded to the nearest integer. Fragment detections within 0.5 bp are accepted to belong to the same ribotype, because the machine error in size estimation is below 0.5 bp. This is often the default binning threshold in ARISA and TRFLP software (Dunbar *et al.* 2001). However, we found a few exceptions to the 1bp bin width rule where biological variation in sequence length within an OTU was greater than 1 bp. When

OTU sequence length variation indicated greater than 1 bp size variation for an OTU with 97% similarity of sequence identity we expanded the 1 bp ribotype bin to include ribotypes known to belong to the same OTU. Binned ribotype abundance data have been archived with the Bonanza Creek LTER http://www.lter.uaf.edu/data_b.cfm and were used in multivariate analysis of fungal communities.

REFERENCES

1.

Fisher, M.M. & Triplett, E.W. (1999). Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. *Appl. Environ. Microbiol.*, 65, 4630-4636.

2.

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

3.

Huang, X. & Madan, A. (1999). CAP3: A DNA Sequence Assembly Program. *Genome Research*, 9, 898-877.

4.

Rambaut, A. (2002). *Sequence Alignment Editor*. Available at: http://tree.bio.ed.ac.uk/software/seal/. Last accessed 12 December 2010.

5.

Rambaut, A. (2009). *FigTree: Phylogenetic tree production*. Available at: http://tree.bio.ed.ac.uk/software/figtree/. Last accessed 11 February 2012.

6.

Timling, I., Dahlberg, A., Walker, D.A., Gardes, M., Charcosset, J.Y., Welker, J.M *et al.* (2012). Distribution and drivers of ectomycorrhizal fungal communities across the North American Arctic. *Ecosphere*. http://dx.doi.org/10.1890/ES12-00217.1

7.

White, T.J., Bruns, T., Lee, S. & Taylor, J.W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications* (eds. Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J.). Academic Press, New York (New York, USA), pp. 315-322.

8.

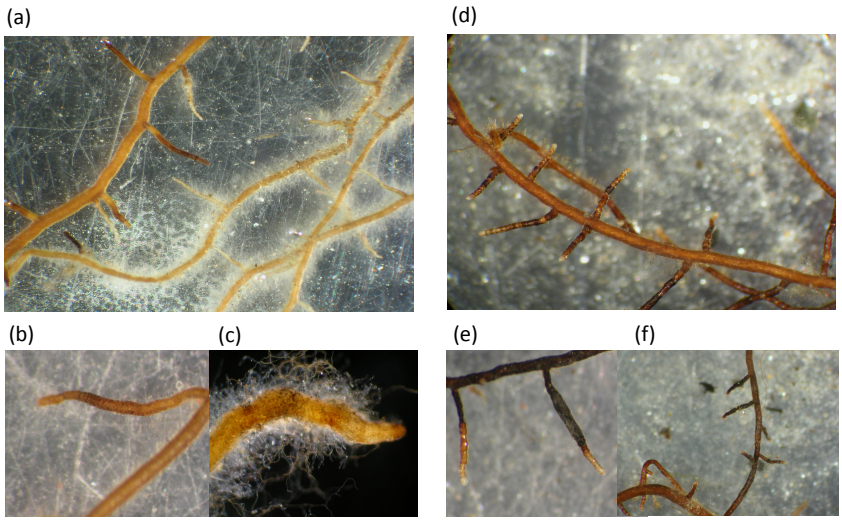
Zwickl, D.J. (2006). *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Ph.D thesis. The University of Texas at Austin, Austin (Texas, USA), pp 1-114.

Appendix 3.4. Operational Taxonomic Units at 97% sequence similarity for fungi associated with seedlings inoculated with soils from the Anaktuvuk River Fire.

GenBank Accession	Operational Taxonomic Unit	Ecology	Best Match GenBank Accession	Best match Description	Identity (%)	Coverage (%)	Bits
KF660543	OTU1	Pathogen	HQ115698.1	*Pyrenochaeta cava*	99.2	90	955
KF660544	OTU2	DSE/Endophyte	AB190400.1	*Phialophora lagerbergii*	99.8	89	1132
KF660545	OTU3	Pathogen	HM208713.1	*Pyrenochaeta* sp.	93	81*	664
KF660546	OTU4	Saprotroph	GQ152144.1	*Articulospora tetracladia*	95	63*	843
KF660547	OTU5	Pathogen	JN689972.1	*Phoma* sp.	96.2	86*	890
KF660548	OTU6	Pathogen	FJ903342.1	*Paraphoma* sp.	100	90	1039
KF660549	OTU7	Endophyte/Mycorrhizae/Pathogen/Saprotroph	AY706325.1	Helotiaceae sp.	92.6	97	726
KF660550	OTU8	Endophyte/DSE	EF093162.1	*Phialocephala* sp.	99	61*	987
KF660551	OTU9	Pathogen	JN28117.1	*Pyrenochaeta cava*	99.5	78	833
KF660552	OTU10	Pathogen	HM595513.1	*Coniochaeta* sp.	100	91	1065
KF660553	OTU11	Endophyte/DSE	JX630499.1	*Cadophora finlandica*	98	99	1555
KF660554	OTU12	Pathogen	AY337712.1	*Phoma herbarum*	100	100	1100
KF660555	OTU13	Endophyte/DSE	AY394906.1	*Phialocephala fortinii*	99.7	92	1118
KF660556	OTU14	Pathogen	EU218894.1	Ceratobasidiaceae sp.	90.9	96	803
KF660557	OTU15	Endophyte/DSE	AF486119.1	*Phialophora finlandica*	98	99	1576
KF660558	Singlet1	Saprotroph	AF106538.1	Orbiliales sp.	81	100	760
KF660559	Singlet3	Endophyte/DSE	AB190400.1	*Phialophora lagerbergii*	92.7	95	657
KF660560	Singlet5	Endophyte/Saprotroph	JF908572.1	*Pezizella* sp.	96	84*	791
KF660561	Singlet6	Endophyte/DSE	JQ272328.1	*Phialocephala* sp.	94	100	1321
KF660562	Singlet7	Endophyte/Mycorrhizae/Pathogen/Saprotroph	AY204609.1	Helotiaceae sp.	89.8	80	465
KF660563	Singlet8	Endophyte/DSE	AY394906.1	Vibrisseaceae sp.	92.3	100	715
KF660564	Singlet9	Pathogen	AY154682.1	*Alternaria* sp.	100	99	1174
KF660565	Singlet10	Pathogen	HQ324780.1	*Phaeosphaeria* sp.	95.5	93	831
KF660566	Singlet11	Pathogen	HQ839784.1	*Verticillium dahlia*	99.1	93	1065
KF660567	Singlet12	Endophyte/DSE	DQ826739.1	*Exophiala pisciphil*	99.5	96	1224
KF660568	Singlet13	ERM	AM887700.1	*Rhizoscyphus erica*	99.5	97	1098
KF660569	Singlet14	Pathogen	HQ324780.1	*Phaeosphaeria* sp.	98	97	1756
KF660570	Singlet15	Endophyte/Saprotroph	JX415336.1	*Cudoniella* sp.	97	98	998
KF660571	Singlet16	Endophyte/Pathogen/Saprothroph	JX164065.1	*Cladosporium* sp.	98	68*	1119
KF660572	Singlet17	Endophyte/Pathogen/Saprobe	DQ247778.1	Nectriaceae sp.	99.8	95	1055
KF660573	Singlet19	Pathogen	AF52674.1	*Setomelanomma* sp.	97.6	89Ψ	924
KF660574	Singlet20	Endophyte	EF016382.1	Herpotrichiellaceae sp.	89.9	100	637
KF660575	Singlet22		AY997048.1	Fungi sp.	87.5	18	119
KF660576	Singlet23	Endophyte/Saprotroph	JF908572.1	*Pezizella* sp.	96	69	793
KF660577	Singlet25	Pathogen	GQ458019.1	*Debaryomyces* sp.	100	100	1285
KF660578	Singlet27	DSE/Endophyte	AB190400.1	*Phialophora lagerbergii*	98.2	95	1037
KF660579	Singlet29	Pathogen	EU552496.1	*Candida parapsilosis*	97.5	100	1023
KF660580	Singlet30	ECM/Pathogen/Saprotroph	AY217135.1	Basidiomycota	86	31	174

*Coverage lower than 90% due to short subject sequence

Appendix 3.5. Photographs of typical morphologies of seedling root systems: a-c alder; d-f spruce.



Appendix 3.6. Description of final solutions for Nonmetric Multidimensional Scaling ordinations of fungal composition for all seedlings and treatment x species combinations.

				Variance Represented			
Ordination	Dimension of solution	stress	Final Instability	Total	Axis 1	Axis 2	Axis 3
All seedlings	3	3.75	0.04	87.8%	26.0%	30.70%	31.0%
Treatment x Spruce	3	5.57	0.03	94.3%	20.3%	60.60%	13.40%
Treatment x Alder	3	4.16	0.03	97.20%	28.70%	43.90%	24.60%

Chapter 4

Shrub-ectomycorrhizal-seedling interactions facilitate tree establishment after wildfire at the Alaskan boreal treeline[3]

ABSTRACT

Ectomycorrhizal fungi (EMF) are critical symbionts of all boreal tree species. These mycobionts are potentially limiting to tree-seedling establishment beyond current treeline in tundra. Although climatically driven increases in wildfire frequency and extent have been hypothesized to increase vegetation transitions from tundra to boreal forest, fire reduces EMF inoculum. We investigated whether ectomycorrhizal shrubs that resprout after fire maintain their mycobionts and whether these shrub EMF taxa colonize and enhance the growth of tree seedlings that establish after wildfire. Seedlings and adjacent shrubs shared EMF taxa, and there were strong correlations between the structure of seedling and shrub EMF communities. These results suggest a common mycorrhizal network that links resprouting post-fire shrubs with tree seedlings that establish after wildfire. Shrub taxon, distance to the nearest shrub, and fire severity influenced the similarity between seedling and shrub fungal communities. EMF composition was correlated with seedling biomass and C:N ratio and was one of the strongest explanatory variables predicting seedling biomass. Seedling age and fire severity were also important predictors. These results suggest that EMF mycobionts provided by resprouting post-fire vegetation are important to nutrient acquisition and biomass accrual of naturally establishing tree seedlings at treeline and may be a critical source of inoculum for tree seedling establishment beyond current treeline.

[3] Hewitt, R. E., D. L. Taylor. T. N. Hollingsworth, and F. S. Chapin. Plant-fungal interactions after a novel disturbance in the Arctic: implications for shrub and tree migration. Prepared for submission to Ecological Monographs.

INTRODUCTION

Both paleoecological and present-day demographic studies show that understanding the controls over seedling establishment is key to predicting treeline advance (Germino et al. 2002, Lloyd 2005). Yet, the key drivers of successful seedling establishment at and beyond treeline are not clear. Tree seedling establishment is influenced by myriad factors including propagule dispersal, substrate availability, life history traits, and facultative and/or competitive species interactions (Pickett *et al.* 1987). One often-overlooked yet physiologically important factor in seedling establishment is the symbiosis with ectomycorrhizal fungi (EMF), the obligate mycobionts of all boreal tree species (Smith and Read 2008). Given the considerable ecological repercussions of treeline advance into tundra for ecosystem properties, including increased aboveground carbon storage, decreased albedo, and increased latent and sensible feat fluxes (Chapin *et al.* 2005), it is important to understand the relative importance of abiotic and biotic controls over tree-seedling establishment at the margin of their current range.

In the boreal forest and at treeline in Alaska tree seedling recruitment is strongly linked with fire regime (Johnstone et al. 2004, Lloyd et al. 2007). In the last half-century wildfire frequency and extent have increased across the boreal forest and in regions of tundra (Macias Fauria and Johnson 2008, Rocha et al. 2012). Increased prevalence of wildfire in areas with previously rare fire occurrence, i.e. both tundra and treeline, is expected to facilitate vegetation transitions from tundra to boreal forest (Landhausser and Wein 1993) by opening up new, high-quality microsites for seedling establishment and reducing competition from extant vegetation (White 1979). However, fires at treeline and in tundra may have detrimental effects on critical belowground mycobionts, EMF, through combustion and heating of soil and mortality of host plants (Dahlberg 2002). In boreal and temperate regions, severe wildfire activity has been shown

to reduce EMF biomass (Stendell *et al.* 1999) and colonization of roots (Treseder *et al.* 2004) and alter EMF community structure (Baar et al. 1999, Grogan et al. 2000). Changes in mycobiont composition due to wildfire are likely to influence seedling success because of taxon-specific functional traits that benefit host plants (Smith and Read 2008), such as the relationships between EMF taxon, exploration strategy or successional status, and the efficacy of nutrient mobilization and translocation (Last et al. 1987, Hobbie and Agerer 2010).

The potentially negative effects of wildfire on plant-fungal interactions and seedling establishment may be amplified at treeline and in tundra due to low densities or the absence of ectomycorrhizal host plants (Read 1991). Although seedlings can be colonized by spores or sclerotia of the resistant propagule community that survive fire (Baar et al. 1999, Taylor and Bruns 1999), colonization by mycelium hosted by established vegetation is more effective because the fungus is actively foraging. Established plants have a greater ability to compete for soil resources, so seedlings that integrate into a common mycorrhizal network (CMN) with established plants have reduced carbon costs per unit of nitrogen gained (He *et al.* 2003). The function of CMNs depends upon differences in the magnitude of benefits conferred to seedlings by different fungal taxa (Nara 2006a). CMNs that link seedlings to established host plants reflect the lack of host-specificity of many mycobionts (Horton et al. 1999, Nara 2006b). Potential sources of inoculum for tree seedlings after fire at treeline and in tundra are ectomycorrhizal tundra shrubs, some of which have the capacity to resprout after fire and maintain their pre-fire mycorrhizal communities (Hewitt *et al.* 2013).

Although the most common shrubs at treeline and in tundra, *Betula nana* and *Salix* sp., are known to support generalist EMF communities (Timling *et al.* 2012), their capacity to facilitate natural tree seedling establishment at the edge of the boreal forest and beyond treeline

via mycorrhizal linkages is not well understood. The provision of mycelium by resprouting shrubs and the development of CMNs between establishing seedlings and resprouting tundra shrubs could play an important role in tree-seedling establishment success at treeline and promote migration into tundra after fire. This study had three main research goals: 1) to investigate whether seedlings that established after fire share EMF taxa with nearby resprouting shrubs, 2) to examine whether EMF that are compatible with seedlings and shrubs share similar ecological attributes such as EMF exploration type, i.e. foraging morphology, or geographic range, and 3) to determine if EMF communities and specifically taxa that are shared by seedlings and shrubs influence seedling growth responses. These questions ultimately contribute to understanding whether there are compatible mycobionts after fire that support seedling growth at the margin of their current range and provide new insights into drivers of seedling establishment beyond treeline after fire.

METHODS

Study Site

Our study area at treeline in the upland boreal forest of interior Alaska is bounded by the Brooks Range and latitudinal treeline to the north (67˚N), and the Alaska Range to the south (63˚N). Interior Alaska has a continental climate and is underlain by discontinuous permafrost. Sampling was focused in two treeline sites: 1) the uplands of the southern foothills of the Brooks Range at Finger Mountain north of the Yukon River and 2) the White Mountains at Nome Creek located between the Yukon and Tanana Rivers (Figure 4.1). Upland forest cover at both study sites is dominated by black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.), with patches of deciduous broadleaf trees, mainly trembling aspen (*Populus tremuloides* Michx.) and

Alaskan paper birch (*Betula neo-alaskana* Sarg.). Treeline at these sites is dominated by a mix of white spruce (*Picea glauca* Moench) and black spruce. Alpine tundra at both sites is dominated by ericaceous shrubs, dwarf birch, tall shrubs including *Salix* L. sp. and *Alnus viridis* (Chaix) DC., graminoids, and feather mosses. Both sites burned in 2004 when over 2.7 million ha of forest burned across the interior of Alaska. Site data available are at Bonanza Creek Long Term Ecological Research (LTER) [http://www.lter.uaf.edu/data.cfm]).

Field sampling

We sampled 40 pairs of tree seedlings and adjacent shrubs at our Finger Mountain site and 29 pairs of tree seedlings and shrubs at our Nome Creek site in July and August of 2009. At each site along five parallel 1 X 30 m transects we excavated the root systems and aboveground parts of aspen, birch, or spruce seedlings and the closest resprouting dwarf birch (*Betula nana*), tree birch (*Betula neo-alaskana*), willow (*Salix* sp.) or bearberry (*Arctostaphylos uva-ursi*) shrub. Due to low seedling density along the transects we continued to sample seedlings and shrubs by haphazardly searching for tree seedlings. We recorded the substrate and presence of the dominant vegetation within one meter of the focal seedling, the depth of the organic layer where the seedling and shrub were collected, and the distance between each seedling and the nearest shrub. Excavated rootballs were transported back to the University of Alaska Fairbanks (UAF). In 2010 we also harvested the seven white spruce seedlings that had been outplanted 20 years previously (Hobbie and Chapin 1998) in unburned shrub tundra at Toolik Lake, 65 km north of the arctic treeline (Figure 4.1). These seedlings were all that remained alive out of 120 that were originally planted in shrub tundra.

Seedling age and biomass

For all harvested seedlings, aboveground biomass was separated into stems and leaves, dried at 60°C for 48 hours in a drying oven, and weighed. For naturally established seedlings harvested at Nome Creek and Finger Mountain we verified that each seedling established after the wildfire by sanding the base of each seedling stem with 400-600 grit sandpaper and counting growth rings on a sliding bench micrometer. Carbon and nitrogen concentrations for leaf material were obtained by running foliar samples on a Thermo Scientific Delta V interfaced with a Carlo Erba elemental analyzer at the Alaska Stable Isotope Facility, UAF Water & Environmental Research Center.

Fungal sampling and characterization

Roots of both seedlings and shrubs were washed gently with DI water. Roots were traced back to the root crown to ensure plant identity. Intact roots were preserved in RNAlater (Life Technologies Corporation, Foster City, CA, USA) for ~20 weeks before root tip sampling. Root systems were cut into 4 cm segments and floated in ultrapure DI water. Healthy ectomycorrhizal root tips were picked from each segment. In a preliminary trial we found that eighteen tips adequately captured the richness on the root systems for four host plants (36 vs. 18 tips 0.83±1.33 S.E., p=0.55). Eighteen root tips were randomly selected and pooled from the root system and stored in ultrapure water at -80˚ C and lyophilized. In the case of the outplanted seedlings and adjacent shrubs from Toolik Lake, root tips were analyzed individually and then aggregated to provide the EMF composition of each host plant.

DNA extraction and ARISA

Lyophilized pooled samples from treeline sites at Nome Creek and Finger Mountain were ground in lysis buffer using a sterile pestle. DNA was extracted from the pooled root-tip samples from each host plant using the DNEasy Plant Mini Kit (*QIAGEN* Inc., Valencia, California, USA) according to the manufacturer's instructions and following the protocol of Bent and Taylor (2010). Genomic DNA was amplified for Automated Ribosomal Intergenic Spacer Analysis (ARISA) of mycorrhizal community structure. Following the protocol of Bent & Taylor (2010) we mixed 25 µl PCR reactions containing 0.65 mM MgCl2, 0.2 mM dNTPs, 0.05 µM forward primer FAM-ITS1F (CTTGGTCATTTAGAGGAAGTAA; (Gardes and Bruns 1993), labeled on the 5' end with FAM, a fluorescein amidit (Applied Biosystems, Carlsbad CA, USA)), 0.05 µM reverse primer ITS4 (TCCTCCGCTTATTGATATGC; (White et al. 1990), 0.06 mg/ml bovine serum albumin, 0.15 µl JumpStart RED Taq (Sigma-Aldrich, St. Louis, MO, USA), 1X JumpStart RED Taq buffer and 1 µl of genomic DNA. Reaction mixes were prepared in 0.2 µl tubes and thermocycled in an MJ Research PTC-225 thermal cycler as follows: 96°C for 3 min, 35 cycles of 94°C for 30s, 52°C for 30s, then 72°C for 3 min, followed by 72°C for 10 min following protocol of Bent and Taylor (2010). We then made 15 µl cocktails containing 1 µl of the diluted PCR product mixed with 14.25 µl of formamide and 0.75 µl of size standard (MapMarker 1000 X-rhodamine, BioVentures Inc., Murfreesboro, TN, USA). This was heated to 95˚C for 5 minutes, placed on ice, and then run through an ABI 3100 Genetic Analyzer (Applied Biosystems, Carlsbad CA, USA; Pop6, 50cm array, T-RFLP_1500 protocol). In our experience, ARISA is a sensitive and cost-effective method to obtain richness and abundance data for the low diversity EMF communities in our study.

Amplicon cloning and sequencing

For ARISA profiles from pooled tips that yielded a single ribotype, we obtained fungal ITS sequences by direct sequencing. We also obtained direct sequences, rather than ARISA profiles or clone sequences, for each of the 18 root tips sampled from the outplanted seedlings from Toolik Lake. To assign taxonomic identities to ARISA ribotypes we matched ARISA ribotypes with sequence identities from both direct and clone sequences. For direct sequencing we followed the PCR protocol described earlier except we used primers ITS1F and ITS4 without fluorescent labels. For all other samples we produced pooled clone libraries. For each sample (seedling or shrub) we amplified 3 replicates of genomic DNA and used a modified cycling regime as follows 96°C for 3 min, 25 cycles of 94°C for 30s, 52°C for 30s, then 72°C for 3 min, followed by 72°C for 10 min to reduce PCR bias. We then pooled the three PCR products for each pooled sample. Following PCR we measured the concentration of DNA in each pooled sample using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) to determine the lowest total DNA amount for a sample. We then pooled PCR products within site and host species with equal concentrations of DNA i.e. each sample contributed equal concentrations of DNA to the pooled sample. These pooled site X species samples were then run through CHROMA Spin+TE-400 columns (Clontech, Mountain View, CA, USA) to purify the DNA. We used the DNA Clean and Concentrator – 25 kit (Zymo Research, Irvine, CA, USA) to concentrate the DNA. Using the NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) we calculated the concentration of DNA in each pooled concentrated sample and diluted each sample in TE to 25 ng/μl.

We used the TOPO TA Cloning® Kit for Sequencing (Invitrogen, Carlsbad, CA, USA). Amplicons were ligated into pCR®4-TOPO® as follows: 4 µl of amplicon DNA was mixed with 1µl salt solution and 1µl of TOPO plasmid vector pCR®4-TOPO®, vortexed at medium speed and centrifuged at 5 rmp for 20 seconds, incubated for 30 min at room temperature 22-23ºC and then placed on ice. To transform chemically competent cells 2 µl of ligation reaction was added to a vial of competent *E. coli* cells (the One Shot® Competent Cells, Invitrogen, Carlsbad, CA, USA) and incubated on ice for 10 minutes. Cells were transformed using heat shock (42ºC for 30 seconds) and incubated in 250 µl of SOC medium (2% tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose) for one hour. Fifty µl of undiluted and 10x (diluted in ultrapure water) concentrations of cells were then plated on selective medium (Luria-Bertani (LB) agar with 50 mg/ml kanamycin, Fisher Biotech, Hampton, NH, USA) and incubated for 24 hours at 37°C for each site X species library. Cloned cells were selected from grown colonies from each site X species library and used in direct PCR using primers M13F and M13R. We mixed 48 µl PCR reactions containing 25 mM MgCl2, 10 mM dNTPs, 50 µM forward primer M13F (TGT AAA ACG ACG GCC AGT), 50 µM reverse primer M13R (CAG GAA ACA GCT ATG ACC), 0.06 mg/ml bovine serum albumin, 0.25 µl JumpStart RED Taq (Sigma-Aldrich, St. Louis, MO, USA), 1X JumpStart RED Taq buffer and 2 µl of cells. Reaction mixes were prepared in 0.2 µl tubes and thermocycled in an MJ Research PTC-225 thermal cycler as follows: 95°C for 8 min, 30 cycles of 92°C for 30s, 50°C for 45s, then 72°C for 2 min, followed by 72°C for 10 min. PCR product was gel checked and then used in RFLP reactions in order to pick unique clones for sequencing. For each PCR product we made an 8 µl cocktail of 0.8 µl 10X NEBuffer4 (New England BioLabs, Ipswich, MA, USA), 0.20 µl BSA, 6.75µl ultrapure water, and .25µl 10,000 U/ml Hae III enzyme (New England BioLabs,

Ipswich, MA, USA). We added 12 µl of PCR product to the cocktail and incubated at 37 °C for 12 hours. We ran the RFLP digest product out on a one percent agarose gel. We examined the RFLP gels and selected samples with unique RFLP patterns for each site X species library. PCR products for samples with unique RFLP patterns were shipped on wet ice overnight to Functional Biosciences Inc., Madison, WI for Sanger sequencing. Sequence lengths did not always exactly match the ARISA fragment length. Therefore, to directly match DNA sequences with our ARISA profiles ,we performed ARISA on the clone PCR product that was sequenced. ARISA ribotype identities were obtained for two representative PCR samples from each OTU in our sequence dataset.

Bioinformatics

We used GeneMapper 3.7 to read ARISA electropherograms. Fragment length (ribotype) and peak height are taken to represent presence and abundance of fungal taxa, respectively. The peak heights for each fragment were relativized by dividing the fluorescence height for each peak by the total fluorescence for a sample profile (Fisher and Triplett 1999). ARISA ribotypes were binned at 0.5 base pair (bp) threshold, which resulted in a 1.0 bp bin size (Dunbar *et al.* 2001). We used 1.0 bp ARISA ribotype bins as surrogates for "species" in certain fungal community analyses. Binned ARISA ribotype abundance data have been archived with the Bonanza Creek LTER http://www.lter.uaf.edu/data_b.cfm.

Clone and direct sequences were assembled using CodonCode Aligner 3.7 (CodonCode Corporation, Centerville, MA, USA) using PHRED; fasta and qual files for assembled consensus sequences were exported from CodonCode. We used in-house perl scripts to mask low-quality bases, orient, and purge (>3% Ns) sequences. We tested for chimeric sequences with an open-

source chimera checker (Edgar – Uchime) using an in-house bioinformatics tool (Taylor and Houston 2011) and manual inspection of aligned sequences using SeAl alignment software (Rambaut, 2002). We eliminated 18 putative chimeric sequences. We grouped all direct and clone sequences into Operational Taxonomic Units (OTUs) using CAP3 (Huang and Madan 1999) at 97% sequence identity. A representative sequence was selected for each OTU after manual inspection in SeAl. To assign species identities we ran a BLAST search using the representative sequence for each OTU against both the UNITE version 5.0 reference sequence database and the entire nucleotide database in GenBank utilizing the filter for uncultured and environmental samples. We assessed the top matches with the highest bit score from the UNITE and GenBank databases. We considered our query sequence to be identified to the species level when it had >97% sequence similarity and >90% coverage to a reference sequence, genus level with <97% and >93% sequence similarity or incongruence in species level matches of the top hits, family level with <93% and >83%, and order level with <83% sequence similarity (Deslippe et al. 2011, Timling et al. 2012). When the best matches were identified to the same taxonomic level we reported the UNITE Species Hypothesis, which provides a stable name and accession number (Kõljalg et al. 2013). In a few cases the top matches did not meet our criteria for >90% sequence coverage, and in this case we report the best match with the highest bit score and highest sequence coverage. When the top matches were identified at different taxonomic levels, we report the best match with the highest taxonomic specificity.

We used a tiered approach to assign OTU identities derived from Sanger sequencing to ARISA ribotypes. First, OTU identities for direct sequences were matched up with the dominant ribotype in the pooled ARISA profile based on fragment length (Appendix 4.1). Sequence lengths were computed using the DNA stats function under the sequence analysis menu at DNA

2.0 Bioinformatics toolbox (https://www.dna20.com/index.php?pageID=216). The next step was to provide OTU assignments for each ribotype based on sequences from each site X host plant clone library. In a few cases this resulted in the expansion of a ribotype bin to greater than 1 bp. We did not expand bins based on OTU assignments from the different sites. An OTU had to be observed within the site x host plant clone library for it to be assigned to that site. However, within a site a 1 bp ribotype was considered one taxon and the OTU assignment was generalized for the ribotype bin. In four out of ninety-four ribotypes we found that several OTUs matched up with one ARISA ribotype (ribotypes 11, 48, 52, and 60). Because the inclusion of these taxa could inflate our estimates of shared taxa that occur on seedlings and adjacent shrubs, we ran a sensitivity analysis to assess the impact of excluding these taxa from the analysis and found that patterns in EMF composition were robust to the removal of these taxa (Appendix 4.2). All analyses were conducted with this more conservative dataset excluding these four ribotypes.

Statistical analysis

Ordination analysis of vegetation and fungal composition for naturally established seedlings

To examine the vegetation and fungal composition associated with each naturally established focal seedling we used Bray-Curtis ordinations (Bray and Curtis 1957) with the Sørensen distance and the variance-regression method of endpoint selection (Beals 1984). The vegetation ordination was based on presence-absence data for vegetation documented within one meter of each harvested focal seedling in the field. The fungal ordination was based on ribotype abundance relativized for each seedling or shrub sample. For both the vegetation and fungal ordinations, taxa that occurred in less than five percent of the samples were excluded from the ordination (McCune and Grace 2001). We transformed the data using Beal's smoothing to relieve the "zero truncation problem" (McCune and Grace 2001). We tested for correlations

between fungal composition and environmental and fire variables. We specifically tested whether each shrub and seedling species differed in their EMF community composition using the Multiple-Response Permutation Procedure (MRPP) (Berry et al. 1983) (shrubs *Betula nana*= 23 shrubs, *Arctostaphylos uva-ursi* = 9 shrubs, *Salix* sp. =15 shrubs; seedlings *Populus tremuloides*= 34 seedlings, *Picea mariana* = 5 seedlings, *Betula neo-alaskana*. =8 seedlings). For the MRPP, we used the Euclidean distance measure. Because fungal composition was strongly correlated with organic soil depth, a proxy for fire severity, we performed an Indicator Species Analysis (Dufrene and Legendre 1997) to detect ribotypes that were over-represented for a particular fire-severity class (high severity <3 cm residual organic soil depth or low >3 cm residual organic soil depth) (Johnstone and Chapin 2006). For the Indicator Species Analysis we used 4999 permutations in the Monte Carlo test of indicator values for ribotypes. Vegetation and fungal ordination axes represented composition. The vegetation axes were exported and included in the environmental matrix as part of the fungal ordination, and both the fungal and vegetation axes were exported and used in Random Forest analysis. All multivariate analyses of mycorrhizal communities were performed in PC-ORD 6.0 (MJM Software Design, Gleneden Beach, Oregon (USA)).

Analysis of common mycorrhizal networks between seedlings and shrubs

We were interested in whether there was any evidence that seedlings and shrubs shared mycobionts. Therefore, we tested for a relationship between the structure of the fungal communities associated with seedlings and with adjacent shrubs at our treeline sites. We performed the same test with the outplanted seedlings from the Toolik Lake site. We used the Mantel test (Mantel 1967) with 999 Monte Carlo permutations to evaluate the null hypothesis that there was no significant relationship between the fungal community structure of seedlings

and shrubs. The matrices representing seedling and shrub fungal communities from the Nome Creek and Finger Mountain treeline sites had 62 seedlings or shrubs (rows) and 91 ribotypes (columns). For the outplanted seedlings at Toolik Lake the seedling and shrub matrices had 14 seedlings or shrubs (rows) and 41 ribotypes (columns). We also computed Sørensen dissimilarity distances for all seedlings and shrubs. We used matched pairs T-tests to evaluate whether seedlings paired with the adjacent shrub had fungal communities that were more similar than seedlings that were unpaired, i.e., randomly paired with shrubs that were not adjacent to the sampled seedling. We evaluated Spearman correlations to detect collinearity between variables (Appendix 4.3). Data visualization and univariate statistical procedures were performed in JMP 10.0 (SAS Institute Inc, Cary, NC, USA).

To test what fire and host plant variables influenced the similarity between seedling and shrub EMF communities, we used Random Forest regression trees. Random Forest is an ensemble decision tree method that uses an algorithmic approach to make predictions based on the input variables (Breiman 2001b). Multiple decision trees are constructed through a process known as bagging (Breiman 1996), which utilizes a bootstrap sample from a subset of the data (~63% of the data) and the random selection of a subset of the predictor variables. The most important variable from the random subset of predictor variables is then used to produce each node split (Breiman 2001a). The fully-grown (statistical) trees are used to predict the observations excluded from the bootstrap sample, i.e. out-of bag data (~37% of the full dataset). Predictions are calculated by combining the predictions across the forest of regression trees by averaging. The importance of each explanatory variable is determined when permutations of the observed values for a predictor variable in the out-of-bag data are run down the tree to predict the response variable. If an explanatory variable is important, then the permutation of that

variable will have a large effect on the prediction of the response variable (Breiman 2001b). The variable importance is reported as the percent increase in the miscalculation rate, i.e., decrease in accuracy, for each predictor when the predictor is permuted (Breiman 2001b). For each predictor, Random Forest computes the prediction error (mean square error) of a tree in the forest from the permutation of the observed values for a variable in the out-of-bag data (Breiman 2001a). The difference between the classification of observed out-of-bag data and the classification of permuted data is averaged across all trees and divided by the standard error (Breiman 2001a, Cutler et al. 2007).

Random Forest is increasingly used in ecological studies because of its predictive accuracy when modeling data with complex interactions that may not meet the assumptions of parametric analysis (Breiman 2001b, Cutler et al. 2007). In comparison to other regression and classification tree methods, Random Forest does not over-fit the data and through the random selection of explanatory variables it reduces bias (Prasad *et al.* 2006). We utilized the Random Forest method because of its ability to accurately model small samples sizes with potentially noisy predictor variables and the ease of interpretation (Diaz-Uriarte and de Andres 2006, Cutler et al. 2007, Qi 2012). We used partial dependence plots to evaluate the marginal effects of the most important variables predicting similarity between tree and shrub EMF communities. Similarity between fungal communities was evaluated using the Sørensen dissimilarity distance for each seedling-shrub pair. Partial dependence plots depict predicted Sørensen dissimilarity in relation to each explanatory variable after accounting for the average effect of all other explanatory variables. To determine which variables are important to predictions of Sørensen dissimilarity we used the R 3.0.2 package Random Forest (R Development Core Team, 2013, R Foundation for Statistical Computing, Vienna, Austria). Using Random Forest we built 500

regression trees using a random sample of the 47 observations of paired fungal communities. At each node in the trees, two predictor variables were chosen at random from the seven explanatory variables (seedling age, site, seedling taxon, organic depth, vegetation composition, distance between seedling and shrub, and shrub taxon).

Ecological attributes of fungal taxa

One way fungal taxa can be characterized is by their hyphal development patterns. The categorization of exploration type (Agerer 2001) describes the distance at which the hyphae forage (i.e., long, medium, short, or contact strategies) and whether they form rhizomorphs, exploratory and foraging conduits comprised of aggregated hyphae. Exploration strategies have also been related to soil nutrient foraging capabilities (Hobbie and Agerer 2010). Because the exploration strategy, foraging distance, and morphology of mycorrhizal fungi could influence whether a seedling at a given distance is rapidly colonized by inoculum from resprouting vegetation after fire, we conducted an analysis comparing the exploration strategy of shared and unshared fungi on naturally established seedlings. For each ectomycorrhizal taxon we defined the exploration strategy and presence of rhizomorphs using the DEEMY database, an Information System for Characterization and DEtermination of EctoMYcorrhizae, (URL: http://www.deemy.de/) (DEEMY 2014). This analysis was limited in scope because the majority of the taxa in our dataset were not documented at the species level in the DEEMY database. When a species was not found in the database we followed the classifications for genera in Agerer (2001) and Hobbie and Agerer (2010). We used chi square tests to evaluate if exploration strategy was related to whether a taxon was observed on a paired seedling and shrub.

To determine whether the EMF that we observed on seedlings and adjacent shrubs at treeline are present beyond current treeline in Arctic tundra, we documented the geographic

locations of the top 100 GenBank hits with >90% coverage and >97% sequence similarity for each queried OTU that was shared by a seedling and its adjacent shrub. We restricted our reporting to boreal and Arctic tundra regions.

Relationships between fungal composition and seedling attributes

We specifically tested whether fungal composition was correlated with C:N ratio of foliar tissue and biomass of seedlings at Nome Creek and Finger Mountain using Canonical Correspondence Analysis (CCA) (Ter Braak 1986). C:N ratio of foliar tissue from the current growing year reflects the growth strategy and nitrogen limitation of a plant at the time of harvest. C:N ratio may, therefore, be reflective of the importance of specific fungal taxa to seedling success. In the analysis we had one species matrix with 47 seedlings (rows), a reduced number due to the elimination of seedlings that only associated with rare ribotypes, and the nine ribotypes (columns), all ribotypes with over five percent coverage across these seedlings. We exluded all spruce seedlings from these analyses (n=8 seedlings) because their needles were not separated by annual growth increment and therefore could reflect growing conditions prior to 2009. With CCA we rescaled the axes using the Centered with Unit Variance method and optimized the representation of fungal taxa not seedlings. We used the Monte Carlo randomization with 999 randomizations to test whether there was a significant relationship between the environmental variables and fungal composition. We compared the species matrix to an environmental matrix with 47 seedlings (rows) and two columns (C:N and seedling biomass). We assessed correlations between specific fungal taxa and the canonical axes to determine if the presence and abundance of a particular fungal taxon was related to biomass or C:N ratio of foliar tissue.

Our third research goal was to evaluate the relative importance of fungal composition and shared fungal taxa on tree seedling biomass in relation to the other abiotic and biotic explanatory variables. We used the Random Forest regression tree method to identify important associations between seedling biomass and explanatory variables: fire severity, fungal and vegetation variables. We used partial dependence plots to evaluate the marginal effects of the most important variables predicting seedling biomass. Again, we used the R package Random Forest to determine which variables are important to predictions of seedling biomass. Using Random Forest we built 500 regression trees using a random sample of the 69 observations of seedling biomass. At each node in the trees, two predictor variables were chosen at random from the nine explanatory variables (seedling age, site, seedling taxon, organic depth, vegetation composition, fungal composition, percent of shared fungi, distance between seedling and shrub, and shrub taxon).

RESULTS

Vegetation and fungal composition

The sequences from the naturally occurring seedlings and adjacent shrubs at Nome Creek and Finger Mountain were grouped into 53 OTUs, 17 of which were singletons. The sequences from the outplanted seedlings and adjacent shrubs at Toolik Lake were grouped into 41 OTUs of which 20 were singletons. Only seven of the OTUs observed on the outplanted seedlings or shrubs at Toolik Lake were also detected on naturally occurring seedlings or shrubs at our treeline sites (Table 4.1). The majority (75%: 66 of 87 OTUs) of the taxa detected in our sequencing effort belonged to the Basidiomycota (Table 4.1). The vast majority of the fungal sequences (82%) were EMF with a few additional dark septate endophytes (DSE), other

endophytes, and ericoid mycorrhizal fungi (ERM). Many of the EMF taxa that were shared by seedlings and the adjacent shrub were multi- or late-stage fungi, which are generally found on mature host plants of later successional seres and colonize from mycelia, from the genera *Russula*, *Lactarius*, *Cortinarius*, and *Boletus* (Appendix 4.1, Table 4.2).

Bray-Curtis ordinations resulted in a 3-D solution for both the vegetation and the fungal communities associated with naturally occurring tree seedlings. For the vegetation ordination the three axes accounted for 95.9% of the variance in vegetation community structure, with axis one contributing 67.9%, axis two 19.9%, and axis three 7.2%. For the fungal ordination the three axes accounted for 91.3% of the variance in EMF community structure, with axis one contributing 43.2%, axis two 37.8%, and axis three 10.3%. Axis one of the fungal ordination was not highly correlated with environmental or fire variables. Axis two was correlated with the vegetation composition (r^2=0.29), and axis three was correlated with variables related to the depth of the organic horizon, a proxy for fire severity in the boreal forest, measured for all seedlings (r^2=0.22).

Common mycorrhizal networks between seedlings and shrubs

To investigate the presence of CMNs we examined several lines of evidence. Firstly, there was a significant positive association between the structure of the fungal communities on naturally established seedlings and the closest shrub (Mantel Test r= 0.130, p=0.001). Secondly, we compared paired seedlings and shrubs and randomly paired seedlings and shrubs, i.e. unpaired, and found that paired seedlings-and-closest shrubs had more similar fungal communities than seedlings that were paired randomly with shrubs ($T_{(61)}$ = 4.379, p=<0.0001). The Sørensen dissimilarity (0= the same community, 1= no shared taxa) for adjacent seedling-shrub pairs was lower (0.816 ±0.031 S.E.) than the dissimilarity for seedlings and shrubs that

were paired at random (0.96 ±0.031 S.E). Thirdly, on average 31.53% (±0.045 S.E.) of the fungal taxa observed on seedlings were shared with the closest adjacent shrub. Finally, across both treeline sites far more taxa were observed on multiple host-species (i.e. they were generalist EMF) than were observed specifically on seedlings and adjacent shrubs that we sampled (39 out of 94 ribotypes), indicating that our results likely underestimate networks between host plants in our study sites (see Appendix 4.1 for list of host plants associated with each fungal taxon).

We also tested for the presence of CMNs between the outplanted seedlings and the nearest shrub at Toolik Lake. We found no significant correlation between fungal composition for outplanted *P. glauca* seedlings and the closest *Salix* and *B. nana* shrubs (Mantel Test r= 0.081, p=0.173). The Sørensen dissimilarity was not significantly different for outplanted, adjacent seedling-shrub pairs and seedlings and shrubs that were paired at random ($T_{(14)} = 0.480$, p=<0.639). The mean Sørensen dissimilarity for *B. nana* shrubs was 0.836 (paired) and 0.892 (unpaired) ±0.061 S.E and for *Salix* shrubs 0.950 (paired) and 0.926 (unpaired) ±0.044. These patterns of fungal similarity show that in fire-disturbed sites, establishment near a resprouting shrub provides mycobionts with which young seedlings can associate, whereas in unburned habitats the mycobionts of outplanted seedlings show no strong correlation with the mycobionts of the nearest shrub twenty years after outplanting.

We were interested in which variables were most important to predicting the similarity between fungal composition on naturally occurring seedlings and the adjacent shrub. Random Forest analysis indicated that shrub taxon was the most important variable, followed by distance (cm) between the seedling and adjacent shrub, vegetation composition, organic depth, seedling taxon, site, and seedling age, in predicting dissimilarity in fungal communities between paired seedlings and shrubs (Figure 4.2a). *B. nana* shrubs appeared to have slightly more dissimilar

fungal communities compared to the similarity between seedlings and either *Salix* or *A. uva-ursi* (Figure 4.2b). As distance increased between seedlings and shrubs, there was a marked decline in the similarity between fungal communities, especially as the distance approached 50 cm (Figure 4.2c). The effect of distance on fungal similarity reached a plateau beyond a seedling-to-shrub distance of 75 cm. These results suggest that shrubs less than 50-75 cm from a tree seedling had greatest effect on the seedling's EMF community. Fungal communities were predicted to become less similar as the residual organic horizon deepened (Figure 4.2d). Seedling species, site, and age appeared to exert little influence on the dissimilarity between fungal communities with paired shrubs. These Random Forest results were supported by the MRPP results, which indicated that EMF composition did not vary by seedling species (A= 0.029, p=0.073), whereas, EMF composition did vary by shrub species (A= 0.042, p= 0.024).

Geographic ranges and exploration types of shared fungi

Perhaps because of small sample size, we did not detected any relationships between EMF exploration type or rhizomorph formation and whether an EMF taxon was shared between a seedling and its adjacent shrub ($\chi^2_{(17)}$=10.487, p=0.485). Despite this lack of statistical significance, two-thirds (6 out of 9) of fungi that were detected on both seedlings and adjacent shrubs formed rhizomorphs. When we compared taxa with the contact, short, medium, or long exploration types, we found that exploration strategy was not related to whether a taxon is observed on the adjacent host plant ($\chi^2_{(16, 3)}$=2.776 p=0.428). The number of taxa in our dataset that were identified to the genus or species level in the DEEMY database was small and thus limited our analysis of hyphal attributes for each fungal taxon in out dataset.

When we consider exploration strategy and successional stage within the context of fire severity, we found that the taxa that were indicators of low-severity burned patches belonged

primarily to Russulaceae (in the genera *Russula* and *Lactarius*), which are generally multi- or late-stage fungi and have variable exploration strategies and rhizomorph formation (Appendix 4.1). The single ectomycorrhizal taxon that was an indicator of high severity, *Inocybe lacera*, was only observed on *Betula nana* shrubs. This species has a short-distance exploration strategy, does not produce rhizomorphs (Appendix 4.1) and is classified as an early-stage taxon.

From our GenBank sequence comparisons, we found the majority of the fungi (11 out of 17 taxa) that we observed as shared by naturally established seedlings and adjacent shrubs at treeline have also been observed in Arctic tundra (Table 4.2), suggesting the presence of appropriate mycobionts for boreal tree seedlings beyond current treeline.

Relationships between fungal composition and seedling attributes

Fungal composition was significantly correlated with both seedling C:N ratio and biomass (Figure 4.3, CCA Monte Carlo test species-environment correlation C:N species-environment correlation=0.608, p=0.050 and biomass species-environment correlation=0.444, p=0.050). Axis one explained 4.7 % and axis two explained 2.6% of the variance in composition. Although the CCA explains a low percentage of the variance in fungal composition, the Monte Carlo randomization test indicates that the eigenvalues for axis one (C:N) and axis two (biomass) were higher than the range expected by chance (Table 4.3). When we examined correlations between specific taxon abundances and the axes of the ordination, we found that seedlings with high abundance of one taxon, *Russula nitida* (ribotype 66), had lower C:N (Pearson r=0.450, r^2=202). Biomass was not correlated with the abundance or presence of any single taxon, suggesting that the effects of fungal composition on seedling biomass and C:N ratio reflected effects of multiple EMF taxa.

We were interested in the importance of fungal composition in predicting seedling biomass as compared to other fire, environmental and host-plant variables. Analysis with Random Forest showed that seedling age was the most important variable predicting seedling biomass, followed by site, fire severity (organic depth), fungal composition, and vegetation composition (Figure 4.4). The distance between a seedling and resprouting shrub, seedling taxon, percentage fungi shared with a resprouting shrub and the shrub taxon all had less important influences on seedling biomass. The partial dependence plots of age-effects on seedling biomass indicate that age is not a critical predictor of seedling biomass until after three years (Figure 4.5a). Seedling biomass had a bimodal response to fire severity. Seedlings are predicted to have higher biomass at organic depths between three and five cm and then again at organic depths greater than 17 cm (Figure 4.5b). Although distance and the percentage of shared fungi had weaker effects on the prediction of seedling biomass, the partial dependencies of these two variables when other variables are held at their average indicate that biomass increased when the percentage of shared fungi surpassed 20% (Figure 4.5c). The apparent dip in effect of shared fungi on biomass between zero and 20% shared probably reflects the very small sample size of seedlings that shared 20% of their fungal taxa (n=2) compared to the number of seedlings that shared no taxa (n=28) and those that shared greater than 20% (n=32). In contrast to the negative effect of seedling-to-shrub distance on similarity of EMF community composition between seedling and shrub (Figure 4.2c), distances greater than 150 cm had a *positive* effect on seedling biomass (Figure 4.5d). These results suggest that shrubs have both negative effects, possibly due to root competition and positive effects, possibly due to shared fungi.

DISCUSSION

The effects of belowground species interactions on plant community assembly has been a central question in community ecology in the last several decades (Janzen 1970, Connell 1971, Perry et al. 1989, van der Heijden and Horton 2009). Our results are consistent with studies in other ecosystems showing that CMNs between established vegetation and seedlings support seedling establishment (Horton et al. 1999, Nara 2006b). We obtained several lines of support for the presence of CMNs between seedlings and shrubs and the provision of inoculum to seedlings by shrubs. The similarity between seedling and shrub fungal communities was related to recruitment-site factors and fire severity. Fungal composition was related to biomass accrual and in parallel with studies during primary succession (Nara 2006a), we observed the species-level influence of EMF on the nitrogen economy of seedlings. Our sequence analysis of the geographic distributions of fungi shared by seedlings and shrubs showed that EMF have broad distributions across northern biomes, suggesting that EMF compatible with boreal tree seedlings are present beyond the current range limit of boreal trees. These results illustrate that resprouting shrubs not only play a role in the provision of EMF at the range limit of boreal tree species, but also might provide patches with suitable EMF inoculum well beyond treeline in tundra after wildfire.

Our analysis of EMF shared between seedlings and resprouting shrubs revealed that multi- and late-stage fungi were present post-fire in addition to some early-stage taxa. The colonization of seedlings by late-stage EMF after fire contrasts with findings from the temperate zone where post-fire seedlings are primarily colonized by the resistant propagule community, including genera such as *Wilcoxina*, *Tuber*, and *Rhizopogon*, which are distinct from taxa

associated with mature vegetation (Baar et al. 1999, Taylor and Bruns 1999, Grogan et al. 2000). In a sister study, we also observed the persistence of multi- and late-stage taxa after fire on resprouting shrubs in tundra and concluded that the later successional-stage mycobionts survived the wildfire (Hewitt et al. 2013). Ultimately, the successional status of EMF correlates with functional traits related to nutrient capture of increasingly complex substrates over time (Last *et al.* 1987). Therefore, the presence of multi- and late-stage EMF on resprouting shrubs and seedlings indicates ecological resilience of taxonomic diversity and related functional diversity after fire.

The detection of early-, multi-, and late-stage EMF taxa may indicate heterogeneity in inoculum source after fire. In temperate ecosystems, high root densities are associated with root colonization by mycelial dispersal whereas low root densities are associated with root colonization by spore dispersal (Peay et al. 2011). After soil combustion associated with wildfire we would expect root densities to be reduced in a patchy manor related to fire severity. High-severity burned patches may, therefore, foster the germination of early-stage, spore-dispersed taxa; whereas, lower severity patches and resprouting vegetation with higher root densities support the maintenance of multi- and late-stage taxa that colonize roots through mycelial dispersal. Consistent with this framework, we found that *Inocybe lacera*, an early-stage, spore-dispersed taxon (Nara *et al.* 2003), was the only EMF taxon that was a significant indicator of high-severity sites. In contrast, the three EMF taxa that were significant indicators of low-severity sites were all in the genera *Russula* and *Lactarius*, two genera often classified as multi-stage or late-stage fungi (Smith and Read 2008).

In addition to investigating the identities and ecological attributes of EMF shared by naturally established seedlings and nearby resprouting shrubs, we wanted to know which fire,

host-plant and environmental factors may influence whether compatible fungi are shared. The similarity between EMF communities associated with seedlings and adjacent shrubs are primarily influenced by recruitment site and fire severity, emphasizing the role of highly stochastic processes (dispersal, fire severity) in shaping fungal communities on seedlings. Shrub taxon and the distance to the nearest shrub were the two most important variables predicting similarity between seedling and shrub-fungal communities followed by vegetation composition and fire severity. We were surprised to see shrub taxon as an important predictor because none of the host shrubs are known to have particularly narrow fungal specificities (Molina *et al.* 1992). We expected them to fare equally well at surviving fire and supporting generalist EMF taxa that associate with seedlings. However, it appears that *B. nana* supports EMF communities less similar to boreal seedling mycobionts than *Salix* sp. or *A. uva-ursi*. Our field data support results from a growth chamber study where inoculation with soils collected from below *Salix* sp. and *A. uva-ursi* shrubs resulted in greater EMF root colonization of black spruce seedlings than *B. nana* soils (Reithmeier and Kernaghan 2013). It is intuitive that distance to the nearest host plant is influential in structuring fungal communities because of the colonization of a seedling root system by EMF mycelium that are established (Newman 1988). In our study, and in other studies, distance to the EMF source influenced the composition of EMF (Dickie and Reich 2005). The inverse relationship between EMF similarity and organic depth may show decreased taxon abundance or presence with increased disturbance severity and subsequent colonization of root systems by surviving taxa through priority effects (Kennedy *et al.* 2009). In contrast to our observations at treeline, the strong relationship between EMF communities on seedlings and shrubs was not observed in undisturbed tundra at Toolik Lake. This may be the case because the *nearest* shrub may not be as critical when there are well developed CMNs of multiple fungal

genets connecting multiple host plants that have not been disturbed by fire. Another explanation for the observation that EMF composition on outplanted seedlings was not correlated with adjacent shrubs may be that the roots of these outplanted seedlings were colonized with greenhouse contaminants at the time of transplant (two year old seedlings) and therefore were not dependent on networking with established EMF shrubs. However, at the time of harvest, only one common greenhouse contaminant, *Thelophora terrestris*, was detected in low abundances on two outplanted seedlings.

We found compelling evidence that fungal composition is an important driver of seedling biomass. Age, site, fire severity, and fungal composition were the top variables predicting seedling biomass. Site and fire severity are correlated variables (Appendix 4.3), which contributes to the similarity in their ranked variable importance scores and suggests that, although some other site factors may influence seedling biomass, fire-severity is likely the main driver. Previous research in the boreal forest has shown that residual organic depths strongly influence successional trajectories and successful seedling establishment (Johnstone and Chapin 2006, Johnstone et al. 2010). Our results support those findings and also highlight for the first time the importance of fungal symbionts to treeline seedling biomass. Although distance to the nearest shrub was not one of the most important variables predicting biomass, the partial dependence plot depicts a complex relationship between seedlings and adjacent resprouting vegetation. Seedlings that were at a distance greater than 150 cm had a marked increase in predicted biomass. This likely reflects a balance between the negative effects of root competition and positive CMN-effects on seedling biomass within 150 cm (Booth and Hoeksema 2010) and then a dramatically positive effect on seedling biomass when the seedling is beyond the range of root competition and released from the detrimental effects of shrubs.

Regardless of the potentially complex impact of shrubs on seedling biomass, we observed a significant correlation between fungal composition and the nitrogen economy of seedlings in our CCA analysis. These findings are in support of studies during primary succession, where species-level effects on C:N ratio were detected (Nara 2006a). Although the important role of EMF in providing mineral nutrients to host plants is well established, these observations are often made under artificial conditions. Our results show the important role of EMF for *naturally* established seedlings colonized by complex communities of native fungi. In particular, *Russula nitida* was significantly correlated with a decrease in seedling C:N. This taxon has been detected in inorganic nitrogen-rich soils in the boreal forest (Toljander *et al.* 2006) suggesting that it might be effective at mobilizing labile sources of nitrogen, made more available after fire, to host plants.

Our geographic sequence comparisons indicated that mycobionts of tree seedlings at treeline after fire-disturbance were also observed in undisturbed sites on tall and dwarf shrubs in tundra. Similarly, Timling *et al.* (2014) found that 73% of all fungal taxa observed in the southern-most extent of Arctic tundra, the region most likely to undergo conversion from tundra to boreal forest, were detected in the boreal forest. Furthermore, there is continuity between the understory composition in the boreal forest and tundra plant communities (Hollingsworth *et al.* 2006), particularly with regard to EMF hosts such as *Salix* sp. and *Dryas* sp. Together, this evidence suggests that appropriate inoculum is present on the landscape pre-fire, and, when resprouting shrubs are present post-fire, fire severity does not reduce the availability of inoculum for establishing tree seedlings. Moreover, resprouting shrubs support EMF that support seedling biomass accrual and influences seedling nutrient status. From this result, we would expect that in

shrub tundra, where there are abundant EMF host shrubs, EMF nurse shrubs may facilitate forest expansion.

ACKNOWLEDGEMENTS

This research was supported by funding from Alaska EPSCoR (EPS-0701898) and the state of Alaska, a National Science Foundation Graduate Research Fellowship (DGE-0639280 and 1242789), and a Center for Global Change student award to REH. In-kind support was provided by the Bonanza Creek Long-Term Ecological Research site and the U.S. Forest Service PNW Research Station. We thank Dana Nossov for field support and Jamie Hollingsworth for producing Figure 4.1.

REFERENCES

Agerer, R. 2001. Exploration types of ectomycorrhizae - A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza **11**:107-114.

Baar, J., T. R. Horton, A. M. Kretzer, and T. D. Bruns. 1999. Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. New Phytologist **143**:409-418.

Beals, E. W. 1984. Bray-Curtis ordination: and effective strategy for analysis of multivariate ecological data. Advances in Ecological Research **14**:1-55.

Bent, E., and D. L. Taylor. 2010. Direct amplification of DNA from fresh and preserved ectomycorrhizal root tips. Journal of Microbiological Methods **80**:206-208.

Berry, K. J., K. L. Kvamme, and P. W. Mielke. 1983. Improvements in the permutation test for the spatial-analysis of the distribution of artifacts into classes. American Antiquity **48**:547-553.

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

Bray, J. R., and J. T. Curtis. 1957. An Ordination of the Upland Forest Communities of Southern Wisconsin. Ecological Monographs **27**:325-349.

Breiman, L. 1996. Bagging predictors. Machine learning **24**:123-140.

Breiman, L. 2001a. Random forests. Machine learning **45**:5-32.

Breiman, L. 2001b. Statistical modeling: The two cultures. Statistical Science **16**:199-231.

Chapin, F. S., M. Sturm, M. C. Serreze, J. P. McFadden, J. R. Key, A. H. Lloyd, A. D. McGuire, T. S. Rupp, A. H. Lynch, J. P. Schimel, J. Beringer, W. L. Chapman, H. E. Epstein, E. S. Euskirchen, L. D. Hinzman, G. Jia, C. L. Ping, K. D. Tape, C. D. C. Thompson, D. A. Walker, and J. M. Welker. 2005. Role of land-surface changes in Arctic summer warming. Science **310**:657-660.

Connell, J. H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. Pages 298-312 *in* P. J. Den Boer and G. R. Gradwell, editors. Dynamics of Population: Proceedings of the advanced study institute on dynamics of numbers of populations. Center for Agricultural Publishing and Documentation, Wageningen.

Cutler, D. R., T. C. Edwards Jr, K. H. Beard, A. Cutler, K. T. Hess, J. Gibson, and J. J. Lawler. 2007. Random forests for classification in ecology. Ecology **88**:2783-2792.

Dahlberg, A. 2002. Effects of fire on ectomycorrhizal fungi in fennoscandian boreal forests. Silva Fennica **36**:69-80.

DEEMY. 2014. An Information System for Characterization and DEtermination of EctoMYcorrhizae. Ludwig-Maximilians-Universität München, Dept. Biologie I – Systematische Mykologie.

Deslippe, J. R., M. Hartmann, W. W. Mohn, and S. W. Simard. 2011. Long-term experimental manipulation of climate alters the ectomycorrhizal community of *Betula nana* in Arctic tundra. Global Change Biology **17**:1625-1636.

Diaz-Uriarte, R., and S. A. de Andres. 2006. Gene selection and classification of microarray data using random forest. Bmc Bioinformatics **7** 1-3.

Dickie, I. A., and P. B. Reich. 2005. Ectomycorrhizal fungal communities at forest edges. Journal of Ecology **93**:244-255.

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

Dunbar, J., L. O. Ticknor, and C. R. Kuske. 2001. Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities. Applied and Environmental Microbiology **67**:190-197.

Fisher, M. M., and E. W. Triplett. 1999. Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. Applied and Environmental Microbiology **65**:4630-4636.

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

Germino, M., W. Smith, and A. C. Resor. 2002. Conifer seedling distribution and survival in an alpine-treeline ecotone. Plant Ecology **162**:157-168.

Grogan, P., J. Baar, and T. D. Bruns. 2000. Below-ground ectomycorrhizal community structure in a recently burned bishop pine forest. Journal of Ecology **88**:1051-1062.

He, X.-H., C. Critchley, and C. Bledsoe. 2003. Nitrogen transfer within and between plants through common mycorrhizal networks (CMNs). Critical Reviews in Plant Sciences **22**:531-567.

Hewitt, R. E., E. Bent, T. N. Hollingsworth, F. S. Chapin, and D. L. Taylor. 2013. Resilience of Arctic Mycorrhizal Fungal Communities after Wildfire Facilitated by Resprouting Shrubs. Ecoscience **20**:296-310.

Hobbie, E. A., and R. Agerer. 2010. Nitrogen isotopes in ectomycorrhizal sporocarps correspond to belowground exploration types. Plant and Soil **327**:71-83.

Hobbie, S. E., and F. S. Chapin. 1998. An experimental test of limits to tree establishment in Arctic tundra. Journal of Ecology **86**:449-461.

Hollingsworth, T., M. Walker, F. S. Chapin, and A. Parsons. 2006. Scale-dependent environmental controls over species composition in Alaskan black spruce communities. Canadian Journal of Forest Research **36**:1781-1796.

Horton, T. R., T. D. Bruns, and V. T. Parker. 1999. Ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment. Canadian Journal of Botany-Revue Canadienne De Botanique **77**:93-102.

Huang, X., and A. Madan. 1999. CAP3: A DNA Sequence Assembly Program. Genome Research **9**:898-877.

Janzen, D. H. 1970. Herbivores and the number of tree species in tropical forests American Naturalist **104**:501-528.

Johnstone, J., F. S. Chapin, J. Foote, S. Kemmett, K. Price, and L. Viereck. 2004. Decadal observations of tree regeneration following fire in boreal forests. Canadian Journal of Forest Research **34**:267-273.

Johnstone, J. F., and F. S. Chapin. 2006. Effects of soil burn severity on post-fire tree recruitment in boreal forest. Ecosystems **9**:14-31.

Johnstone, J. F., F. S. Chapin, T. N. Hollingsworth, M. C. Mack, V. Romanovsky, and M. Turetsky. 2010a Fire, climate change, and forest resilience in interior Alaska. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **40**:1302-1312.

Kennedy, P. G., K. G. Peay, and T. D. Bruns. 2009. Root tip competition among ectomycorrhizal fungi: Are priority effects a rule or an exception? Ecology **90**:2098-2107.

Kõljalg, U., R. H. Nilsson, K. Abarenkov, L. Tedersoo, A. F. S. Taylor, M. Bahram, S. T. Bates, T. D. Bruns, J. Bengtsson-Palme, T. M. Callaghan, B. Douglas, T. Drenkhan, U. Eberhardt, M. Dueñas, T. Grebenc, G. W. Griffith, M. Hartmann, P. M. Kirk, P. Kohout, E. Larsson, B. D. Lindahl, R. Lücking, M. P. Martín, P. B. Matheny, N. H. Nguyen, T. Niskanen, J. Oja, K. G. Peay, U. Peintner, M. Peterson, K. Põldmaa, L. Saag, I. Saar, A. Schüßler, J. A. Scott, C. Senés, M. E. Smith, A. Suija, D. L. Taylor, M. T. Telleria, M. Weiss, and K.-H. Larsson. 2013. Towards a unified paradigm for sequence-based identification of fungi. Molecular Ecology **22**:5271-5277.

Landhausser, S. M., and R. W. Wein. 1993. Postfire vegetation recovery and tree establishment at the arctic treeline: climate-change-vegetation-response hypotheses. Journal of Ecology **81**:665-672.

Last, F., J. Dighton, and P. Mason. 1987. Successions of sheathing mycorrhizal fungi. Trends in Ecology & Evolution **2**:157-161.

Lloyd, A. H. 2005. Ecological histories from Alaskan tree lines provide insight into future change. Ecology **86**:1687-1695.

Lloyd, A. H., C. L. Fastie, and H. Eisen. 2007. Fire and substrate interact to control the northern range limit of black spruce (*Picea mariana*) in Alaska. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **37**:2480-2493.

Macias Fauria, M., and E. A. Johnson. 2008. Climate and wildfires in the North American boreal forest. Philosophical Transactions of the Royal Society B: Biological Sciences **363**:2315-2327.

Mantel, N. 1967. The detection of disease clustering and generalized regression approach. Cancer Research **27**:209-220.

McCune, B., and J. B. Grace. 2001. Analysis of Ecological Communities. 2nd edition. MjM Software Design, Gleneden Beach, Oregon, USA.

Molina, R., H. Massicotte, and J. M. Trappe. 1992. Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. Pages 357-423 *in* M. F. Allen, editor. Mycorrhizal functioning: An integrative plant-fungal process. Chapman and Hall, New York, New York, USA.

Nara, K. 2006a. Ectomycorrhizal networks and seedling establishment during early primary succession. New Phytologist **169**:169-178.

Nara, K. 2006b. Pioneer dwarf willow may facilitate tree succession by providing late colonizers with compatible ectomycorrhizal fungi in a primary successional volcanic desert. New Phytologist **171**:187-198.

Nara, K., H. Nakaya, and T. Hogetsu. 2003. Ectomycorrhizal sporocarp succession and production during early primary succession on Mount Fuji. New Phytologist **158**:193-206.

Newman, E. 1988. Mycorrhizal links between plants: their functioning and ecological signiÔ¨Åcance. Adv. Ecol. Res **18**:243-270.

Peay, K. G., P. G. Kennedy, and T. D. Bruns. 2011. Rethinking ectomycorrhizal succession: are root density and hyphal exploration types drivers of spatial and temporal zonation? Fungal Ecology **4**:233-240.

Perry, D. A., M. P. Amaranthus, J. G. Borchers, S. L. Borchers, and R. E. Brainerd. 1989. Bootstrapping in Ecosystems: Internal interactions largely determine productivity and stability in biological systems with strong positive feedback. Bioscience **39**:230-237.

Pickett, S., S. Collins, and J. Armesto. 1987. A hierarchical consideration of causes and mechanisms of succession. Vegetatio **69**:109-114.

Prasad, A. M., L. R. Iverson, and A. Liaw. 2006. Newer classification and regression tree techniques: Bagging and random forests for ecological prediction. Ecosystems **9**:181-199.

Qi, Y. 2012. Random Forest for Bioinformatics. Pages 307-323 *in* C. Zhang and Y. Ma, editors. Ensemble Machine Learning. Springer US.

Read, D. J. 1991. Mycorrhizas in ecosystems. Experientia **47**:376-391.

Reithmeier, L., and G. Kernaghan. 2013. Availability of Ectomycorrhizal Fungi to Black Spruce above the Present Treeline in Eastern Labrador. PLoS One **8**.

Rocha, A. V., M. M. Loranty, P. E. Higuera, M. C. Mack, F. S. Hu, B. M. Jones, A. L. Breen, E. B. Rastetter, S. J. Goetz, and G. R. Shaver. 2012. The footprint of Alaskan tundra fires during the past half-century: implications for surface properties and radiative forcing. Environmental Research Letters **7**:044039.

Smith, S. E., and D. J. Read. 2008. Mycorrhizal Symbiosis. third edition. Academic Press, New York, New York, USA.

Stendell, E. R., T. R. Horton, and T. D. Bruns. 1999. Early effects of prescribed fire on the structure of the ectomycorrhizal fungus community in a Sierra Nevada ponderosa pine forest. Mycological Research **103**:1353-1359.

Taylor, D. L., and T. D. Bruns. 1999. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. Molecular Ecology **8**:1837-1850.

Taylor, D. L., and S. Houston. 2011. A Bioinformatics Pipeline for Sequence-Based Analyses of Fungal Biodiversity. Pages 141-155 *in* J.-R. Xu and B. H. Bluhm, editors. Fungal Genomics. Humana Press, New York, New York, USA.

Ter Braak, C. J. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology **67**:1167-1179.

Timling, I., A. Dahlberg, D. A. Walker, M. Gardes, J. Y. Charcosset, J. M. Welker, and D. L. Taylor. 2012. Distribution and drivers of ectomycorrhizal fungal communities across the North American Arctic. Ecosphere **3**:3:art111. http://dx.doi.org/110.1890/ES1812-00217.00211.

Timling, I., D. A. Walker, C. Nusbaum, N. J. Lennon, and D. L. Taylor. 2014. Rich and cold: Diversity, distribution and drivers of fungal communities in patterned-ground ecosystems of the North American Arctic. Molecular Ecology **Accepted**.

Toljander, J. F., U. Eberhardt, Y. K. Toljander, L. R. Paul, and A. F. S. Taylor. 2006. Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. New Phytologist **170**:873-884.

Treseder, K., M. Mack, and A. Cross. 2004. Relationships among fires, fungi and soil dynamics in Alaskan boreal forests. Ecological Applications **14**:1826-1838.

van der Heijden, M. G. A., and T. R. Horton. 2009. Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. Journal of Ecology **97**:1139-1150.

White, P. S. 1979. Pattern, process, and natural disturbance in vegetation. The Botanical Review **45**:229-299.

White, T. J., T. Bruns, S. Lee, and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 *in* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, editors. PCR Protocols: A Guide to Methods and Applications. Academic Press, New York.

FIGURES

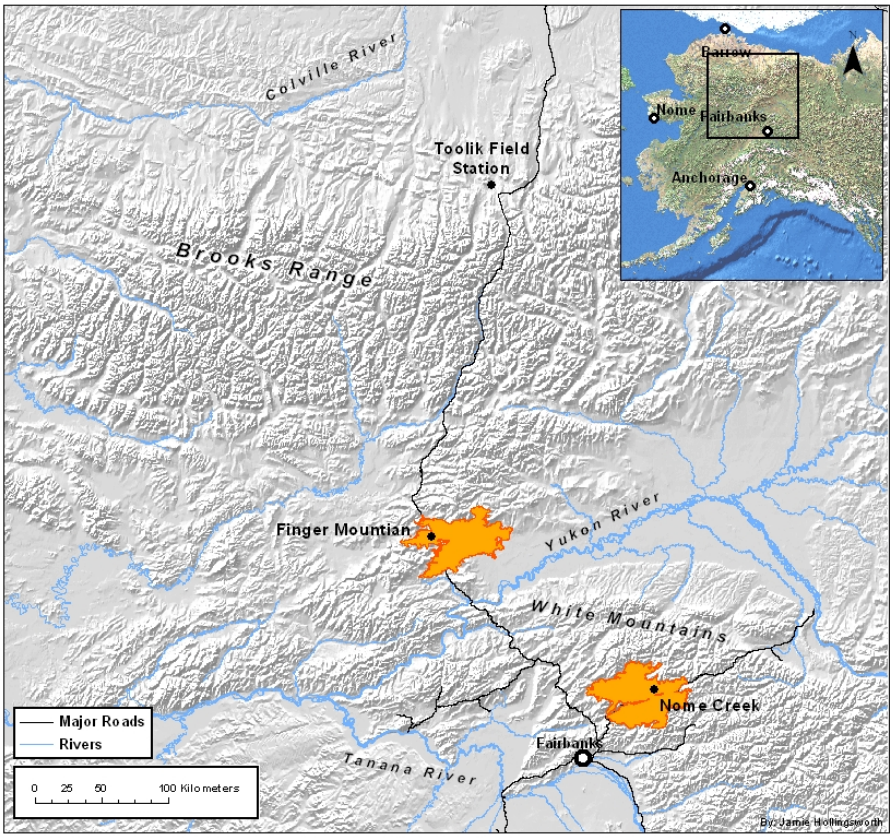


Figure 4.1. A map of our study region with sampling sites at Toolik Lake, where we sampled outplanted seedlings in unburned arctic tundra, and at Finger Mountain and Nome Creek, where we sampled naturally established seedlings at post-fire treeline sites. Finger Mountain and Nome Creek, the burned treeline study sites, are within burn scars from wildfires in 2004 shown as orange polygons.

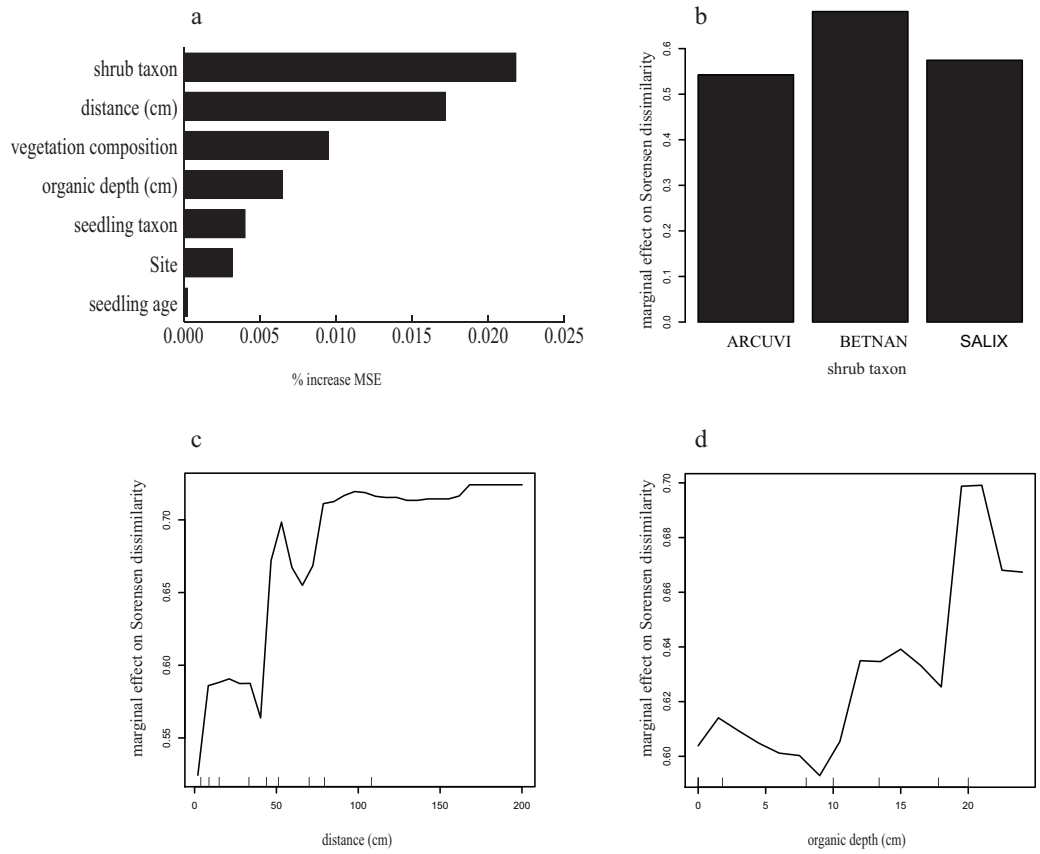


Figure 4.2a-d. Variable importance scores and partial dependence plots from Random Forest regression trees predicting the Sørensen dissimilarity between seedlings and adjacent shrubs. Variable importance scores are derived from the increase in the mean square error when a variable is permuted. Panel a. depicts the relative variable importance for all variables included in our Random Forest model. Partial dependence plots depict predicted dissimilarity in fungal communities in relation to each explanatory variable after accounting for the average effect of all other explanatory variables Panels b-d depict the marginal effect of b. shrub taxon, c. distance between a focal seedling and the closest adjacent shrub, and c. the depth of the residual organic horizon, a proxy for fire severity, at each seedling to the similarity between seedling and shrub EMF composition. ARCUVI=*Arctostaphylos uva-ursi*, BETNAN=*Betula nana.* On the y-axis 0= the same community, 1= no shared taxa.

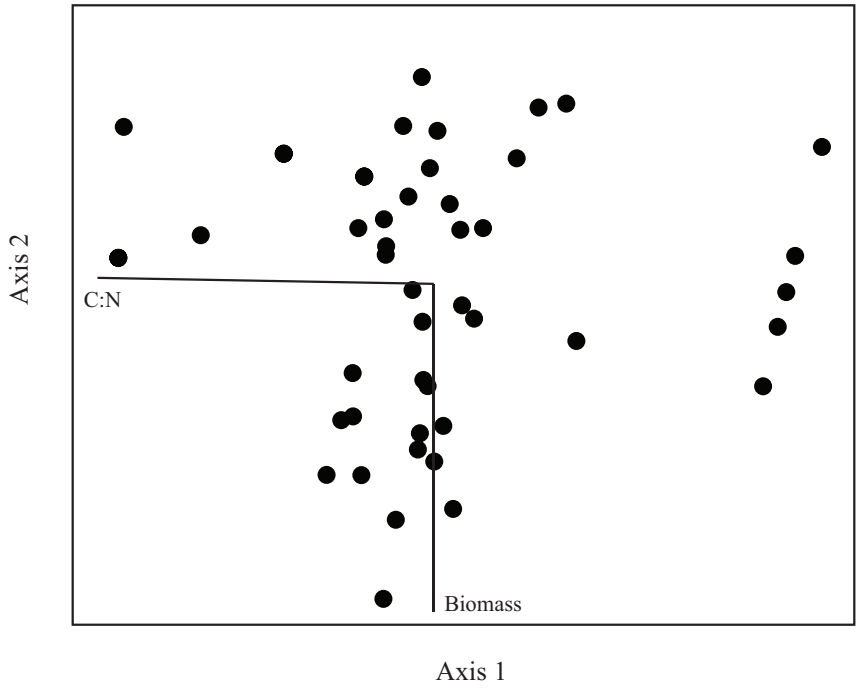


Figure 4.3. Canonical Correspondence Analysis ordination of dominant fungal ribotypes in environmental space using LC (linear combination) scores. Biplot vectors show direction and magnitude of the correlation between species composition and foliar C:N and biomass for seedlings harvested at Finger Mountain and Nome Creek.

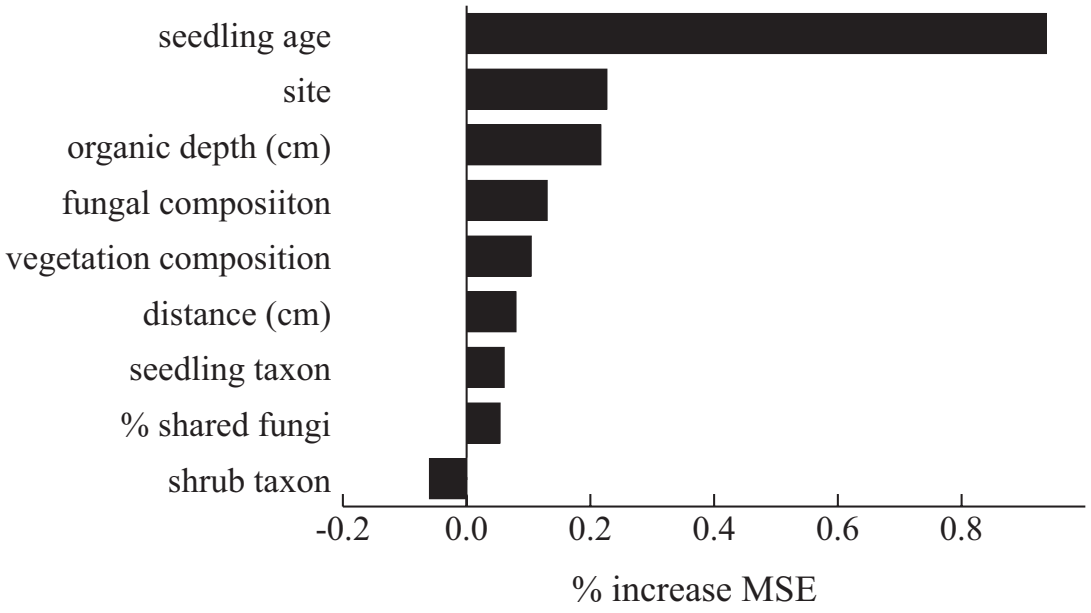


Figure 4.4. Variable importance scores resulting from Random Forest regression trees predicting seedling biomass. Variable importance scores are derived from the increase in the mean square error when a variable is permuted. Percentage shared fungi is the percentage of fungal taxa shared between a seedling and the adjacent shrub.

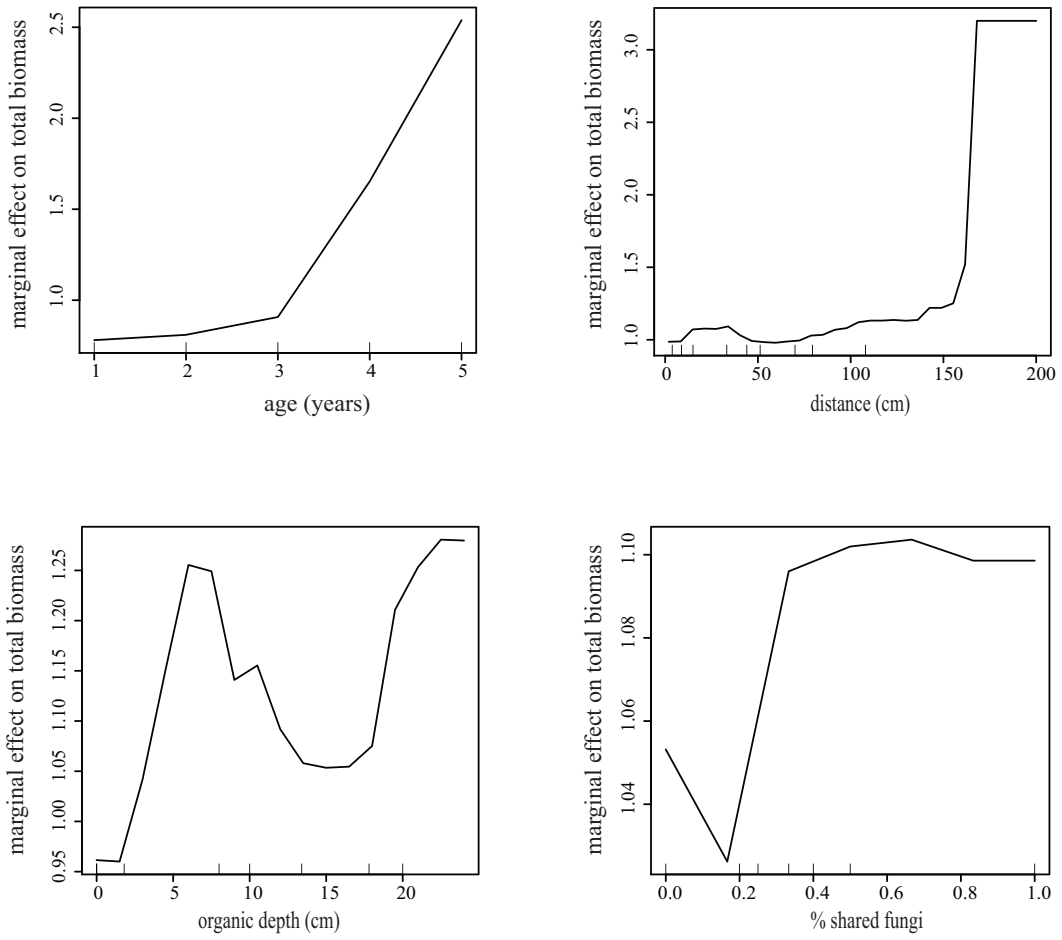


Figure 4.5a-d. Partial dependence plots from Random Forest regression trees predicting seedling biomass in relation to a. seedling age (years), b. the distance between a seedling and the closest resprouting shrub, d. the percent of EMF taxa shared between a seedling and the closest resproutning shrub. Partial dependence plots depict predicted biomass in relation to each explanatory variable after accounting for the average effect of all other explanatory variables.

TABLES

Table 4.1. EMF Operational Taxonomic Units (OTUs) detected on the root systems of seedlings and adjacent shrubs at Nome Creek, Finger Mountain, and Toolik Lake. Phylum B= Basidiomycota; A= Ascomycota. Guild EMF= ectomycorrhizal fungus, DSE= dark septate endophyte, ERM= ericoid mycorrhizal fungus. Identity (%) is the sequence similarity between the query sequence and the hit sequence from the GenBank or UNITE database. Coverage is the percentage of the top match sequence that matches up with the query sequence.

Database	OTU	Reference	Taxon	Phylum	Guild	Bit Score	Identity(%)	Coverage
GenBank	OTU2[ab]	JQ712012.1|	*Thelophora terrestris*	B	EMF	1197	99	100
GenBank	OTU3[ab]	AJ889968.1	*Pseudotomentella tristis*	B	EMF	1254	100	100
GenBank	OTU4[a]	AJ534916.1|	*Tomentella* sp.	B	EMF	1062	96	97
UNITE	OTU5[a]	AF325596|SH105145.05FU	*Cortinarius alboviolaceus*	B	EMF	926	97	90
GenBank	OTU6[a]	JQ888186.1	*Lactarius vietus*	B	EMF	1286	99	100
GenBank	OTU7[ab]	AB671499.2	*Phialocephala fortinii*	A	DSE	1589	99.21	100
GenBank	OTU8[a]	HM189788.1	*Laccaria* cf. *laccata*	B	EMF	1210	98.4	100
GenBank	OTU9[ab]	AY061696.1	*Russula* sp.	B	EMF	1277	100	100
UNITE	OTU10[a]	JX045670|SH166458.05FU	*Cortinarius aurantioba*	B	EMF	1164	99	90
GenBank	OTU11[a]	JQ088277.1	*Meliniomyces variabilis*	A	ERM	1020	99.29	100
UNITE	OTU12[a]	UDB003315|SH112369.05FU	*Tomentella terrestris*	B	EMF	1088	98	90
UNITE	OTU13[a]	JQ888195|SH104545.05FU	*Russula aeruginea*	B	EMF	1098	98	90
GenBank	OTU14[a]	AJ534914.1	*Tomentella* sp.	B	EMF	1177	100	97
GenBank	OTU16[a]	AB598090.1	*Helotiales*	A	unk.	987	98.57	100
GenBank	OTU17[a]	HQ604315.1	Inocybe sp.	B	EMF	1020	93.42	100
GenBank	OTU18[a]	EU862204.1	*Clavulina* cf. *amethystina*	B	EMF	1271	99.86	100
GenBank	OTU19[a]	JN943924.1	*Tylospora asterophora*	B	EMF	1149	100	100
GenBank	OTU20[a]	AM084698.1	*Cenococcum geophilum*	A	EMF	955	99.24	100
UNITE	OTU21[a]	FJ013060|SH113360.05FU	*Pustularia*	A	EMF	793	96	85
GenBank	OTU22[a]	HQ215816.1	*Tomentella* sp.	B	EMF	1090	100	90
GenBank	OTU24[a]	JF908219.1	*Inocybe striaepes*	B	EMF	1059	99	91
UNITE	OTU25[a]	GU452530|SH111021.05FU	Ceratobasidiaceae	B	EMF	1150	99.83	89

Table 4.1. continued

GenBank	OTU26[ab]	HM189968.1	*Tomentella* sp.	B	EMF	1116	98	98
GenBank	OTU27[a]	DQ069035.1	Leptodontidium sp.	A	DSE	931	99	92
GenBank	OTU28[a]	KC750231.1	*Boletus edulis*	B	EMF	1393	100	100
GenBank	OTU29[ab]	AY394885.1	*Meliniomyces bicolor*	A	ERM	1520	97.64	100
GenBank	OTU32[a]	JQ753774.1	*Tomentellopsis submollis*	B	EMF	1194	98.38	100
GenBank	OTU33[a]	DQ097872.1	*Lactarius glyciosmus*	B	EMF	1303	99	100
UNITE	OTU34[a]	JN580836|SH115122.05FU	*Inocybe lapponica*	B	EMF	1124	100	87
GenBank	OTU35[a]	AB190393.1	*Cadophora finlandica*	A	DSE	924	97.94	97
UNITE	OTU36[a]	AF268894|SH105234.05FU	*Cortinarius* sp.	B	EMF	894	96	92
UNITE	OTU37[ab]	UDB011115|SH101144.05FU	*Russula vinososordida*	B	EMF	1158	98	90
GenBank	OTU38[a]	AM887700.1	*Rhizoscyphus* sp,	A	Endophyte/DSE	863	95.1	100
UNITE	OTU40[b]	UDB001659|SH099706.05FU	*Tomentella* sp.	B	EMF	1051	98	91
UNITE	OTU41[b]	UDB011117|SH102330.05FU	*Russula renidens*	B	EMF	1172	100	91
UNITE	OTU42[b]	UDB001641|SH113568.05FU	*Russula versicolor*	B	EMF	1189	99	95
UNITE	OTU43[b]	UDB000104|SH102894.05FU	*Laccaria laccata*	B	EMF	1118	98	91
UNITE	OTU45[b]	AY033087|SH119554.05FU	*Phialocephala fortinii*	A	DSE	866	98	93
UNITE	OTU46[b]	UDB013733|SH107552.05FU	*Sebacina* sp.	B	EMF	856	95	91
GenBank	OTU47[b]	JX907815.1	*Piloderma* sp.	B	EMF	1055	99	96
GenBank	OTU48[b]	JQ711843.1	Sebacinaceae	B	EMF	730	88.35	100
GenBank	OTU49[b]	JQ711917.1	*Tomentella bryophila*	B	EMF	1059	97	100
GenBank	OTU50[b]	JN580813.1	*Inocybe borealis*	B	EMF	1040	99	90
UNITE	OTU51[b]	FN669174|SH108557.05FU	*Clavulina* sp.	B	EMF	995	95	91
UNITE	OTU52[b]	UDB003173|SH101292.05FU	*Hebeloma* sp.	B	EMF	989	95	91
GenBank	OTU53[b]	U83482.1|TSU83482	*Tomentella* sp.	B	EMF	974	96	100
GenBank	OTU54[b]	AM087275.1	*Cenococcum geophilum*	A	EMF	1338	97.12	100
GenBank	OTU55[a]	gHQ604430.1	*Inocybe lacera*	B	EMF	1236	100	100
GenBank	OTU56[a]	HQ157840.1	*Meliniomyces vraolstadiae*	A	ERM	874	97.82	100
GenBank	OTU57[a]	KF241543.1	*Lactarius rufus*	B	EMF	1332	100	100
GenBank	Singleton6[a]	JQ711816.1	*Amphinema byssoides*	B	EMF	1009	97.62	100
GenBank	Singleton9[a]	GU234047.1	*Russula violaceoincarnata*	B	EMF	1116	97.13	96

Table 4.1. continued

GenBank	Singleton10[a]	JX630675.1	*Tomentella* aff. *badia*	B	EMF	1066	96	100
GenBank	Singleton14[a]	EU998918.1	*Articulospora tetracladia*	A	Endophyte	941	97.63	100
GenBank	Singleton28[a]	EU998918.1	*Articulospora* sp.	A	Endophyte	863	95.09	100
UNITE	Singleton29[a]	UDB015636|SH106769.05FU	Thelophoraceae sp.*	B	EMF	1007	96	90
GenBank	Singleton31[a]	EU883432.1	*Tetracladium* sp..	A	Endophyte	983	98.91	100
GenBank	Singleton32[a]	JQ711930.1	*Piloderma sphaerosporum*	B	EMF	1164	99.69	100
UNITE	Singleton33[a]	AY524845|SH117250.05FU	*Phialocephala sphaeroides*	A	DSE	896	98	89
GenBank	Singleton36[b]	HQ157840.1	*Meliniomyces vraolstadiae*	A	ERM	874	98	100
GenBank	Singleton37[b]	AF430254.1	*Hebeloma velutipes*	B	EMF	1088	97	100
UNITE	Singleton38[b]	HM190119|SH113987.05FU	Helotiales	B	EMF	753	90	96
GenBank	Singleton39[b]	HQ604214.1	*Inocybe* sp.	B	EMF	948	94	100
UNITE	Singleton43[b]	JQ711813|SH112374.05FU	*Tomentella* sp.	B	EMF	938	95	93
UNITE	Singleton44[b]	UDB003179|SH101293.05FU	*Hebeloma* sp.	B	EMF	975	94	94
GenBank	Singleton45[b]	AF272926.1|AF272926	*Tomentella lateritia*	B	EMF	1007	99	89
UNITE	Singleton46[b]	UDB000993|SH102123.05FU	*Peziza* sp.	A	EMF	741	94	91
UNITE	Singleton47[b]	UDB008226|SH105784.05FU	*Entoloma* sp.	B	EMF	1168	94.03	100
UNITE	Singleton48[b]	UDB011683|SH100143.05FU	*Lactarius trivialis*	B	EMF	1237	99	95
GenBank	Singleton49[b]	GU234147.1	*Lactarius uvidus*	B	EMF	1249	100	100
GenBank	Singleton50[b]	JN225936.1	Helotiales	A	unk.	623	91.38	92
GenBank	Singleton53[b]	AB671499.2	*Phialocephala fortinii*	A	DSE	414	96.68	100
UNITE	Singleton55[b]	UDB000231|SH112640.05FU	*Tomentella* sp.	B	EMF	731	94	99
GenBank	Singleton56[b]	AY669581.1	*Cortinarius palustris* var. *sphagneti*	B	EMF	1146	97	99
UNITE	Singleton57[b]	UDB013733|SH107552.05FU	*Sebacina* sp.	B	EMF	951	97	91
GenBank	Singleton58[b]	HQ157883.1	*Meliniomyces vraolstadiae*	A	ERM	837	96.64	99
GenBank	Singleton59[b]	JQ665490.1	*Sebacina epigaea*	B	EMF	977	98	97
UNITE	Singleton60[b]	U83480|SH112490.05FU	*Tomentella ramosissima*	B	EMF	969	98	85
UNITE	Singleton61[b]	FN429013|SH105144.05FU	*Cortinarius casimiri*	B	EMF	963	99	90
UNITE	Singleton62[a]	UDB002549|SH101149.05FU	*Russula violaceoincarn*	B	EMF	910	97	100
GenBank	Singleton63[a]	JX436967.1	*Russula* sp.	B	EMF	1092	100	84
GenBank	Singleton64[a]	HQ604208.1	*Inocybe_subcarpta*	B	EMF	1149	98.47	100
GenBank	Singleton65[a]	FJ876170.1	*Russula claroflava*	B	EMF	929	96.3	97
UNITE	Singleton68[a]	UDB001230|SH165610.05FU	*Cortinarius diasemospermus var. leptospermus*	B	EMF	973	99	87
UNITE	Singleton69[a]	HQ604208.1	*Inocybaceae* sp.	B	EMF	641	84.47	100
GenBank	Singleton71[a]	AF418637.1	*Russula decolorans*	B	EMF	1116	100	100
GenBank	Singleton72[a]	AB634267.1	*Thelephora terrestris*	B	EMF	440	99	96

[a] observed on naturally established seedling
[b] observed on outplanted seedling

Table 4.2. Distribution of GenBank matches in the boreal forest and beyond treeline in Arctic tundra for fungi shared by seedlings and adjacent shrubs. The top 100 GenBank matches at the species level (>90% coverage of ITS1-5.8s-ITS2) gene region with >97% sequence similarity) were considered. Ribotypes with multiple OTU assignments and those that did not have an OTU assignment (ribo51, ribo25, ribo15) were excluded from this analysis. We recognize that some taxa, e.g. *Cortinarius* sp., generally require higher stringency for species level matches, ie. 99% similarity; for OTU 36 sequence matches in the boreal region match at 99% and those in the Arctic match at 98% similarity

			Biome		Host Plant					
OTU	# of seedling-shrub pairs	Name	Arctic tundra	Boreal Forest	*Arctosaphylos uva-ursi*	*Betula nana*	*Betula neo-alaskana*	*Picea glauca*	*Populus tremuloides*	*Salix* sp.
OTU2	7	*Thelephora terrestris*		+	+	+	+	+	+	+
OTU37	6	*Russula vinososordida*	+			+	+		+	+
OTU29	4	*Meliniomyces bicolor*	+	+		+	+		+	+
OTU57	3	*Lactarius rufus*		+		+	+		+	+
OTU34	2	*Inocybe lapponica*	+						+	+
OTU9	2	*Russula* sp.	+	+		+		+	+	
Singleton14	1	*Gyoerffyella rotula*	+	+		+		+	+	
OTU11	1	*Meliniomyces variabilis*	+	+		+		+		
Singleton6	1	*Amphinema byssoides*		+		+			+	
OTU36	1	*Cortinarius* sp.	+	+		+	+		+	+
OTU19	1	*Tylospora asterophora*		+	+		+		+	
OTU25	1	Ceratobasidiaceae		+		+			+	
OTU12	1	*Tomentella terrestris*	+	+		+		+	+	
OTU33	1	*Lactarius glyciosmus*	+	+					+	+
OTU6	1	*Lactarius vietus*	+	+		+	+		+	
OTU28	1	*Boletus edulis*		+	+				+	
OTU7	1	*Phialocephala fortinii*	+	+		+		+		

Table 4.3. Monte Carlo test results for Canonical Correspondence Analysis of EMF composition and seedling biomass and C:N ratio

Axis	Environmental variable	Real Data	Randomized Data				Real Data	Randomized Data			
		Eigenvalue	Mean	Minimum	Maximum	p	Species-environemtn correlation	Mean	Minimum	Maximum	p
1	C:N	0.325	0.211	0.055	0.492	0.0591	0.608	0.479	0.243	0.724	0.0501
2	Biomass	0.178	0.093	0.002	0.239		0.444	0.317	0.05	0.535	

Appendix 4.1. Fungal ARISA ribotypes detected on the root systems of seedlings and adjacent resprouting shrubs. ARCUVI=*Arctostaphylos uva-ursi*, BETNAN=*Betula nana*, POPTRE=*Populus tremuloides*, BETNEO=*Betula neo-alaskana*.

Ribotype	Fragment length	abundance	OTU	Best match description	Host plant	Site	Exploration type	Rhizomorphs
1	480	1			POPTRE	Finger Moutnain		
2	548	1			POPTRE	Finger Moutnain		
3	561	1			SALIX	Finger Moutnain		
4	567	5	OTU20	*Cenococcum geophilum*	BETNAN, POPTRE	Finger Moutnain, Nome Creek	short	no
5	584	2			BETNAN, PICEA	Finger Moutnain		
6	590	1			POPTRE	Nome Creek		
7	591	1			BETNEO	Finger Moutnain		
8[bc]	591-592	5	Singleton14	*Articulospora tetracladia*	BETNAN, PICEA, POPTRE	Nome Creek		
9[d]	591-592	5	Singleton28	*Articulospora* sp.	PICEA, POPTRE	Finger Moutnain		
10	593	2			POPTRE	Nome Creek		
11[ab]	593-594	18	OTU35	*Cadophora finlandica*	BETNEO, PICEA, POPTRE, SALIX	Finger Moutnain		
			OTU38	*Rhizoscyphus* sp.				
			OTU56	*Meliniomyces vraolstadiae*				
			Singleton33	*Phialocephala sphaeroides*				
12	595	1			BETNAN	Nome Creek		
13	595	2	Singleton31	*Tetracladium* sp.	BETNAN, POPTRE	Finger Moutnain		
14	598	1			POPTRE	Nome Creek		
15[b]	600	4			ARCUVI, BETNAN, PICEA, POPTRE	Nome Creek		
16	601	1	OTU27	*Leptodontidium* sp.	POPTRE	Nome Creek		
17	601	1			SALIX	Finger Moutnain		
18	603	5	OTU7	*Phialocephala fortinii*	BETNAN, BETNEO, PICEA, SALIX	Finger Moutnain		
19	603	2	OTU16	Helotiales	BETNAN, BETNEO	Nome Creek		
20	604	1			POPTRE	Nome Creek		
21	605	1	OTU21	*Pustularia* sp.	POPTRE	Nome Creek		
22[b]	609-610	2	OTU11	*Meliniomyces variabilis*	BETNAN, PICEA	Finger Moutnain		
23	612	1			POPTRE	Nome Creek		
24	616	1			POPTRE	Finger Moutnain		
25[b]	619-620	5			BETNAN, BETNEO, PICEA, POPTRE	Finger Moutnain		
26	630	1			POPTRE	Finger Moutnain		
27[b]	633	2	Singleton6	*Amphinema byssoides*	BETNAN, POPTRE	Nome Creek	medium-fringe	yes
28	636	1	Singleton68	*Cortinarius diasemospermus* var. *leptospermus*	POPTRE	Finger Moutnain	medium-fringe*	yes*
29[b]	638	7	OTU36	*Cortinarius* sp.	BETNAN, BETNEO, POPTRE, SALIX	Finger Moutnain	medium-fringe*	yes*
30	641	1	OTU5	*Cortinarius alboviolaceus*	PICEA	Finger Moutnain	medium-fringe*	yes*
31	649	1			BETNAN	Finger Moutnain		
32	654	1			BETNAN	Finger Moutnain		
33[b]	668-672	8	OTU19	*Tylospora asterophora*	ARCUVI, BETNEO, POPTRE	Nome Creek	short	no
34	671	1			BETNEO	Finger Moutnain		
35	672	1			SALIX	Finger Moutnain		
36	677	1			POPTRE	Nome Creek		
37	680	1			POPTRE	Nome Creek		
38	682	1			POPTRE	Finger Moutnain		
39	683	1	Singleton32	*Piloderma sphaerosporum*	POPTRE	Finger Moutnain	medium-fringe*	yes*
40	684	1			POPTRE	Nome Creek		
41	692	1	Singleton69	Inocybaceae	PICEA	Finger Moutnain	short*	no*
42	697	1			BETNAN	Finger Moutnain		
43[b]	697-698	3	OTU34	*Inocybe lapponica*	POPTRE, SALIX	Finger Moutnain	short*	no*
44[b]	700	2	OTU25	Ceratobasidiaceae	BETNAN, POPTRE	Nome Creek		
45	701-702	2	OTU24	*Inocybe striaepes*	BETNAN, POPTRE	Nome Creek	short*	no*
46	703	2			BETNAN, POPTRE	Nome Creek		
47[b]	706-707	17	OTU2	*Thelephora terrestris*	ARCUVI, BETNAN, BETNEO, PICEA, POPTRE, SALIX	Finger Moutnain, Nome Creek	medium-smooth	yes
48[ab]	708-711	40	OTU4	*Tomentella* sp.	ARCUVI, BETNAN, BETNEO, PICEA, POPTRE, SALIX	Finger Moutnain, Nome Creek	m,s/c*	yes*
			OTU13	*Russula aeruginea*			medium-smooth	yes
			OTU14	*Tomentella* sp.			m,s/c*	yes*
			OTU22	*Tomentella* sp.			m,s/c*	yes*
			OTU26	*Tomentella* sp.			m,s/c*	yes*
			Singleton10	*Tomentella* aff. *badia*			m,s/c*	yes*
			Singleton29	Thelophoraceae sp.*				
			Singleton64	*Inocybe subcarpta*			short	no
			Singleton72	*Thelephora terrestris*			medium-smooth	yes
49	712	1			BETNAN	Nome Creek		
50[b]	714	3	OTU12	*Tomentella terrestris*	BETNAN, PICEA, POPTRE	Nome Creek	m,s/c*	yes*
51[b]	722-723	3			ARCUVI, POPTRE	Nome Creek		
52[ab]		14	OTU3	*Pseudotomentella tristis*	BETNAN, PICEA, POPTRE, SALIX	Finger Moutnain		
			OTU32	*Tomentellopsis submollis*				
			OTU41	*Russula renidens*			m,s/c*	y/n*
53[c]	728-729	5	OTU55	*Inocybe lacera*	POPTRE	Nome Creek	short	no
54	731	1	OTU10	*Cortinarius aurantioba*	BETNAN	Finger Moutnain	medium-fringe	yes
55	733	1	OTU17	*Inocybe* sp.	BETNEO	Nome Creek	short	no
56	733-734	3			BETNAN, POPTRE	Finger Moutnain		
57	734	1	Singleton62	*Russula violaceoincarn*	BETNAN	Nome Creek	m,s/c*	y/n*
58	735	2	Singleton9	*Russula violaceoincarnata*	BETNAN, BETNEO	Nome Creek	m,s/c*	y/n*
59	737	1			POPTRE	Nome Creek		
60[ab]	738-739	8	OTU18	*Clavulina* cf. *amethystina*	BETNAN, PICEA, POPTRE, SALIX	Finger Moutnain		
			OTU8	*Laccaria laccata*			medium-smooth*	y/n*
61	738	1			POPTRE	Nome Creek		
62	739	2			POPTRE	Nome Creek		
63[bd]	743-744	24	OTU37	*Russula vinososordida*	BETNAN, BETNEO, POPTRE, SALIX	Finger Moutnain	m,s/c*	y/n*
64	743-744	3	Singleton63	*Russula* sp.	BETNAN, POPTRE	Nome Creek	m,s/c*	y/n*
65	745	2			BETNAN, POPTRE	Finger Moutnain		
66[bd]	746-747	8	OTU9	*Russula nitida*	BETNAN, PICEA, POPTRE	Finger Moutnain	m,s/c*	y/n*
67	748	2	Singleton71	*Russula decolorans*	BETNAN, POPTRE	Nome Creek	contact	no
68	749	1	Singleton65	*Russula* sp.	BETNAN	Finger Moutnain	m,s/c*	y/n*
69	758	1			BETNAN	Finger Moutnain		
70[b]	762	2	OTU33	*Lactarius glyciosmus*	POPTRE, SALIX	Finger Moutnain	m,s/c*	no
71[bd]	768	5	OTU57	*Lactarius rufus*	BETNAN, BETNEO, POPTRE, SALIX	Finger Moutnain	m,s/c*	yes
72	769	1			POPTRE	Finger Moutnain		
73[b]	770-772	5	OTU6	*Lactarius vietus*	BETNAN, BETNEO, POPTRE	Nome Creek	m,s/c*	y/n*
75	791	1			POPTRE	Nome Creek		
76[b]	801	2	OTU28	*Boletus edulis*	ARCUVI, POPTRE	Nome Creek	long	yes
77	839	1			POPTRE	Finger Moutnain		
78	866	1			POPTRE	Nome Creek		
79	879	1			BETNAN	Finger Moutnain		
80	906	1			SALIX	Finger Moutnain		
81[b]	920	2	OTU7	*Phialocephala fortinii*	BETNAN, PICEA	Finger Moutnain		
82	927-929	17	OTU29	*Meliniomyces bicolor*	BETNAN, BETNEO, POPTRE, SALIX	Finger Moutnain, Nome Creek		
83	931	1			POPTRE	Finger Moutnain		
84	932	1			POPTRE	Nome Creek		
85	933	1			POPTRE	Finger Moutnain		
86	960	1			POPTRE	Finger Moutnain		
87	993	1			POPTRE	Nome Creek		
88	993	1			POPTRE	Finger Moutnain		
89	1041	1			POPTRE	Finger Moutnain		
90	1048	1			POPTRE	Nome Creek		
91	1078	1			POPTRE	Nome Creek		
92	1078	1			BETNAN	Finger Moutnain		
93	1087	1			POPTRE	Nome Creek		
94	1124	1			BETNEO	Finger Moutnain		
95	1135	1			BETNAN	Finger Moutnain		

Appendix 4.1. continued

[a] excluded from analyses because multiple OTUs were assigned to one ribotype

[b] observed on seedling and adjacent shrub

[c] indicator species of high-severity patches (< 3 cm residual organic soil)

[d] indicator species of low-severity patches (> 3 cm residual organic soil)

[*] means described at genus level in DEEMY (2014).

Appendix 4.2. Sensitivity analysis to determine the effect of removing ribotypes with more than one Operational Taxonomic Unit (OTU) assignment.

To assign phylogenetically explicit taxonomic identities to ARISA ribotypes we made direct matches between DNA sequences and ARISA ribotypes based on ribotype fragment length (described in methods). For four of ninety-four ribotypes we found that several OTUs matched up with one ARISA ribotype (ribotypes 11, 48, 52, and 60, see Appendix 4.1). Because the inclusion of these taxa could inflate our estimates of shared taxa that occur on seedlings and adjacent shrubs, we ran a sensitivity analysis to assess the impact of excluding these taxa from the analysis. First we used the Mantel test (Mantel 1967) with 999 Monte Carlo permutations to evaluate the null hypothesis that there was no significant relationship between the fungal community structure for the full matrix (Matrix A) and the matrix with ribotypes 11, 48, 52, and 60 removed (Matrix B). To format symmetrical matrices we eliminated the fungal community profiles for seven seedlings and shrub pairs where the fungal community profile of one of the pair was characterized by ribotypes 11, 48, 52, and/or 60. The resulting OTU matrix had 124 seedlings and shrubs (rows) with 95 ARISA ribotypes (columns) for Matrix A and Matrix B had 91 ARISA ribotypes (columns). The Mantel Test indicated that there was a positive association between the Matrix A and Matrix B ($r = 0.69$, $p=0.001$). This suggests that the removal of the four ribotypes does not significantly change the composition and structure of the fungal community. We also evaluated Bray Curtis ordinations of seedling fungal communities for Matrix A and Matrix B and found that more of the variance in fungal composition was explained for Matrix B. Both ordinations had a three dimensional solution. For Matrix B the three axes represented 91.3% of the variance in fungal community structure, with axis one contributing

43.2%, axis two 37.8%, and axis three 10.3%. Whereas, for Matrix A the three axes represented 74.7% of the variance in fungal community structure, with axis one contributing 41.7%, axis two 19.1%, and axis three 7.5%. We concluded that using Matrix B composition in our analysis adequately represented fungal composition of our data and that this was the most appropriate and conservative approach given that Matrix B may underestimate linkages between seedlings and shrubs.

Appendix 4.3. Spearman correlations between variables. Significant correlations are bold.

Variable	Variable	Spearman ρ	Prob>|ρ|
Sorensen Dissimilarity	Jaccard Dissimilarity	0.9384	**<.0001**
Fungal Composition	Jaccard Dissimilarity	0.1451	0.3305
Fungal Composition	Sorensen Dissimilarity	0.0711	0.6348
Vegetation Composition	Jaccard Dissimilarity	-0.0923	0.4754
Vegetation Composition	Sorensen Dissimilarity	-0.0523	0.6865
Vegetation Composition	Fungal Composition	-0.0711	0.6349
Age	Jaccard Dissimilarity	-0.108	0.4035
Age	Sorensen Dissimilarity	-0.0841	0.5155
Age	Fungal Composition	-0.1161	0.4371
Age	Vegetation Composition	-0.0686	0.5757
Distance (cm)	Jaccard Dissimilarity	0.3263	**0.0097**
Distance (cm)	Sorensen Dissimilarity	0.3561	**0.0045**
Distance (cm)	Fungal Composition	-0.0508	0.7345
Distance (cm)	Vegetation Composition	-0.3782	**0.0014**
Distance (cm)	Age	0.2269	0.0609
Organic depth (cm)	Jaccard Dissimilarity	-0.0129	0.9204
Organic depth (cm)	Sorensen Dissimilarity	0.0404	0.7554
Organic depth (cm)	Fungal Composition	-0.2248	0.1288
Organic depth (cm)	Vegetation Composition	0.554	**<.0001**
Organic depth (cm)	Age	0.1528	0.21
Organic depth (cm)	Distance (cm)	-0.0331	0.7871

Chapter 5

General Conclusions

The role of fire in treeline advancement with a warming climate

A warming climate has been directly related to treeline advance globally (Harsch et al. 2009). Changes in climate are also directly related to fire regime dynamics in the boreal forest and tundra biomes (Hu et al. 2010, Kelly et al. 2013). It has been hypothesized that fire-effects may prove to be a greater driver of species migrations than the direct effect of warming (Dale et al. 2001). In Alaska and western Yukon Territory, fire regime is tightly coupled with tree seedling recruitment, forest stand development, and the northward migration of lodgepole pine (Johnstone and Chapin 2003, Johnstone and Chapin 2006, Johnstone et al. 2010). Increases in fire severity and extent and decreases in fire return interval are therefore hypothesized to facilitate tree migration and shrub expansion in tundra. Fire, however, can reduce or alter the composition of ectomycorrhizal fungi (EMF) (Cairney and Bastias 2007), the obligate mycobionts of all boreal trees and many tundra shrubs (Molina et al. 1992) that are predicted to expand in the next century.

EMF composition and abundance have been related to successful seedling establishment both within and beyond the current range limit of host plants in other ecosystems (Horton et al. 1999, Nara 2006b, Nunez et al. 2009). In many controlled experiments and in some field investigations EMF are physiologically important to seedling establishment and growth (Smith and Read 2008), life-history stages critical to forecasting future advance or retreat in treeline position (Harsch and Bader 2011). However, the effect of mycobionts on tree seedling establishment and treeline dynamics has been largely overlooked.

In Alaska the impacts of fire on successional trajectories at treeline forest sites and in tundra are not well understood. In order to make accurate predictions of the rate and magnitude of future changes in boreal-tundra landcover in the next decades, it is important to understand the interacting factors that can influence tree seedling recruitment at treeline. My dissertation examined the role that ectomycorrhizal fungi (EMF) may play in post-fire tree seedling recruitment and treeline dynamics. The primary goals of this research were to 1) determine how fire severity affects fungal inoculum associated with resprouting vegetation (Chapter 2, 4) and in soils (Chapter 3) and 2) to assess the effects of post-fire inoculum on tree and shrub seedling performance for plant species expected to expand in tundra over the next century (Chapter 3, 4). With my plot-level studies I was able to elucidate important EMF inoculum sources at and beyond treeline (Chapter 2, 4) and test the relative importance of biotic (EMF) and abiotic (fire and environment) factors that determine seedling growth at treeline after fire (Chapter 4). This research revealed the physiological importance of mycorrhizal fungi to tree seedling growth (Chapter 3, 4) and variation in inoculum potential across the treeline and tundra landscape after fire (Chapter 2, 3, 4). Together, these studies provide a framework to integrate our mechanistic understanding of post-fire plant-fungal interactions into predictions of treeline movement in response to future scenarios of change.

Fire severity effects on plant-fungal interactions with implications for tree and shrub migration

There is strong evidence from boreal forest research that shows a positive effect of fire-severity on seedling establishment with the exposure of mineral soils (Johnstone and Chapin 2006, Johnstone et al. 2010). This has led to the hypothesis that fire will likely facilitate aforestation of tundra by opening up new, high-quality microsites and killing plant competitors (Landhausser and Wein 1993). Contrastingly, fungal communities are often depressed post-fire

when fires kill the majority of host plants or there is severe combustion of the soil (Dahlberg 2002, Cairney and Bastias 2007). I examined the interacting role of fire severity and fungal inoculum on seedling growth for two plant species (alder and spruce) expected to expand in tundra with future warming and fire (Chapter 3). Seedlings were inoculated with soils collected from across a fire-severity gradient in the largest tundra fire recorded in the Arctic, the 2007 Anaktuvuk River Fire (ARF). I found strong relationships between seedling growth and fungal composition, fire severity, and abundance of pathogenic fungi and dark septate endophytes (DSE). This research emphasized the potentially important role of DSE as beneficial mycobionts for seedlings, as has been suggested in other studies (Newsham 2011), in the absence of EMF, and the inhibitory role pathogens may play in seedling growth. These results suggest that effects of fire severity on soil biota may dampen or even reverse the expected increases in tree and shrub establishment after fire. Extrapolating from what we detected in the growth chamber study to the field, I speculate that the negative effects of fire-severity on soil biota will be more pronounced on the landscape when the detrimental effects of pathogens or the reduced, positive effects of DSE and EMF outweigh positive substrate effects on seedling growth and survival. Although we could not test this in the growth chamber study (Chapter 3), in the field we found that fire severity and fungal composition were the two most important predictors of naturally established seedling biomass at treeline (Chapter 4). For naturally established seedlings, fire-severity was a more important predictor of seedling biomass than fungal composition. This suggests that responses of both abiotic, e.g., substrate quality, and biotic, e.g., fungal composition, aspects of fire-severity influence seedling biomass. In turn, the influence of post-fire fungal composition on seedling biomass appears to be context-dependent in relation to burn severity.

The importance of post-fire resprouting vegetation in supporting fungal communities

After low-severity fires, vegetation can resprout from belowground stems. Vegetation that resprouts after fire, such as understory and tundra shrubs, could maintain their mycobionts on the rootstock that survived fire and therefore provide mycelial inoculum to seedlings. In other disturbance-prone systems, existing vegetation facilitates revegetation through the provision of mycelium (Nara 2006a, Nara 2006b). In some of these cases, the ability of seedlings to integrate into common mycorrhizal networks with shrubs increases their biomass and nitrogen uptake (Nara 2006a). Because there is continuity between the understory composition in the boreal forest and tundra plant communities (Hollingsworth *et al.* 2006), particularly with regard to EMF hosts such as *Salix* sp. and *Betula nana* shrubs that can resprout after fire, I investigated how fire severity affects fungal communities associated with shrubs that resprout and whether these resprouts are potential fungal nurse plants that support fungi compatible with seedlings.

To explore the role of resprouting shrubs in post-fire treeline dynamics, I investigated fire-severity effects on mycorrhizal communities associated with resprouting EMF shrubs in tundra and at treeline (Chapters 2, 4). In tundra I detected mycobionts that were primarily EMF with some DSE, ERM, and one putative pathogen (Chapter 2). There was a surprising *lack* of differentiation of mycobionts into fire-specialists and fire-sensitive fungi cross the ARF fire-severity gradient, which contrasted with findings from temperate and boreal studies after severe or frequent wildfire events (Baar et al. 1999, Grogan et al. 2000, Dahlberg 2002). My detection of multi- and late- stage EMF taxa associated with resprouting EMF host plants was observed in tundra (Chapter 2) and at treeline sites (Chapter 4), indicating that the resprouting life history strategy of some EMF shrubs confers resilience of dominant mycorrhizal fungi to fire

disturbance. These findings suggest that EMF shrubs maintain mycorrhizal diversity and function on the landscape after fire across the treeline ecotone.

Finally, I investigated whether the EMF taxa maintained by resprouting shrubs colonized and enhanced the growth of tree seedlings that established after wildfire at treeline in Alaska (Chapter 4). I found that common mycorrhizal networks (CMNs) at treeline likely linked resprouting shrubs with tree seedlings that established after wildfire similar to findings from primary sucesssion (Nara 2006b) and in mature forest stands (Horton et al. 2005). Fungal composition was related to seedling biomass and C: N ratio, as has been observed in a few other field studies (Nara and Hogetsu 2004, Dickie and Reich 2005, Nara 2006a). I also found that distance between seedlings and shrubs was one of the most important predictors of the similarity in fungal composition. Together, these results suggest that EMF mycobionts provided by resprouting post-fire vegetation are important to nutrient acquisition and biomass accrual of naturally establishing tree seedlings at treeline (Chapter 4). When considering the severely limited EMF inoculum provided by soils in the ARF (Chapter 3), this additional evidence (Chapter 4) suggests that resprouting EMF shrubs may be a critical source of inoculum for tree seedling establishment beyond current treeline and in turn, EMF shrubs likely facilitate seedling establishment and aforestation of tundra.

In summary, these empirical studies showed 1) that soils beyond current treeline were not sources of abundant mycorrhizal inoculum, and instead fire severity influenced tree and shrub performance through pathogenic effects or potentially beneficial effects of root endophytes; 2) shrubs that resprouted after wildfire in tundra and at treeline were sources of EMF inoculum; and 3) resprouting shrubs supported fungi that associated with boreal tree seedlings and may facilitate seedling establishment due to the positive influence of fungal symbionts on seedling

nutrition and biomass. Together the results from this dissertation show that EMF host shrubs in tundra are sources of compatible inoculum for boreal tree seedlings and may promote seedling recruitment through growth and nutrient–effects regardless of predicted changes in the fire disturbance regime under conditions of a warming climate.

Integration of plot-level research into landscape modeling of tree migration

Based on my field observations, experiments, and the literature, I developed a conceptual model of abiotic and biotic controls over seedling establishment in order to assess treeline ecosystem vulnerability to climate-induced changes in fire activity. In collaboration with the Scenarios Network for Alaska & Arctic Planning (SNAP), this conceptual framework is being implemented in the spatially explicit fire-climate-vegetation landscape model, ALaska FRame-based EcoSystem Code (ALFRESCO) (Rupp et al. 2000, Rupp et al. 2001). ALFRESCO 2.0 simulates vegetation succession and fire occurrence (Figure 5.1) across Alaska and the western Yukon Territory (Breen et al. 2013, Gray et al. 2013). This modeling exercise links mechanistic investigations of regional patterns of seedling establishment to model projections of sub-continental biome shifts after fire. These landscape-modeling scenarios directly address how future fire regime may influence treeline dynamics. In this section, I describe this modeling approach as a basis for exploring the landscape implications of my empirical results.

Modification of state transitions from tundra to spruce in ALFRESCO 2.0

In collaboration with the SNAP Treeline and Vegetation Dynamics working group, I refined the steps that affect the state transition of a 1 km^2 cell from tundra to spruce due to colonization by seedlings (Breen et al. 2013). This state transition is influenced by fire

occurrence and severity, proximity to a seed source, and favorable conditions for germination, establishment and growth (Figure 5.2). My dissertation research informed the development of the mycorrhizal modifiers that influence seedling establishment and growth (Figure 5.3).

Incorporating mycorrhizal effects on seedling establishment and growth

Based on my experimental and observational studies, we emphasized in the model the role of shrubs as an important source of inoculum on the tundra landscape. We used shrub densities from the Viereck (1992) vegetation classification to estimate inoculum potentials for three tundra classes represented in the model domain: shrub tundra, graminoid tundra, and wet sedge tundra. We view these tundra classes as having a gradient of host plant densities that could support mycorrhizal fungi and therefore mycorrhizal effects on seedling establishment (Figure 5.4). We also parameterized the effects of fire severity on fungal inoculum potential as part of the fire history modifier. There are five classes of fire severity: unburned, low burn severity, moderate burn severity, high crown severity with low surface severity, and high crown severity with high surface severity. Of these burn classes, we expect only the high-severity classes to have a strong effect on inoculum potential. Fire-severity effects on fungal symbionts are related to the degree to which host plants are killed and the amount of soil combusted (Dahlberg 2002). Inoculum potential in relation to pre-fire vegetation class and fire-severity class will then modify the seed: seedling ratio, a proxy for seedling establishment, and seedling growth rates. State transitions from tundra to spruce occur when substantial growth of spruce trees surpasses a basal area threshold.

Future work

My empirical studies indicate that EMF are physiologically important to seedling growth and nutrient status and that post-fire plant fungal interactions influence seedling performance. To determine whether post-fire EMF-seedling interactions influence landscape-level patterns of treeline movement, landscape modeling will be utilized to evaluate the influence of plant-fungal interactions on tree migration and forest development. My results suggest that the accuracy of forecasted state transitions from tundra to forest in ALFRESCO will be improved by incorporating plant-fungal interactions. This will be accomplished by parameterizing state transitions to include fungal effects on the establishment and growth of spruce seedlings. We will assess the influence of EMF rules on vegetation state transitions. We expect that simulations that include EMF-effects on seedling establishment will improve the biological accuracy of predictions of landscape patterns of treeline movement.

Summary and management implications

From my field and growth chamber investigations there are two main findings 1) there is variation in inoculum potential after fire with hot-spots of EMF inoculum compatible with tree seedlings in the close vicinity of resprouting shrubs and 2) fungal inoculum is physiologically important to tree seedling growth in a species-specific manner. Our studies suggest that the outcomes of post-fire plant-fungal interactions are contingent on pre-fire vegetation cover, i.e. presence and abundance of EMF hosts and their ability to resprout. Thus, from the treeline ecotone out into tundra, we can anticipate future seedling recruitment success in relation to

abundance of post-fire inoculum sources, i.e. resprouting shrubs, with higher recruitment in shrub tundra compared to graminoid or wet sedge tundra.

From the results I suggest that land and fire managers who desire to promote aforestation will need to consider mycorrhizal effects. One example of this is the consideration of availability of compatible mycobionts under management practices of assisted migration (Kranabetter et al. 2012). The effects of compatible mutualists on plant migrations has been considered with naturally established plants (Perry et al. 1990). Within the forested landscape, foresters have found that application of EMF inoculum promotes tree seedling establishment and productivity where inoculum is scarce (Trappe 1977, Castellano et al. 1985). These results hold strong parallels with our field studies after fire at treeline where heterogeneity in EMF inoculum composition affects seedling productivity. Thus, variation in EMF inoculum on the landscape may limit tree seedling establishment, but the provision of compatible inoculum could enhance forest recruitment.

Both the mechanistic underpinnings of our planned modeling efforts and model outputs can be used to improve our understanding of tree seedling establishment and growth post-fire at and beyond treeline. The mechanistic understanding of controls over seedling establishment enhances both the short-term accuracy of predicting revegetation within the first decade after fire and in forecasting fire effects and flammability across the treeline ecotone and into tundra on a multi-decadal time scale. Outputs from ALFRESCO 2.0 simulations will represent decision-support tools for land managers in the state of Alaska because they will show the spatially explicit likelihood of conversion from tundra to forest. The empirical studies and planned model outputs from this dissertation research will inform land and fire managers about treeline

sensitivity to fire and vulnerability of forest ecotones to future change in the face of high uncertainty about direct and indirect effects of warming.

REFERENCES

Baar, J., T. R. Horton, A. M. Kretzer, and T. D. Bruns. 1999. Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. New Phytologist **143**:409-418.

Breen, A. L., A. E. Bennett, R. E. Hewitt, T. N. Hollingsworth, H. Genet, E. Euskirchen, T. S. Rupp, and A. D. McGuire. 2013. Tundra fire and vegetation dynamics: simulating the effect of climate change on fire regimes in Arctic ecosystems. American Geophysical Union.

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

Castellano, M. A., J. M. Trappe, and R. Molina. 1985. Inoculation of container-grown Douglas-fir seedlings with basidiospores of *Rhizopogon vinicolor* and *R. colossus*: effects of fertility and spore application rate. Canadian Journal of Forest Research **15**:10-13.

Dahlberg, A. 2002. Effects of fire on ectomycorrhizal fungi in fennoscandian boreal forests. Silva Fennica **36**:69-80.

Dale, V. H., L. A. Joyce, S. McNulty, R. P. Neilson, M. P. Ayres, M. D. Flannigan, P. J. Hanson, L. C. Irland, A. E. Lugo, C. J. Peterson, D. Simberloff, F. J. Swanson, B. J. Stocks, and B. M. Wotton. 2001. Climate change and forest disturbances. Bioscience **51**:723-734.

Dickie, I. A., and P. B. Reich. 2005. Ectomycorrhizal fungal communities at forest edges. Journal of Ecology **93**:244-255.

Gray, S. T., A. W. Bennett, W. R. Bolton, A. L. Breen, T. Carman, E. Euskirchen, H. Genet, E. Jafarov, J. Jenkins, T. Kurkowski, M. Lindgren, T. Martin, S. McAfee, A. D. McGuire, S. Marchenko, R. Muskett, S. Panda, J. Reynolds, A. Robertson, V. Romanovsky, T. S. Rupp, K. Timm, and Y. Zhang. 2013. Using Integrated Ecosystem Modeling to Understand Climate Change. Alaska Park Science **12**:1-17.

Grogan, P., J. Baar, and T. D. Bruns. 2000. Below-ground ectomycorrhizal community structure in a recently burned bishop pine forest. Journal of Ecology **88**:1051-1062.

Harsch, M. A., and M. Y. Bader. 2011. Treeline form - a potential key to understanding treeline dynamics. Global Ecology and Biogeography **20**:582-596.

Harsch, M. A., P. E. Hulme, M. S. McGlone, and R. P. Duncan. 2009. Are treelines advancing? A global meta-analysis of treeline response to climate warming. Ecology Letters **12**:1040-1049.

Hollingsworth, T. N., M. Walker, F. S. Chapin, and A. Parsons. 2006. Scale-dependent environmental controls over species composition in Alaskan black spruce communities. Canadian Journal of Forest Research **36**:1781-1796.

Horton, T. R., T. D. Bruns, and V. T. Parker. 1999. Ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment. Canadian Journal of Botany-Revue Canadienne De Botanique **77**:93-102.

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

Hu, F. S., P. E. Higuera, J. E. Walsh, W. L. Chapman, P. A. Duffy, L. B. Brubaker, and M. L. Chipman. 2010. Tundra burning in Alaska: Linkages to climatic change and sea ice retreat. Journal of Geophysical Research-Biogeosciences **115**:G04002.

Johnstone, J. F., and F. S. Chapin. 2003. Non-equilibrium succession dynamics indicate continued northern migration of lodgepole pine. Global Change Biology **9**:1401.

Johnstone, J. F., and F. S. Chapin. 2006. Effects of soil burn severity on post-fire tree recruitment in boreal forest. Ecosystems **9**:14-31.

Johnstone, J. F., T. N. Hollingsworth, F. S. Chapin, and M. C. Mack. 2010. Changes in fire regime break the legacy lock on successional trajectories in Alaskan boreal forest. Global Change Biology **16**:1281-1295.

Kelly, R., M. L. Chipman, P. E. Higuera, I. Stefanova, L. B. Brubaker, and F. S. Hu. 2013. Recent burning of boreal forests exceeds fire regime limits of the past 10,000 years. Proceedings of the National Academy of Sciences **110**:13055-13060.

Kranabetter, J., M. Stoehr, and G. O'Neill. 2012. Divergence in ectomycorrhizal communities with foreign Douglas-fir populations and implications for assisted migration. Ecological Applications **22**:550-560.

Landhausser, S. M., and R. W. Wein. 1993. Postfire vegetation recovery and tree establishment at the arctic treeline: climate-change-vegetation-response hypotheses. Journal of Ecology **81**:665-672.

Molina, R., H. Massicotte, and J. M. Trappe. 1992. Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. Pages 357-423 *in* M. F. Allen, editor. Mycorrhizal functioning: An integrative plant-fungal process. Chapman and Hall, New York, New York, USA.

Nara, K. 2006a. Ectomycorrhizal networks and seedling establishment during early primary succession. New Phytologist **169**:169-178.

Nara, K. 2006b. Pioneer dwarf willow may facilitate tree succession by providing late colonizers with compatible ectomycorrhizal fungi in a primary successional volcanic desert. New Phytologist **171**:187-198.

Nara, K., and T. Hogetsu. 2004. Ectomycorrhizal fungi on established shrubs facilitate subsequent seedling establishment of successional plant species. Ecology **85**:1700-1707.

Newsham, K. K. 2011. A meta-analysis of plant responses to dark septate root endophytes. New Phytologist **190**:783-793.

Nunez, M. A., T. R. Horton, and D. Simberloff. 2009. Lack of belowground mutualisms hinders Pinaceae invasions. Ecology **90**:2352-2359.

Perry, D. A., J. G. Borchers, S. L. Borchers, and M. P. Amaranthus. 1990. Species migrations and ecosystem stability during climate change - the belowground connection. Conservation Biology **4**:266-274.

Rupp, T. S., F. S. Chapin, and A. M. Starfield. 2001. Modeling the influence of topographic barriers on treeline advance at the forest-tundra ecotone in northwestern Alaska. Climatic Change **48**:399-416.

Rupp, T. S., A. M. Starfield, and F. S. Chapin. 2000. A frame-based spatially explicit model of subarctic vegetation response to climatic change: comparison with a point model. Landscape Ecology **15**:383-400.

Smith, S. E., and D. J. Read. 2008. Mycorrhizal Symbiosis. third edition. Academic Press, New York, New York, USA.

Trappe, J. M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. Annual Review of Phytopathology **15**:203-222.

Viereck, L. A., C. T. Dyrness, A. R. Batten, and K. J. Wenzlick. 1992. The Alaska vegetation classification. U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station., Portland, Oregon, USA.

FIGURES

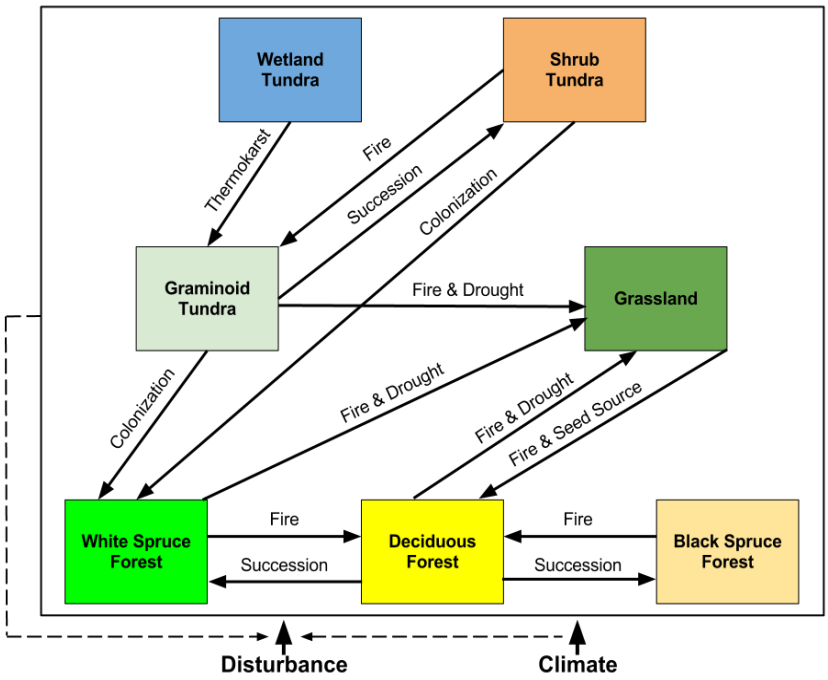


Figure 5.1. Conceptual diagram of the processes affecting state transitions in ALFRESCO 2.0. Arrows indicate causal relationships (Breen et al. 2013, Gray et al. 2013).

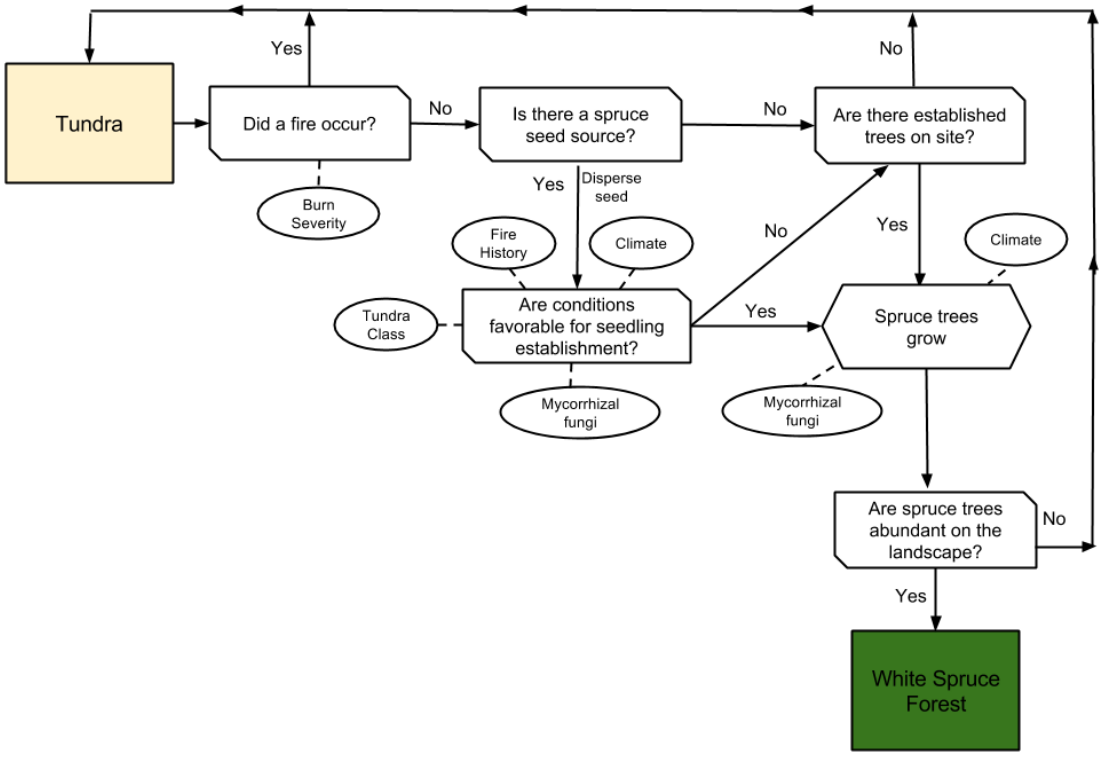


Figure 5.2. Conceptual diagram of the processes affecting state transitions from tundra to spruce in ALFRESCO 2.0. Arrows indicate the progression from one step in the transition process to the next step. The pathway to the right represents infilling of a 1 km^2 pixel as long as there are spruce trees in the pixel and conditions are favorable. This is indicated by the different hexagonal polygon. Figure modified from work by the ALFRESCO 2.0 Team (Breen et al. 2013, Gray et al. 2013).

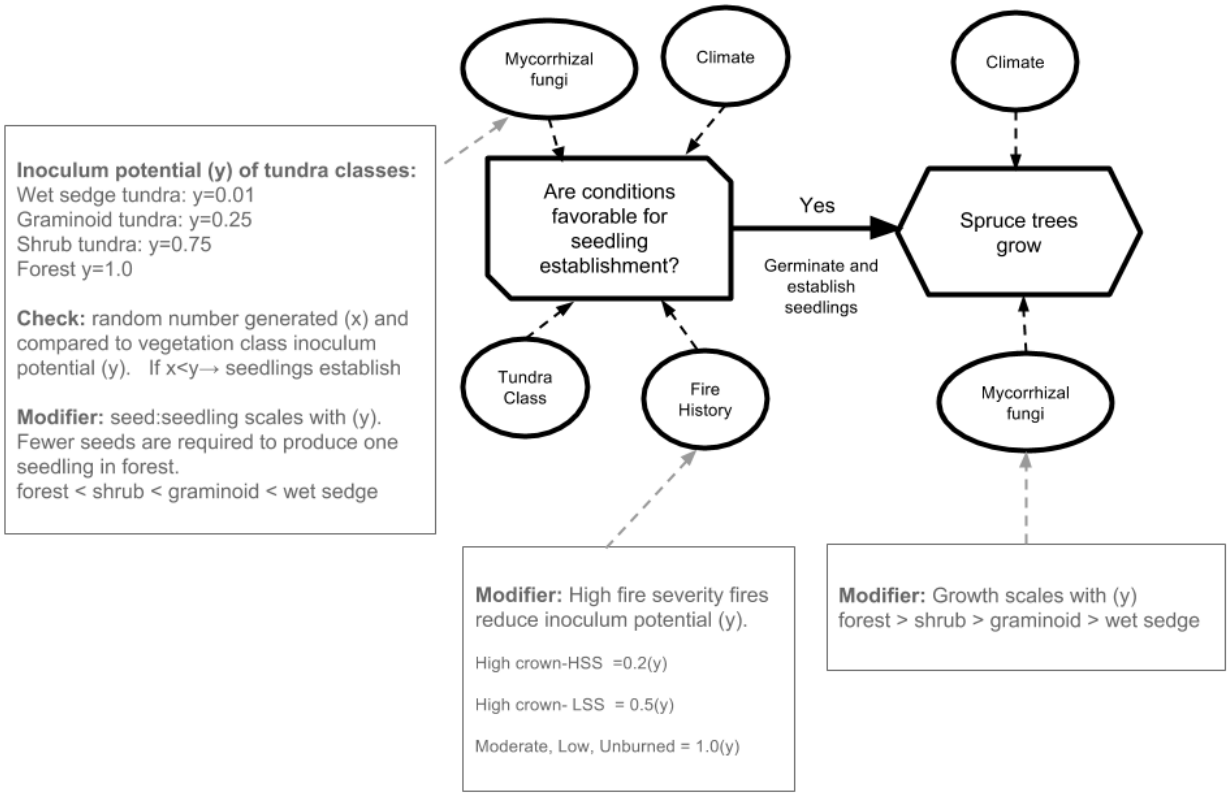


Figure 5.3. Conceptual diagram of the influence of mycorrhizal fungi on tree seedling establishment and growth. The arrows show the progression from addressing ecological factors for one life history stage to the next. Fire-severity classes are as follows: 0=unburned, 1=low burn severity, 2=moderate burn severity= 3= high crown severity with low surface severity, and 4= high crown severity with high surface severity. Tundra classes are based on a highly modified output originating from the North American Land Change Monitoring System. We assigned inoculum potential scores to the tundra classes based on shrub densities reported by Viereck et al. (1992).

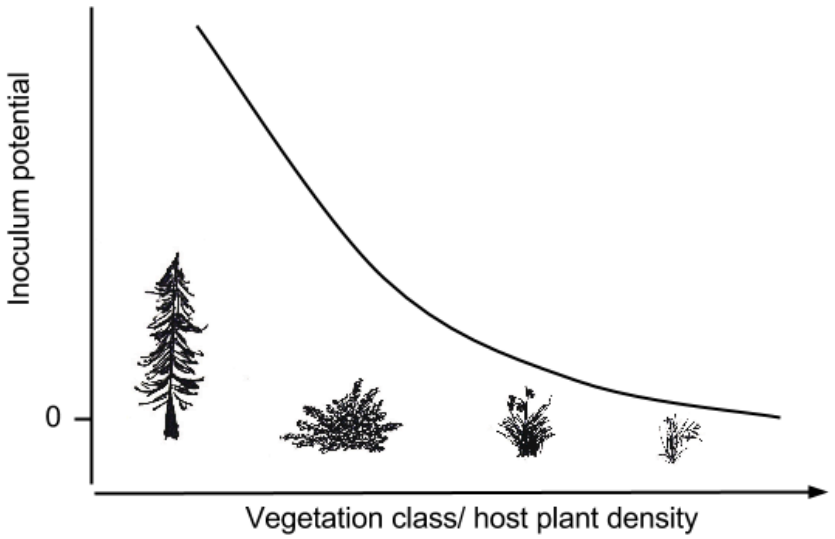


Figure 5.4. Conceptual diagram of inoculum potential in relation to EMF host-plant density across vegetation classes in ALFRESCO 2.0. Inoculum potential declines from spruce forest, to shrub tundra, to graminoid tundra, and is the lowest in wet sedge tundra. Shrub densities in these vegetation classes are derived from the Alaska Vegetation Classification (Viereck et al. 1992).