In late November and early December 2016, following a record drought in the southern Appalachian region of Tennessee and North Carolina, United States, severe wildfires, referred together as the Chimney Tops 2 fire, burned portions of the Great Smoky Mountains National Park (GSMNP). Following this fire, numerous sporocarps of so-called pyrophilous fungi appeared in the fire zone (see for example, Miller et al., 2017; Matheny et al., 2018). The causes of post-fire sporocarp formation are largely unknown but could include heat-induced changes to soil pH, increases in soil carbon, heat activation of spores, lack of competition from other less heat-tolerant fungi, or fruiting in response to loss of a nutritional host. The natural history of pyrophilous fungi in the absence of wildfires is also largely unknown. In the absence of fire, the pyrophilous basidiomycete Pholiota carbonicola (P. highlandensis) may be endophytic with moss (Raudabaugh et al., 2020), and some Morchella species (morels) may be dormant in soils as sclerotia (Miller et al., 1994). For the majority of pyrophilous species, however, the natural history is unknown.

Sphaerosporella is one of several genera/species of pyrophilous fungi. Although reportedly present in unburned areas, Sphaerosporella brunnea is more frequently observed in burned areas (Molina et al., 1992; Motiejūnaitė et al., 2014) and areas recovering from fire (Rifai, 1968; Egger, 1986; Molina et al., 1992; Miller et al., 1998). It is considered to be a primary colonizer after fire (Meotto and Carraturo, 1988) and is a common contaminant in greenhouses, especially in autoclaved soils. Yao and Spooner (1996, p. 386)
reported the habitat of S. "brunnea" as "new or old burnt ground and charcoal heaps, sometimes amongst mosses". *Sphaerosporella "brunnea"* has been reported from Eurasia, Australia, and North America (Rifai, 1968; Schumacher, 1982; Yao and Spooner, 1996), but the taxonomy and placement of *Sphaerosporella* taxa is insecure (Appendix S1), and the epithet *S. "brunnea"* has been used without validation for what is likely several taxa.

*Sphaerosporella "brunnea"* has also been described as ectendomycorrhizal (Molina et al., 1992; Yu et al., 2001) or ectomycorrhizal (Danielson, 1984). *Sphaerosporella* mycorrhizae on *Pinus banksiana* (Jack pine) was described as forming "poorly developed mantles", "dichotomously branched, glabrous to floccose, ochraceous (light brownish yellow) but darkening with age, having large diameter hyphae (4–14 µm wide) and with a well-developed Hartig net (Danielson, 1984, pp. 454–455). The ability to form mycorrhizae was not shared by related taxa *Anthracobia melaloma*, *Trichophaea* (Sphaerospora) *minuta*, *T. contradicta*, and *T. ambulans* in inoculation tests of containerized Jack pine (Danielson, 1984). *Sphaerosporella "brunnea"* appears to have a broad host range and was reported as ectomycorrhizal on *Pinus banksiana*, *Picea*, *Larix*, and *Populus* (Danielson, 1984) and *Quercus robur* and *Castanea sativa* (as *Castanea satina* in title) (Meotto et al., 1992) and was identified by sequences from root tips from a *Quercus ilex* forest in northern Spain (de Román and de Miguel, 2005).

*Sphaerosporella hinnulea* was originally described as 'on soil amongst grass', and examination of the type specimen showed fragments of charcoal (Yao and Spooner, 1996, p. 386). *Sphaerosporella hinnulea* has been reported from Europe (Petersen, 1970) and North America (MyCoPortal, 2019).

In this paper, we further evaluate the multiple roles of *Sphaerosporella* fungi following wildfires in a southern Appalachian forest habitat and identify four clades of pyrophilous fungi within *Sphaerosporella*.  

### MATERIALS AND METHODS

#### Selection of burned areas for study

Numerous scattered small and large wildfires occurred within the larger GSMNP burn perimeter. Burn intensity was assigned based on tree scorch, tree death, and residual soil organic layer as follows: (1) lightly burned: some leaf debris left, soil organic layer present, scorch marks less than 1 foot (30.48 cm) and tree survival high; (2) moderately burned: most leaf debris gone and scorch marks on trees more extensive than one foot above the forest floor, some tree death with spotty burn holes; and (3) severely burned: no surface organic material remaining and soil organic layer destroyed, tree trunks/roots mostly burned. For the Chimney Tops 2 fire, areas that were severely burned were predominantly steep slopes and *Pinus pungens* and *Pinus rigida* ridgetop stands.

Study areas were selected by walking along trails within the burn perimeter and selecting for severely burned, moderately burned, lightly burned, and unburned areas for subsequent sampling and observation. Selected areas were: (1) the Cove Hardwood Loop Trail (35.6362N; 83.4945W: unburned control and generally unburned to lightly burned areas with small areas of severely burned soils); (2) Balsam Quiet walkway (35.6460N; 83.5098W: moderately burned); (3) Twin Creeks (35.6876N; 83.5015W: unburned control and moderately to severely burned adjacent area); (4) Baskins Creek Trail (35.6794N; 83.4773W: unburned control, moderately burned, and severely burned areas). Two more severely burned areas were added in June 2017: (5) Cove Mountain Trail (35.6944N; 83.5332W: severely burned); and (6) Two Mile Lead Trail (35.6755N; 83.4970W: severely burned). Adjacent to Two Mile Lead Trail was a moderately burned site, the Old Sugarlands Trail-Bullhead Trail Spur (35.6749N; 83.4905W) with patches of severely burned soil. Occasionally, ancillary collections were made in other sites (e.g., Cherokee Orchard, Rainbow Falls Trail) by the research team and additional collectors from the Great Smoky Mountains National Park.

#### Soil samples

To track changes in the abundance of *Sphaerosporella* and other pyrophilous fungi in soils, soil samples were collected from all areas listed above except the two areas that were added to the study in June, 2017 (Two Mile Lead and Cove Mountain Trail). Monthly soil sampling began in February of 2017 and continued with sampling every 3 months through December 2018. A 5-m² area was marked from each area sampled. Within that area, a 1-m² area was selected at random and a 2.5 × 12 cm plug was removed from each corner and from the center with an augur. No attempt was made to separate soil layers. Plugs were mixed thoroughly and transported to the laboratory on ice in a cooler where they were sieved to remove rocks and roots and then frozen at −80°C for storage. Selection of a 1-m² site within each 5-m² area was random and changed with each sampling period.

#### Ascocarp collections

To evaluate patterns of *Sphaerosporella* ascocarp formation following the December 2016 Chimney Tops II fire, we surveyed all study areas every 2 weeks between February and August 2017, then monthly through 2018. Since fungal ascocarps are ephemeral, ascocarps were collected when they were found, often in and around burn holes (areas in which tree trunks and roots burned below the soil line leaving large holes). Each collection comprising several ascocarps was assigned a Fire Survey Number or an ANM collection number (Appendix S2). Collections were accessioned into the University of Tennessee herbarium (TENN) or the Illinois Natural History Survey herbarium (ILLS) at the University of Illinois, Urbana-Champaign. Data for each collection are available in MyCoPortal (MyCoPortal, 2019).

#### Pine seedlings

*Pinus pungens* is a fire-adapted Appalachian endemic, growing in small, isolated, and declining populations on ridge top outcrops. Three of these populations (Baskins Creek, Cove Mountain, and Two Mile Lead) were severely burned in the 2017 fire. Following the fire, burned trees dropped remaining needles, and serotinous cones dropped seeds, which germinated in May–June 2017. To evaluate whether germinating pine seedlings had acquired mycorrhizae, we collected emerging *Pinus pungens* seedlings beginning June–July 2017 with as much root ball as practical. Seedlings were stored in plastic bags in a cooler for transport. Seedling roots were washed and examined under a dissecting microscope at 8–10× for mycorrhizal root tips. Putative ectomycorrhizal root tips were photographed using a Leica EZ4W system (Leica Microsystems, Buffalo Grove, IL,
USA). Up to four root tips per plant were removed and processed for DNA extraction. Selection of root tips was based on color and branching of the mycorrhizal root tip to obtain as wide a variation as possible. Each pine seedling was assigned a pine number, pressed, and dried. For larger seedlings, a branch and the root system were pressed. In 2017, each mycorrhiza was assigned a separate fire survey number; in 2018, mycorrhizae from the same plant were assigned a fire survey number followed by letters a–d to indicate separate root tips from the plant (Appendix S2). In 2017, pine seedlings were evaluated for mycorrhizae only, and endophytes were evaluated on a randomly collected set of pine needles. In 2018, each pine seedling was evaluated for both mycorrhizae and endophytes (below).

Pine needle collection and culture

To evaluate whether germinating pine seedlings had acquired endophytic fungi, *Pinus pungens* and *P. rigida* needles were collected during July 2017 (Baskins Creek, Two Mile Branch), and during April–May 2017 and September–October 2018 (Baskins Creek, Two Mile Branch, and Cove Mountain). In 2017, two or three needles were selected randomly from 50 *Pinus pungens* seedlings and *P. rigida* coppicing pine shoots (not seedlings collected for mycorrhiza). These were sectioned into 1-cm³ pieces and surface-sterilized. Surface sterilization was by either (1) 30% v/v hydrogen peroxide for 30 s followed by rinsing in sterile distilled water or (2) 90% v/v ethanol for 30 s, 10% Cloroxy for 2 min, then 75% v/v ethanol for 2 min (U'Ren and Arnold, 2016) then placed on sterile malt extract agar (MEA; 15 g/L Difco malt extract, 20 g/L Bacto Agar: Thermo-Fisher Scientific, Waltham, MA, USA). Mycelium growing from the cut ends of needles was isolated and subcultured on MEA.

In 2018, we wished to compare needle endophytes and mycorrhizae from the same 1-year-old seedlings. Ten *Pinus pungens* trees were sacrificed at each of the three severely burned *P. pungens* sites in May and again in September–October (Baskins Creek, Cove Mountain, and Two Mile Branch). Two needles were taken from the bottom and the top of each plant, cut into sections, sterilized as above (U’Ren and Arnold, 2016), and needle segments were cultured on MEA. Voucher cultures for 2017 and 2018 were stored at 8°C on MEA slants. In some cases, cultures were subcultured in 15 mL potato dextrose (PD) broth (24 g PD/L; Thermo-Fisher Scientific, Waltham, MA, USA). Mycelium growing from the cut ends of needles was isolated and subcultured on MEA.

To determine fungal identity and population variability, a fungal molecular barcode (Schoch et al., 2012) was obtained for ascomycetes, mycorrhizae, and endophyte cultures. For DNA extractions from *Sphaerosporella* ascomycetes, individual ascomycetes were removed from soil using a Leica EZ4W dissecting microscope and placed in sterile distilled water. Remaining soil was gently removed from the ascomycetes with watchmaker’s forceps and dissecting needles. A single ascomycete was placed in 25 µL extraction buffer (0.1 M Tris HCl, 0.25 M KCl, 810 µM EDTA, pH 9.5–10; procedure developed by the Vilgalys Lab, Duke University). For assessment of mycorrhizae, each putative mycorrhizal root tip was placed in 25 µL extraction buffer. For endophyte cultures, DNA was extracted by scraping the surface of cultures to obtain a 1-mm³ piece of tissue, which was then suspended in 25 µL extraction buffer. Alternately, if cultures were grown in PD broth, a 1-mm³ piece was cut from the fungal mat and placed in extraction buffer. Some cultures were extracted using an Omega E.N.Z.A. HP fungal DNA kit (Omega Bio-Tek, Norcross, GA, USA).

Tissues in extraction buffer were macerated with a sterile pipette tip and heated to 95°C for 10 min. Twenty-five microliters of filter-sterilized 3% w/v bovine serum albumin (BSA heat shock fractionated: Sigma-Aldrich, St. Louis, MO, USA) was added to each tube and vortexed. One microliter of the final solution was used in a PCR reaction. Each PCR reaction mix consisted of 12.5 µL Thermo Scientific Dream Taq Hot Start Green Master Mix (2X, Thermo Fisher Scientific, Waltham, MA, USA), 0.3 µL of each forward and reverse primer (10 µM), 1 µL DNA and 11 µL ddH2O. Primers ITS1F, ITS4, ITS2, and ITS3 were used in various combinations to amplify the whole nrITS region or fragments of the region (White et al., 1990; Gardes and Bruns, 1996). The ITS PCR cycle was 5 min at 94°C followed by 40 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C. The final extension was 3 min at 72°C. PCR products were confirmed by gel electrophoresis. Five microliters of the PCR product was treated with ExoSAP-IT (Thermo-Fisher Scientific) using the manufacturer’s directions. Sanger dideoxy sequencing reactions were performed using BigDye Terminator 3.1 (Thermo Fisher Scientific) using the manufacturers’ directions but with cycles increased to 35. Sanger sequencing was performed by the University of Tennessee UT Genomics Core, College of Arts and Sciences.

Sanger sequences were manually aligned and corrected using the software package GCG (Womble, 2000). Sequences from GenBank falling between 90–100% identity with the post-fire *Sphaerosporella* clades were also downloaded and aligned with GSMNP post-fire *Sphaerosporella* sequences. GenBank sequences whose alignment indicated membership in one of the four post-fire GSMNP *Sphaerosporella* clades were included in the phylogenetic analysis (usually 97–99% ITS sequence identity in a blast search of GenBank). Remaining sequences (usually 91–92% identity in blast search) belonged to a clade that was sister to GSMNP post-fire *Sphaerosporella*. Two sequences from this sister clade were used as the outgroup. Maximum likelihood phylogenetic analyses were performed in Geneious 11.0.3 (Biomatters, Auckland, New Zealand) using the PhyML program and a general time reversible model of substitution with an estimated gamma distribution parameter and proportion of invariable sites and with four substitution rate categories. Five-hundred bootstrap iterations were performed. For clade identifications of *Sphaerosporella* sequences in soils, *Sphaerosporella* sequences from Illumina sequencing (below) were aligned with those obtained from ascomycetes, mycorrhizae, and endophyte cultures. Because the taxonomy of *Sphaerosporella* is unclear, groups identified by PhyML analysis were assigned clade numbers rather than taxonomic names.

Molecular methods: soils

DNA was extracted from 0.25 g of frozen soil from each site at each sampling time using the Qiangen DNeasy PowerSoil kit (Qiagen, Germantown, MD, USA). DNA was quantified fluorometrically (Qubit, Invitrogen, Carlsbad, CA, USA) and normalized to 20 ng/µL for subsequent PCR. We included PCR controls (amplification but
FIGURE 1. PhyML analysis of ribosomal ITS sequences from ascocarps (green), pine ectomycorrhizae (blue), and pine endophyte cultures (pink). Bootstrap support for 500 bootstrap replications is given to the left of each supported node.
no DNA) for each 96-well PCR plate. These negative controls were also sequenced, with no evidence of laboratory contamination. For PCR, we used Illumina TruSeq V3 indices (Illumina, San Diego, CA, USA) linked to ITS2 rDNA fungal-specific primers (5.8S-Fun/ITS4-Fun; Taylor et al., 2016). These primers allow for the detection of the largest diversity of fungi while also restricting nontarget species (e.g., plants and animals). Reactions contained 20.5 µL of platinum PCR Supermix (Invitrogen), 1.25 µL of each primer (10 µM), 0.5 µL of BSA (20 µg/mL), and 2 µL of DNA. All PCRs were performed in triplicate with a hot start at 94°C for 3 min; 25 cycles of 94°C for 45s, 50°C for 1 min, 72°C for 90 s; and a final extension step of 72°C for 10 min. Triplicate PCRs were combined and cleaned with Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA) and quantified with a Qubit fluorometer. Samples were pooled in equal amounts and sequenced on an Illumina MiSeq v3 (2 × 250bp PE run) at the Center for Environmental Biotechnology sequencing core at the University of Tennessee, Knoxville. Sequences are deposited in the NCBI Sequence Read Archive under BioProject PRJNA605421.

Sequences were processed with DADA2 v. 1.10.6 (Callahan et al., 2016) in R v. 3.5.0 (R Core Team, 2019). Error rates were estimated for each read direction (two for forward reads and five for reverse), and sequences were truncated to maintain only high-quality DNA (250 bp forward and 210 bp reverse). Sequences were merged with at least 15 bp of overlap and taxonomy was assigned using the RDP UNITE training set (Cole et al., 2014). This process resulted in 2,783,693 initial reads. After chimera checking, the remaining 1,394,626 reads were clustered into 24,814 amplicon sequence variants (ASVs) and used for all subsequent analyses. ASVs are the narrowest designation of fungal operational taxonomic units (OTUs) because they rely on 100% DNA sequence read identity. However, ecological patterns of fungal assemblages are robust to using ASV versus more traditional 97% DNA similarity for species classification (Glassman and Martiny, 2018; Gardner et al., 2019).

RESULTS

Phylogenetic relationships between clades of Sphaerosporella

ITS sequences of Sphaerosporella fell into four putative groups (Fig. 1). Clades 1 and 2 were the most frequently recovered and were present in all three severely burned sites (Baskins Creek, Two Mile Lead, and Cove Mountain; Fig. 2). Clades 1, 2, and 4 each contained sequences from asccarps, mycorrhizae, and endophytes, although asccarps for clade 4 were represented by a single GenBank accession (MF066094; Czech Republic, from a “burnt place”). Clade 3 was represented by a single asccarp. Clade 4 contained four sequences derived from roots of a single pine tree plus sequences from GenBank. Sequences recovered from Illumina MiSeq analysis belonged predominantly to Clades 1 and 2, and a single sequence belonged to Clade 3. Two additional sequences from soil represented an unknown clade or an aberration. Tests to determine whether the observed clades were due to coalescence rather than species divergence (Rosenberg’s $P_{AB}$ statistics and the $P_{RD}$ statistic) generally supported species divergence rather than random coalescence as an explanation for the observed clades (Appendix S1).
Ascocarps

Approximately 6 months after the fire, *Sphaerosporella* ascocarps were found in severely burned “hot spots” within moderately burned areas and within severely burned *Pinus pungens* or *Tsuga canadensis* areas, usually in and around burn holes and occasionally in association with young regenerating mosses and liverworts (Fig. 3). Ascocarps were collected on the Old Sugarlands Trail–Bullhead Trail spur, from the severely burned Twin Creeks *Tsuga canadensis* site, and from the severely burned Baskins Creek and Cove Mountain *Pinus pungens* areas (Appendix S2). Most ascocarps of confirmed *Sphaerosporella* were collected between 7 June and 27 August 2017 and less frequently in June, July, and August 2018 (Fig. 4). One collection (ANM2957) was made in October 2017. By mid-summer 2017, *Sphaerosporella* sporocarps were covered with white mycelium, possibly due to a second contaminating fungus or from their own spores. The latter

![Image of Sphaerosporella ascocarps and mycorrhizae](image)

**FIGURE 3.** *Sphaerosporella* ascocarps and mycorrhizae. (A) *Sphaerosporella* clade 1 ascocarps. Collection Fire Survey 73. (B) *Sphaerosporella* Clade 3 ascocarps. Collection Fire Survey 359. (C) *Sphaerosporella* Clade 1 mycorrhiza. Collection Fire Survey 503a. Forceps tips are 0.5 cm long. (D) *Sphaerosporella* Clade 2 mycorrhiza. Collection Fire Survey 462a.
explanation is likely since Sanger sequencing from mycelium-covered sporocarps produced clean *Sphaerosporella* sequences with no indication of a second contaminating fungus.

**Mycorrhizae**

*Sphaerosporella* ectomycorrhizae (ECM; Figs. 2, 3C–D, 4) were recovered from the roots of 28/159 *Pinus pungens* seedlings (17.6%) growing in severely burned areas in years 1 and 2 of the study. In two cases (Pines 19, 110), sequences representing both *Sphaerosporella* Clades 1 and 2 were recovered from the same root system. A fourth clade of *Sphaerosporella* was recovered from roots of a single pine seedling. For 8/28 seedlings, *Sphaerosporella* was the only genus recovered on the sapling root system. For the remaining seedlings, the root system was shared with other mycorrhizal species, but there was no discernable association pattern or bias with respect to other mycorrhizal species (Appendix S3). *Sphaerosporella* Clade 1 and Clade 2 sequences were recovered from *Pinus pungens* roots from Baskins Creek, Two Mile Lead, and Cove Mountain severely burned areas (Fig. 2).

**Endophytes**

Forty-five fungal cultures were obtained from randomly collected needles of *Pinus pungens* seedlings and from a coppicing shoot of *Pinus rigida* in 2017. Of these, four were *Sphaerosporella*. In 2018, *Sphaerosporella* cultures were obtained from 26 of 1340 needle fragments from 67 pine seedlings. Cultures of Clade 1 were rapid growing, diffuse, and white, covering the plate with fine hyphal threads in a 4–5-day-old subculture. Cultures of Clade 2 were tan. *Sphaerosporella* Clade 1 endophytes were recovered from pine needles at Baskins Creek, Two Mile, and Cove Mountain locations. *Sphaerosporella* Clade 2 endophytes were recovered only from needles from Two Mile Lead (Appendix S3).

**Soil samples**

Soil samples produced 1,394,626 amplicon sequence variants (ASVs), of which 5115 (0.37%) fell within *Sphaerosporella*; however, these were concentrated in soil samples from the Baskins Creek *Pinus pungens* and to a lesser degree Twin Creeks *Tsuga canadensis* severely burned areas. In the Baskins Creek area, the percentage of recovered sequences that were *Sphaerosporella* ranged from 1 to 8%. Illumina sequencing primarily recovered *Sphaerosporella* Clades 1 and 2. A single sequence representing Clade 3 was also recovered. A very small number of sequences were obtained from moderately burned areas but not from unburned control areas. Clade 1 sequences first appeared in soils in May–June 2017 and persisted through March 2018. Clade 2 sequences first appeared in May 2017 and persisted through December 2017 (Fig. 5). These data are consistent in timing and location with *Sphaerosporella* ascocarp formation, which began June 2017 (Fig. 4).

**DISCUSSION**

Several authors have reported that certain pyrophilous species naturally occur as mycorrhizae (Egger and Paden, 1986a; Vrålstad et al., 1998), root pathogens (Weir, 1915; Egger and Paden, 1986b), endophytes (Matheny et al., 2018; Raudabaugh et al., 2020), or soil

![FIGURE 4. Date of appearance of *Sphaerosporella* ascocarps, endomycorrhizae (ECM) and endophytes (Endo). (A) *Sphaerosporella* Clade 1. (B) *Sphaerosporella* Clade 2.](image-url)
saprobes (Warcup, 1990). The dual mycorrhizal and endophytic post-fire role of *Sphaerosporella*, however, coupled with an affinity for fruiting on severely burned ground is unusual among pyrophilous species. While the presence of fungal mantle-like tissue on root tips does not guarantee that all *Sphaerosporella* taxa are mycorrhizal (Tedersoo et al., 2010), *Sphaerosporella* is widely believed to be an ectomycorrhizal or ectendomycorrhizal (Molina et al., 1992). The isolation of putative *Sphaerosporella* endophytes from needles of *P. pungens* has not been previously reported.

Unexpectedly, there was no correlation between endophytic and mycorrhizal infections on the same plant. The experimental design for 2018, in which both needle endophytes and mycorrhizae were sampled on a plant-by-plant basis, enabled us to answer the question “Is mycorrhizal infection with *Sphaerosporella* a necessary prerequisite to *Sphaerosporella* endophytes?” The almost complete lack of overlap between presence of *Sphaerosporella* mycorrhizae on pine roots and *Sphaerosporella* in cultures growing from needle segments suggests that the infection processes of roots and needles are separate (Appendix S3). The one exception was Pine 137 where cultures of *Sphaerosporella* Clades 1 and 2 were isolated as endophytes and *Sphaerosporella* Clade 1 was isolated from the roots. The infection process in roots may depend on interaction with *Sphaerosporella* in the soil, while the infection process in needles may be through a different process, perhaps by vectored or airborne propagules entering through stomates. An alternate scenario would be that the initial infection began at the root and progressed to the needles with subsequent elimination from the roots. Finally, it is possible that a root infection with *Sphaerosporella* was not accompanied by an observable mantle and so was not detected during root sampling.

There was also no correlation between *Sphaerosporella* mycorrhizal infections and infections on the same root system with other mycorrhizal species (Appendix S3), suggesting that infection with other mycorrhizal species neither enhanced nor repressed mycorrhizal infection by *Sphaerosporella*.

**Origins of post-fire Sphaerosporella**

It is probable that the intense fires in *Pinus pungens* and *Tsuga canadensis* stands destroyed fungi in the top few inches of soil but identification of *Sphaerosporella* sequences as endophytes of *Pinus rigida* shoots coppicing from deep roots suggests that this pine may serve as a possible reservoir for *Sphaerosporella* in heavily burned *Pinus pungens* zones. In arctic boreal forests, there is some evidence of mycobiont sharing between resprouting shrubs and tree seedlings that could aid in re-establishment of trees (Hewitt et al., 2017). In vitro, *Sphaerosporella* grows rapidly and can propagate by hyphae and conidia (Sánchez et al., 2014); such abilities would aid its establishment as a mycobiont on the roots of *P. pungens* sapling roots. Another possible route to post-fire establishment of *Sphaerosporella* might be through needle drop. After the fires, the burned soil in *Pinus pungens* habitats was initially covered with needles dropped by damaged trees. If these needles harbored *Sphaerosporella* endophytes, needle drop could serve as an alternate source of the fungus for infection of new seedlings.

Finally, heat-resistant ascospores have been known for some time, especially within the food industry, and a large number of fungi are known to survive pasteurization temperatures although temperature resistance may depend on substrate (Goddard, 1935). Thus, one would expect ascospores of some species to survive wildfires, and with reduced competition from non-heat-tolerant species, to propagate quickly across substrates. In some species, ascospore germination is triggered or enhanced by heat. Wicklow (1975) noted that two Sordariomycetes, *Coniochaeta discospora* and *C. tetraspora*, were present in soil dilutions after prairie fires but not before and noted that both produce spores that are activated only after exposure to heat. Warcup and Baker (1963) noted that the number of colonies of *Aspergillus fischeri* var. *glaber* and *Humicola glaber* increased after a heat treatment and that *Peziza praetervisa* increased after a heat treatment and that *Trichophaeabundans* required heat treatment for germination. *Trichophaeabundans* is considered a close relative of *Sphaerosporella*.

In this study, *Sphaerosporella* was identified from among endophytes and mycorrhizae of new pine seedlings in two severe fire zones, Baskins Creek (*Pinus pungens*) and Twin Creeks (*Tsuga canadensis*). In soil samples, *Sphaerosporella* nrITS sequences were predominantly recovered from the severely burned areas of Baskins Creek (*Pinus pungens* zone) and Twin Creeks (mixed *Tsuga canadensis* and hardwoods) beginning in May and June 2017 (soil samples were not taken at Two Mile Lead and Cove Mountain). This finding agrees with both

![Figure 5. Incidence of *Sphaerosporella* 1 and 2 in combined Baskins Creek and Twin Creeks soil samples.](Image)
the timing and locations of asccocarps as well as the appearance of mycorrhizae on *P. pungens* roots (Figs. 4, 5). *Sphaerospora* was not observed in adjacent unburned control areas. No *Sphaerospora* sequences were recovered from soil samples taken from the Twin Creeks unburned control site close to the Twin Creeks severely burned site nor from the Baskins Creek unburned control site. *Sphaerospora* ASVs were recovered only rarely from other sites and at very low levels. Taken together, the data suggest either a post-fire soil inoculation by *Sphaerospora* asccocarps or alternately, dormant spores that were triggered to germinate by heat but were too few for DNA signatures to be recovered by Illumina sequencing.

The very rapid growth of *Sphaerospora* in culture, the reported production of conidiospores (Sánchez et al., 2014) and the role of *Sphaerospora* as an early broad-host-range mycorrhizal colonizer/endophyte makes *Sphaerospora* an interesting candidate for post-fire remediation but much remains to be done to evaluate infection origins, growth rates in post-fire soils, and the range of host species that it will infect.

**ACKNOWLEDGMENTS**

This study was partially supported by awards from the National Science Foundation for "Collaborative Research: A Survey of Post-Fire Ascomycete and Basidiomycete Fungi in an Eastern Deciduous Forest" to the University of Tennessee (DEB 1733750) and the University of Illinois (DEB 1733854). We thank Paul Super, Science Coordinator at the Great Smoky Mountains National Park, for facilitating collection permits (study GRSM-01294, permit GRSM-2017-SCI-1294), and the Great Smoky Mountains National Park and Friends of the Smokies for a grant to fund endophyte and mycorrhizal work in 2018. We are extremely grateful to Jessica Moore and Friends of the Smokies for a grant to fund endophyte and mycorrhizal work in 2018. We are extremely grateful to Jessica Moore for PCR amplification and preparation of soil DNAs for Illumina sequencing. We thank two reviewers and the associate editor for their insightful comments.

**AUTHOR CONTRIBUTIONS**

K.W.H., A.C., P.B.M., A.N.M., and T.I. carried out fieldwork associated with this project. Identifications were carried out by R.H.P., A.N.M., T.I., and P.B.M. Sacrifice of trees, documentation of mycorrhizae, and Sanger sequencing was done by A.C. and K.W.H. Illumina sequencing and analyses of data was done by S.K. K.W.H. wrote the manuscript.

**DATA AVAILABILITY**

Sequence exemplars for each clade are available from NCBI, GenBank. Illumina data was deposited in the NCBI Sequence Read Archive under BioProject PRJNA605421. Specimens are available from TENN and ILLS. Specimen data are available from MyCoPortal: https://mycoportal.org/portal/index.php.

**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the supporting information tab for this article.

**APPENDIX S1. Taxonomic considerations.**

**APPENDIX S2. Sphaerospora ascomata, mycorrhizae and endophytes identified in this study.**

**APPENDIX S3. Lack of overlap between Sphaerospora mycorrhizae and endophytes, 2018 collections.**

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