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5   **Title:** Identifying functional impacts of heat-resistant fungi on boreal forest recovery after wildfire

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7   **Authors:** Nicola J Day[1][¥], Steven G Cumming[2], Kari E. Dunfield[3], Jill F Johnstone[4,5], Michelle C

8   Mack[6], Kirsten A Reid[1][‡], Merritt R Turetsky[7], Xanthe J Walker[6], Jennifer L Baltzer[1]

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10 [1]Biology Department, Wilfrid Laurier University, Waterloo, Ontario, Canada

11 [2]Department of Wood and Forest Sciences, Laval University, Quebec City, Quebec, Canada

12 [3]School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada

13 [4]Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

14 [5]Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, Alaska, USA

15 [6]Center for Ecosystem Science and Society, Northern Arizona University, Flagstaff, Arizona, USA

16 [7]Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada

17 [¥]Present address: School of Science, Auckland University of Technology, Auckland, New Zealand

18 [‡]Present address: Department of Geography, Memorial University of Newfoundland, St. John's,

19 Newfoundland and Labrador, Canada

20

21 **Corresponding author:** Nicola J. Day. Email: njday.ac@gmail.com.

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25

26 **Abstract**

27 Fungi play key roles in carbon (C) dynamics of ecosystems: saprotrophs decompose organic

28 material and return C in the nutrient cycle, and mycorrhizal species support plants that accumulate

29 C through photosynthesis. The identities and functions of extremophile fungi present after fire can

30 influence C dynamics, particularly because plant-fungal relationships are often species-specific.

31 However, little is known about the function and distribution of fungi that survive fires. We aim to

32   assess the distribution of heat-resistant soil fungi across burned stands of boreal forest in the

33   Northwest Territories, Canada, and understand their functions in relation to decomposition and

34   tree seedling growth. We cultured and identified fungi from heat-treated soils and linked sequences

35   from known taxa with high throughput sequencing fungal data (Illumina MiSeq, ITS1) from soils

36   collected in 47 plots. We assessed functions under controlled conditions by inoculating litter and

37   seedlings with heat-resistant fungi to assess decomposition and effects on seedling growth,

38   respectively, for black spruce (*Picea mariana*), birch (*Betula papyrifera*), and jack pine (*Pinus*

39   *banksiana*). We also measured litter decomposition rates and seedling densities in the field without

40   inoculation. We isolated seven taxa of heat-resistant fungi and found their relative abundances

41   were not associated with environmental or fire characteristics. Under controlled conditions,

42   *Fayodia gracilipes* and *Penicillium arenicola* decomposed birch, but no taxa decomposed black

43   spruce litter significantly more than the control treatment. Seedlings showed reduced biomass

44   and/or mortality when inoculated with at least one of the fungal taxa. *Penicillium turbatum* reduced

45   growth and/or caused mortality of all three species of seedlings. In the field, birch litter

46   decomposed faster in stands with greater pre-fire proportion of black spruce, while black spruce

47   litter decomposed faster in stands experiencing longer fire-free intervals. Densities of seedlings

48   that had germinated since fire were positively associated with ectomycorrhizal richness while there

49   were fewer conifer seedlings with greater heat-resistant fungal abundance. Overall, our study

50   suggests that extremophile fungi present after fires have multiple functions and may have

51   unexpected negative effects on forest functioning and regeneration. In particular, heat-resistant

52   fungi after fires may promote shifts away from conifer dominance that are observed in these boreal

53   forests.

54

55   **Introduction**

56   Soil fungi form an important link between aboveground and belowground pools of carbon (C) in

57   forests (Baldrian, 2017). These heterotrophs can acquire C by decomposing organic material

58   (saprotrophs), which influences belowground C, and by forming symbiotic relationships with plant

59   roots and enhancing plant growth (mycorrhizas), thereby affecting aboveground C (Smith and

60   Read, 2008). Non-mycorrhizal fungi, such as pathogens and endophytes, can also colonise roots

61   and either decrease or increase plant growth (Jarosz and Davelos, 1995; Agrios, 2005; Porras-

62   Alfaro and Bayman, 2011). Plant-fungal interactions are sometimes species-specific (Bent et al.,

63   2011; Treseder et al., 2014; Sterkenburg et al., 2015); saprotrophic fungal taxa can have affinities

64   for litter from different plant species (Treseder et al., 2014) and a fungal taxon that promotes

65   growth of one plant species may reduce growth of another species (Klironomos, 2002). A single

66   fungal taxon or isolate can also have multiple functions (Shah et al., 2016; Smith et al., 2017),

67   making it challenging to draw conclusions about function from DNA sequences alone.

68   Experimental studies can enhance knowledge of fungal functions and elucidate how direct and

69   indirect effects of fires on fungal communities may impact aboveground and belowground C.

70   Boreal forests are an important C sink; an estimated one-third of global terrestrial C is stored

71   in these forests and most of this is in the soils (Pan et al., 2011). The majority of C combusted in

72   boreal forest fires is from the soil (Boby et al., 2010; Rogers et al., 2014; Walker et al., 2018a).

73   Thus, accumulation of belowground C between consecutive fires is critical to maintaining these

74   soils as net C sinks (Andrieux et al., 2018; Walker et al., 2019). Accumulation of deep organic soil

75   layers in boreal forests is often attributed to slow decomposition rates due to dominance of plants

76   with recalcitrant material, mainly conifers, combined with cold temperatures that limit microbial

77   activity, such as bacteria and fungi (Prescott et al., 2000; Fenton et al., 2005). However, fungi are

78   key drivers of accumulation of soil organic matter in boreal forests (Kyaschenko et al., 2017). In

79   terms of accumulation of aboveground C, fungi and other microbes influence plant growth and

80   community structure (Hewitt et al., 2013; Duhamel et al., 2019). Although microbes play central

81   roles in C cycling in boreal forests (Kyaschenko et al., 2017), our knowledge of the impact of

82   boreal wildfires in these ecosystems on soil microbial functioning is limited.

83   Fire activity is projected to increase in many regions of the world with climate change, as is

84   already observed in boreal forests of western North America (Kasischke and Turetsky, 2006;

85   Wotton et al., 2017; Coops et al., 2018). Increased fire intensity, fire severity, and frequency may

86   be causing state changes in dominant vegetation and function in boreal forests (Fenton, 2016;

87   Johnstone et al., 2016). In boreal forest stands, fire often causes 100% tree mortality and there is a

88   flush of recruitment of tree seedlings in the initial years following fire that sets the successional

89   trajectory for future decades (Johnstone et al., 2004; Shenoy et al., 2011). There are increasing

90   observations of shifts away from black spruce (*Picea mariana*) dominance toward jack pine (*Pinus*

91   *banksiana*) or deciduous species such as paper birch (hereafter birch; *Betula papyrifera/B.*

92   *alaskana*) or trembling aspen (*Populus tremuloides*) (Johnstone et al., 2010; Boiffin and Munson,

93   2013; Whitman et al., 2018). Post-fire soil microbial communities could affect this change through

94 differential positive or negative impacts on regenerating plant communities with implications for

95 plant successional trajectories and accumulation of aboveground C. Likewise, post-fire microbial

96 communities may differentially influence litter decomposition depending on plant species, with

97 implications for organic soil layers and sequestration of belowground C (Laganière et al., 2010;

98 Treseder et al., 2014). Fire characteristics including fire intensity, fire severity, and fire return

99 interval can influence post-fire microbial community structure (Glassman et al., 2016; Day et al.,

100 2019), necessitating an enhanced understanding of the impacts of fire on soil microbial community

101 structure and the associated coupled plant-soil responses.

102   Fire is a strong filter on biotic communities, even in fire-adapted systems, and may promote

103 survival or germination of extremophiles such as heat-resistant fungi (Suryanarayanan et al.,

104 2011). For example, nearly 40 macrofungal species that need fire to produce spores, most of which

105 are saprotrophs, have been identified in Fennoscandian boreal forests (Dahlberg, 2002).

106 Saprotrophs are generally less susceptible to fires than mycorrhizas and need less time to recover

107 after fire (Treseder et al., 2004; Sun et al., 2015; Holden et al., 2016). However, heat-resistant

108 spores of the ectomycorrhizal fungus *Rhizopogon olivaceotinctus* enables it to increase in

109 abundance after fire in montane forests in California (Izzo et al., 2006; Peay et al., 2009; Glassman

110 et al., 2016). Fires that burn deeper into the soil organic layer may expose fire-adapted spores from

111 a previous fire cycle that have declined in frequency or abundance through succession. Fungi may

112 also be insulated from heat in organic soil layers and survive via mycelial networks or within roots

113 (Hewitt et al., 2017; Birnbaum et al., 2019). In the upper soil layers, species-specificity of plant-

114 fungal interactions means that the identities of fungi that survive fires could have functional

115 implications for the rate and trajectory of forest recovery and its associated C storage. Moreover,

116 decomposition rates are often related to factors that drive microbial community structure and

117 activity, such as fire severity, fire frequency, pre-fire stand type (leading to different litter types),

118 and temperature (Zhang et al., 2008; Prescott, 2010; Treseder et al., 2014; Aaltonen et al., 2019)

119 but their relative importance in field settings is poorly understood.

120 An unprecedentedly large fire event in the Northwest Territories (NWT), Canada provided an

121 opportunity to evaluate the role of fire in structuring fungal communities and their functions.In

122 2014, 2.85 Mha of boreal forest in the NWT burned (Walker et al., 2018a), the largest annual area

123 burned in the NWT since records began in 1965 (Canadian Interagency Forest Fire Centre, 2014).

124 In the year immediately following fire, we conducted DNA sequencing on soils from 47 plots that

125 were dominated by conifers before the fire, and found that fire severity altered fungal community
126 composition and caused declines in fungal taxon richness (Day et al., 2019). In this study, we build
127 on this work to assess the distribution of heat-resistant soil fungi across the burned landscape and
128 understand their functions in relation to decomposition and tree seedling growth. We isolated and
129 identified heat-resistant fungi from soils and inoculated litter of conifer and deciduous species with
130 these fungal isolates in biochamber studies to assess their potential to function as saprotrophs. We
131 also tested the effects of heat-resistant fungal taxa on seedling growth, either positive or negative,
132 to assess their potential functions as endophytes or plant pathogens. We did a decomposition
133 experiment with conifer and deciduous litter over 24 mos. in the field at 30 of the 47 plots that
134 varied in pre-fire species composition and fire history. We also identified and counted all tree
135 seedlings that had regenerated one year after the fire in all 47 plots.

136   We asked:

137   (1) How are heat-resistant fungi distributed across the landscape after fire? We hypothesised
138 that such fungi would be relatively more abundant in areas that experienced fire more recently
139 because there has been less opportunity for succession away from these heat-adapted taxa. Heat-
140 resistant fungi may increase with increased fire severity because soil combustion may expose
141 dormant structures in deeper soil layers and heat may promote spore gemination after fire;

142   (2) How do heat-resistant fungi impact decomposition and plant growth of coniferous and
143 deciduous tree seedlings? Our hypothesis was that most heat-resistant fungi would be saprotrophs
144 because these are common after fire in boreal forests in high throughput sequencing studies (e.g.,
145 Sun et al., 2015; Day et al., 2019). Saprotrophic activity would be evidenced by decomposition
146 (mass lost) when inoculated with heat-resistant fungi on litter under controlled conditions. Since
147 fungi are key for forest regeneration after fires, we further hypothesised that many heat-resistant
148 fungi would promote growth of tree seedlings under controlled conditions, even if they were not
149 specifically mycorrhizal;

150   (3) How does spatial variation in abiotic factors and heat-resistant fungi contribute to
151 decomposition rates and seedling densities in the field? Our hypothesis was that deciduous (birch)
152 litter would decompose faster than coniferous (black spruce) litter, and that spatial variation in
153 mean annual temperature would be the best predictor of decomposition rates for both species
154 (Cornwell et al., 2008; Zhang et al., 2008). Since we expected many heat-resistant fungi to be
155 saprotrophs (Q2), we hypothesised that litter decomposition rates of both tree species would be

156 faster where these were more abundant. In contrast, we expected seedling densities to be more
157 related to other aspects of the fungal community, such as total richness or ectomycorrhizal
158 richness.

159

160 **Methods**

161

162 *Study Area*

163

164 This study spanned across the Taiga Plains ecozone in the Northwest Territories (NWT), Canada.
165 The Taiga Plains is a mix of undulating glacial till and peatlands with permafrost in wetter areas
166 (Ecosystem Classification Group, 2009). It is characterised by open, slow-growing forest
167 dominated by black spruce (*Picea mariana* (Mill) BSP) and/or jack pine (*Pinus banksiana* Lamb.)
168 with birch (*Betula papyrifera* Marsh./*B. neoalaskana* Sargent, J. Arnold Arbor) and trembling
169 aspen (*Populus tremuloides* Michx.) in well-drained areas. All sample locations are within the
170 discontinuous permafrost zone (Zhang et al., 1999), and in the Low Subarctic ecoregion
171 (Ecosystem Classification Group, 2009). Mean annual temperatures in Hay River (60.82°N, -
172 115.79°W) and Yellowknife (62.45°N, -114.37°W) are -2.5°C and -4.3°C, respectively. Mean
173 annual precipitation in Hay River and Yellowknife is 336 mm and 228 mm, respectively (1981-
174 2010; Environment and Climate Change Canada, 2018).

175

176 *Field methods*

177

178 This study draws upon an extensive set of research sites that were established to understand the
179 impact of fires throughout the NWT in the years immediately following the 2014 fires. A total of
180 221 permanent sampling plots were established across gradients of pre-fire landcover, moisture
181 class (site drainage) and fire characteristics, described in Walker et al. (2018a, 2018b, 2019); this
182 study focuses on a subset of these plots on the Taiga Plains. Each plot comprised two parallel 30
183 m transects spaced 2 m apart (60 m^2), running due north. All seedlings were identified and counted
184 in 1 m × 1m quadrats at 0, 12, and 24 m along the east transect line in June-August 2015.
185   To assess fire severity, burn depth, pre-fire soil organic layer (SOL) thickness, and
186 proportion SOL combusted were estimated from measurements in the 2014 burned plots combined

187 with calibrations from other plots in the NWT that had not burned since 1965 (Walker et al.,
188 2018b). Briefly, we measured SOL depth at 10-20 points in both unburned and burned plots. In
189 plots with black spruce, burn depth was estimated as the height of adventitious roots above the
190 residual SOL (Boby et al., 2010) on 10 trees per plot plus an offset (~3 cm) based on the depth of
191 adventitious roots from the green moss surface measured in unburned stands (Walker et al.,
192 2018b). Pre-fire SOL in black spruce forests was calculated by summing the depth of the residual
193 SOL thickness and burn depth. In plots where only jack pine was present, burn depth was based
194 on moisture class-specific estimates of residual SOL thickness compared to SOL depth in
195 unburned plots. Pre-fire SOL thickness in jack pine forests was calculated by summing residual
196 SOL and burn depth. Our metric of fire severity was the proportion of SOL combusted, which was
197 calculated as burn depth/pre-fire SOL thickness (Walker et al., 2018b).

198 We identified and counted every tree in the 60 m^2 plots to assess pre-fire stand composition,
199 including fallen trees killed by fire. Black spruce was the dominant tree species in these forests
200 and stand type was characterised as the proportion of black spruce stems within the plot. This pre-
201 fire stand type metric represents a gradient of black spruce to jack pine.

202 To estimate the time after last fire prior to 2014 at each plot, we collected basal tree discs or
203 cores just above the root collar of five trees of each dominant conifer representing the dominant
204 size class in each plot. Samples were sanded with progressively finer grits then scanned and rings
205 were counted in Cybis CooRecorder v.7.8 (Larsson, 2006) or WinDendro 2009 (Regent
206 Instruments Canada Inc. 2009). The rapid germination of tree seedlings post-fire (Greene and
207 Johnson, 1999) means that these tree ages can be used to reliably estimate the time after last fire
208 in boreal forests. In most cases, tree ages within plots were clearly structured by recruitment
209 cohorts and clustered within 20 years of each other (see Walker et al., 2018b for details). These
210 data are available online (Walker et al., 2018c).

211

212 *Soil fungal communities*

213

214 At 47 plots (39 plots that burned in 2014 and eight plots in mature forests that did not burn in
215 2014), soil fungal communities were assessed using high throughput sequencing (Illumina MiSeq;
216 Fig. 1; Day et al., 2019). Plots were chosen to represent a range of environmental and fire
217 conditions in terms of stand type, fire severity, and stand age (Table S1). The methods and

218   description of these fungal communities are fully described in Day et al. (2019). Briefly, the top 5

219   cm soil was collected for analysis of fungal communities adjacent to quadrats at 0, 12, and 24 m

220   along the east transect for a total of 141 samples (47 plots × three samples per plot). DNA was

221   extracted and information on the full fungal communities was assessed using Illumina (MiSeq:

222   ITS 1 region, primers ITS1F and ITS2). Sequencing and bioinformatics processing was performed

223   by McGill University and Génome Québec Innovation Centre (Montreal, Québec, Canada). A total

224   of 13,424,680 paired-end reads passed the control quality steps and were assembled. Reads were

225   clustered at 97% similarity to obtain operational taxonomic units (OTUs). We removed three

226   samples with very low reads (<5000 sequences) and rare OTUs that occurred in two or fewer of

227   these 138 samples, and rarefied to 4300 reads per sample. The resulting dataset had 138 samples

228   with a mean of 42,999 reads per sample (range 4,316-191,316) and 266 OTUs per sample (range

229   62-627). Sequences were deposited to DDBJ/ENA/GenBank under the BioSample

230   SAMN09289836 of BioProject PRJNA447993.

231

232   *Isolation and identification of heat-resistant fungi from soils*

233

234   Of the 47 plots sampled for DNA sequencing, 12 plots representing different moisture classes were

235   chosen to isolate heat-resistant fungi via culturing (xeric: three plots; subxeric: one plot; mesic-

236   subxeric: four plots; mesic: three plots; mesic-subhygric: one plot; Fig. S1). Soil samples were

237   transported on ice to the University of Guelph and processed within 1 wk of collection. Heat-

238   resistant fungi were cultured from soil samples (top 5 cm) following standard methods (Nguyen

239   and Seifert, 2008). Soil samples were subjected to heat for 45 min in an 80°C water bath, inverting

240   every 10 min to distribute heat, then cooled at room temperature for 30 min. Under aseptic

241   conditions, 1000 µl of the soil solution was mixed with 100 ml half-strength potato dextrose agar

242   with 4 mg chloramphenicol (PDA-C; Oxoid Ltd, Thermo Fisher Scientific, Basingstoke, England),

243   then poured into Petri plates. Plates were sealed with parafilm and incubated upright at room

244   temperature in the dark. Cultures that grew were isolated into pure culture over 2 wks.

245   Fungal isolates were identified to taxa using a combination of DNA sequencing and

246   morphological assessments. We initially categorised each isolate into morphotypes based on

247   macroscopic characteristics such as shape and colour. One fungal isolate from each morphotype

248   was cultured in PDA-C for DNA extraction using the Macherey-Nagel Nuceleospin Mini kit using

249   CTAB lysis buffer (Macherey-Nagel and Co. KG, Düren, Germany). Extracted DNA from each

250   representative morphotype was amplified by PCR (primers ITS5 and ITS4). The PCR mix was

251   composed of 500 nM of each primer, 4 µl 5X buffer 25 mM magnesium chloride 200 nM

252   dinucletotide triphosphate mix 1.25 units of GoTaq Flexi polymerase enzyme (Promega, Madison,

253   Wisconsin, USA), and 1 µl fungal template DNA. Total volume of the mix was made up to 20 µl

254   with sterile DNase free water. The PCR program consisted of an initial cycle of DNA denaturation

255   at 95°C for 10 min, followed by 35 cycles of 95°C for 15 s, annealing at 49.5°C for 30 s, elongation

256   at 72°C for 90 s, followed by a 7 min elongation at 72°C. PCR products were purified using the

257   GenElute PCR Clean-Up Kit (Sigma-Aldrich, Saint Louis, Missouri, USA) and sequenced by

258   Sanger sequencing at the Genomics Facility in the Advanced Analysis Centre at the University of

259   Guelph (http://www.uoguelph.ca/~genomics/).

260   We entered sequences into the BLAST tool to find closely related sequences in GenBank

261   (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Identifications from Genbank were verified to genus or

262   species using microscopy for all sequenced and unsequenced isolates of each morphotype.

263   Voucher specimens of each isolate are available in long-term culture collections at the University

264   of Guelph (K. E. Dunfield) and the Canadian Collection of Fungal Cultures (DAOMC 251855 –

265   251868). Sequences have been deposited in Genbank (MN410597-MN410606). Of the isolates,

266   seven heat-resistant fungal taxa were identified (see Results). In some cases, multiple isolates that

267   were thought to be different morphotypes according to macroscopic characteristics were

268   determined to be the same fungal species from sequences. Specifically, three isolates thought to

269   be different morphotypes were identified as *Penicillium fuscum* from sequences and five isolates

270   thought to different morphotypes were identified as *P. turbatum*. Since morphologically similar

271   fungi can have different sequence identities or functions and we did not sequence all isolates, our

272   fungal identities may be considered the minimum that were actually present.

273

274   Incubation study: Saprotrophic activity of heat-resistant fungi

275

276   We did an incubation study to assess the saprotrophic activity of each of the seven heat-resistant

277   fungal taxa on a conifer (black spruce) and a deciduous (birch) species. Senescing leaves of black

278   spruce were collected from multiple locations across the Taiga Plains in the NWT in late July 2016

279   by vigorously shaking and rubbing branches of live trees. Senescing leaves of birch were collected

280   by picking yellow or brown senescent leaves from trees or leaves that had obviously recently fallen

281   to the ground in mid-August. Litter was dried at 60°C for 3 d after discarding those with signs of

282   disease or herbivory and shipped in paper bags to Wilfrid Laurier University.

283   The experimental design was fully factorial with 10 replicates per plant-fungal taxa

284   combination, including a control. There were eight fungal treatments: *Coniochaeta* sp., *Fayodia*

285   *gracilipes*, Leotiomycetes sp., *Penicillium turbatum, P. fuscum, P. arenicola, P. spinulosum,* and

286   a control treatment for a total of 160 experimental units. One fungal isolate of each species was

287   used for all fungal inoculation experiments due to growth chamber space limiting our ability to

288   test all isolates, meaning that we do not have data on within-fungal taxa variation in function. Litter

289   was autoclaved in bags for 75 min at 121°C and then placed in a drying oven at 55°C for 3 d. For

290   each species, 400 mg of litter were weighed into 7×7 cm nylon mesh litterbags (500 µm); the

291   birch leaves, including petioles, were roughly cut into 2-5 cm pieces with flame-sterilised scissors

292   to fit into litterbags. After filling, litterbags were again autoclaved for 20 min and stored covered

293   at room temperature overnight. One isolate of each heat-resistant fungal taxa had been cultured in

294   the dark at room temperature for approximately 3 wks on PDA-C. Under aseptic conditions, fungal

295   cultures were cut into 5 mm square pieces and five pieces of fungi from the growing edge were

296   placed into each bag. The control treatment consisted of pieces of PDA-C from uninoculated

297   plates. Each litterbag was put into a bleach-disinfected container, rinsed with autoclaved deionised

298   water with moistened sterile sand, and covered with a perforated lid, following methods from

299   studies with comparable aims (Day and Currah, 2011; Foudyl-Bey et al., 2016). Containers were

300   placed in an incubator in darkness at 23°C and 60% humidity. Containers were randomised at the

301   beginning and again 5 wks into the 10 wk experiment. At the end of the experiment, litter was

302   removed from litterbags, dried at 60°C for 5 d, and weighed.

303

304   Seedling growth effects of heat-resistant fungi

305

306   To test the effects of heat-resistant fungi on seedling growth, we did a growth chamber trial with

307   black spruce, jack pine, and birch. Efforts to germinate trembling aspen were unsuccessful so this

308   species was excluded. Seeds were collected throughout the Taiga Plains in 2015 and stored in

309   airtight containers at 4°C. Seeds were germinated in 50:50 sterile soil:sand mix for 4 wks in a

310   growth chamber with 20/4 hours of day/night at 21/18°C. Trials showed that surface-disinfecting

311   seeds resulted in low germination rates, so seeds were not treated prior to germination.

312   We implemented a fully factorial growth chamber experiment, with 10 replicates per plant-

313   fungus combination: *Coniochaeta* sp., *Fayodia gracilipes*, Leotiomycetes sp., *Penicillium*

314   *turbatum, P. fuscum, P. arenicola, P. spinulosum*, and a control treatment for a total of 240

315   experimental units. Each unit was a Conetainer™ (Stuewe and Sons, Oregon, USA). Sieved soil

316   from burned areas in the NWT was autoclaved twice and rested for 1 wk to allow off-gassing.

317   Conetainers™ were bleach-disinfected, rinsed, and partially filled with a 1:1 homogenised mix of

318   sand and soil. Heat-resistant fungi were cultured in the dark at room temperature for 3 wks on

319   PDA-C. Under aseptic conditions, fungal cultures were cut into 5 mm square pieces and five pieces

320   of fungi from the growing edge was placed in each conetainer™. The control treatment contained

321   pieces of PDA-C from uninoculated plates. One seedling was added to each conetainer™, covered

322   with a layer of soil and sand mix, and topped with sterile sand. Conetainers™ were spatially

323   randomised within a growth chamber at the beginning and 5 wks into the 10 wk experiment.

324   Seedlings were watered with reverse-osmosis purified water once a day. At harvest, shoots and

325   roots were separated, dried at 60°C for 3 d, and then weighed.

326

327   In situ *litter decomposition rates*

328

329   At 30 plots (Fig. S1), we did an *in situ* litterbag experiment to assess post-fire decomposition rates

330   of two widespread tree species in boreal forests, black spruce and birch, using the same litter as

331   the incubation study described above. These plots were selected to incorporate a range of

332   conditions in stand type (dominated by black spruce, jack pine, or mixed), fire severity, and stand

333   age (Table S1); 17 plots overlapped with where fungal sequencing had been undertaken (described

334   above).

335   Litterbags (10 cm × 10 cm) were made from nylon mesh (0.5 mm). For black spruce and

336   birch, 1 g dried leaves were weighed into individually numbered bags and heat-sealed closed. At

337   the end of August 2016, we placed five litterbags of each species, spaced 5 m apart, at each of the

338   30 plots. Bags were placed on the burned soil surface and secured with a peg through a wire loop

339   attached to the bag. Most litterbags were collected 24 mos. after deployment (150 litterbags per

340   species) but at eight plots we deployed additional litterbags collected after 12 mos. (40 litterbags

341   per species). Immediately following collection, litterbags were kept on ice and shipped to Wilfrid

342   Laurier University. Bag contents were dried at 60°C for 3 d and then weighed. While our mesh

343   size excluded macrofauna, our *in situ* decomposition rates necessarily include effects of

344   microfauna or microbes other than fungi.

345

346   *Data analyses*

347

348   All statistical analyses were conducted in R v.3.6.0 (R Core Development Team, 2019), using

349   packages nlme (Pinheiro et al., 2019), lme4 (Bates et al., 2015), ggeffects (Lüdecke, 2018),

350   DHARMa (Hartig, 2019), lsmeans (Lenth, 2016), and boot (Davison and Hinkley, 1997; Canty

351   and Ripley, 2019). Functions within the "tidyverse" were used for basic data manipulations and

352   graph production (Wickham, 2017), with extensions in egg (Auguie, 2018). R code is provided in

353   supplementary material.

354

355   1. How are heat-resistant fungi distributed across the landscape after fire?

356

357   To understand how the isolated heat-resistant fungal taxa were distributed across the landscape,

358   we combined the Sanger sequences of each heat-resistant fungus with the high throughput

359   sequencing data from 138 quadrats in 47 plots (Day et al., 2019). We put all Sanger sequences

360   from the heat-resistant fungi into one FASTA file and used command "closed_ref" in USEARCH

361   v.10 to perform closed-reference clustering against the MiSeq sequences (Edgar, 2010). Sanger

362   sequences were matched with OTUs from Illumina at 97% similarity. The distribution across the

363   landscape of each heat-resistant fungal taxon was visualised on a map using ArcGIS.

364   Correlation tests between richness of total fungi, saprotrophs, and plant pathogens against

365   heat-resistant fungal richness were done at the quadrat and plot scales using the Spearman

366   coefficient due to non-normality. Saprotrophs and plant pathogen OTUs were based on functional

367   assignments from published literature using FUNGuild (Nguyen et al., 2016). We retained OTUs

368   with functions considered "probable" and "highly probable" within guilds and were not assigned

369   to multiple guilds of either plant pathogens (24 OTUs), saprotrophs (217 OTUs), or

370   ectomycorrhizas (152 OTUs). *P*-values were corrected for multiple tests on the same experimental

371   units using the false discovery rate.

372   To assess drivers of distributions of heat-resistant fungi, we undertook a generalised linear

373   mixed effects model with the summed number of reads of heat-resistant fungi per sample as the

374   response variable and a random effect for plot (three quadrat-level samples per plot). We summed

375   reads of all heat-resistant fungal taxa. The model assumed a negative binomial distribution to

376   account for over-dispersion with logarithmic link. We included predictors that we hypothesised

377   would be related to the distributions of heat-resistant fungi: fire severity, stand age, and pre-fire

378   stand type. All predictor variables were pairwise uncorrelated ($r<0.7$). Predictors were centred and

379   standardised prior to inclusion in the models.

380

381   2. How do heat-resistant fungi impact decomposition rates and plant growth of coniferous and

382   deciduous tree seedlings?

383

384   2a. Decomposition: To assess saprotrophic activity of the heat-resistant fungi for birch and black

385   spruce litter, we calculated decomposition rate as percent mass lost: (initial dry weight – dry weight

386   at time of collection) / initial dry weight) $\times 100$. This was used as the response in a linear model

387   for each tree species. Fungal taxon was the predictor, comparing decomposition rate of each taxon

388   against that of the uninoculated control treatment. Three experimental units were removed from

389   the birch model due to sand causing large values for dry weights or contamination (one

390   Leotiomycetes, two in the control treatment). Results are presented as the mean percent difference

391   for each fungal treatment from the control treatment, based on 999 bootstrap samples. A post-hoc

392   Tukey-Kramer analysis was used to assess differences in effects between fungal taxa.

393

394   2b. Seedling growth: To test the effects of heat-resistant fungi on seedling growth, we modelled

395   total dry biomass and root-shoot ratio for each tree species: black spruce, jack pine, and birch.

396   Seedling growth was assessed for each tree species using a linear model with fungal taxon as the

397   predictor, comparing plant growth in each taxon against that of the uninoculated control treatment.

398   Only two of the 10 replicates for the birch-*P. turbatum* treatment survived, so this was excluded

399   from the analyses. Although 10 replicates of the birch-*F. gracilipes* treatment survived, a labelling

400   error reduced the number of experimental units to eight. Results are presented as the mean percent

401   difference for each fungal treatment from the control treatment, based on 999 bootstrap samples.

402   *P*-values were corrected and a post-hoc Tukey-Kramer analysis was used as above.

403

404   3. How does spatial variation in abiotic factors and heat-resistant fungi contribute to decomposition

405   rates and seedling densities in the field?

406

407   We assessed decomposition rates of black spruce and birch litter by calculating percent mass lost

408   as above. Using the litterbags that were collected at 24 mos. for each species, we modelled drivers

409   of decomposition rates using linear mixed effects models with plot as the random effect. Two and

410   one litterbags were not recovered for black spruce and birch respectively. Predictors included in

411   the models were fire severity, stand age, stand type, and mean annual temperature. We estimated

412   spatial variation in mean annual temperature for each plot for 2017 from Climate WNA (Wang et

413   al., 2012). All predictor variables were pairwise uncorrelated ($r<0.7$). Predictors were centred and

414   standardised prior to inclusion in the models. An outlier in birch litter was omitted from this model

415   because the sample was contaminated with sand, making it heavy and leading this point to have

416   high leverage in the model.

417   To assess how fungal communities impacted *in situ* litter decomposition rates, we used data

418   from the 17 plots where we had both these pieces of information. Since litterbags were not paired

419   spatially with the samples for sequencing, we used the mean decomposition rate per plot as the

420   response variable. We ran separate models for each predictor at the plot level. Heat-resistant fungal

421   relative abundance was the total number of reads of all heat-resistant fungi isolated above. Fungal

422   OTU richness was the total number of all fungal OTUs at the plot. Saprotroph richness was the

423   number of OTUs in this category based on FUNGuild (Nguyen et al., 2016). General linear models

424   were run specifying a gaussian response. One outlying plot with high leverage was removed from

425   all black spruce models. *P*-values were corrected as above.

426   Since we had fungal sequencing spatially paired with seedling densities (per m^2) in three

427   quadrats at each plot, we used these data to assess how fungal communities corresponded to

428   seedling densities in the field. There were no seedlings in the unburned plots and two samples were

429   removed from the sequencing data due to too few sequences for a total of 115 quadrats in 39 plots.

430   We ran five separate generalised linear mixed effects models, one for each predictor of fungal

431   community structure: heat-resistant fungal relative abundance, and fungal OTU richness variables

432   calculated as above except at the quadrat level. Fungal plant pathogen richness and

433   ectomycorrhizal richness were the number of OTUs in each of these categories in each sample,

434   based on FUNGuild (Nguyen et al., 2016). Models were run as a Poisson response and plot was

435   the random effect in each model. We re-ran these models with the response as seedling densities

436   of the dominant conifers black spruce and jack pine. *P*-values were corrected as above.

437

438   **Results**

439   *1. How are heat-resistant fungi distributed across the landscape after fire?*

440

441   We isolated a seven unique heat-resistant fungal taxa from soils from burned boreal forest stands

442   in the NWT (Table S2). Six of the seven heat-resistant fungal taxa isolated were in

443   Pezizomycotina, the major subphylum of Ascomycota. The fungal taxa were *Fayodia gracilipes*,

444   *Penicillium arenicola, P. fuscum, P. spinulosum, P. turbatum*, *Coniochaeta* sp., which could only

445   be identified to genus, and an isolate in class Leotiomycetes. *Penicillium turbatum* was the most

446   commonly isolated fungus, isolated from 7/12 plots. Detection of the heat-resistant fungi across

447   the wider plot network showed that at least one of the seven heat-resistant taxa were present in all

448   but four of the 47 plots (Fig. 2; Table 1). Taxa that were locally abundant by being present in many

449   quadrats within plots, and in many plots so they were widespread across the landscape: *P. fuscum*,

450   Leotiomycetes sp., and *F. gracilipes*. Those that were locally rare by being present in few quadrats

451   within plots and in few plots so they were rare across the landscape were *P. arenicola, Coniochaeta*

452   sp., and particularly *P. spinulosum* that was only detected in one quadrat. *P. turbatum* fell between

453   these two categories, where it was in a moderate number of quadrats and plots.

454   Spearman rank correlations between heat-resistant fungal richness against richness of total

455   fungi and against saprotrophs were positive and significant at the quadrat scale (Fig. S2). There

456   was a positive relationship between plant pathogen richness and heat-resistant fungal richness at

457   the plot scale. The mixed models showed no significant relationships between our *a priori*

458   predictors (stand age, pre-fire stand type, fire severity) and the total relative abundance of heat-

459   resistant fungi (Table 2).

460

461   *2. How do heat-resistant fungi impact decomposition and plant growth of coniferous and*

462   *deciduous tree seedlings?*

463

464   2a. Decomposition: At the end of the incubation experiment assessing saprotrophic ability of heat-
465   resistant fungi, all taxa except *Coniochaeta* sp. and Leotiomycetes sp. colonised birch litter, while
466   all taxa except Leotiomycetes sp. colonised black spruce litter (Table S5). On black spruce litter,
467   no fungi showed saprotrophic activity: that is, decomposition rates did not differ between
468   inoculated treatments and the control treatment for this species (Figs. 3 and S4; Table S3). The
469   Tukey-Kramer post-hoc test showed no significant differences in decomposition rates between
470   fungal taxa (Table S4). On birch litter, *F. gracilipes* and *P. arenicola* showed significant
471   saprotrophic activity (Fig. 3; Table S3). The post-hoc test showed that *F. gracilipes* caused faster
472   decomposition rates than all other taxa except *P. arenicola* (Table S4). *Penicillium arenicola*
473   caused faster decomposition rates than *P. fuscum*. Control treatments of both black spruce and
474   birch showed background rates of decomposition (Table S4; Fig. S4).

475

476   2b. Seedling growth: In the seedling growth experiment, black spruce had significantly reduced
477   biomass when inoculated with all fungal taxa except *P. fuscum* and *P. spinulosum*, relative to the
478   control treatment (Figs. 4 and S5; Table S6). Seedlings inoculated with either Leotiomycetes sp.
479   or *P. fuscum* had significantly greater biomass than those inoculated with *P. turbatum* (Table S7).
480   No taxa caused significant differences in root-shoot ratios relative to the control treatment (Table
481   S6). *Penicillium arenicola* was the only taxon that was recovered from surface disinfected black
482   spruce roots even though it did not exert any significant growth effects on this species (Table S8).
483   For birch seedlings, *P. turbatum* caused mortality of all but two individuals (Fig. S3). Birch
484   seedlings inoculated with *P. spinulosum* had significantly lower biomass relative to the control
485   treatment (Fig. 4; Table S6). Seedlings inoculated with either Leotiomycetes sp. or *F. gracilipes*
486   had significantly greater biomass than those inoculated with *P. spinulosum* (Table S7). No taxa
487   caused significant differences in root-shoot ratios relative to the control treatment (Table S6). The
488   growth effects of *P. spinulosum* and *F. gracilipes* occurred even though these taxa were not
489   recovered from surface disinfected birch roots (Table S8).
490   All seven fungal taxa significantly reduced biomass of jack pine seedlings (Fig. 4; Table S6)
491   and there were no among-taxon differences in their effects (Table S7). No taxa caused significant
492   differences in root-shoot ratios relative to the control treatment (Table S6). *Coniochaeta* sp.,
493   Leotiomycetes sp., *P. arenicola*, and *P. spinulosum* were all recovered from surface disinfected
494   jack pine roots (Table S8).

495

3. How does spatial variation in abiotic factors and heat-resistant fungi contribute to decomposition rates and seedling densities in the field?

498

Birch leaf litter decomposed more rapidly than black spruce in the field at eight plots after 12 mos. (t-test: t=8.71; P<0.001) and at 30 plots after 24 mos. (t-test: t=18.11; P<0.001). Birch litter had mean decomposition rate (mass lost) of 35% of its initial mass (range: 25-44%) after 12 mos. and 45% (range: 33-58%) after 24 mos.. In contrast, the decomposition rate of black spruce was 24% on average (range: 15-35%) after 12 mos. and 34% (range: 26-55%) after 24 mos. (Fig. S6).

Drivers of decomposition rates after 24 mos. differed among species (Fig. 5; Table S9). Decomposition of black spruce litter was faster in older plots (those that had a longer fire-free interval). Decomposition of birch litter was faster with greater pre-fire proportion of black spruce. In contrast with our hypothesis, spatial variation in mean annual temperature had no significant effect on decomposition rates of either species. The random effect shows that there was high among-plot variation in decomposition rates for both species (>2 SDs; Table S9).

For the models of mean plot *in situ* decomposition rates as a response in relation to three predictors of fungal community structure (heat-resistant fungal relative abundance, fungal OTU richness, saprotroph richness), no relationships were significant (Table 3). In contrast, for the models for fungal community effects on seedling densities, there was a positive significant relationship between ectomycorrhizal richness and seedling densities of all species and for conifer seedlings (Table 4); this effect was also significant for plant pathogen richness. For conifers, there were significantly fewer seedlings with greater relative abundance of heat-resistant fungi (Table 4). The high variance in the random effect shows there was relatively high among-plot variation (>2 SDs; Table 4).

519

Discussion

Knowledge of extremophile distributions and functions can aid understanding of how ecosystems recover under harsh conditions or after severe events. Our study has provided an investigation of the identity, prevalence, and function of heat-resistant fungal taxa in recently burned boreal forest stands of the NWT of Canada, and demonstrated their contribution to some aspects of pools of aboveground and belowground C. The incubation study showed that two of the seven heat-resistant

526 fungal taxa could function as saprotrophs but only on birch and not on black spruce litter.
527 Unexpectedly, all seven isolated heat-resistant fungi reduced seedling growth of at least one of
528 birch, black spruce, or jack pine, relative to the uninoculated control treatment. The conifers were
529 particularly negatively affected in the controlled experiments, while in the field there were fewer
530 conifer seedlings with greater abundance of these heat-resistant fungi. This suggests that fungi can
531 shape forest regeneration after fires and may contribute to declines in conifer regeneration in some
532 situations.

533

534 *Heat-resistant fungal taxa*
535 Our sequence of *F. gracilipes*, the only Basidiomycete cultured, matched 100% with that isolated
536 from soil in a stand of *Picea abies* and *Populus alba* in Russia at 60°N. This taxon has been isolated
537 as a saprotroph from agricultural soils in Michigan, USA (Thorn et al., 1996). Here, we found *F.*
538 *gracilipes* to be locally abundant within plots and widely distributed across the plot network,
539 saprotrophic on birch, and reduced biomass of conifer seedlings. At the other end of the scale,
540 *Coniochaeta* sp. was rare within plots and across the plot network, reduced conifer seedling
541 biomass, and colonised roots of jack pine and birch. This genus is known to produce antifungal
542 and antibacterial compounds and has been isolated from bogs and fens in Ireland and the USA
543 (Asgari et al., 2007; Thormann and Rice, 2007). The ability of these saprotrophic taxa to colonise
544 roots but not necessarily alter plant biomass demonstrates both the species-specificity involved in
545 plant-fungal interactions and the plasticity of functions of a single fungal taxon (e.g., Smith et al.,
546 2017) .
547 Our sequence of Leotiomycetes matched a species hypothesis in the UNITE database for
548 *Leohumicola* sp., a genus that has been isolated from soils experiencing regular fires in eastern
549 Canada (Hambleton et al., 2005; Kõljalg et al., 2013). Our results support this as a fire-adapted
550 group that is root-associated, since the Leotiomycetes isolate colonised roots of birch and jack pine
551 but did not colonise litter. However, it significantly reduced biomass of conifer seedlings.
552 *Leohumicola* spp. contain ericoid mycorrhizas that can survive fires via thick-walled, heat-resistant
553 spores called aleurioconidia (Hambleton et al., 2005; Nguyen and Seifert, 2008). This group may
554 therefore support regeneration of ericoid plants in the understory, such as *Vaccinium vitis-idaea*,
555 which was commonly resprouting in our plots (White, 2018). These different effects of fungal taxa
556 on plant growth are thought to promote aboveground diversity (Bever et al., 2010).

557   *Penicillium* spp. are ecologically diverse and globally ubiquitous, with many known to be
558   heat-resistant (Houbraken and Samson, 2011; Visagie et al., 2014). Members of this genus are
559   common in peatlands and have been frequently isolated from soils in Canadian boreal forests
560   (Summerbell, 2005; De Bellis et al., 2007; Thormann and Rice, 2007). Our sequence for *P.*
561   *arenicola* closely matched those recovered from soils in Norman Wells, NWT, and pine forests in
562   Ukraine and Ontario (Peterson et al., 2010). This taxon was sparsely distributed, colonised litter
563   and roots of all taxa, was saprotrophic on birch litter, and reduced biomass of black spruce
564   seedlings. While *P. spinulosum* is considered a saprotroph, we did not observe this effect in our
565   study. Instead, this taxon colonised roots of jack pine and reduced seedling growth of all three tree
566   species. This was unexpected since *P. spinulosum* is associated with ectomycorrhizal roots of black
567   spruce in Ontario boreal forests (Summerbell, 2005). This taxon was only present in one quadrat
568   at one of our 47 plots. Little is known of *P. turbatum* in terms of function, but this taxon had the
569   most negative effects on seedlings in terms of both survival and biomass across tree species.

570   The lack of relationship between the relative abundance of heat-resistant fungi and fire
571   severity, as the proportion of the organic horizon combusted, lends support to our previous
572   speculation that many fungi in these soils survived fires rather than dispersing in after the fires
573   (Day et al., 2019). While fungal propagules close to the soil surface may be susceptible to mortality
574   due to fire, fungi without adaptations to high heat likely survive deeper down (Baar et al., 1999;
575   Certini, 2005; Cairney and Bastias, 2007). This may be particularly important in deep organic soils
576   that provide insulation against high temperatures. Fungi can also survive high temperatures within
577   wood (Carlsson et al., 2012; Kuo et al., 2015) and roots (e.g., Hewitt et al., 2013). Future studies
578   investigating how these mechanisms of survival mediate fungal community structure and function
579   after fire would help us better understand ecosystem recovery after wildfires and the patchiness in
580   local and regional distributions. This would require pre-fire fungal information.

581   In this study, most of our heat-resistant fungal taxa were either locally infrequent and rare
582   across the plot network across the landscape, or locally abundant within plots and widely
583   distributed across the plot network; these latter taxa were often also in the unburned plots.
584   However, our DNA-based methods do not indicate if these taxa were active. Recent
585   comprehensive work on abundance-occupancy relationships using fungal fruiting bodies revealed
586   that most fungi are rare but also that locally rare fungi can be abundant at larger scales (Gange et
587   al., 2019).

588   By definition, heat-resistant fungi are those that can survive 75°C for at least 30 min
589   (Nguyen and Seifert, 2008; Samson et al., 2010). It is likely that there are other heat-resistant fungi
590   in our plots but they were not detected due to isolates growing over each other on plates, media
591   selectivity, and presence of unculturable taxa. This may contribute to why we found no significant
592   predictors of heat-resistant fungal abundance across the plots. Our methods were not designed to
593   isolate mycorrhizas so it was not surprising that these were not isolated. Other studies
594   experimentally inferring effects of fire on microbial communities have used temperatures between
595   45 and 75°C (Izzo et al., 2006; Peay et al., 2009), up to 500°C, where temperatures above 400°C
596   resulted in complete fungal mortality (Bárcenas-Moreno and Bååth, 2009). These high
597   temperatures may reflect those at the very surface of soils (Certini, 2005). Nonetheless, our study
598   has shed light on the distribution and ecological functions of extremophile fungi in the
599   environment.

600

601   *Post-fire fungi reduced seedling biomass and conifer seedling densities*
602   Our study suggests that functions of extremophile fungi that are present post-fire may not
603   necessarily promote ecosystem recovery in the way we may expect. A surprising result was that
604   all seven heat-resistant fungi significantly reduced biomass of seedlings of at least one tree species
605   relative to the uninoculated control treatment. Many inoculated fungi were not re-isolated from
606   roots of seedlings but still reduced biomass, suggesting indirect effects on plant growth such as
607   nutrient turnover or via secondary metabolites. For example, *P. spinulosum* reduced birch growth
608   but was not recovered from surface-disinfected roots. The conifers were particularly susceptible
609   to negative effects of heat-resistant fungi, and this transferred to the field where there were fewer
610   conifer seedlings with greater abundance of these fungi. Further exploration is warranted on the
611   effect of fungi that may contribute to observed declines in conifer regeneration after wildfires in
612   some boreal forests (Johnstone et al., 2010; Reid, 2017). Future studies could pre-inoculate
613   seedlings with heat-resistant fungi where isolated functions have been tested under controlled
614   conditions, such as the ones isolated in our study, and transplant them into the field to better
615   elucidate the roles of these fungi in forest regeneration.
616   At this stage, we cannot consider these heat-resistant fungal taxa plant pathogens because no
617   seedlings showed disease symptoms and Koch's postulates have not been fulfilled (tests used to
618   verify the identity and cause of pathogenicity). Richness of OTUs considered plant pathogens in

619   FUNGuild were positively related to seedling densities in the field, which highlights the limitations

620   in defining plant-fungal interactions based on taxonomy alone. Our wider sequencing data show a

621   positive relationship between fungi that are considered pathogenic and fire severity (Fig. S7), a

622   pattern similarly observed in the Alaskan tundra (Hewitt et al., 2016). This might be expected as

623   pathogens have adaptations to survive adverse conditions or long periods of dormancy without a

624   host (Agrios, 2005) that may also confer ability to survive high heat. We need a better

625   understanding of pathogens after fire in natural systems and their role in determining forest

626   regeneration relative to other factors. If high fire severity promotes survival of fungi that reduce

627   plant growth to the point that forest regeneration or plant communities are compromised, this could

628   reduce aboveground C in terms of plant biomass and impact nutrient cycling.

629   In the field, the positive effect of ectomycorrhizal richness on seedling densities supports this

630   as an important mutualism for forest regeneration (Grogan et al., 2000; Treseder et al., 2004; Peay

631   et al., 2009). Understanding fungal functions from sequencing alone is complicated by the ability

632   for single fungal isolates to obtain C in multiple ways and have multiple functions, which may be

633   modified by other microbial interactions in the field (Schäfer et al., 1989; Shah et al., 2016; Smith

634   et al., 2017). While we did not assess differences between isolates of single taxa in our study, our

635   single isolate of *F. gracilipes* was a saprotroph on birch but also reduced growth of black spruce

636   and jack pine. How these multifunctionalities play out in the field is unclear. For example,

637   *Neurospora crassa*, a well-known and ubiquitous heat-resistant saprotroph, is also capable of

638   endophytic and pathogenic life stages within the same host, *Pinus sylvestris* (Kuo et al., 2015).

639   Ectomycorrhizas can also be saprotrophs (Shah et al., 2016), making them particularly important

640   for C cycling by decomposing organic matter and influencing aboveground C stocks by providing

641   nutrients for plant growth.

642

643   *Implications of fire-induced shifts in vegetation*

644   *In situ*, birch litter decomposed faster where there was greater proportion of pre-fire black spruce.

645   Put another way, birch decomposed better in black spruce-dominated plots relative to jack pine-

646   dominated plots. This pattern could reflect moisture availability, a limiting factor in microbial

647   activity, because black spruce stands have high surface moisture (Fig. S8). However, if moisture

648   was the underlying mechanism then we expect a positive relationship for both species, which was

649   not the case (Tables S10 & S11). Others suggest that poor drainage and cool conditions reduce

650 decomposition rates in black spruce-dominated plots regardless of litter inputs (Preston et al.,
651 2014), which contrasts with our observations in this post-fire environment. A study of mesic boreal
652 forests suggests that litter inputs were more important than climatic differences along a latitudinal
653 gradient for accumulation of soil organic matter (Kohl et al., 2018). This could explain the lack of
654 significance of mean annual temperature in our study. Alternatively, mean annual temperature may
655 not have been a good predictor of decomposition rates because the gridded data were too coarse
656 or low variation in this variable.

657   Fire-induced shifts from black spruce to deciduous or jack pine dominance are being
658 observed across the NWT (Reid, 2017; Whitman et al., 2018) and North American boreal forests
659 (Johnstone et al., 2010; Boiffin and Munson, 2013; Hart et al., 2019). Shifts from black spruce to
660 jack pine, the dominant shift on the Taiga Plains where our study occurred (Reid, 2017; Whitman
661 et al., 2018), may not alter C pools very much because these conifers have similar litter quality
662 (Preston et al., 2014). However, while shifts towards deciduous dominance may alter litter quality
663 and lead to faster decomposition rates and potentially reduce belowground C, aboveground C can
664 increase, resulting in overall similar net C balance but differential distribution (Alexander et al.,
665 2012; Alexander and Mack, 2016). During decomposition, C can be retained in mineral soil
666 (Prescott, 2010), in undecomposed mycelial networks (Clemmensen et al., 2013), or in soil fauna.
667 Soil warming, which may be enhanced post-fire, can alter fungal communities and reduce resource
668 allocation to decomposition activities (Asemaninejad et al., 2017; Romero-Olivares et al., 2019).
669 Thus, the association between decomposers, CO_2 or CH_4 fluxes, and accumulation of soil organic
670 matter is not always clear (Allison et al., 2010; Kyaschenko et al., 2017). Mycorrhizas can
671 sequester large amounts of C by transferring C from photosynthate into deep soil layers
672 (Clemmensen et al., 2013, 2015). Fire-induced plant mortality and long-term changes in
673 mycorrhizal communities could therefore have greater relative importance for driving C dynamics
674 than changes in saprotrophs or surface decomposition.

675

676 *Black spruce decomposed faster in stands with longer fire-free intervals*
677 The positive relationship observed between stand age and *in situ* black spruce decomposition rates
678 could reflect recovery or succession of microbial community function over time (Holden et al.,
679 2013; Clemmensen et al., 2015; Sun et al., 2015). Our previous work showed detectable
680 differences in fungal community composition in different stand ages at these plots, even after fire

681 (Day et al., 2019). The present study showed no relationship between stand age and heat-resistant
682 fungi, but as mentioned above we likely did not isolate all heat-resistant fungi that were present.
683 Although none of the heat-resistant fungi were saprotrophic on black spruce in the incubation
684 study, black spruce litter decomposed faster in the field where these fungi were more abundant.
685 These results may seem counterintuitive but the decomposition rates encompass effects of all
686 organisms able to enter litterbags, including fungi, bacteria, and microfauna. While we autoclaved
687 the litter to sterilise it in the incubation study to be able to clearly assess the effect of the inoculated
688 fungi, this increased the C:N ratio of both species (Table S12). Other sterilisation techniques that
689 have less impact on the chemical composition of litter could be considered for future studies. While
690 it is unfortunate that we had background decomposition in the uninoculated control treatment, we
691 can assume that all treatments experienced this background level so our experiment showed that
692 some fungi were able to decompose above that of the background level.

693   Heat-resistant fungi may decline with time since fire, as has been observed in *Rhizopogon*
694 *olivaceotinctus* in California, which fills a short-term disturbance niche after fire (Peay et al., 2009;
695 Glassman et al., 2016). Fungal succession may be inhibited as fire frequency increases, which
696 could lead to greater prevalence or dominance of heat-resistant fungi over the long term.
697 Information on pre-fire fungal communities would help us better understand changes in heat-
698 resistant fungi with changes in fire frequency, which is seldom available (Glassman et al., 2016).
699 Pre-fire data would also facilitate being able to partition fungi that survived fire from those that
700 dispersed in after fire.

701

702 *Conclusion*

703 Our study provides information on the distribution and function of extremophile heat-resistant
704 fungi in a fire-adapted forested ecosystem. We investigated how fire impacts soil fungi and the
705 potential implications for accumulation of C via decomposition and tree seedling growth. We
706 found the distribution of heat-resistant fungi after fires are commonly locally abundant and widely
707 distributed, including in unburned areas, or locally rare and sparse across the landscape. All heat-
708 resistant fungi reduced biomass of seedlings at least one tree species in controlled experiments.
709 Lower conifer seedling densities where heat-resistant fungi were more abundant in the field
710 suggests that these fungi may play a role in structuring plant communities after fire by reducing
711 conifer regeneration. The greater *in situ* decomposition rates of black spruce litter in stands with

longer fire-free intervals suggests this is linked to recovery or succession of microbial communities of the NWT. While DNA sequencing studies provide a useful overview of microbial community structure, understanding functional consequences of fire on fungal communities can aid predictions of decomposition and plant growth, and aboveground and belowground C sequestration as fire activity increases in many regions.

Author contributions

NJD and JLB conceived the study. NJD, SGC, JFJ, MCM, MRT, and XJW designed field sampling. NJD, KAR, and XJW collected field samples and data. NJD undertook all lab work and experiments, and data analyses. NJD wrote the first draft of the manuscript. All co-authors edited the manuscript.

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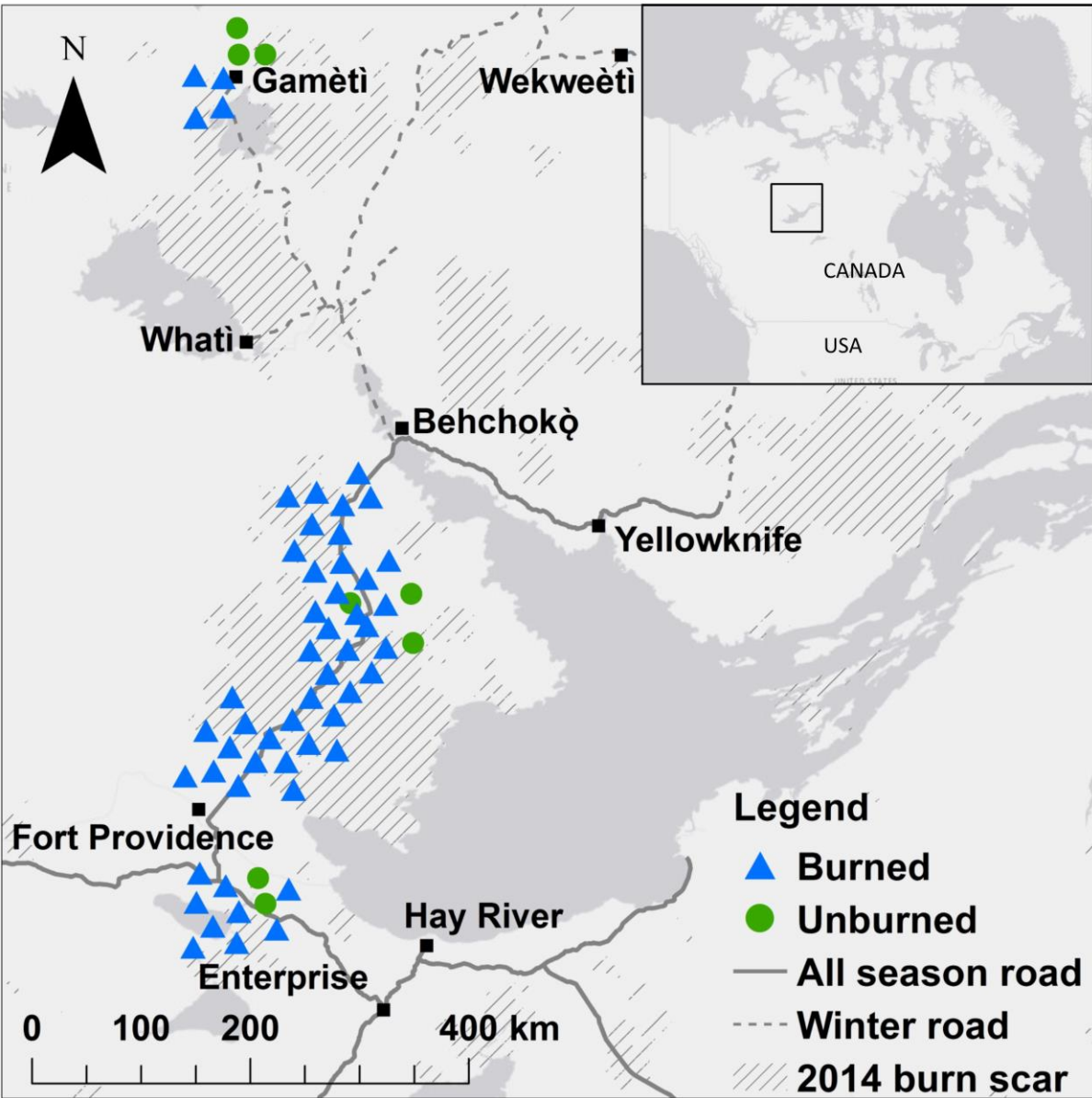
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1046

Figures

1050 Fig. 1. Location of 47 plots where soil samples were collected for analysis of soil fungal
1051 communities (Illumina MiSeq) and seedlings were counted one year after fire in boreal forest
1052 stands of black spruce and jack pine on the Taiga Plains in the Northwest Territories, Canada.
1053 Blue triangles show plots that burned in 2014, green circles show plots that did not burn in 2014
1054 (unburned). Hashed areas show 2014 burn scars and dark grey areas represent water bodies.
1055 Points are jittered to reduce overlap but were at least 100 m from the road. There were three
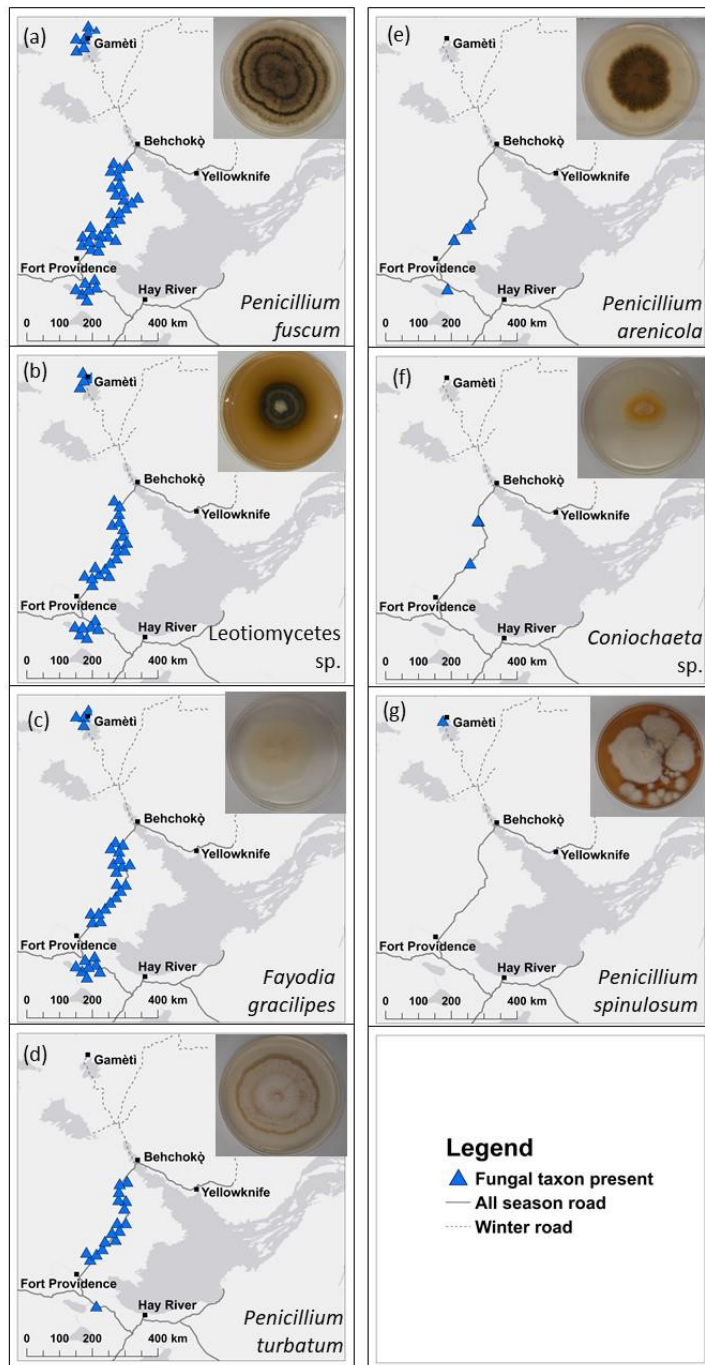1056 quadrats at each plot.

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Fig. 2. Map of distributions of seven of heat-resistant fungal taxa across 47 plots in boreal forests
in the Northwest Territories, Canada. Blue triangles show locations of (a) *Penicillium fuscum*, (b)
Leotiomycetes sp., (c) *Fayodia gracilipes*, (d) *P. turbatum*, (e) *P. arenicola*, (f) *Coniochaeta* sp.,
and (g) *P. spinulosum*. Insets are photos of cultured fungi. Distribution information is based on
matching Sanger sequences of the isolated heat-resistant fungal taxa with operational taxonomic
units from high throughput sequencing (Illumina, MiSeq) sequences at 97% similarity. Points are
jittered to reduce overlap but were at least 100 m from the road. All 47 plots are shown in Fig. 1.
Details of cultures are in Table S2. There were three quadrats at each plot.

1068
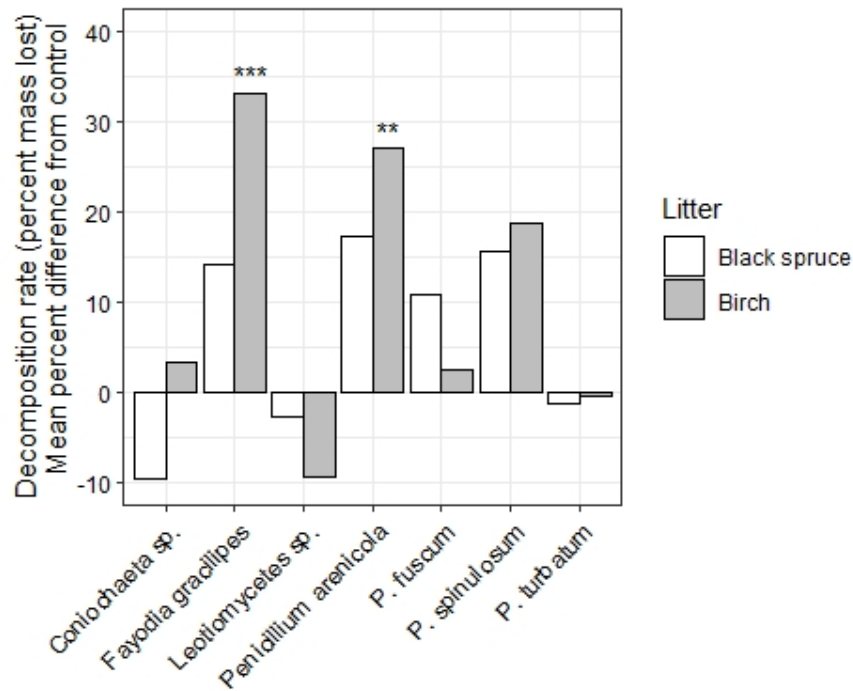
1069 Fig. 3. Saprotrophic activity of seven heat-resistant fungal taxa, as measured by decomposition
1070 rates measured as mass lost of black spruce (white bars) and birch (grey bars) litter over 10 wks
1071 incubation in controlled conditions. Results are presented as the mean percent difference for each
1072 fungal treatment from the uninoculated control treatment, from 999 bootstrap samples. Values
1073 above zero indicate greater decomposition in inoculated samples relative to the control treatment.
1074 Asterisks indicate significant effects of inoculation relative to the control treatment based from
1075 linear models (**$P<0.01$; ***$P<0.001$; Table S3).
1076
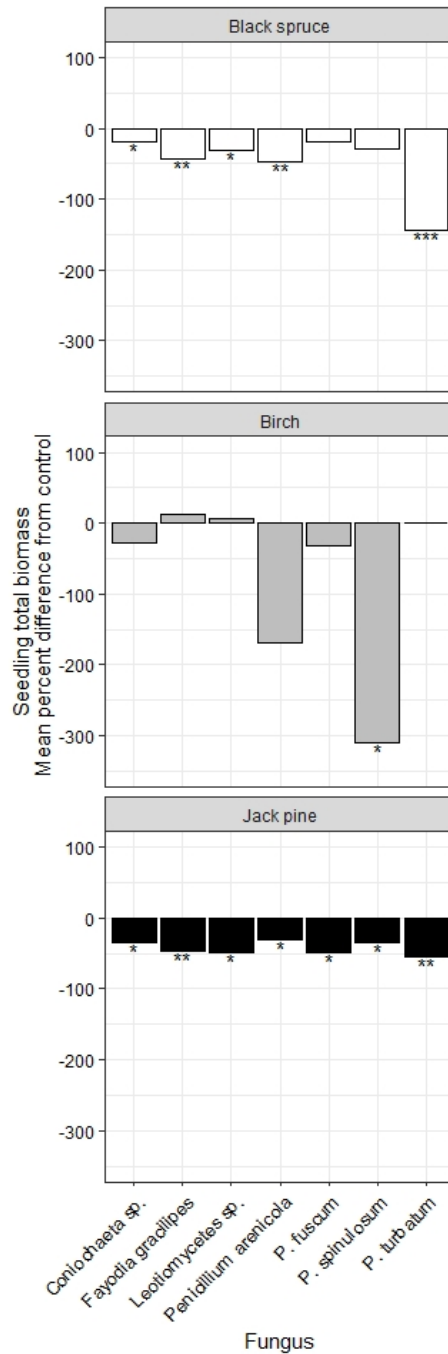
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1078
1079 Fig. 4. Effects of seven heat-resistant fungi on seedling total biomass of black spruce (white
1080 bars), birch (grey bars), and jack pine (black bars) in growth chamber over 10 wks. Results are
1081 presented as the mean percent difference for each fungal treatment from the uninoculated control
1082 treatment, based on 999 bootstraps. Values above zero indicate greater biomass when the fungus
1083 was inoculated, relative to the control. Asterisks indicate statistically significant differences
1084 relative to the control treatment from linear models (*$P<0.05$; **$P<0.01$; ***$P<0.001$; Table
1085 S6).
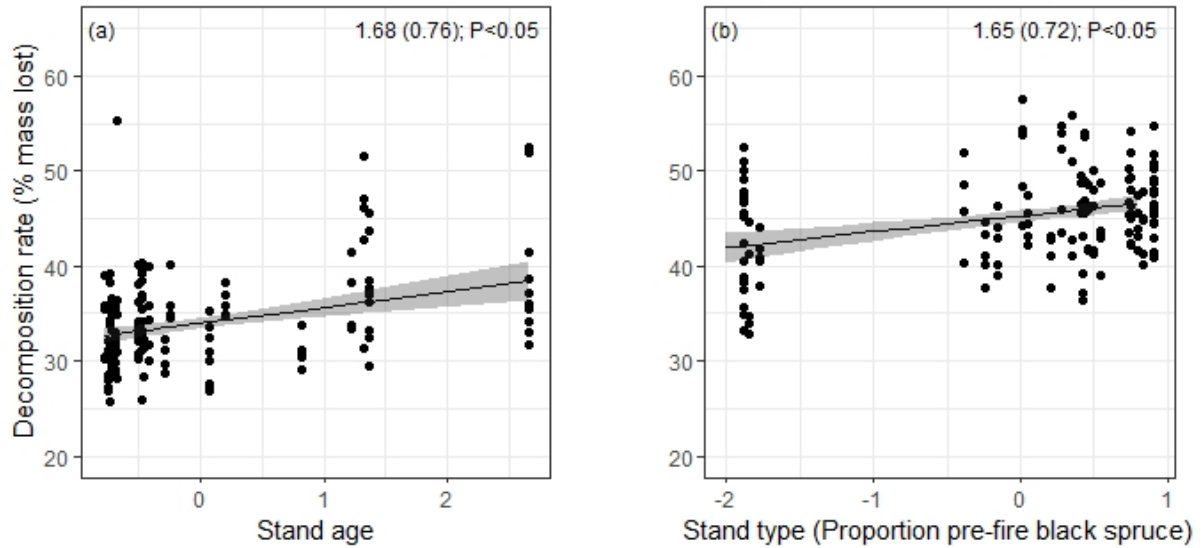
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Fig. 5. Model-based predictions of *in situ* decomposition rates of litter of (a) birch against pre-fire stand type (proportion pre-fire black spruce stems), and (b) black spruce against stand age based on percent mass lost in litterbags over 24 mos. at 30 plots in burned boreal forests, Northwest Territories, Canada. The y-axes show predicted values from linear mixed effects models, with standardised predictors on the x-axes. Estimated coefficients, standard errors, and *P*-values are shown. Full model summaries are reported in Table S9.

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1096 **Tables**

1097

1098 Table 1. Frequencies and relative abundances of heat-resistant fungal taxa across burned boreal
1099 forest stands, Northwest Territories, Canada. Frequency across 138 quadrats in 47 plots, and the
1100 mean and range of the relative abundance (number of reads) per plot are shown. Information is
1101 based on matching Sanger sequences of the isolated heat-resistant fungal taxa with operational
1102 taxonomic units from high throughput sequencing (Illumina, MiSeq) sequences at 47 plots at
1103 97% similarity. Taxa are presented in declining order of plot frequency.

Fungal taxon	Quadrat frequency (n=138)	Plot frequency (n=47)	Mean abundance per plot (range)	Plot frequency in unburned (n=8)
Penicillium fuscum	99	43	151 (0-1086)	8
Fayodia gracilipes	58	31	278 (0-3309)	4
Leotiomycetes sp.	49	31	296 (0-3601)	4
P. turbatum	30	17	7 (0-97)	1
P. arenicola	6	5	<1 (0-6)	0
Coniochaeta sp.	3	3	<1 (0-12)	0
P. spinulosum	1	1	<1 (0-3)	0

1104

Table. 2. Summary of negative binomial mixed-effects model of relative abundance of seven heat-resistant fungi from 47 plots in boreal forests of the Northwest Territories, Canada. Plot was the random effect (SD: 1.44). The dispersion parameter was 0.51. SE: standard error of the mean.

Predictor	Estimate	SE	Z	P
(Intercept)	4.43	0.25	17.54	<0.001
Fire severity	0.00	0.25	-0.02	0.985
Stand age	-0.03	0.25	-0.10	0.921
Pre-fire stand type	0.02	0.25	0.06	0.949

Table 3. Summary of linear models of plot-level *in situ* litter decomposition against predictors of fungal community structure: heat-resistant fungal relative abundance, fungal operational taxonomic unit (OTU) richness, and saprotroph OTU richness, based on high throughput sequencing at 17 plots in burned boreal forests, Northwest Territories, Canada. One outlying plot was removed from all black spruce models. Fungal OTUs were assigned as saprotrophs based on FUNGuild. *P*-values were corrected for multiple tests on the same experimental units. Significant effect (P<0.05) is in bold. SE: standard error of the mean.

Litter species	Predictor	Estimate	SE	t	P
Black spruce	(Intercept)	33.76	0.58	58.46	<0.001
	Heat-resistant fungal abundance	-0.94	0.60	-1.57	0.887
	(Intercept)	33.76	0.62	54.35	<0.001
	Fungal OTU richness	0.31	0.64	0.49	0.919
	(Intercept)	33.76	0.62	54.51	<0.001
	Saprotroph OTU richness	0.35	0.64	0.54	0.716
Birch	(Intercept)	44.89	1.05	42.97	<0.001
	Heat-resistant fungal abundance	-1.17	1.08	-1.09	0.441
	(Intercept)	44.89	1.08	41.38	<0.001
	Fungal OTU richness	-0.03	1.12	-0.03	0.977
	(Intercept)	44.89	1.08	41.37	<0.001
	Saprotroph OTU richness	0.05	1.12	0.04	0.977

Table 4. Summary of linear mixed-effect models of quadrat seedling densities (per m^2) for all species and for conifers only against predictors of fungal community structure: heat-resistant fungal relative abundance, fungal operational taxonomic unit (OTU) richness, pathogen OTU richness, and ectomycorrhizal OTU richness, based on high throughput sequencing in burned boreal forests, Northwest Territories, Canada. OTUs were assigned as plant pathogens and ectomycorrhizas based on FUNGuild. Plot SD shows the among-plot standard deviation in intercepts from the random effect. P-values were corrected for multiple tests on the same experimental units. Significant effects ($P<0.05$) are in bold. SE: standard error of the mean.

	Predictor	Estimate	SE	t	P	Plot SD
All seedlings	Intercept	-0.24	0.51	-0.46	0.735	2.68
	Heat-resistant fungal abundance	0.00	0.04	-0.01	0.989	
	Intercept	-0.24	0.51	-0.47	0.735	2.69
	Fungal OTU richness	0.05	0.05	1.06	0.735	
	Intercept	-0.24	0.51	-0.48	0.686	2.67
	Pathogen OTU richness	**0.18**	**0.05**	**3.79**	**<0.01**	
	Intercept	-0.24	0.51	-0.48	0.686	2.68
	Ectomycorrhizal richness	**0.14**	**0.05**	**2.93**	**<0.05**	
Conifer seedlings	Intercept	-0.31	0.49	-0.64	0.550	2.58
	Heat-resistant fungal abundance	**-0.24**	**0.05**	**-4.67**	**<0.001**	
	Intercept	-0.29	0.49	-0.60	0.550	2.58
	Fungal OTU richness	0.05	0.05	0.91	0.550	
	Intercept	-0.31	0.49	-0.62	0.550	2.56
	Pathogen OTU richness	**0.24**	**0.05**	**4.67**	**<0.001**	
	Intercept	-0.30	0.49	-0.60	0.550	2.58
	Ectomycorrhizal OTU richness	**0.14**	**0.05**	**2.90**	**<0.05**	

Supplementary material for manuscript *Identifying functional impacts of heat-resistant fungi on boreal forest recovery after wildfire*

Authors: Nicola J Day, Steven G Cumming, Kari E. Dunfield, Jill F Johnstone, Michelle C Mack, Kirsten A Reid, Merritt R Turetsky, Xanthe J Walker, Jennifer L Baltzer
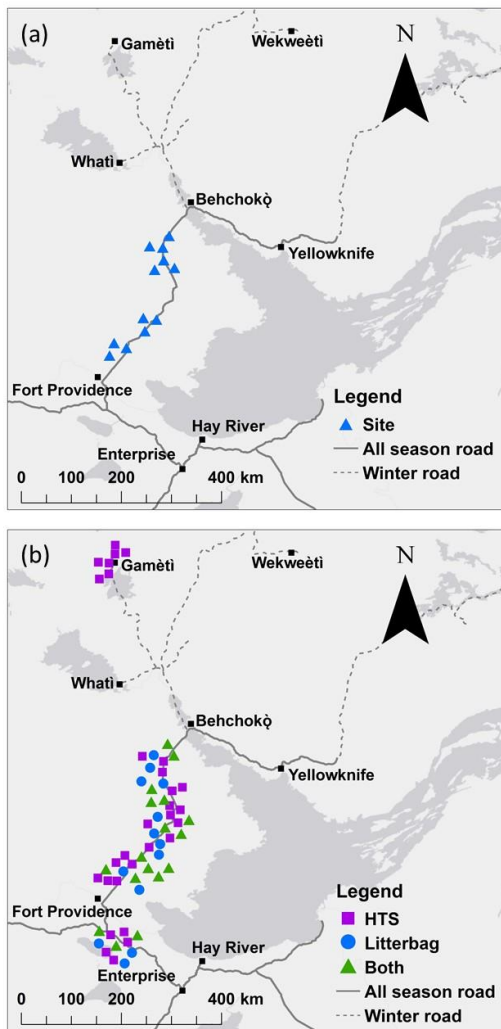
Supplementary figures

Fig. S1. Locations of plots in boreal forest stands of black spruce and jack pine on the Taiga Plains in the Northwest Territories, Canada. Maps show (a) locations of 12 plots from which soils were collected for culturing of heat-resistant fungi (blue triangles), and (b) locations of 30 plots where litterbags of black spruce and birch were located for in situ decomposition rates (blue circles), where high-throughput sequencing was conducted (purple squares), and where there were both litterbags and high-throughput sequencing occurred (n=17). Points are jittered to mitigate overlap but were at least 100 m from the road. See Fig. 1 for burn scars. Note that litterbags were at a total of 30 plots and high-throughput sequencing were at a total of 47 plots. There were three quadrats at each plot.
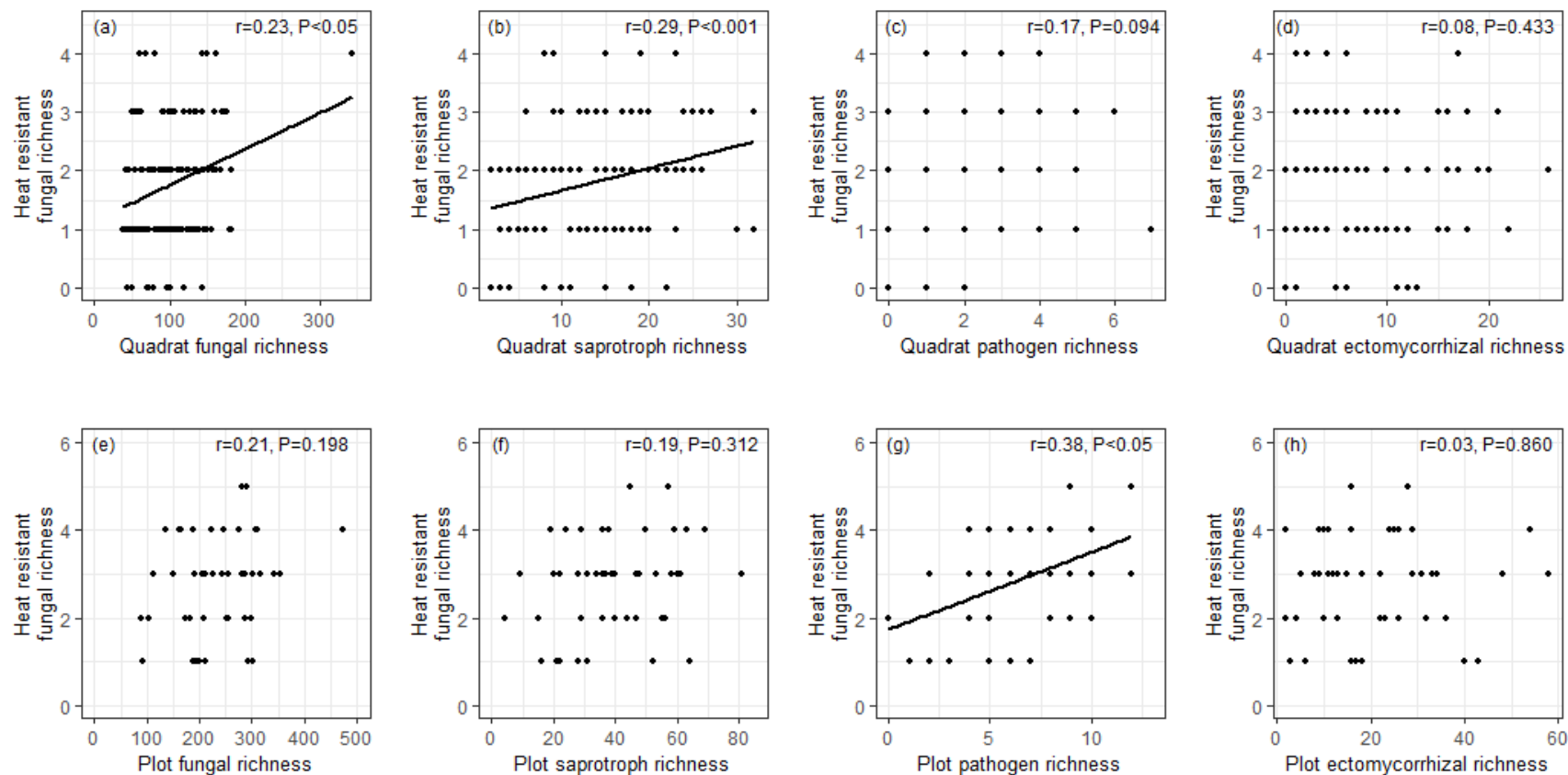
Fig. S2. Correlations between measures of fungal richness and heat resistant fungal richness for (a) quadrat total fungal richness, (b) quadrat fungal saprotroph richness, (c) quadrat plant pathogen richness, (d) quadrat ectomycorrhizal richness, and (e) plot total fungal richness, (f) plot fungal saprotroph richness, (g) plot fungal plant pathogen richness, and (h) plot ectomycorrhizal richness. Information is based on matching Sanger sequences of the isolated heat-resistant fungal taxa with operational taxonomic units from high throughput sequencing (Illumina, MiSeq) sequences at 47 plots at 97% similarity. Operational taxonomic units to functions were assigned using FUNGuild. Spearman's correlation coefficients (r) and false discovery rate-corrected *P*-values are shown. For (a) and (e) the direction and significance of relationships are unchanged when the outlier is removed. There were three quadrats at each plot.
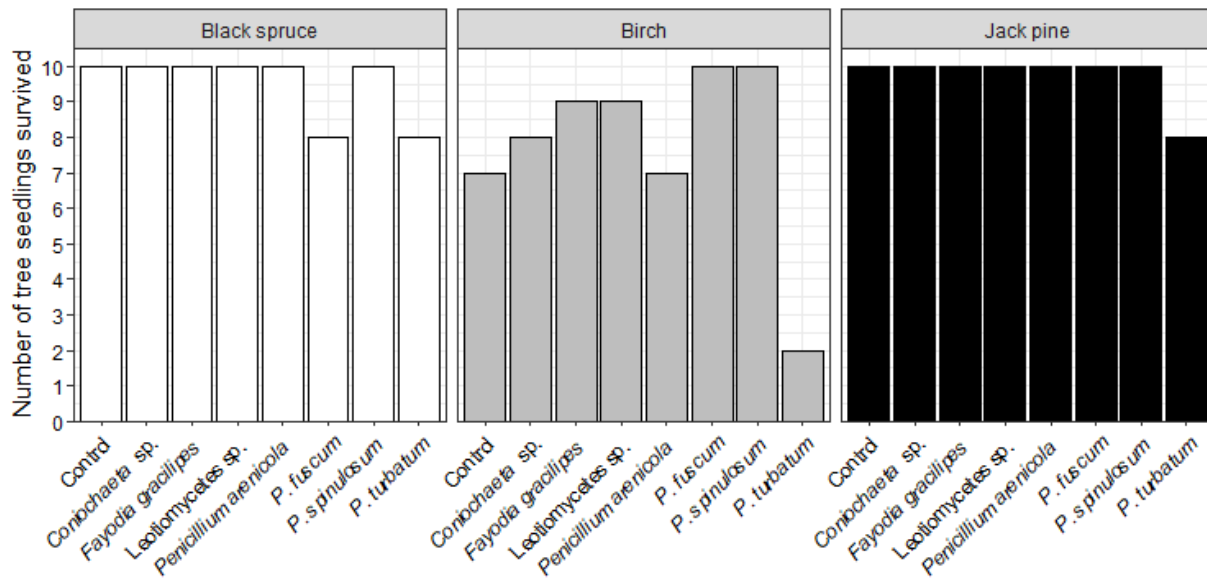
Fig. S3. Survival of seedlings of black spruce, birch, and jack pine inoculated with seven heat-resistant fungal taxa in growth chamber experiment over 10 weeks. There were 10 replicates for each plant-fungal combination at the beginning of the experiment. Control is the uninoculated treatment.
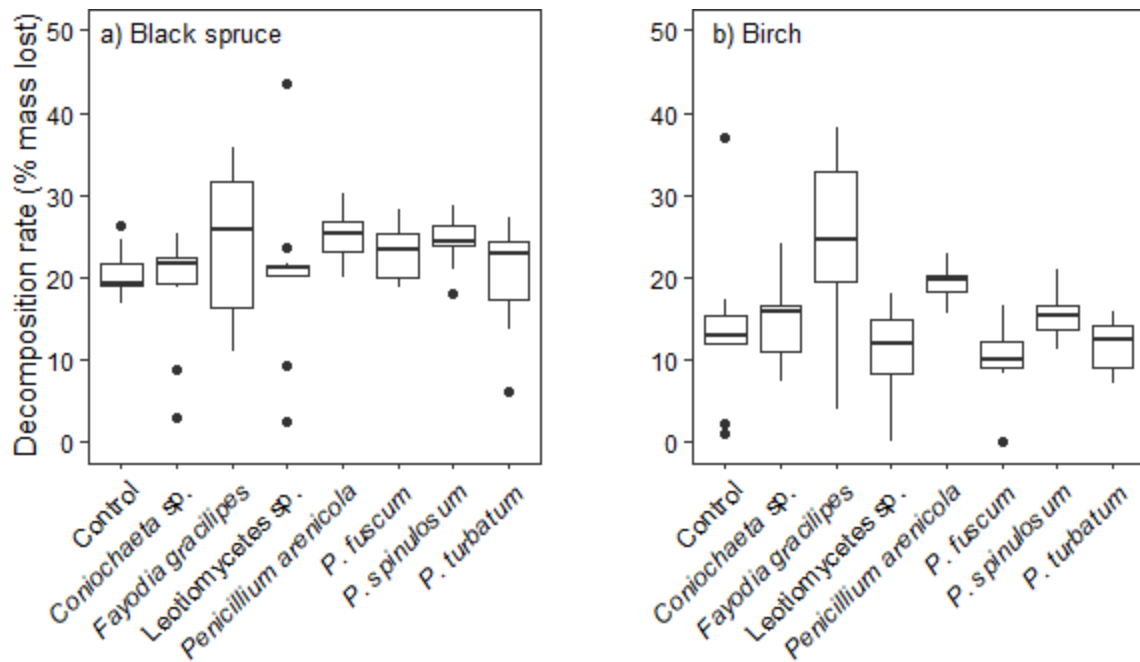
Fig. S4. Boxplots of raw data showing saprotrophic activity of seven heat-resistant fungal taxa, as measured by decomposition rates measured as mass lost of (a) black spruce and (b) birch litter over 10 weeks incubation in controlled conditions.
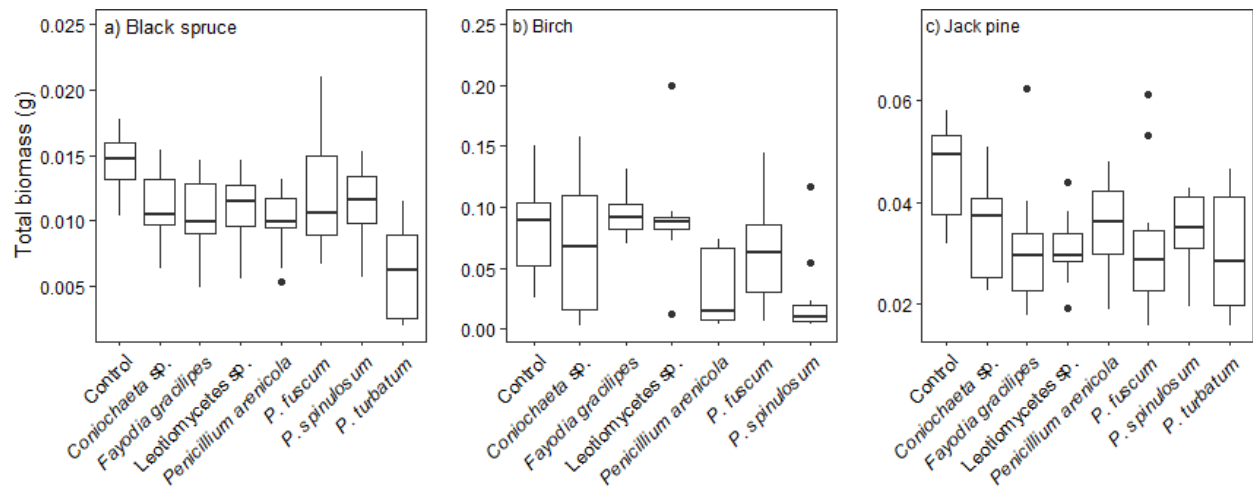
Fig. S5. Boxplots of raw data showing effects of seven heat-resistant fungi on seedling total biomass of (a) black spruce, (b) birch, and (c) jack pine in growth chamber over 10 weeks.
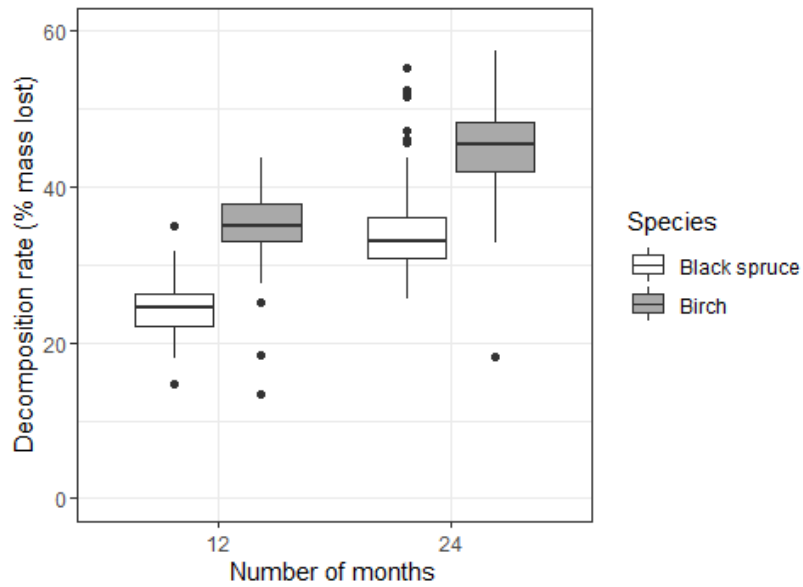
Fig. S6. Boxplot for *in situ* litter decomposition study showing percent mass lost for birch and black spruce litter after 12 months (40 litterbags at 8 plots) and 24 months (150 litterbags at 30 plots) in burned boreal forests in the Northwest Territories, Canada.
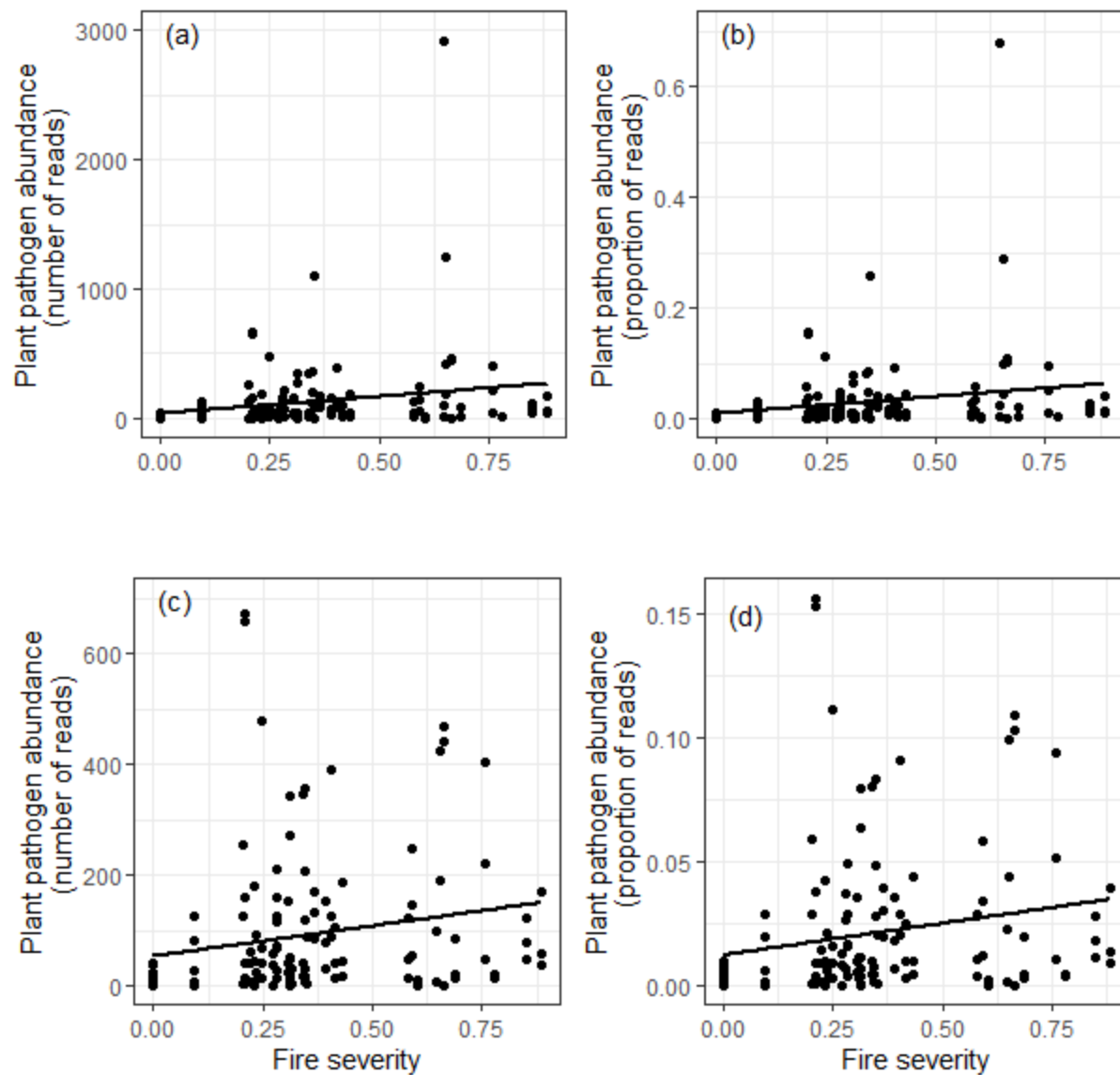
Fig. S7. Relationships between fire severity and fungal pathogen relative abundance in terms of number of reads (a) & (c) and proportion of reads (b) & (d) for 47 plots in burned boreal forests of the Northwest Territories, Canada. (c) and (d) show the relationships with very large values removed. Fungal pathogens were determined from high throughput sequencing data and linking operational taxonomic units to function using FUNGuild. Fire severity is measured as proportion soil organic layer combusted. Fire severity of zero denotes unburned plots. Spearman correlation coefficients: (a) r=0.21; (b) r=0.21; (c) r=0.20; (d) r=0.20.
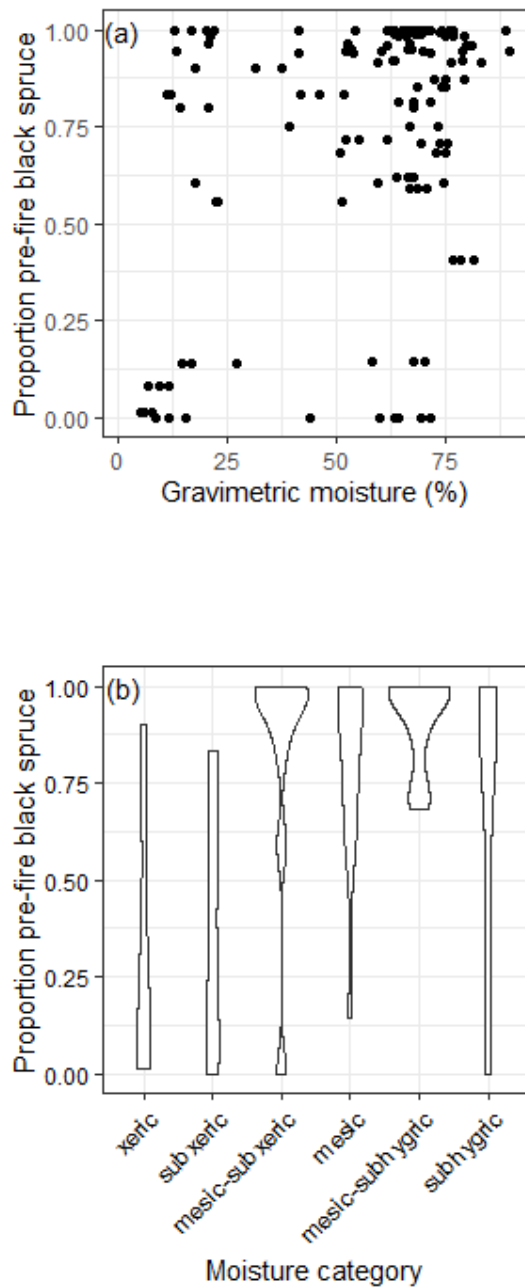
Fig. S8. Relationships between (a) gravimetric soil moisture (%) and (b) moisture category and proportion pre-fire black spruce stems at 47 plots in boreal forests of the Northwest Territories, Canada. Plot locations are the same as where soil fungal communities were characterised by high throughput sequencing. Gravimetric soil moisture was measured from a point sample in 2015 in the top 5 cm of soil and calculated from wet and dry weights. Moisture category was assessed for each plot in the field according to soil drainage and landscape position, with xeric as the driest and subhygric as the wettest.

Supplementary tables

Table S1. Mean, minimum, and maximum values for environmental and fire variables for the high throughput sequencing study (47 plots), the *in situ* decomposition study (30 plots), and where heat-resistant fungi were cultured from soils (12 plots) in boreal forests, Northwest Territories, Canada. The 12 plots where heat-resistant fungi were isolated were nested within the 47 plots where high throughput sequencing was done. 17 plots where the *in situ* decomposition study took place overlapped with the 47 high throughput sequencing plot (Fig. S1). Mean annual temperature is based on gridded data and was only downloaded for the 30 plots for the *in situ* decomposition study.

	High throughput sequencing plots (n=47)	*In situ* decomposition plots (n=30)	Heat-resistant fungal isolations (n=12)
Stand type (proportion of pre-fire stems black spruce)	0.75 (0, 1)	0.70 (0, 1)	0.64 (0.01, 0.95)
Stand age (years)	108 (58, 232)	112 (70, 232)	91.6 (71, 145)
Fire severity (Proportion soil organic layer combusted)	0.34 (0, 0.88)	0.52 (0.09, 1)	0.45 (0.23, 0.85)
Mean annual temperature in 2017 (ºC)	N/A	-2.0 (-2.7, -1.1)	N/A

Table S2. Sequenced fungal isolates that were cultured from heat-treated soils from burned boreal forest stands, Northwest Territories, Canada. Isolate code is the unique identifier used in this study. Identification is the fungal taxon based on sequences and morphology. Genbank accession is the code associated with the sequence deposited in genbank. DAOMC is the number associated with the culture in the Canadian Collection of Fungal Cultures. Closest genbank and max identity are shown. Species hypotheses and blast results from the UNITE database are shown (UNITE Community 2017). OTU is the operational taxonomic unit that was the closest match between the Sanger sequences from the cultured fungi and that of the Illumina sequences at 47 plots at 97% similarity. The *F. gracilipes* sequence matched two OTUs equally. *denotes isolates used in the incubation and growth chamber experiments

Isolate code	Identification	Genbank accession	DAOMC	Unite SH	Reference sequence	E value	Percent identity	OTU
*PENtur_ZF46-18B_HRF1	*Penicillium turbatum*	MN410596	251861	SH1529984.08FU	EF434072	0.0	100.00	898562
PENtur_ZF20-11A_HRF5	*Penicillium turbatum*	MN410599	251863	SH1529984.08FU	EF434072	0.0	99.82	898562
PENtur_ZF20-11B_HRF10	*Penicillium turbatum*	MN410603	251862	SH1529984.08FU	AF034454	0.0	99.82	898562
*FAYgra_ZF46-35C_HRF2	*Fayodia gracilipes*	MN410597	251855	SH1553066.08FU	KC176299	0.0	100.00	210980, 847195
*PENfus_ZF20-11B_HRF3	*Penicillium fuscum*	MN410598	251865	SH1529988.08FU	KJ508319	0.0	99.82	982916
PENfus_ZF46-18A_HRF11	*Penicillium fuscum*	MN410604	251868	SH1529988.08FU	KJ508319	0.0	99.64	982916
PENfus_ZF46-35B_HRF13	*Penicillium fuscum*	MN410605	251866	SH1529988.08FU	AF033411	0.0	100.00	982916
*PENspi_ZF46-18B_HRF8	*Penicillium spinulosum*	MN410601	251858	SH1529988.08FU	LN901135	0.0	99.64	715550
*PENare_ZF20-34C_HRF9	*Penicillium arenicola*	MN410602	251859	SH1532429.08FU	GU092963	0.0	99.82	385200
*CONsp_ZF20-11B_HRF7	*Coniochaeta* sp.	MN410600	251857	SH1645174.08FU	KJ188672	0.0	98.82	947267
*LEOsp_ZF46-18C_HRF14	Leotiomycetes sp.	MN410606	251856	SH1564438.08FU	EU292368	0.0	99.81	253574

Table S3. Model summaries from linear models assessing decomposition of birch and black spruce litter inoculated with seven heat-resistant fungi in incubation experiments over 10 weeks. Coefficient estimates for each fungal taxon are relative to uninoculated controls. Significant effects (P<0.05) are in bold. SE: standard error of the mean.

Plant	Fungus	Estimate	SE	*t*	*P*
Black spruce	Intercept (control)	0.08	0.01	10.16	<0.001
	Coniochaeta sp.	-0.01	0.01	-0.51	0.611
	Fayodia gracilipes	0.01	0.01	1.32	0.192
	Leotiomycetes sp.	0.00	0.01	0.03	0.977
	Penicillium arenicola	0.02	0.01	1.53	0.129
	P. fuscum	0.01	0.01	0.90	0.374
	P. spinulosum	0.02	0.01	1.36	0.179
	P. turbatum	0.00	0.01	-0.02	0.987
Birch	Intercept (control)	0.04	0.01	5.60	<0.001
	Coniochaeta sp.	0.01	0.01	1.31	0.194
	Fayodia gracilipes	**0.05**	**0.01**	**5.04**	**<0.001**
	Leotiomycetes sp.	0.00	0.01	0.02	0.988
	Penicillium arenicola	**0.03**	**0.01**	**3.17**	**<0.01**
	P. fuscum	0.00	0.01	-0.31	0.759
	P. spinulosum	0.02	0.01	1.68	0.098
	P. turbatum	0.00	0.01	0.35	0.728

Table S4. Summary of Tukey-Kramer post-hoc analyses to test differences between effects of each heat-resistant fungal taxon on decomposition rates of black spruce and birch litter in incubation experiments over 10 weeks. Significant contrasts (P<0.05) are in bold. SE: standard error of the mean.

Litter species	Contrast	Estimate	SE	*Z*	*P*
Black spruce	*Coniochaeta* sp. - *F. gracilipes*	-5.21	2.85	-1.83	0.601
	Coniochaeta sp. - Leotiomycetes sp.	-1.54	2.85	-0.54	0.999
	Coniochaeta sp. - *P. arenicola*	-5.83	2.85	-2.05	0.451
	Coniochaeta sp. - *P. fuscum*	-4.01	2.85	-1.41	0.855
	Coniochaeta sp. - *P. spinulosum*	-5.33	2.85	-1.87	0.572
	Coniochaeta sp. - *P. turbatum*	-1.41	2.85	-0.49	1.000
	F. gracilipes - Leotiomycetes sp.	3.67	2.85	1.29	0.904
	F. gracilipes - *P. arenicola*	-0.62	2.85	-0.22	1.000
	F. gracilipes - *P. fuscum*	1.20	2.85	0.42	1.000
	F. gracilipes - *P. spinulosum*	-0.12	2.85	-0.04	1.000
	F. gracilipes - *P. turbatum*	3.80	2.85	1.33	0.887
	Leotiomycetes sp. - *P. arenicola*	-4.29	2.85	-1.51	0.805
	Leotiomycetes sp. - *P. fuscum*	-2.47	2.85	-0.87	0.989
	Leotiomycetes sp. - *P. spinulosum*	-3.79	2.85	-1.33	0.888
	Leotiomycetes sp. - *P. turbatum*	0.13	2.85	0.05	1.000
	P. arenicola - *P. fuscum*	1.82	2.85	0.64	0.998
	P. arenicola - *P. spinulosum*	0.50	2.85	0.18	1.000
	P. arenicola - *P. turbatum*	4.42	2.85	1.55	0.779
	P. fuscum - *P. spinulosum*	-1.32	2.85	-0.46	1.000
	P. fuscum - *P. turbatum*	2.60	2.85	0.91	0.985
	P. spinulosum - *P. turbatum*	3.92	2.85	1.37	0.869
Birch	*Coniochaeta* sp. - *F. gracilipes*	**-9.81**	**2.48**	**-3.96**	**<0.01**
	Coniochaeta sp. - Leotiomycetes sp.	3.41	2.55	1.34	0.885
	Coniochaeta sp. - *P. arenicola*	-4.88	2.48	-1.97	0.502
	Coniochaeta sp. - *P. fuscum*	4.26	2.48	1.72	0.675
	Coniochaeta sp. - *P. spinulosum*	-0.97	2.48	-0.39	1.000
	Coniochaeta sp. - *P. turbatum*	2.53	2.48	1.02	0.972
	F. gracilipes - Leotiomycetes sp.	**13.22**	**2.55**	**5.19**	**<0.001**
	F. gracilipes - *P. arenicola*	4.93	2.48	1.99	0.491
	F. gracilipes - *P. fuscum*	**14.07**	**2.48**	**5.68**	**<0.001**
	F. gracilipes - *P. spinulosum*	**8.84**	**2.48**	**3.57**	**<0.01**
	F. gracilipes - *P. turbatum*	**12.34**	**2.48**	**4.98**	**<0.001**
	Leotiomycetes sp. - *P. arenicola*	**-8.29**	**2.55**	**-3.26**	**<0.05**

	Leotiomycetes sp. - *P. fuscum*	0.85	2.55	0.33	1.000
	Leotiomycetes sp. - *P. spinulosum*	-4.38	2.55	-1.72	0.676
	Leotiomycetes sp. - *P. turbatum*	-0.88	2.55	-0.34	1.000
	P. arenicola - P. fuscum	**9.14**	**2.48**	**3.69**	**<0.01**
	P. arenicola - P. spinulosum	3.92	2.48	1.58	0.763
	P. arenicola - P. turbatum	7.41	2.48	2.99	0.056
	P. fuscum - P. spinulosum	-5.23	2.48	-2.11	0.409
	P. fuscum - P. turbatum	-1.73	2.48	-0.70	0.997
	P. spinulosum - P. turbatum	3.50	2.48	1.41	0.853

Table S5. Assessment of colonisation of sterilised birch and black spruce litter by seven heat-resistant fungal taxa after incubation for 10 weeks. Colonisation was assessed at the end of the experiment from fungal growth from surface-disinfected litter on potato dextrose agar over 7 days.

	Litter colonised? i.e. fungal growth observed?	
Fungal taxon	Black spruce	Birch
Coniochaeta sp.	**Yes**	No
Fayodia gracilipes	**Yes**	**Yes**
Leotiomycetes sp.	No	No
Penicillium arenicola	**Yes**	**Yes**
P. fuscum	**Yes**	**Yes**
P. spinulosum	**Yes**	**Yes**
P. turbatum	**Yes**	**Yes**

Table S6. Summary from linear models assessing total biomass and root-shoot ratio of birch, black spruce, and jack pine inoculated with seven heat-resistant fungi in growth chamber over 10 weeks. Coefficient estimates for each fungus are relative to uninoculated controls. All but two birch seedlings died when inoculated with *Penicillium turbatum* so this effect was not included in the model. Significant effects ($P<0.05$) are in bold, based on corrected values for multiple tests on the same experimental units. Black spruce coefficients are presented to 3 d.p. due to seedlings being very small. SE: standard error of the mean.

Plant	Fungus	Total biomass				Root-shoot			
		Estimate	SE	*t*	*P*	Estimate	SE	*t*	*P*
Black spruce	Intercept (control)	0.014	0.001	14.39	<0.001	0.71	0.09	7.51	<0.001
	Coniochaeta sp.	**-0.004**	**0.001**	**-2.47**	**<0.05**	-0.08	0.13	-0.63	0.654
	Fayodia gracilipes	**-0.004**	**0.001**	**-3.06**	**<0.01**	-0.07	0.13	-0.52	0.692
	Leotiomycetes sp.	**-0.003**	**0.001**	**-2.39**	**<0.05**	-0.10	0.13	-0.78	0.588
	Penicillium arenicola	**-0.005**	**0.001**	**-3.24**	**<0.01**	-0.26	0.13	-1.96	0.096
	P. fuscum	-0.002	0.002	-1.51	0.217	0.00	0.14	-0.01	0.994
	P. spinulosum	-0.003	0.001	-2.27	0.053	-0.05	0.13	-0.39	0.748
	P. turbatum	**-0.008**	**0.002**	**-5.52**	**<0.001**	-0.15	0.14	-1.08	0.415
Birch	Intercept (control)	0.08	0.02	5.04	<0.001	0.63	0.09	6.81	<0.001
	Coniochaeta sp.	-0.01	0.02	-0.50	0.766	-0.05	0.13	-0.37	0.766
	Fayodia gracilipes	0.01	0.02	0.55	0.766	-0.06	0.13	-0.47	0.766
	Leotiomycetes sp.	0.01	0.02	0.41	0.766	0.04	0.12	0.30	0.766
	Penicillium arenicola	-0.05	0.02	-2.07	0.153	0.08	0.13	0.58	0.766
	P. fuscum	-0.02	0.02	-0.80	0.766	-0.06	0.12	-0.46	0.766
	P. spinulosum	**-0.06**	**0.02**	**-2.69**	**<0.05**	0.08	0.12	0.63	0.766
Jack pine	Intercept (control)	0.05	0.00	13.59	<0.001	1.11	0.08	14.55	<0.001
	Coniochaeta sp.	**-0.01**	**0.00**	**-2.38**	**<0.05**	0.07	0.11	0.67	0.676
	Fayodia gracilipes	**-0.01**	**0.00**	**-3.02**	**<0.05**	-0.09	0.11	-0.81	0.612
	Leotiomycetes sp.	**-0.02**	**0.00**	**-3.15**	**<0.05**	-0.05	0.11	-0.47	0.730

	Penicillium arenicola	**-0.01**	**0.00**	**-2.26**	**<0.05**	-0.03	0.11	-0.31	0.784
	P. fuscum	**-0.01**	**0.00**	**-2.96**	**<0.05**	-0.10	0.11	-0.93	0.573
	P. spinulosum	**-0.01**	**0.00**	**-2.44**	**<0.05**	0.03	0.11	0.28	0.784
	P. turbatum	**-0.02**	**0.01**	**-3.12**	**<0.01**	-0.06	0.11	-0.49	0.730

Table S7. Summary of Tukey-Kramer post-hoc analyses to test differences between effects of each heat-resistant fungal taxon on total biomass of black spruce, birch, and jack pine seedlings in growth chamber experiments over 10 weeks. Significant contrasts (*P*<0.05) are in bold. SE: standard error of the mean.

Seedling species	Contrast	Estimate	SE	Z	P
Black spruce	*Coniochaeta* sp. - *F. gracilipes*	0.00	0.00	0.58	0.999
	Coniochaeta sp. - Leotiomycetes sp.	0.00	0.00	-0.08	1.000
	Coniochaeta sp. - *P. arenicola*	0.00	0.00	0.77	0.995
	Coniochaeta sp. - *P. fuscum*	0.00	0.00	-0.82	0.992
	Coniochaeta sp. - *P. spinulosum*	0.00	0.00	-0.20	1.000
	Coniochaeta sp. - *P. turbatum*	0.00	0.00	3.19	0.031
	F. gracilipes - Leotiomycetes sp.	0.00	0.00	-0.67	0.998
	F. gracilipes - *P. arenicola*	0.00	0.00	0.18	1.000
	F. gracilipes - *P. fuscum*	0.00	0.00	-1.37	0.871
	F. gracilipes - *P. spinulosum*	0.00	0.00	-0.79	0.994
	F. gracilipes - *P. turbatum*	0.00	0.00	2.64	0.143
	Leotiomycetes sp. - *P. arenicola*	0.00	0.00	0.85	0.990
	Leotiomycetes sp. - *P. fuscum*	0.00	0.00	-0.74	0.996
	Leotiomycetes sp. - *P. spinulosum*	0.00	0.00	-0.12	1.000
	Leotiomycetes sp. - *P. turbatum*	**0.00**	**0.00**	**3.27**	**0.024**
	P. arenicola - *P. fuscum*	0.00	0.00	-1.54	0.784
	P. arenicola - *P. spinulosum*	0.00	0.00	-0.97	0.978
	P. arenicola - *P. turbatum*	0.00	0.00	2.46	0.211
	P. fuscum - *P. spinulosum*	0.00	0.00	0.63	0.999
	P. fuscum* - *P. turbatum	**0.01**	**0.00**	**3.80**	**0.004**
	P. spinulosum - *P. turbatum*	0.01	0.00	3.38	0.017
Birch	*Coniochaeta* sp. - *F. gracilipes*	-0.02	0.02	-1.06	0.939
	Coniochaeta sp. - Leotiomycetes sp.	-0.02	0.02	-0.95	0.964
	Coniochaeta sp. - *P. arenicola*	0.04	0.02	1.64	0.656
	Coniochaeta sp. - *P. fuscum*	0.01	0.02	0.29	1.000
	Coniochaeta sp. - *P. spinulosum*	0.05	0.02	2.25	0.268
	F. gracilipes - Leotiomycetes sp.	0.00	0.02	0.17	1.000
	F. gracilipes - *P. arenicola*	0.06	0.02	2.62	0.121
	F. gracilipes - *P. fuscum*	0.03	0.02	1.39	0.807
	F. gracilipes* - *P. spinulosum	**0.07**	**0.02**	**3.28**	**0.018**
	Leotiomycetes sp. - *P. arenicola*	0.06	0.02	2.60	0.126
	Leotiomycetes sp. - *P. fuscum*	0.03	0.02	1.30	0.851

	Leotiomycetes sp. - *P. spinulosum*	**0.07**	**0.02**	**3.33**	**0.015**
	P. arenicola - P. fuscum	-0.03	0.02	-1.45	0.777
	P. arenicola - P. spinulosum	0.01	0.02	0.45	0.999
	P. fuscum - P. spinulosum	0.04	0.02	2.08	0.362
Jack pine	*Coniochaeta* sp. - *F. gracilipes*	0.00	0.00	0.64	0.998
	Coniochaeta sp. - Leotiomycetes sp.	0.00	0.00	0.77	0.995
	Coniochaeta sp. - *P. arenicola*	0.00	0.00	-0.12	1.000
	Coniochaeta sp. - *P. fuscum*	0.00	0.00	0.58	0.999
	Coniochaeta sp. - *P. spinulosum*	0.00	0.00	0.06	1.000
	Coniochaeta sp. - *P. turbatum*	0.00	0.01	0.88	0.988
	F. gracilipes - Leotiomycetes sp.	0.00	0.00	0.13	1.000
	F. gracilipes - P. arenicola	0.00	0.00	-0.76	0.995
	F. gracilipes - P. fuscum	0.00	0.00	-0.07	1.000
	F. gracilipes - P. spinulosum	0.00	0.00	-0.58	0.999
	F. gracilipes - P. turbatum	0.00	0.01	0.27	1.000
	Leotiomycetes sp. - *P. arenicola*	0.00	0.00	-0.89	0.987
	Leotiomycetes sp. - *P. fuscum*	0.00	0.00	-0.19	1.000
	Leotiomycetes sp. - *P. spinulosum*	0.00	0.00	-0.70	0.997
	Leotiomycetes sp. - *P. turbatum*	0.00	0.01	0.15	1.000
	P. arenicola - P. fuscum	0.00	0.00	0.70	0.997
	P. arenicola - P. spinulosum	0.00	0.00	0.18	1.000
	P. arenicola - P. turbatum	0.01	0.01	0.99	0.976
	P. fuscum - P. spinulosum	0.00	0.00	-0.51	1.000
	P. fuscum - P. turbatum	0.00	0.01	0.33	1.000
	P. spinulosum - P. turbatum	0.00	0.01	0.82	0.992

Table S8. Assessment of colonisation of roots of birch and black spruce that had been inoculated by seven heat-resistant fungal taxa after 10 weeks of seedling growth. Colonisation was assessed at the end of the experiment from fungal growth from surface-disinfected litter on potato dextrose agar over 7 days.

	Fungal growth observed? i.e. roots colonised?		
Fungal taxon	Black spruce	Birch	Jack pine
Coniochaeta sp.	No	**Yes**	**Yes**
Fayodia gracilipes	No	No	No
Leotiomycetes sp.	No	**Yes**	**Yes**
Penicillium arenicola	**Yes**	**Yes**	**Yes**
P. fuscum	No	**Yes**	No
P. spinulosum	No	No	**Yes**
P. turbatum	No	**Yes**	No

Table S9. Summaries from linear mixed effects models assessing fire and forest stand drivers of *in situ* litter decomposition rates of birch and black spruce in litterbags over 24 months at 30 plots in burned boreal forests, Northwest Territories, Canada. Standardised values for the predictors are shown. Plot was the random effect (black spruce SD: 2.27; birch SD 3.77). Significant effects (*P*<0.05) are in bold. SE: standard error of the mean.

Litter	Predictor	Estimate	SE	*t*	*P*
Black spruce	Intercept	33.99	0.54	62.93	<0.001
	Fire severity	-0.13	0.62	-0.21	0.837
	Stand age	**1.68**	**0.76**	**2.21**	**<0.05**
	Stand type	0.45	0.65	0.70	0.492
	Mean annual temperature	0.61	0.84	0.72	0.475
Birch	Intercept	45.25	0.60	75.08	<0.001
	Fire severity	0.50	0.69	0.72	0.478
	Stand age	0.70	0.85	0.83	0.417
	Stand type	**1.65**	**0.72**	**2.27**	**<0.05**
	Mean annual temperature	-0.02	0.93	-0.02	0.989

Table S10. Summary of mixed effect models assessing plot moisture category as a predictor of *in situ* decomposition rates of birch and black spruce after 24 months at 30 plots in burned boreal forests, Northwest Territories, Canada. Moisture category was the predictor with the driest category, xeric, as the reference level. Plot was the random effect (black spruce SD: 4.20; birch SD 4.20).. SE: standard error of the mean.

Litter species	Moisture category	Estimate	SE	*t*	*P*
Black spruce	Intercept (Xeric)	31.26	1.63	19.16	<0.001
	Subxeric	3.28	3.05	1.07	0.293
	Mesic-subxeric	3.39	2.04	1.67	0.109
	Mesic	2.25	2.14	1.05	0.303
	Mesic-subhygric	4.04	2.31	1.75	0.093
	Subhygric	4.44	3.05	1.46	0.158
Birch	Intercept (Xeric)	42.47	1.75	24.21	<0.001
	Subxeric	4.83	3.28	1.47	0.154
	Mesic-subxeric	2.58	2.19	1.18	0.249
	Mesic	2.97	2.30	1.29	0.210
	Mesic-subhygric	4.02	2.48	1.62	0.119
	Subhygric	2.32	3.28	0.71	0.487

Table S11. Summary of Tukey-Kramer post hoc contrasts for moisture category as a predictor of in situ decomposition rates of black spruce and birch litter after 24 months at 30 plots in burned boreal forests, Northwest Territories, Canada.

Litter species	Contrast	Estimate	SE	t	P
Black spruce	Xeric - Subxeric	-3.28	3.05	-1.07	0.887
	Xeric -Mesic-subxeric	-3.39	2.04	-1.67	0.565
	Xeric - Mesic	-2.25	2.14	-1.05	0.895
	Xeric - Mesic-subhygric	-4.04	2.31	-1.75	0.513
	Xeric - Subhygric	-4.44	3.05	-1.46	0.694
	Subxeric - Mesic-subxeric	-0.12	2.85	-0.04	1.000
	Subxeric - Mesic	1.03	2.93	0.35	0.999
	Subxeric - Mesic-subhygric	-0.76	3.05	-0.25	1.000
	Subxeric - Subhygric	-1.16	3.65	-0.32	1.000
	Mesic-subxeric - Mesic	1.14	1.85	0.62	0.989
	Mesic-subxeric - Mesic-subhygric	-0.65	2.04	-0.32	1.000
	Mesic-subxeric - Subhygric	-1.05	2.85	-0.37	0.999
	Mesic – Mesic-subhygric	-1.79	2.14	-0.84	0.958
	Mesic - Subhygric	-2.19	2.93	-0.75	0.974
	Mesic-subhygric - Subhygric	-0.40	3.05	-0.13	1.000
Birch	Xeric - Subxeric	-4.83	3.28	-1.47	0.685
	Xeric -Mesic-subxeric	-2.58	2.19	-1.18	0.841
	Xeric - Mesic	-2.97	2.30	-1.29	0.788
	Xeric - Mesic-subhygric	-4.02	2.48	-1.62	0.595
	Xeric - Subhygric	-2.32	3.28	-0.71	0.979
	Subxeric - Mesic-subxeric	2.25	3.07	0.73	0.976
	Subxeric - Mesic	1.86	3.15	0.59	0.991
	Subxeric - Mesic-subhygric	0.81	3.28	0.25	1.000
	Subxeric - Subhygric	2.51	3.92	0.64	0.987
	Mesic-subxeric - Mesic	-0.38	1.98	-0.19	1.000
	Mesic-subxeric - Mesic-subhygric	-1.43	2.19	-0.65	0.985
	Mesic-subxeric - Subhygric	0.27	3.07	0.09	1.000
	Mesic – Mesic-subhygric	-1.05	2.30	-0.46	0.997
	Mesic - Subhygric	0.65	3.15	0.21	1.000
	Mesic-subhygric - Subhygric	1.70	3.28	0.52	0.995

Table S12. Mean values (±SD) for carbon, nitrogen, and C:N ratio for dried, non-autoclaved litter and autoclaved litter of birch and black spruce. Values are based on three samples of each litter type. Samples were homogenised and ground to a fine powder. Up to 2 mg was weighed into tin capsules. Measurements were taken using a Perkin Elmer 2400 CHNS Analyser, (Guelph, ON, Canada) with acetanilide standards.

	C		N		C:N	
Litter species	Non-autoclaved	Autoclaved	Non-autoclaved	Autoclaved	Non-autoclaved	Autoclaved
Birch	46.6 ± 1.1	46.6 ± 2.54	1 ± 0.09	0.87 ± 0.06	46.7 ± 2.97	53.4 ± 1.5
Black spruce	46.6 ± 1.81	49 ± 0.44	0.76 ± 0.02	0.72 ± 0.03	61.1 ± 3.59	68.1 ± 2.35