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

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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.
Keywords: Arctic tundra, *Betula nana*, fire, fungal internal transcribed spacer (ITS) region, mycorrhizal community structure, nurse plant.

Résumé : On s'attend à ce que des changements dans le régime des feux dans la toundra causés par le climat modifient l'abondance et la distribution des arbustes à travers l'Arctique. Cependant, la manière dont le feu pourrait avoir un effet indirect sur la performance des arbustes en perturbant les symbiotes mycorhiziens est peu connue. Nous avons utilisé des outils moléculaires, dont ARISA et le séquençage des ITS fongiques, pour caractériser les communautés de mycorhizes dans des rejets d'arbustes de *Betula nana* à travers un gradient de sévérité de feu à la suite du plus grand incendie ayant jamais eu lieu dans la toundra arctique de l'Alaska (juillet-octobre 2007). Les effets du feu sur la composition fongique étaient dépendants de l'échelle de résolution taxonomique. Les variations dans la composition de la communauté fongique étaient corrélées avec la sévérité du feu. La richesse fongique et l'abondance relative des taxons dominants diminuaient avec une augmentation de la sévérité du feu. Toutefois, contrairement aux régions tempérées et boréales où les feux d'origine naturelle sont fréquents, les champignons mycorhiziens observés sur des rejets d'arbustes dans la toundra n'étaient pas clairement différenciés entre espèces spécialistes du feu et espèces sensibles au feu. En effet, les champignons dominants, incluant des taxons caractéristiques des derniers stades successionnels, étaient présents indépendamment de la sévérité du feu. Il est probable que la stratégie d'histoire de vie des arbustes de la toundra qui comprend la production de rejets confère une résilience aux champignons mycorhiziens dominants face aux perturbations par le feu en maintenant dans le paysage après feu une source d'inoculum. En nous basant sur ces résultats, nous suggérons que la production de rejets par les arbustes pourrait faciliter la régénération de la végétation après feu et potentiellement l'expansion d'arbres et d'arbustes dans des scénarios de réchauffement climatique et d'augmentation des feux dans la toundra arctique.
Mots-clés : *Betula nana*, espaceur transcrit interne (région ITS) fongique, feu, plante compagne, structure de la communauté de mycorhizes, toundra arctique.

Nomenclature: Annotated Checklist of the Panarctic Flora, online. Index Fungorum Partnership, online.

Introduction

Fire frequency and severity play a strong role in regulating shrub regeneration and distribution in the Arctic (Racine *et al.*, 2004; Lantz, Gergel & Henry, 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

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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 y (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, one of the 4 dominant vascular plants in northern Alaskan tundra (Walker *et al.*, 2005), exhibits increased productivity in response to warming (Bret-Harte, Shaver & Zoerner, 2001) and is the only dominant species that is obligately ectomycorrhizal (Molina *et al.*, 1992). While dwarf shrubs such as *B. nana* resprout from below-ground 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, some fire-specialist fungi, primarily those 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). Other fire-sensitive fungi, primarily those 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, Mack & Cross, 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, Donaldson & Last, 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 an 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 have an impact on 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 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

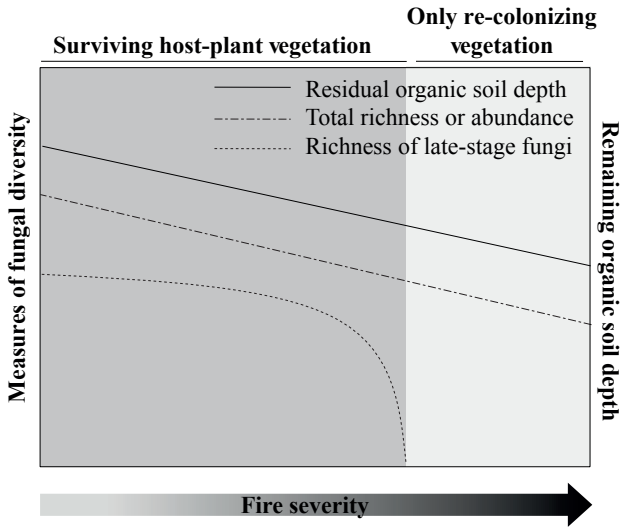

FIGURE 1. Hypothesized changes in measures of fungal community composition across a tundra fire-severity gradient. Late-stage fungi are likely K-strategists and predominantly basidiomycetes.

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.

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*, *Carex bigelowii*), evergreen ericoid mycorrhizal shrubs (*Ledum palustre* and *Vaccinium vitis-idaea*), deciduous ectomycorrhizal shrubs (*Betula nana* and *Salix pulchra*), 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, 2 growing seasons after the fire, we visited 14 burned sites within the ARF burn scar corresponding to different fire severities and 1 unburned site near the burn scar (15 sites total, Table I). Sites were chosen to represent a continuous fire-severity gradient, based on the composite burn index (CBI), a fire-severity metric, between 2 endpoints from unburned to severely burned. The ARF burn scar is accessible only by helicopter in the summer or snow machine in winter. Therefore, we chose 3 logistically accessible sites to sample intensively: 1 unburned road-accessible site near the ARF burn scar (site 1) and 2 sites with helicopter access, 1 moderately burned site (site 104) and 1 high burn-severity site (site 101) (Table I). At each intensive site we harvested 10 *B. nana* resprouts along 5 parallel 1- × 30-m transects 10 m apart. Two shrubs were sampled from each transect, with the first sampling location randomly determined and the second located 10 m from the first. To increase the spatial extent of our study and potentially better represent the

TABLE I. Site descriptions for 15 sampling sites grouped by fire-severity classes within or adjacent to the Anaktuvuk River Fire burn scar.

Fire Severity [a]	Site [b]	No. samples	Location	Site description [c] [d]
Unburned	**1**	10	69.032°N, 148.833°W	Unburned tundra 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 tussock tundra 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* in the inter-tussock space; 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*; 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 tundra 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 tundra with *Eriophorum* sp. tussocks, *Saussurea angustifolia* D.C., *B. nana* shrubs, and combusted sphagnum mosses; 2C2A
	37	1	69.271°N, 150.753°W	Moist ridgetop tussock 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	High severity dry site with *Equisetum* sp., graminoids, *Salix* sp., *B. nana* shrubs, and exposed mineral soil; 2C2C
	69	1	69.019°N, 150.412°W	Hill top high severity moist tussock tundra site with *B. nana*, *L. palustre*, and *Eriophorum* sp. tussocks; 2C2A

[a] Fire-severity classes are based on Composite Burn Index (CBI), Normalized Burn Ratio Index (dNBR) values, and site descriptions.

[b] Bold site numbers denote intensively sampled sites, while extensively sampled sites are not bold.

[c] We classified vegetation communities using the Viereck *et al.* (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.

[d] 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).

richness of taxa found across the 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 I). Harvested shrub root balls were approximately 30 cm in diameter, with the root crown in the centre 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 1 week of harvest. Roots were traced back to the root crown and stored for 8 months at 4 °C in RNAlater (Life Technologies Corporation, Foster City, California, 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 y in RNAlater at room temperature. Mycorrhizal morphology and anatomy were also preserved (D. L. Taylor, 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 3 points and averaged across a site). Fire-severity variables included CBI (measured in the field in 30 m radius plots 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 I). Detailed methods for data collection and summarized fire, soil, and environmental site data are presented in Jandt *et al.* (2012). Environmental data sets 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, 10 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 40× 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 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 6 shrubs demonstrated that 18 tips were sufficient to represent the richness of fungal amplicons present on a root system (18 tips *versus* 36 tips: $T_{(6)} = -1.00$, $P = 0.423$; Appendix I). Hence, sampling more tips would not have drastically changed community composition results. For that reason, 18 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 I). 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 and 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 8 bases, which enable them to work with the USER Friendly Cloning Kit (New England Biolabs, Ipswich, Massachusetts, USA). The resulting primer sequences are as follows, with the additional bases underlined: USER-ITS1F, GGAGACAUCTTGGTCATTTAGAGGAAGTAA (Gardes & Bruns, 1993); USER-ITS4, GGGAAAGUTCCTCCGCTTATTGATATGC (White *et al.*, 1990). Each reaction mix (8.5 µL) was electrophoretically separated for 15 min on an agarose gel in TBE buffer (0.8% agarose, 100 V, 40 min) 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.) following the manufacturer's instructions and ligated into pNEB205A (New England Biolabs). Ligation reactions were then used

for the transformation of competent *E. coli* (One Shot MAX Efficiency DH5α-T1R Competent Cells, Invitrogen, Grand Island, New York, USA) following the manufacturer's instructions (USER Friendly Cloning Kit, New England Biolabs). Growth of colonies on plates was verified, and then plates containing at least 13 well-separated white colonies (1 per sample) were shipped on wet ice overnight to a facility for plasmid purification and sequencing (Functional Biosciences Inc., Madison, Wisconsin, USA). Twelve or 13 clones were sequenced for each shrub.

Cloned sequences were assembled using Codoncode Aligner 3.7 (CodonCode Corporation, Dedham, Massachusetts, USA) using PHRED. We used in-house perl scripts to mask low-quality bases (cutoff Q20) and 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 1) length of overlap between the query and the hit and 2) % 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 II; Table I).

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 1 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 III, Table Ia), 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 III, Table Ib), 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 the data for OTU abundance, 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 2 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 data set. 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, Kvamme & Mielke, 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 overrepresented 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 neighbour 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, which was performed 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 × 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 1 of the original 45 shrubs). These sequences were grouped into 52 OTUs (Appendix II; Table I). 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%), and 11 taxa to family, order, or class (21%); 1 taxon we could identify only to subphylum (2%). The dominant OTUs represented 4 different functional groups: EMF, DSE, ERM, and pathogens (Table II). As in other mycorrhizal studies, our 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; Table IIIa). The final solution of the ordination for the OTU data set yielded 2 dimensions with a final instability of 0.034. The final stress for the OTU data set was 6.145, suggesting a good ordination with low risk of drawing false inferences (Clarke, 1993; McCune & Grace, 2002). The 2 axes represented 93.2% of the variance in fungal community structure, with axis 1 contributing 49.9% and axis 2, 43.4%. Axis 1 was correlated with variables that reflect fire severity (CBI and dNBR) and latitude (Figure 2; Table IIIa). By contrast, axis 2 represents a complex acidity gradient, as indicated by correlations with elevation and mineral soil pH (Figure 2; Table IIIa). 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 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 (±8.65 SE) and 12.2 for fungi in the Ascomycota (±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.

Table II. Classification and presence of the 10 most abundant operational taxonomic units (OTUs) across fire-severity classes.

OTU abundance	Best match description	Type [a]	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	x
13	*Meliniomyces variabilis*	ERM			x	x
12	*Inocybe borealis*	EMF	x			

[a] EMF = ectomycorrhizal fungi, ERM = ericoid mycorrhizal fungi, DSE = dark septate endophyte.

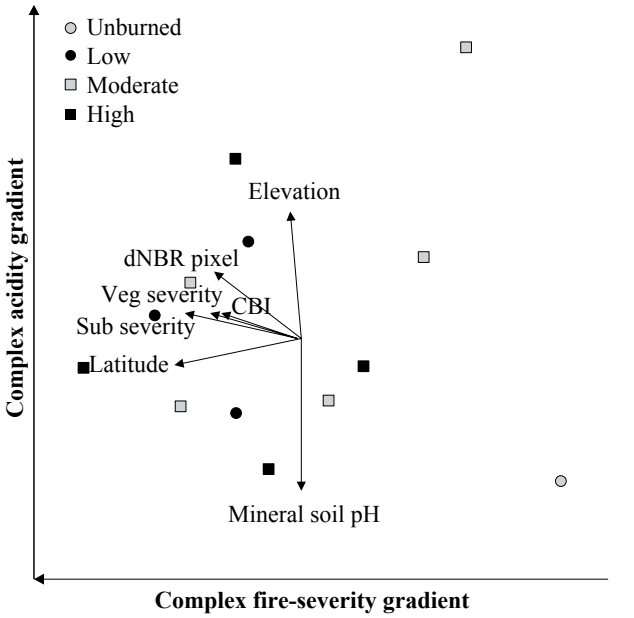

FIGURE 2. NMDS ordinations of mycorrhizal community structure: biplot of important environmental variables significantly correlated ($r^2 \geq 0.200$) with OTU mycorrhizal community structure for unburned, low, moderate, and high severity sites. The vectors show the direction and strength of correlations 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.

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 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 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 1 putative pathogen) occurred in multiple fire-severity classes (Table II), 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 1), late stage-fungi were present across fire-severity classes (Table II). 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, *e.g.*, *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, along with 1 putative pathogen in the genus *Chalara* (Table II). 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 1 in the ordination biplot. Almost all of the dominant OTUs had strong positive correlations with axis 1, representing sites along the decreasing fire-severity gradient (Table IIIb). 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 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 follows 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 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 2 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, Baar & Bruns, 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 6 months to 2 y after fire (Cairney & Bastias, 2007). However, other studies have observed the prevalence of ascomycete fire-fungi in the second growing season after fire (Grogan, Baar & Bruns, 2000) and their persistence for up to 6 y after fire (Visser, 1995).

TABLE III. 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)	Complex fire-severity gradient Axis 1 [a]		Complex acidity gradient Axis 2 [a]	
Environmental variables	*r*	r^2	*r*	r^2
Latitude	–0.631	**0.398**	–0.309	0.095
Slope	0.029	0.001	0.340	0.116
Elevation	–0.227	0.051	0.553	**0.306**
Burn severity substrate	–0.519	**0.270**	0.244	0.060
Burn severity vegetation	–0.494	**0.244**	0.258	0.067
CBI	–0.487	**0.237**	0.283	0.080
Mineral soil pH	0.111	0.012	–0.685	**0.470**
Average organic soil depth	0.351	0.123	0.331	0.109
Active layer depth	0.090	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

b)		Complex fire-severity gradient Axis 1 [a]		Complex acidity gradient Axis 2 [a]	
Phylum [b]	Operational taxonomic unit	*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 chaetospira*	–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**

[a] Bold r^2 values have significant correlations with NMS axes.

[b] Fungal phyla are as follows: A = Ascomycota and B = Basidiomycota.

The dominant EMF in our study were all Basidiomycota in the genera *Russula*, *Lactarius*, and *Inocybe*, which are abundant across arctic habitats, are 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 II), some of the dominant fungi, such as *Russula decolorans* and *Lactarius glyciosmus*, are known to occur in late successional seres. 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, dispersed spores, or fire-resistant propagules (Baar *et al.*, 1999). In contrast with studies from more fire-prone temperate zones, 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

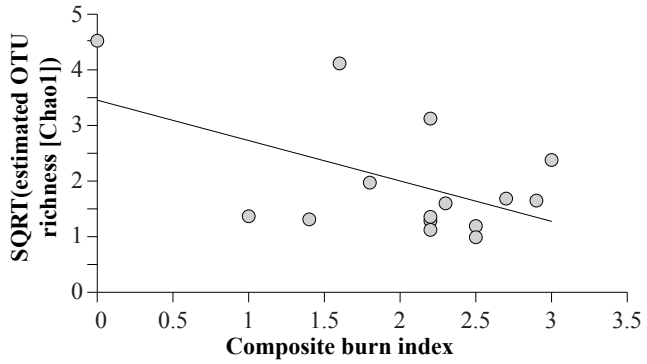

FIGURE 3. Fungal richness decreased with increasing burn severity. Regression of the estimated OTU richness (Chao1) sampled across sites and rarified to an individual shrub ($F_{15, 1} = 4.7751$, $P = 0.0478$, $R^2 = 0.269$). CBI increased with increasing fire severity and is a composite measure of substrate and vegetation burn severity.

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

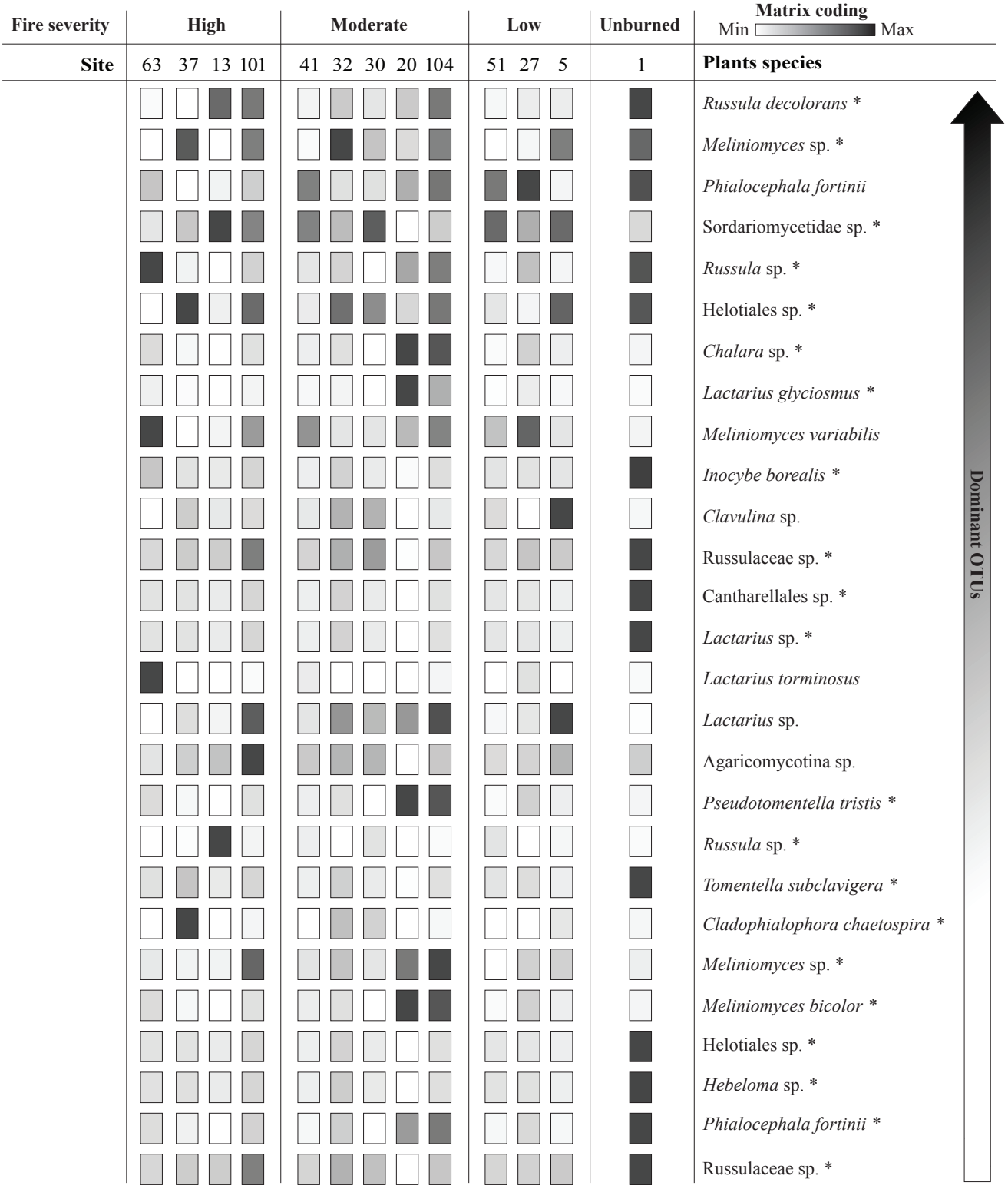

FIGURE 4. Two-way cluster analysis dendrogram of OTU abundance across sampled sites in different burn severities. Abundance of OTUs is shown in grey-scale, where the white squares show where taxa are absent and the filled, black squares show the highest abundance of the OTU. * denotes taxa that were 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.

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 1 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 that found in other post-fire studies (Dahlberg *et al.*, 2001; Smith *et al.*, 2005; Hamman, Burke & Stromberger, 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 2 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 they 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 (E. A. Miller, unpubl. data), which may mean that the lower OTU richness on severely burned sites can be explained through a species–area relationship (Peay *et al.*, 2007) or through competitive interactions among fungi for root tips (Kennedy, Peay & Bruns, 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 that in 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 y may have selected against fire-specialist fungi, and 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 & 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, and 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,b). In tundra, the presence and density of resprouting *B. nana* host plants appear 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 neighbouring ectomycorrhizal boreal forest tree species as warming continues at high latitudes.

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Appendix I

APPENDIX I. 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 to sample 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, California, USA), and the reverse primer ITS4, TCCTCCGCTTATTGATATGC (White *et al.*, 1990), using MapMarker 1000 X-rhodamine size standard (BioVentures, Murfreesboro, Tennessee, USA) and following the protocol of Bent *et al.* (2011). Samples were run on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, New Jersey, USA; Pop6, 50-cm array, T-RFLP_1500 protocol).

BIOINFORMATICS

We used GeneMapper 3.7 (Applied Biosystems, Foster City, New Jersey, 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, Ticknor & Kuske, 2001). We used 1 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).

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 6 shrubs demonstrated that 18 tips were sufficient to represent the richness of fungal amplicons present on a root system (18 tips *versus* 36 tips, $T_6 = -1.00$, $P = 0.423$). 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 data set for each shrub. ARISA profiles will represent rare taxa, while sequences from the clone libraries could potentially capture only 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 2 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 had 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 data sets (Mantel test site matrices $r = 0.681$, $P = 0.001$). For subsequent analyses we used the OTU data set 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., Cary, North Carolina [USA]) with the exception of the multivariate analysis of mycorrhizal communities, which was performed in PC-ORD 6.0 (MJM Software Design, Gleneden Beach, Oregon [USA]).

Appendix II

APPENDIX II, TABLE I. 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 (%) [a]	Overlap [b]	Bit score [c]	GenBank accession [d]
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.	100.00	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*	100.00	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.	100.00	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	100.00	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

[a] Identity (%) = overall fraction of identical positions across high scoring segment pairs.

[b] Overlap = length of the hit participating in alignment without the gaps.

[c] Bit scores: higher scores indicate the greater significance of the alignment between the query sequence and the hit sequence.

[d] GenBank accession = the assigned GenBank accession number for the representative OTU sequence.

Appendix III

APPENDIX III, TABLE I. Correlations between variables: a) environmental and fire variables, 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