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RESEARCH ARTICLE

Botany

Fungal endophytes of an endangered tree vary with stress and microenvironment in an ex situ conservation nursery

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Abstract

Premise: Plants in ex situ conservation nurseries acquire diverse fungal associates that may be moved among nurseries or into the wild during outplanting, including fungal endophytes that contribute to a broad range of functions and occur in leaves, sometimes alongside pathogens. To improve understanding of fungal symbionts in a plant of high conservation concern, we characterized foliar fungal endophytes of *Torreya taxifolia*, one of the world's most threatened conifers, in an ex situ conservation nursery.

Methods: We used culture-based and culture-free approaches to characterize fungal endophytes in leaves of *T. taxifolia* over 2 years and evaluated how endophytes varied spatially and as a function of environmental, plant-specific, and edaphic factors. We also contrasted them with fungi in other plants (local species and species cultivated at a regional scale) and with soil fungi.

Results: Culture-free methods revealed species-rich and phylogenetically diverse foliar fungal endophytes of *T. taxifolia* that vary spatially, reflecting symbiont acquisition from nearby plants, environmental factors, and plant stress. Endophyte community composition is subject to both stochasticity and temporal turnover and differs markedly from fungal communities in soils and other plants in the area.

Conclusions: Our study provides novel insights into factors that can shape fungal endophyte communities for a critically endangered tree species. In addition to identifying local determinants of endophytic symbioses, our work illustrates that plants in conservation nurseries host rich foliar fungal communities of potential importance in plant germplasm protection.

KEYWORDS

Cupressales, endophytic fungi, gymnosperms, microbiome, pathogens, symbiosis, threatened species, Torreya taxifolia

Estimates of extinction risk to plants typically focus on abundant and widely distributed species for which global occurrence data are robust (Meyer et al., 2016). When adjusted for endemism, estimates suggest that up to 40% of vascular plant species are at risk for extinction under a changing climate and associated stresses (Nic Lughadha et al., 2020). Methods to conserve imperiled plants include habitat management, mitigation or exclusion of pathogens and herbivores, and collection and banking of seeds (Thormann et al., 2006). Increasingly, ex situ cultivation and conservation horticulture also have proved essential for propagating species away from biotic and abiotic stressors that threaten wild plants (Abeli et al., 2020). Successful ex situ conservation maintains germplasm, genetic diversity, and population sizes for rare or threatened plants, generating individuals that may be planted in the native range or other settings under comprehensive conservation plans (McGowan et al., 2017; Forgiarini et al., 2023).

Plants grown outside their native range or in a nursery may acquire pathogens that differ from those in their natural environment, with the potential to be transferred into wild plant communities at outplanting (e.g., Kovács et al., 2021; Nakamura et al., 2023; see also Edson et al., 1996; Rooney-Latham et al., 2015; Simamora et al., 2018; Stanley and Bodley, 2020; Kline et al., 2022). In some cases, plant health in ex situ nurseries may be enhanced by inoculation with

symbiotic microbes, including fungi distinct from those found under natural conditions (Solaiman and Hirata, 1997; Estaún et al., 2003; Navarro García et al., 2011; Ortas, 2012; Sebastiana et al., 2013; Salomon et al., 2022; Nerva et al., 2023). Over time, plants in nursery environments can also acquire microbes, including symbionts, without having visible symptoms, leading to a range of potentially beneficial or negative impacts (e.g., Fernández et al., 2013). The contamination risks at outplanting are unclear when long-lived plant tissues accumulate a diversity of horizontally transmitted infections in nurseries that persist and can spread when hosts are translocated, but for which identities or potential roles are not known.

The critically endangered conifer *Torreya taxifolia* Arn. (Florida torreya, stinking cedar; Cupressales, Taxaceae) exemplifies plant decline following a confluence of biotic and climate-relevant stressors, and ex situ nurseries play an important role in its conservation (Smith and Pence, 2017). *Torreya taxifolia* persists in the wild today as a glacial relict, but it previously numbered in the hundreds of thousands as a dominant gymnosperm in some North American forests (Schwartz et al., 1995). It was considered common until assessments in the 1960s revealed decline over multiple decades (Godfrey and Kurz, 1962). The remaining wild population is restricted to limestone bluffs in the Apalachicola River basin of northern Florida and southwestern Georgia, United States, where it occurs in relatively cool, humid, and shaded ravines (Schwartz and Hermann, 1992). Fewer than 1000 mature individuals remained in the wild when last assessed (Spector et al., 2011), and current estimates are less than 600 after Hurricane Michael reduced canopy cover in the area by 80%–90% in 2018 (USFWS, 2020).

Such environmental stressors exacerbate additional threats to the extant population, including the fungal pathogen *Fusarium torreyae* (Hypocreales: Nectriaceae), which can kill *T. taxifolia* at juvenile and adult stages, stunt growth, and decrease seed viability (Smith et al., 2011; Aoki et al., 2013). Due in part to the negative impact of *F. torreyae* (Smith and Trulock, 2010), *T. taxifolia* is now considered the rarest conifer in North America and one of the most endangered conifers worldwide (Farjon, 2010). Urgent conservation needs have sparked efforts to produce healthy germplasm of *T. taxifolia*, including in ex situ nurseries (Coffey et al., 2024). Because *F. torreyae* may be transmitted vertically via seed (see Trulock, 2012), it has the potential to occur in nursery settings if seedstock is infected, or to move with seeds or seedlings during translocation. *Fusarium torreyae* can also establish in leaves of *T. taxifolia* that have been wounded (see Trulock, 2012), and examination of leaves is important for detecting the pathogen before the appearance of symptoms such as stem cankers.

Foliar endophytic fungi (defined by their asymptomatic occurrence in leaves; hereafter, endophytes) may protect leaves against pathogens and abiotic stress that contribute to plant disease or dysfunction (e.g., Arnold et al., 2003; Hubbard et al., 2014; Zahn and Amend, 2017; Egan et al., 2021; Zhou et al., 2021). However, endophytes may also negatively impact plant health and resilience, by increasing plant susceptibility to disease or environmental stress (see Carroll, 1988; Slippers and Wingfield, 2007; Busby et al., 2016; Ridout and Newcombe, 2016; Christian et al., 2017; Giauque et al., 2019). Understanding the composition of endophytes in leaves of *T. taxifolia* is therefore important for defining the types of symbioses present and for the health of this imperiled species. Characterizing foliar endophytic fungi in a conservation setting can help us to clarify potential risks of forward contamination, while also highlighting local-scale factors that may drive the assembly of endophyte communities, which are largely acquired via horizontal transmission in leaves of long-lived plants (Schulz and Boyle, 2005; De Silva et al., 2019; Martínez-Arias et al., 2021; Oita et al., 2021a; U'Ren et al., 2024).

Here we characterized foliar fungal endophytes in apparently healthy *T. taxifolia* trees planted in an ex situ conservation nursery. In the first year of the study (2021), we focused on trees that represent a range of wild germplasm lines. In the second year (2023), we evaluated how endophytes vary spatially and as a function of environmental, plant-specific, and edaphic factors, and we determined whether endophyte communities in *T. taxifolia* are recruited from fungi occurring in soil or as endophytes of plants in the region. We predicted (1) that endophytes of *T. taxifolia* should differ from soilborne fungi in the same environment, consistent with biotic filtering of fungi as foliar symbionts from the local species pool in soils; (2) *Fusarium torreyae* should not be detected in leaves or nursery soils, consistent with conservation practice to exclude this pathogen in ex situ propagation of apparently healthy plants; (3) endophyte communities should shift over time based on measures of community structure, consistent with stochastic assembly of endophyte communities in a regionally naïve host planted recently in the area (i.e., not priorly exposed to local fungi); (4) communities of endophytes should be relatively homogenous across the small scale of the nursery, consistent with local airborne transmission of horizontally transmitted propagules; (5) endophyte communities in *T. taxifolia* should resemble those in related trees of the region, consistent with local transfer of available fungal symbionts.

MATERIALS AND METHODS

In November 2017, 108 seedlings of *T. taxifolia* were planted in-ground in an outdoor, uncovered, approximately 30 m × 30 m ex situ conservation nursery managed jointly by the Sarah P. Duke Gardens and Duke Forest near Durham, North Carolina, United States (ca. 36.0° N, 79.0° W, 125 m a.s.l.). The study area is ca. 1000 km north-northeast of the relict native range of *T. taxifolia*. Mature *T. taxifolia* planted for horticultural and conservation purposes occur rarely in the region and not in proximity to the nursery. The

nursery is surrounded by mixed deciduous forest consisting of native trees (dicotyledonous trees: *Acer*, *Carya*, *Fagus*, *Fraxinus*, *Liriodendron*, *Liquidambar*, *Nyssa*, *Populus*, *Quercus*, and *Ulmus*; coniferous trees: *Juniperus* and *Pinus*). Many of these genera co-occur naturally with *T. taxifolia* in its endemic range (Schotz and Evans, 2015).

Individuals of *T. taxifolia* were approximately 1 year old when planted in the nursery. Nine genetic backgrounds were included from seed passaging by the Atlanta Botanical Garden, with two genotypes predominant (Figure 1). Seedlings were planted in a grid with approximately ~3 m spacing (Figure 1). Plants were mulched and caged above- and

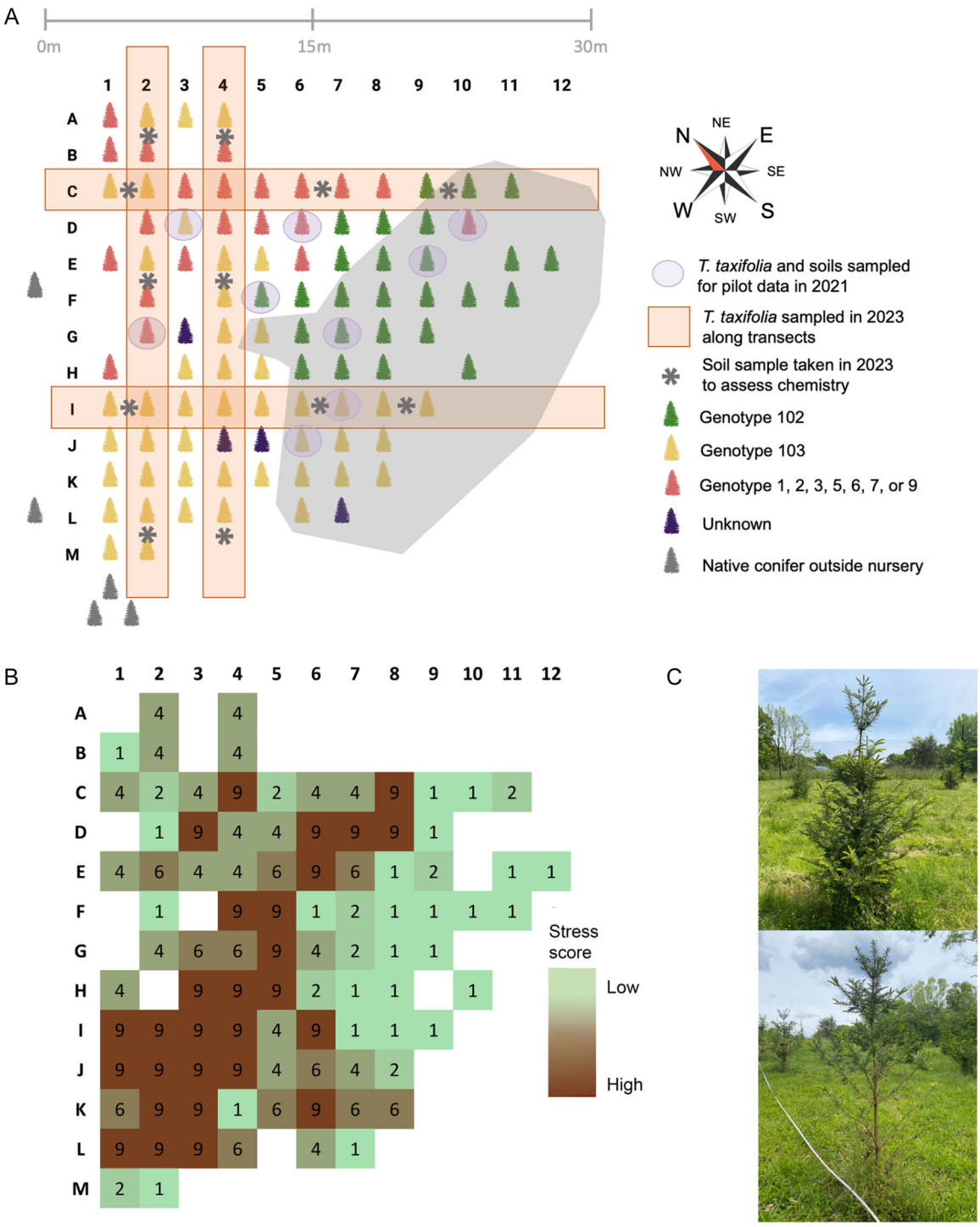

FIGURE 1 Study site, sampling structure, and stress in an ex situ conservation nursery of *Torreya taxifolia* near Durham, North Carolina, USA. (A) Schematic of nursery. Grey indicates extent of shade from native deciduous tree canopy. (B) Stress scores for *T. taxifolia* in the nursery (Appendix S1). (C) Representative *T. taxifolia* with low stress (top panel, tree C2, stress score = 2) and high stress (bottom panel, tree J4, stress score = 9).

belowground with hardware cloth to prevent herbivory, and clean tap water was supplied routinely until plants were established. Following mortality of some individuals, plants of additional genotypes were planted in 2018 (Figure 1). We collected tissues for endophyte characterization in 2021 and 2023, a time sufficient for horizontally transmitted endophytes to establish in leaves (see Arnold et al., 2003; Arnold and Herre, 2003; Christian et al., 2020).

Sample collection and site characterization

In September 2021, we collected 15 healthy, mature leaves from each of nine *T. taxifolia* individuals, including three of each of the three most common genotypes in the nursery (Figure 1). We measured the height of each individual (62–186 cm; Appendix S1). Within 6 h of collection, we rinsed leaves in tap water and cut them into 2-mm^2 segments, which we surface-sterilized (95% ethanol for 10 s; 0.5% NaOCl for 2 min; 70% ethanol for 2 min; Arnold and Lutzoni, 2007). We selected segments haphazardly from each individual for culture-based and culture-free analyses (below). Concurrently, we collected soils from the drip line on the north, south, and east side of each individual from which leaves were collected (Appendix S1). We removed grass and debris before using a 33-cm long soil corer to collect soils at a starting depth of 4 cm. The soils were relatively dry at collection due to an extended period without rainfall and were dried fully after 24 h at 22°C in loosely covered Petri dishes. Each sample was homogenized and partitioned for culture-based and culture-free analyses (below).

In April 2023, we repeated leaf sampling as above, but included 46 *T. taxifolia* individuals along transects (Figure 1; Appendix S1). Our sampling included seven *T. taxifolia* genotypes and a broader range of environmental variation than in 2021 (Figure 1). Seven of nine *T. taxifolia* individuals sampled in 2021 were included (plant D10 had died, and D6 was excluded inadvertently). We measured height (29–223 cm) and canopy diameter at the widest point (18–138 cm), which we ordinated by principal components analysis (PCA). We used the first principal component (PC1), which explained ca. 89.6% of variation in plant height and diameter, as a proxy for plant vigor (Appendix S2). We categorized sun exposure across the nursery by observing shade-requiring groundcover, and categorized soil wetness/dryness based water retention following rainfall. We collected soil at three points along each transect (12 samples, Figure 1; Appendix S1), stored them at 4°C for 24 h prior to drying at 60°C for 24 h, and sent the dried soils to Motzz Laboratories (Phoenix, AZ, USA) for measurements of pH, electrical conductivity, calcium, magnesium, sodium, potassium, zinc, iron, copper, manganese, nickel, NO_3-N, PO_4-P, sulfate, boron, free lime, exchangeable sodium percentage, and cation exchange capacity (Appendix S3). We used PCA to generate two principal components that captured major variation in soil chemistry (soil PC1 and soil PC2: combined, 69.1%; Appendix S2). Finally, we collected mature, healthy leaves from native conifer trees occurring naturally along the northwest to southwest edge of the nursery, including four individuals of *Juniperus virginiana* (Cupressaceae, Cupressales) and one *Pinus taeda* (Pinaceae, Pinales) (Figure 1; Appendix S1). That area had a moderately open canopy and relatively sunny and dry conditions compared to eastern to southeastern areas of the nursery, where mature deciduous trees formed a contiguous canopy. Leaves were prepared as above for culture-free analysis.

In April 2024, we concluded the study by recording the condition of all *T. taxifolia* in the nursery via two measures: canopy openness (loss of lush interior growth, resulting in an open and irregular canopy) and needle drop (loss of green needles). Each measure ranged from 1 (no or few gaps, and no or few dead needles) to 3 (major gaps or numerous dead needles) (Figure 1; Appendix S1). The measures were multiplied to generate stress values (1, low, to 9, severe) (Figure 1; Appendix S1). We used PCA to relate stress to environmental factors (sun exposure, soil dryness) and plant vigor as a function of distance to the exposed edge of the nursery (Figure 1; Appendix S4). Sun exposure, soil dryness, and stress were all greatest near the exposed edge, where plant vigor was lowest (Appendix S4).

Culture-based analyses

With *T. taxifolia* leaves and soils collected in 2021, we used a culture-based approach for four reasons: to determine whether *F. torreyae* could be detected in the nursery via culturing, to compare inferences from culture-based vs. culture-free approaches (below) to inform future studies, to generate a culture library for future work, and to generate a data set comparable to a previous survey of endophytes in wild *T. taxifolia* (Lee et al., 1995). We processed leaves by placing 48 surface-sterilized leaf segments from each individual on 2% malt extract agar (MEA) and dichloran chloramphenicol peptone agar (DCPA), a *Fusarium*-selective medium (Andrews and Pitt, 1986). Plates were incubated at 22°C and checked daily for 1 month. We accessioned emergent fungi as living vouchers at the Robert L. Gilbertson Mycological Herbarium (ARIZ; BES010–BES066, Appendix S5). We ground dry soil in sterile 1.5-mL tubes with ~100 μL of sterile, stainless-steel beads (NextAdvance, Troy, NY, USA; speed 6, 30 s), mixed the ground soil with 0.05% sterile water agar in a 1:100 dilution, and used a sterile spreader to place 1 mL of each sample on DCPA (2 plates/soil sample; 27 samples: 9 plants × 3 soil samples/plant). Plates were incubated at 22°C and checked daily for 10 days. Fungi from soil were grouped by colony size, color, texture, aerial hyphae, and general appearance, and transferred to axenic culture before DNA extraction and vouchering in sterile water at ARIZ (TOS001–TOS119; Appendix S5).

We used the Extract-N-Amp Plant Kit (Sigma-Aldrich, St. Louis, MO, USA) to extract total genomic DNA from cultures. We followed the manufacturer's protocol except

that we used sterile micropestles to grind fungal mycelium in a 1.5-mL tube with 100 μL of Extraction Solution. We heated tubes at 95°C for 10 min before adding 100 μL of Dilution Solution. We used the PCR protocol of Arnold and Lutzoni (2007) with primers ITS1F and LR3 to amplify the nuclear ribosomal internal transcribed spacers and 5.8S region (ITS) and an adjacent portion of the ribosomal large subunit (LSU). We visualized products in 1% agarose gels with SYBR Green (Invitrogen, Waltham, MA, USA) and cleaned PCR products with a modified ExoSAP-IT protocol (Thermo Scientific, Waltham, MA, USA) (1 μL of ExoSAP per 15 μL reaction). Bidirectional Sanger sequencing was completed at the University of Arizona Genetics Core. We assembled reads and called bases using phred and phrap (Ewing et al., 1998) with orchestration by Mesquite v2.01 (Maddison and Maddison, 2007). We edited sequences with Sequencher v4.5 (Gene Codes, Ann Arbor, MI, USA). We defined operational taxonomic units (OTUs) by clustering ITS sequences based on 95% sequence similarity in TBAS v. 2.3 (Carbone et al., 2019). We aligned concatenated ITS (ITS1 and ITS2) and partial LSU sequences to a reference alignment containing members of every class of Fungi, which we analyzed with the evolutionary placement algorithm (EPA) in RAxML with the nucleotide substitution model GTR with Gamma in TBAS (Carbone et al., 2019). This analysis placed sequences in existing clades within the fungal tree of life without altering the topology of the reference tree. We compared ITS-partial LSU sequences for *Fusarium* against a nucleotide alignment of reference sequences of *F. torreyae* and other *Fusarium* species (Appendix S6).

Culture-free analyses

With *T. taxifolia* leaves collected in 2021 and 2023, soil collected in 2021, and leaves of native conifers collected at the northwest to southwest edge of the nursery in 2023, we used a culture-free approach to characterize fungal communities for four purposes: to evaluate whether *F. torreyae* could be detected from leaves of *T. taxifolia* or associated soil; to compare inferences with the culture-based data set; to examine variation in endophyte communities across the nursery, considering spatial, edaphic, environmental, and plant-specific factors such as stress and vigor; and to compare endophytes of *T. taxifolia*, soilborne fungi, endophytes of local conifers, and endophytes of diverse native- and non-native plants in the region (below).

To process leaves collected in 2021 and 2023, we placed 96 freshly surface-sterilized segments per plant per year into sterile 2% w/v cetyltrimethylammonium bromide buffer (CTAB: 24 segments per 750-μL buffer; see U'Ren and Arnold, 2017). Tubes were incubated at ca. 22°C for 72–96 h and stored at −80°C. Before DNA extraction, we decanted CTAB from each tube, lyophilized leaf segments for 48 h, and homogenized them with stainless steel beads on a FastPrep24 tissue lyser (MP Biomedicals, Irvine, CA, USA). We extracted total genomic DNA with the DNEasy 96 Plant Kit (Qiagen, Bethesda, MD, USA; catalog no. 69181) from four sets of 24 segments per individual per collection. We pooled the four extractions per plant before PCR (U'Ren and Arnold, 2017). For soils (2021), we pooled three replicates from each individual into a single, 0.3-g composite sample per plant and extracted DNA via the Powersoil Kit (Qiagen).

We used PCR to amplify the ITS locus with consensus sequence-tagged primers ITS1F and ITS4 (Integrated DNA Technologies, Coralville, IA, USA; see U'Ren and Arnold, 2017). Each 15-μL reaction contained 1 μL of template, 7.5 μL of high-fidelity polymerase (Phusion Flash, Thermo Scientific), 0.15 μL of each primer from 50 μM stocks (0.5 μM per reaction), 0.75 μL of bovine serum albumin, and 5.45 μL of nuclease-free water. An initial PCR with 26 cycles (PCR1) was run for three DNA aliquots per sample as described by Oita et al. (2021a). Products were pooled by sample identity and diluted with sterile, nuclease-free water based on band strength following gel electrophoresis (as above). Diluted PCR1 products were used for a second PCR (PCR2) with nine cycles to add unique sample barcodes (U'Ren and Arnold, 2017; Oita et al., 2021a). Products were quantified by QuBit (Thermo Scientific) and pooled at equivalent molecular weights, resulting in 20 ng of PCR2 product per sample in the final library. Amplicon size was estimated by fragment analysis, pools were cleaned twice with MagBio beads (MagBio Genomics, Gaithersburg, MD, USA) at 0.6× concentration to remove primer dimers, and the final library was quantified by qPCR with the Kapa Library Quantification Kit for Illumina on S1P (Roche Sequencing Solutions, Pleasanton, CA, USA) at the University of Idaho IBEST Genomics and Bioinformatics Research Core. Paired-end sequencing was conducted at IBEST on the Illumina MiSeq platform (2×300 flow cell for 600 cycles) with V3 chemistry and a spiked-in 15% diverse PhiX library (D. New, University of Idaho, personal communication).

Negative controls

The PCR reaction mixtures were prepared in a dedicated laminar flow hood that was decontaminated before and after use, and in which no PCR products were handled. We used designated pipettes sterilized with UV and treated with RNAse Away (Thermo Scientific) with filter tips. The library was prepared in a designated biosafety cabinet. Extraction and PCR blanks were included on each sequencing run.

Positive controls

We used mock communities to assess consistency between Illumina runs, evaluate potential primer bias, and examine the relationship of biological abundance to read abundance. The first community contained equimolar concentrations of

genomic DNA from 31 fungal species representing diversity across the fungal tree of life (Appendix S7; see also Oita et al., 2021a). The second contained DNA from the same species but at differing concentrations (Appendix S7). Mock communities were sequenced five times per run and were prepared with an extraction blank that was amplified and sequenced as above as an additional negative control.

Bioinformatics

Raw sequences were demultiplexed by IBEST via bcl2fastq to remove the PhiX library and further demultiplexed to split by sample with a custom Python script (D. New, University of Idaho, personal communication). We assessed sequence quality with FastQC (Andrews, 2010) and MultiQC (Ewels et al., 2016). We trimmed sequences to 230 bases (b) for forward reads (i.e., the first 230 b of ITS1) with a maximum expected error of 1.0 using a consensus of quality scores and read retention estimates from VSEARCH v2.13.1 (Rognes et al., 2016). We used forward reads for analyses due to higher quality (mean phred scores >30 up to 230 b vs. 160 b for reverse reads). We used VSEARCH to dereplicate, denoise, and remove chimeric sequences.

Our cumulative sampling (*T. taxifolia* leaves in 2021 and 2023; soils in 2021; leaves of five native conifers in 2023) generated 1,866,046 raw reads. After initial quality control, we retained 1,563,784 reads, which we clustered into OTUs based on 95% sequence similarity (U'Ren et al., 2019; Oita et al., 2021b; Garcia et al., 2024). We assigned taxonomy with the SINTAX algorithm in VSEARCH 2.13.3 (Rognes et al., 2016) using the UNITE database for fungi (version 8.3; Abarenkov et al., 2023). We detected 2319 OTUs prior to evaluating positive and negative controls. In turn, mock communities generated 123,836 raw reads and 112,648 reads after initial quality control. We eliminated spurious OTUs based on a threshold that resulted in complete resolution of the mock community (i.e., a read threshold of ≤7 per OTU per sample, which yielded OTU richness that matched the known number of species in the community). This threshold excluded the rarest 2% of reads per sample, resulted in zero OTUs in most negative controls, and reduced the data set to 1604 OTU. Six additional OTUs were removed due to presence in negative controls, resulting in 1598 OTUs. Sequences not assigned to fungi by UNITE were excluded (Appendix S8). The final data set consisted of 1421 OTUs, including members of 12 phyla, 37 classes, 88 orders, and 162 families (Appendix S9). Completeness of sampling was evaluated via species accumulation curves and bootstrap estimates of total species richness (Appendix S10).

Data analyses

Counts of OTUs, taxonomic assignments, and sample metadata were concatenated into a single data set with phyloseq in RStudio 4.0.2 (McMurdie and Holmes, 2013). We estimated observed species richness in phyloseq (Rognes et al., 2016) and Faith's phylogenetic diversity (Faith, 1992) with btools (Battaglia, 2016). We measured diversity as Shannon's index and Fisher's alpha, which are complementary for communities with rare taxa (Mills and Wassel, 1980; Shaw et al., 2008; Feranchuk et al., 2018). Unless otherwise specified, the following analyses were done with the culture-free data set.

We used TBAS v. 2.3 (Carbone et al., 2019) to integrate ITS1 sequences into a phylogenetic context. One representative sequence per OTU was integrated into an alignment representing all known classes of fungi and placed by EPA with RAxML in TBAS as above (Carbone et al., 2019). Given the short length and variability of ITS1, some OTUs could not be placed with high support; therefore, metrics of phylogenetic diversity included only those OTUs placed in fungi by TBAS (1124 of 1421 OTU, 79%). The OTUs excluded by TBAS were confirmed by nucleotide BLAST to be fungal and were retained for all other analyses (Appendix S8).

We used branch lengths from the phylogenetic analysis to calculate phylogenetic species evenness (PSE), phylogenetic species clustering (PSC), mean pairwise distance (MPD), and mean nearest taxon distance (MNTD) (Helmus et al., 2007) in the R package picante (Kembel et al., 2010). Phylogenetic species evenness refers to evenness of species abundances across a phylogeny (PSE = 1 when all species are equally abundant and equally distributed across all branches of a phylogenetic tree). Phylogenetic species clustering refers to the degree to which species are related (PSC = 1 when species are as distantly related as possible given the structure of the tree). Mean pairwise distance refers to mean pairwise phylogenetic distances separating species in a community, and mean nearest taxon distance refers to the mean of phylogenetic distances between each species and its nearest relative in a community. We used standardized effect sizes of MNTD (sesMNTD or mntd.obs.z) to calculate the nearest taxon index (NTI) to inform aspects of community assembly. Negative sesMNTD values represent phylogenetic overdispersion consistent with stochastic drivers, whereas NTI <−2 or >2 suggests competition or strong selection (Webb, 2000; Helmus et al., 2007; Kembel et al., 2010; Llames et al., 2017).

We used linear regression to evaluate variation in endophyte richness, diversity, and phylogenetic diversity as a function of distance to the exposed edge of the nursery, which captured stress, vigor, soil dryness, and sun exposure (Appendix S4). Including *T. taxifolia* genotypes (see Figure 1) and soil chemistry (soil PC1 and PC2) did not explain additional variation, so they were excluded. Community structure was visualized using nonmetric multidimensional scaling (NMDS) and compared by permutational multivariate analyses of variance (PERMANOVA) in the R package vegan (Oksanen, 2010). We used the Bray–Curtis index to compare soil fungi (culture-free data, 2021) with endophytes of *T. taxifolia* (culture-free, 2021). We used presence–absence data (Jaccard index) to assess interannual variation in

endophyte communities of *T. taxifolia* (culture-free, 2021 vs. 2023) and to compare soil fungi (culture-free, 2021) with foliar endophytes from *T. taxifolia* (culture-free, 2021 and 2023). We scrutinized evidence for interannual variation by analyzing beta-dispersion in vegan (Oksanen, 2010) coupled with evaluation of species accumulation curves (Appendix S10). Use of presence–absence data avoided spurious differences due to variation in sequencing depth between Illumina runs. Interannual comparisons were complemented by indicator species analysis in PAST4 (Hammer et al., 2001).

We used the Bray–Curtis index to evaluate spatial structure of endophyte communities in *T. taxifolia* across the nursery (culture-free, 2023) and to compare them with endophytes of native conifers at the nursery edge. For each host in transects 2 and 4 (Figure 1), we used the R package betapart (Baselga and Orme, 2012) to assess nestedness and turnover of communities across the nursery. Nestedness (0, low nestedness, to 1, high nestedness) occurs when less-rich communities represent subsets of richer communities, rather than turnover.

To place endophytes in a regional context, we used the Jaccard index to compare endophytes of *T. taxifolia* (culture-free, 2021) with endophytes in a diversity of native and non-native plants sampled opportunistically and with similar sampling depth in Durham, North Carolina (culture-free, as above; for details, see Oita et al., 2021a). The regional data set included endophytes of native Cupressales (*Juniperus virginiana*), non-native Cupressales (*Cunninghamia lanceolata, Metasequoia glyptostroboides, Torreya nucifera*), and native and non-native plants in other lineages, including seed-free vascular plants (*Thelypteris* sp., *Equisetum* sp.), Ginkgophyta (*Ginkgo biloba*), and angiosperms (*Illicium* sp., *Magnolia grandiflora*) (S. Oita, unpublished data; Oita et al., 2021a). We natural-log transformed Jaccard index values for comparisons of pairwise dissimilarity, which we analyzed with a Wilcoxon rank-sum test.

RESULTS

Leaves of healthy *T. taxifolia* harbored rich fungal communities in the context of an ex situ conservation nursery, especially when evaluated via a culture-free approach. Our culture-based survey detected few endophytes in culture, low richness, and a predominance of Xylariaceae, including on the putatively *Fusarium*-selective medium DCPA (overall, 25 OTU and 57 isolates; isolation frequency = 7.0% of leaf segments; Appendix S5). However, culture-free analyses of the same leaves detected ca. 13-fold higher richness of endophytes (328 OTU among 149,583 reads, encompassing 3 phyla, 17 classes, 35 orders, and 58 families from nine *T. taxifolia* individuals; Figure 2; Appendix S9). Dothideomycetes, Eurotiomycetes, and Sordariomycetes were especially common (Figures 2 and 3; Appendix S9). Bootstrap estimates indicated that approximately 84% of the anticipated species richness of endophytes was detected.

In alignment with our first prediction (1), endophytes of *T. taxifolia* differed from fungi in soils collected concurrently in the nursery. No OTUs detected by culturing from *T. taxifolia* leaves were detected by culturing from soil (Appendix S5). Most fungi isolated on DCPA from soil were *Fusarium*, but other taxa were detected readily (Appendix S5). Culture-free analysis detected rich communities in soil (541 OTUs among 196,848 reads, including 12 phyla, 27 classes, 59 orders, 89 families), representing approximately 84% of expected species richness (Appendices S9, S10). The richness of soil fungi exceeded that of endophytes by approximately 2-fold when assessed via the culture-free approach, and soilborne and endophytic fungi in the nursery differed significantly in composition (Figure 2; Appendix S11: Jaccard distances, PERMANOVA $F_{1,\ 63} = 5.91$, $P < 0.001$). In sum, only 74 OTUs were shared between *T. taxifolia* leaves and soils in 2021, though four of these were at high abundance in both leaves and soils from clades containing known saprotrophs: OTU4, putatively *Melanodothis* sp.; OTU32, Pyrenochaetopsidaeceae sp.; OTU37, Pleosporaceae sp.; and OTU216, a species in the Didymellaceae. Comparisons with the larger data set for leaves of 46 *T. taxifolia* in 2023 (759 OTU, 1,054,211 reads; 3 phyla, 22 classes, 55 orders, 101 families) were similar, as described below.

Consistent with our second prediction (2), we did not detect *Fusarium torreyae* (or its species complex; see Zhou et al., 2016; Armer et al., 2024) by culturing or culture-free analyses of leaves or soil (Appendices S5, S6 and S9). We did not detect any *Fusarium* by culturing from leaves, including on DCPA, but identified one endophytic *Fusarium* by culture-free analysis (OTU 114; *F. sambucinum* species complex per Laraba et al. [2021], detected in seven *T. taxifolia* individuals). In soil, we isolated *F. ficicrescens* and *F. fujikuroi* (*F. fujikoroi* species complex), *F. graminearum* (*F. sambucinum* species complex; Armer et al., 2024), and *F. nisikadoi* (*F. nisikadoi* species complex) on DCPA (Appendices S5, S9).

In alignment with our third prediction (3), communities of endophytes in *T. taxifolia* differed in several respects between study years. Culture-free analyses of leaves of the same individuals sampled in 2021 and 2023 revealed marked differences in endophyte community composition (PERMANOVA $F_{1,13} = 1.73$, $P < 0.001$) (Figure 3). Dispersion did not differ significantly between years ($F_{1,12} = 0.23$, $P = 0.63$) (Appendix S12). Overall, 142 OTUs were shared between sampling years, with ca. 54% turnover between the 2021 and 2023 samples from the same trees. The most prevalent classes in both years were Dothideomycetes, Eurotiomycetes, and Sordariomycetes, but sampling in 2023 detected Pezizomycetes for the first time (Figures 2 and 3; Appendix S9). Indicator species analysis revealed different putative pathogens and saprotrophs overrepresented in either year (Appendix S12). Diversity and PSE were lower in 2023 than in 2021, reflecting a decrease in the evenness of species abundances and a less-even distribution of taxa across the fungal phylogeny, respectively (Appendix S13).

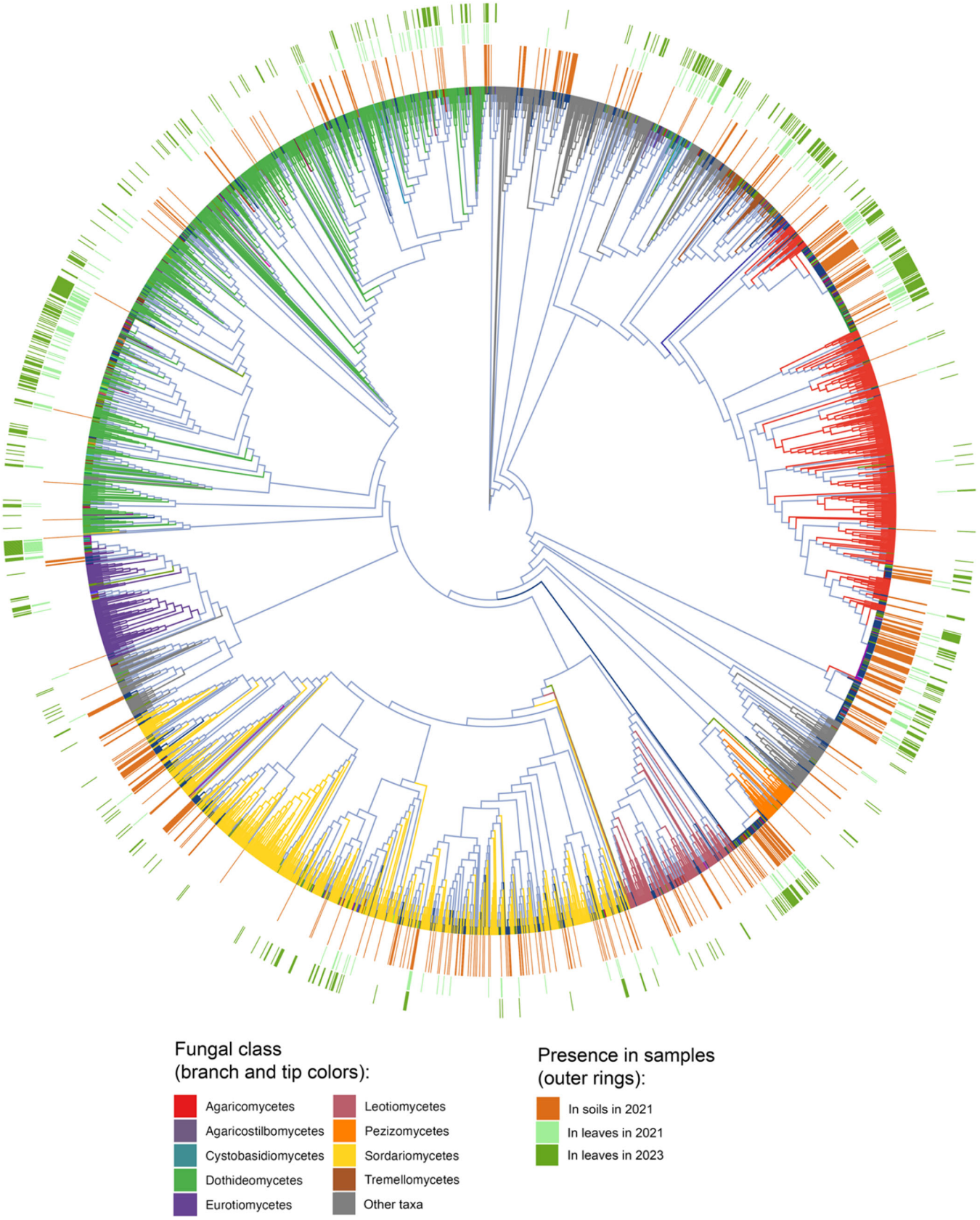

FIGURE 2 Phylogenetic placement and richness, diversity, and composition of endophytes of *Torreya taxifolia* and soil fungi. T-BAS placement within fungi of the 2021 culture-free dataset for soil fungi and the 2021 and 2023 culture-free datasets for endophytes of *T. taxifolia*. Branch colors show major fungal classes. For comparisons of richness, diversity, and composition, see Appendix S11.

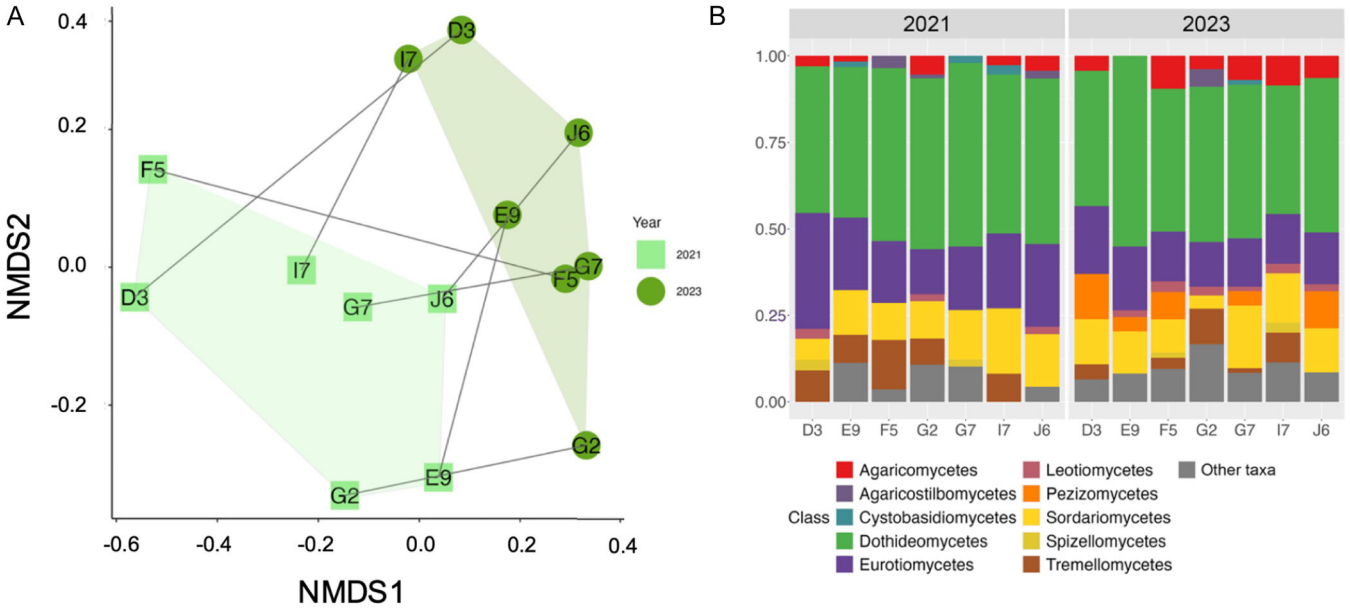

FIGURE 3 Endophytes of *Torreya taxifolia* differed over 2 years. (A) Endophytes in the same *T. taxifolia* individuals differed between 2021 and 2023 (NMDS; Jaccard distances; stress = 0.15). Mock communities from 2021 and 2023 did not differ meaningfully, such that the result shown here likely does not reflect variation among sequencing runs. For analysis of beta dispersion and indicator species, see Appendix S12. (B) Class-level composition of endophytes (number of OTU per class) in the same *T. taxifolia* individuals differed between 2021 and 2023. Pezizomycetes were not detected in 2021 despite being observed in the mock community from that year. For details, see Appendix S9. For comparisons of richness, diversity, and phylogenetic measures between years, see Appendix S13.

Similarly, endophyte communities trended toward lower MPD in 2023, aligning with decreased phylogenetic evenness in 2023 (Appendix S13). Values for PD, MNTD, and PSC did not differ significantly between years, such that the novel detection of Pezizomycetes in 2023 may have been offset by decreases in evenness as a whole (Figure 3; see also Appendix S13). In both sampling years, endophyte communities reflected signatures of stochasticity. We observed negative standardized effect sizes for MNTD and low *P*-values for endophytes of *T. taxifolia* in 2021 and 2023 (Appendix S14), consistent with higher relatedness among endophytes within each year than expected by chance alone, stochastic elements of community assembly, and limited signatures of dispersal limitation. We did not find strong evidence from NTI that competition or selection due solely to environmental pressure could explain endophyte communities in *T. taxifolia* (Appendix S14).

In contrast to our fourth prediction (4), communities of endophytes were not homogenous across the small scale of the nursery. Richness and diversity of endophytes in healthy leaves decreased as a function of distance from the nursery's northwest edge (i.e., decreased as stress decreased, under wetter and more shaded conditions) (Figure 4). The slope of decrease was especially steep for individuals planted more recently (red lines on Figure 4). Phylogenetic diversity did not decrease significantly with distance from the nursery edge, but trended negatively, especially for recently planted individuals (Appendix S16). Moreover, composition of endophyte communities varied markedly across the nursery (PERMANOVA $F_{1,45} = 4.14$, $P = 0.006$), with variation well explained when communities were clustered on the basis of stress and position (Figure 5; $F_{3,50} = 12.21$, $P = 0.001$). Generally, *T. taxifolia* individuals in columns 1–4 of the nursery, which were planted in areas with more sun and drier soils, exhibited high stress (mean stress score = 6.1, 95% confidence interval = 4.8–7.3; see Figure 1; Appendix S1). Endophyte communities fell into two clusters within this set: endophytes of *T. taxifolia* that exhibited relatively high stress and were planted in a sunny area (mean stress = 5.5, 95% CI = 3.6–7.3), and endophytes of *T. taxifolia* that exhibited high stress in a sunny, dry area near the nursery's northwest edge (mean stress = 6.9, 95% CI = 4.9–8.7) (red and gold clusters, Figure 5). Endophytes of trees in wetter, shaded conditions with lower stress formed a third group (mean stress score = 3.3, 95% CI = 1.6–5.0) (green cluster, Figure 5). Community structure along transects C and I indicated community turnover rather than nestedness (Simpson dissimilarity = 0.83; nestedness-resultant fraction of Sorensen dissimilarity = 0.05).

In partial support of our fifth prediction (5), endophyte communities in *T. taxifolia* resembled those in local plants, but only to a limited degree. Overall, 22% of endophyte OTUs observed in *T. taxifolia* were detected in conifers adjacent to the nursery, which represented nearly 67% of all OTUs observed in those conifers. However, this distinction varied spatially. Endophytes of *T. taxifolia* close to the northwest edge of the nursery, where those conifers were located, did not differ significantly from those in the conifers adjacent to the nursery ($P = 0.68$). In contrast, endophytes of *T. taxifolia* farther from the northwest edge had

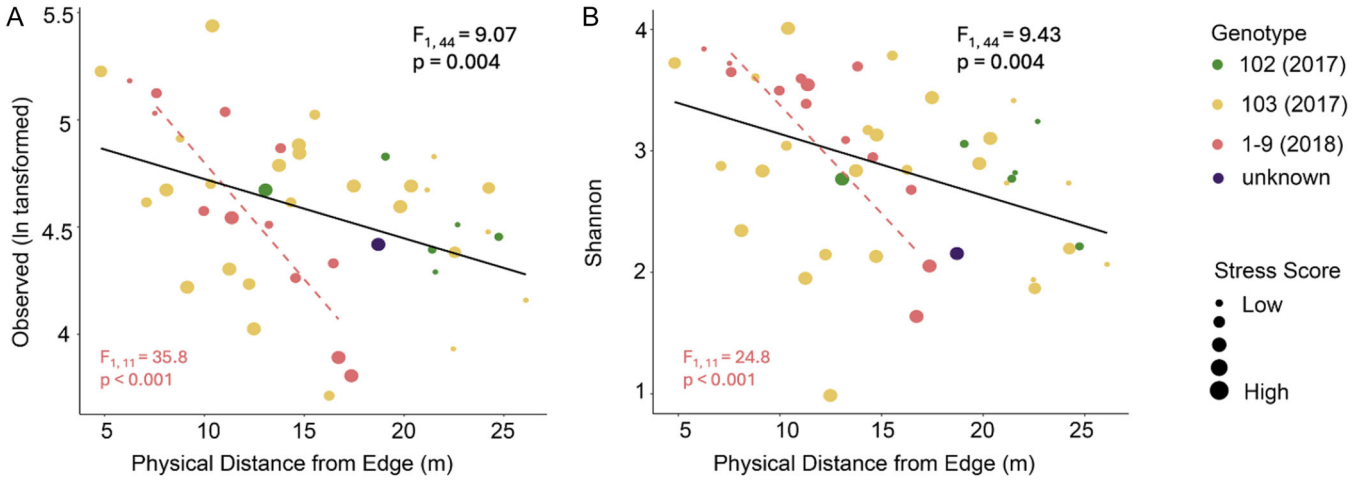

FIGURE 4 Spatial variation in endophyte richness and diversity. (A) Species richness (ln-transformed) and (B) diversity (Shannon index) of endophytes declined with increasing distance from the nursery's northwest edge. Heatmaps visualizing richness and phylogenetic diversity across the plot can be found in Appendix S15. Results for diversity as defined by Fisher's alpha and phylogenetic diversity are shown in Appendix S16.

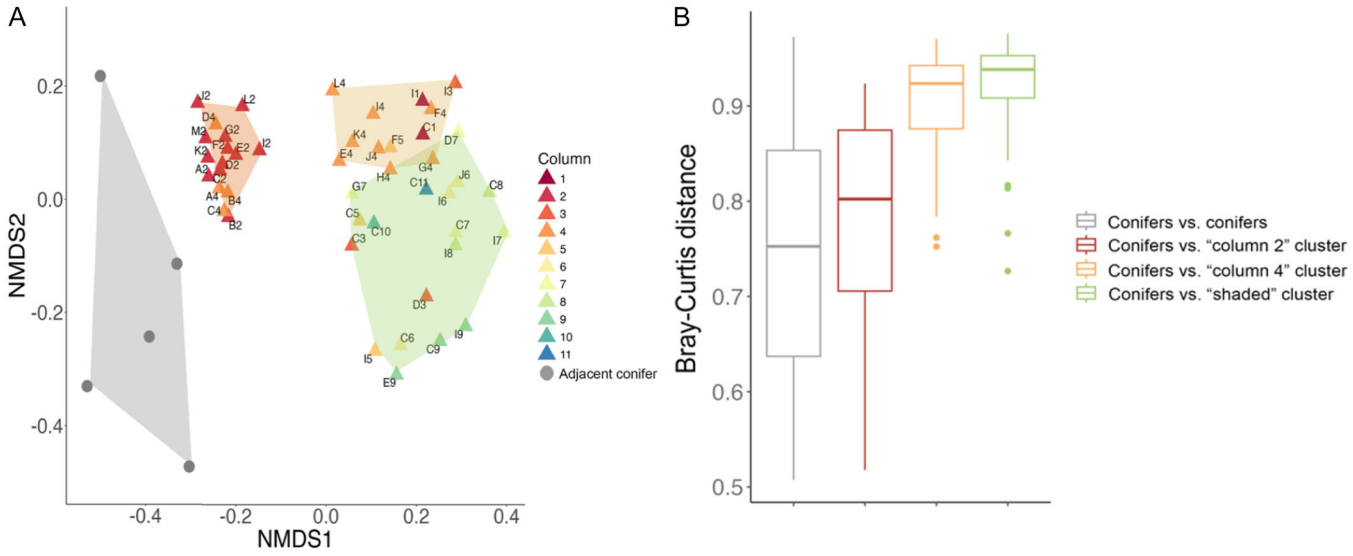

FIGURE 5 Composition of endophyte communities in *Torreya taxifolia* varied across the conservation nursery as a function of location and stress. (A) Spatial variation in endophyte communities is revealed by NMDS (Bray-Curtis distances). The red cluster consists primarily of *T. taxifolia* in column 2 of the nursery, characterized by relatively high stress and sun exposure, drier soil, and closest to the sunny edge where native conifers occur. The gold cluster consists primarily of *T. taxifolia* in column 4 of the nursery, where sun exposure and stress are high and soils are relatively dry, but distance from the edge is moderate. The green cluster consists primarily of *T. taxifolia* in columns 6–11 of the nursery, with low sun exposure, moister soil, lower stress, and the greatest distance to the nursery's northwest edge. (B) Pairwise comparisons of endophyte community distances (Bray–Curtis) of native conifers compared to *T. taxifolia* reveal spatial structure. Heatmaps visualizing these community structure differences can be found in Appendix S15.

distinct communities ($P = 0.001$) (Tukey's honest significant difference tests) (Figure 5). We did not detect evidence for stress as a sole explanatory factor in describing such differences (PERMANOVA, $F_{4,45} = 1.17$, $P = 0.24$). Instead, physical proximity to native conifers was relevant, consistent with local deposition of endophyte propagules (effect of distance to the exposed edge: $F_{1,45} = 4.2$, $P = 0.004$).

More broadly, endophytes of *T. taxifolia* were largely distinct from local and regional pools of fungal diversity (Jaccard distances, comparing *T. taxifolia* endophytes with those of all other plants: $F_{4,73} = 2.67$, $P < 0.001$; vs. soil fungi, $F_{5,82} = 3.15$, $P < 0.001$). In addition to the 22% of endophytes in *T. taxifolia* detected in conifers near the nursery, 11% of the endophytes in *T. taxifolia* were detected in other Cupressales in the region, 13% were detected in non-Cupressales, and 7% were found in soils in the nursery. Thus, ca. 50% of *T. taxifolia* endophytes were unique to that species. Endophyte composition varied markedly among *T. taxifolia* individuals in the nursery, exceeding intraspecific variation in other plants in the region and consistent with stochastic aspects of assembly (Figure 6).

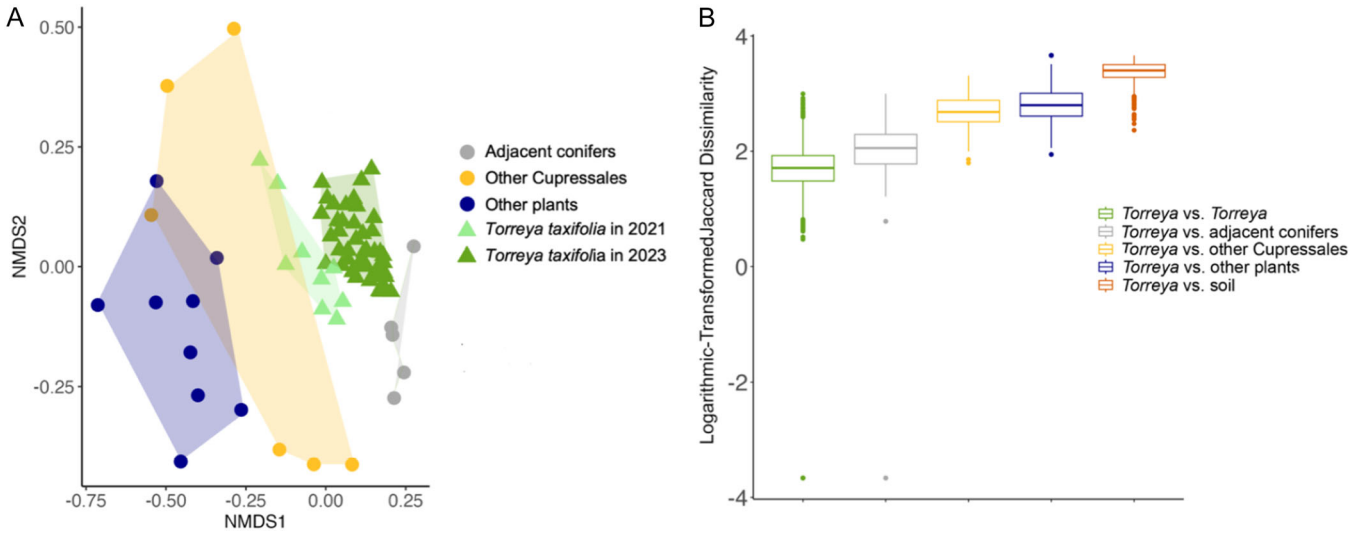

FIGURE 6 Endophytes of *Torreya taxifolia* were distinct from those in other plant taxa in the study area. (A) Endophytes of *T. taxifolia* differed from those in other native and non-native plants cultivated in the area. (B) Results of pairwise comparisons of dissimilarity for endophytes of *T. taxifolia* to endophytes of other regional hosts. Jaccard dissimilarity is ln-transformed, with 0 = no similarity, and more negative values indicating greater similarity. Comparisons to nursery soils and overlap with other local and regional fungi can be found in Appendix S17.

DISCUSSION

Plant-associated fungi are intrinsic to plant survival and resilience, especially under stress conditions (Trivedi et al., 2020; Aimone et al., 2023). Although management and exclusion of pathogens have been recognized as important in plant conservation (Dinoor and Eshed, 1984; Stanley and Bodley, 2020), diverse fungal symbionts are now being integrated into plant conservation plans (Porras-Alfaro and Bayman, 2011; Zahn and Amend, 2017; Mertin et al., 2023). Little is known about factors that may shape fungal symbiont communities in many rare plants. These knowledge gaps motivated our characterization of endophytic fungi in leaves of one of the world's most threatened conifers, *T. taxifolia*. In addition to providing an overview of fungi that can associate with this tree species in a given nursery, we used an ex situ setting to explore the factors shaping endophyte communities in a rare and regionally naïve plant species. We found that *T. taxifolia* hosts diverse endophytic symbionts when cultivated in an ex situ conservation nursery outside its relict range. When analyzed via a culture-free approach, rich endophyte communities are found that vary across a small nursery setting that display signatures of impact caused by environmental factors such as sun exposure, soil dryness, plant stress, and acquisition of local endophytes from nearby native plants. We found interannual shifts in endophytic community composition, differences between communities of endophytic and soilborne fungi, and stochasticity in endophyte community assembly. Endophytes of *T. taxifolia* include some species that occur in other hosts in the area, yet fungal communities are distinctive overall from those of local plants.

When cultivated outside their native range, rare plants can acquire distinctive symbionts relative to those they encounter naturally (e.g., Sikes et al., 2016). Little is known about the endophytes associated with *T. taxifolia* in its relict range; only one study has been published on this topic. Lee et al. (1995) isolated ~30 fungi from wild *T. taxifolia*, including *Trichoderma, Cladosporium*, and *Pestalotiopsis microspora*. They speculated that *P. microspora*, which causes canker development and was isolated from chlorotic leaves, might represent an introduction via forestry in the area (Lee et al., 1995). This perspective is consistent with our interpretation that *T. taxifolia* can readily acquire local fungi as foliar infections (including potential pathogens; Appendix S6). *Pestalotiopsis microspora* likely represents a species complex, and records for it in GenBank may include some misidentifications (Gazis et al., 2011; see also Deng et al., 2024); however, we did not detect this species in this study. We also did not detect *Trichoderma* or *Cladosporium*.

Our results show that culture-based and culture-free approaches are complementary in surveying endophytes, as found in previous work (e.g., U'Ren et al., 2019; Oita et al., 2021b). Notably, both approaches have potential use in surveys of rare plants. The culture-based approach yields a collection that supports species descriptions, genomic characterization, and experiments relevant to plant growth promotion, resilience, or biological control, while the culture-free approach provides a more expansive view of endophyte communities, which can help detect details of community structure when paired with positive controls that clarify primer bias and link biological abundance to read abundance.

We observed an abundance of *Fusarium* in our samples, as is typical of conifer nurseries (Dobbs et al., 2023; Larsson et al., 2023). However, we did not detect *F. torreyae* or its more general species complex. Failure to detect a pathogen has two shortcomings in this study. First, it is not perfect

evidence of absence, and second, ITS sequences do not always have sufficient resolution to differentiate reliably among *Fusarium* species. A more effective approach might have been to screen our DNAs for diagnostic portions of the RNA polymerase II gene (*RPB2*) unique to *F. torreyae* (Dreaden et al., 2024), which we recommend for future work. More generally, there may be potential for endophytes found here to act as latent or opportunistic pathogens (e.g., see Ridout and Newcombe, 2018), as discussed in detail by Newcombe (2011) in the context of integrating endophytes into conservation. This is also an area for further study in our system. Nonetheless, the observed recruitment of putative pathogens points to the importance of phytosanitation in general, and specifically the evaluation of plants and plant products in conservation nurseries prior to outplanting or sharing of plants, soils, or seeds.

Torreya taxifolia in its relict range is an understory tree like many other Taxaceae (Price, 1990). We found that individuals growing in sunnier conditions with drier soil exhibited higher stress and had richer and more diverse endophyte communities than those grown in more shaded, wetter conditions. We expected that relatively few endophytes could survive horizontal transmission in areas of high solar irradiance and lower moisture, which would result in lower species richness in exposed areas, but this was a pattern we did not detect (see also Arnold and Herre, 2003; Oita et al., 2021b). The plants likely experiencing the highest stress were located close to native conifers beside the nursery and had endophyte communities that most resembled the endophyte communities of those native conifers. It is not clear whether stress led to more ready recruitment of local endophytes as a stress mycobiome, or if proximity to the conifers promoted establishment of fungi from those hosts for other reasons. However, we observed leaf litter from *Pinus* and to a lesser extent, *Juniperus* in the crowns of *T. taxifolia* closest to the nursery's edge, consistent with local deposition of plant tissues that may contain endophytes. Sampling endophytes across ex situ conservation nurseries, and in the wild, would broaden the scope for understanding foliar fungal symbionts and their dynamics.

In the future, sampling this and other ex situ nurseries over time provides an opportunity to study the temporal dynamics of endophyte establishment in one of the world's rarest conifers. Plants in nurseries often exhibit dynamic changes in endophyte assemblages on short timescales (Larsson et al., 2023), and younger plants are more variable in their endophyte assemblages than are mature individuals (e.g., Bell-Dereske and Evans, 2021). Endophyte communities also often vary with host age, suggesting the potential for succession or other shifts in endophyte communities as the trees mature (see Espinosa-Garcia and Langenheim, 1990; Oono et al., 2015; Yu et al., 2021). The overall pattern of stochasticity in endophyte communities detected here may be consistent with an early successional stage coupled with exposure to novel symbiont communities.

In general, endophytic fungi must be compatible with host defenses, secondary chemistry, and other biotic filters imposed by host plants (see Saunders et al., 2010). Thus, a subset of the endophyte community in a given host is often distinctive, and host taxa typically differ in the relative abundances of generalists (Lamit et al., 2014; Sapkota et al., 2015; Latz et al., 2021; U'Ren et al., 2024). Although the population genetic structure of *T. taxifolia* individuals in the wild has been characterized, the relatedness of the individuals studied here is unknown. The remaining wild population of *T. taxifolia* has experienced bottlenecks due to plant mortality and limited seed set; there is low genetic diversity coupled with distinct population structure in wild individuals (Eserman et al., 2022; Dreaden et al., 2024). Thus, our current study may have encompassed limited genetic diversity of the remaining wild plants, potentially explaining why we did not detect a signature of plant genotype on endophyte communities (in contrast, see Ek-Ramos et al., 2013; He et al., 2019; Latz et al., 2021). Future studies incorporating genetic relatedness could assess how endophyte communities vary with plant genotype in this and other threatened plants.

Endophytes also pose risks and benefits to conservation efforts. Limited genetic variation in extant populations of rare plants makes breeding for resilience a challenge, and so endophytes are being considered as innovative tools for incorporating pathogen resistance into plants of conservation concern (Hawkes et al., 2023). Some endophytes contribute to pathogen suppression. Examples include antifungal activity of endophytes in needles of *Picea* (Tanney 2017; Anderson Stewart et al., 2019) and *Pinus* (McMullin et al., 2018). Some nurseries already use endophytes as modulators of plant health, including for drought resilience (Zhou et al., 2021) and improvements in water use, biomass, and salinity tolerance (Ballesteros et al., 2024). These observations point to the need for further studies to characterize endophyte diversity and function for threatened plants under a variety of environmental conditions, and to integrate endophytes into management plans and conservation strategies. One step forward is to use the culture-free data set to develop targeted culture-based approaches that can generate libraries of desirable strains to test plant–fungal interactions and conservation potential. Because four of every 10 vascular plant species are threatened with extinction under pressure from rapidly shifting climate regimes and associated changes in abiotic and biotic stress (Nic Lughadha et al., 2020), it is important to integrate symbionts into plant conservation plans.

AUTHOR CONTRIBUTIONS

B.E.S.: Methodology, data curation, formal analysis, investigation, writing original draft, review and editing, visualization. F.L.: Conceptualization, methodology, investigation, review and editing, supervision. A.R.: Conceptualization, methodology, resources, investigation, funding acquisition. S.O.: Investigation, data curation, resources, review and editing. S.M.S.: Investigation and review. A.E.A.: Conceptualization, methodology, data

curation, formal analysis, investigation, writing original draft, review and editing, supervision, funding acquisition.

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DATA AVAILABILITY STATEMENT

Cultures are accessioned as living vouchers at the Robert L. Gilbertson Mycological Herbarium at the University of Arizona (ARIZ) under accession numbers BES010–BES066 (foliar endophytes) and TOS001–TOS118 (soil fungi). Sanger sequences from cultures were uploaded to GenBank as accessions PQ576763–PQ576915. Amplicon sequences were uploaded to the GenBank Sequence Read Archive as BioProject PRJNA1303223, accessions SRR34916168–SRR34916236.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Samples collected in 2021 (Table S1, leaves of *T. taxifolia*; Table S2, soils) and 2023 (Table S3, leaves of *T. taxifolia* and local conifers), and Table S4) observations of stress (2024).

Appendix S2. Results of PCA for soil chemical variables and tree height and diameter.

Appendix S3. Soil characteristics for samples collected in April 2023.

Appendix S4. PCA for host and environmental variables.

Appendix S5. Cultures obtained from leaves of *T. taxifolia* and soils in September 2021.

Appendix S6. Table S1, *Fusarium* reference ITS-nrLSU sequences and strains detected in this study, and Table S2, NEXUS file regarding sequence alignment for *Fusarium*.

Appendix S7. Mock community for Illumina sequencing (see Oita et al., 2021a).

Appendix S8. Sequences determined to be non-fungal or not successfully placed in phylogenetic analysis of fungi.

Appendix S9. Summary (Table S1) and details (Table S2) for results of culture-free analyses of soil and leaves in 2021, and leaves in 2023.

Appendix S10. Species accumulation curves.

Appendix S11. Comparisons of richness, diversity, and composition of *T. taxifolia* endophytes and soil fungi.

Appendix S12. Beta dispersion and indicator species for *T. taxifolia* endophytes in each year of the study.

Appendix S13. Diversity measures of *T. taxifolia* endophytes differed over 2 years.

Appendix S14. Mean nearest taxon distances (MNTD) for endophytes of *T. taxifolia* and soil fungi.

Appendix S15. Spatial variation in Fisher's alpha and phylogenetic diversity.

Appendix S16. Heatmaps detailing diversity and community structure across the nursery.

Appendix S17. Details on community overlap of *T. taxifolia* endophytes with local and regional fungi.

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