Many foliar endophytic fungi of *Quercus gambelii* are capable of psychrotolerant saprotrophic growth

Emily Weatherhead, Emily Lorine Davis, Roger T. Koide* 
Department of Biology, Brigham Young University, Provo, UT, United States of America

*rogerkoide@byu.edu

**Abstract**

Many endophytic fungi have the potential to function as saprotrophs when living host tissues senesce and enter the litter pool. The consumption of plant litter by fungi obviously requires moisture but, in the arid, western USA, the native range of *Quercus gambelii* Nutt., most of the precipitation occurs during the coldest months of the year. Therefore, we hypothesized that the endophytic fungi of *Q. gambelii* have the potential to function as psychrotolerant saprotrophs, which we defined in this study as an organism capable of significant growth on leaf litter at 5˚C. We further hypothesized that a tradeoff exists between growth of endophytic fungi at 5˚C and at 17˚C such that fungal isolates are either cold- or warm-temperature specialists. Consistent with our first hypothesis, we found that 36 of our 40 isolates consumed leaf litter at 5˚C, but there was a surprisingly high degree of variability among isolates in this ability, even among isolates of a given species. Contrary to our second hypothesis, there was no tradeoff between saprotrophic growth at 5˚C and saprotrophic growth at 17˚C. Indeed, the isolates that grew poorly as saprotrophs at 5˚C were generally those that grew poorly as saprotrophs at 17˚C. By virtue of being endophytic, endophytic fungi have priority in litter over decomposer fungi that colonize plant tissues only after they enter the litter pool. Moreover, by virtue of being psychrotolerant, some endophytic fungi may function as saprotrophs during the cold months of the year when moisture is temporarily available. Therefore, we suggest that some endophytic fungi of *Q. gambelii* could play significant ecosystem roles in litter decomposition and nutrient cycling.

**Introduction**

In arid ecosystems, microbe-mediated litter decomposition and nutrient cycling can be limited by moisture [1], but this may depend on the time of year as some arid ecosystems are only seasonally dry. In the Great Basin desert of the western United States, for example, inadequate moisture frequently limits microbial decomposition of litter in the warmer months of the year, but it may not limit decomposition in the winter and early spring when evaporation is low and precipitation is relatively high [2]. Because moisture is least limiting in the winter and early spring, microbial decomposition of litter in the Great Basin of the western United States
requires that some saprotrophic microorganisms have significant metabolic activity under cold conditions [3,4]. Such microorganisms are referred to as psychrotolerant, psychrotrophic or psychrophilic [5,6]. We hereafter refer to this phenomenon as psychrotolerance.

Endophytic fungi very commonly colonize living plant tissues [7]. In some cases the host plant benefits from their colonization, which may improve seed germination [8], vegetative growth [9] and tolerance to stresses such as drought [10,11], extreme temperature [12,13], salinity [13,14], heavy metals [15] and herbivory [16,17]. Moreover, upon senescence of the host tissues they occupy, many endophytic fungi are capable of obtaining nutrition saprotrophically [18–23]. Indeed, by colonizing living plant tissues, endophytic fungi are essentially pre-colonizing litter. Therefore, endophytic fungi are among the first with access to litter as a source of energy and nutrients [21,24]. Priority access to a resource frequently results in a competitive advantage [25–27]. Thus, in litter, endophytic fungi may have a competitive advantage over decomposer fungi that colonize the plant tissues only after they senesce [28].

If an endophytic fungus were strictly biotrophic, it would only have to maintain activity during the warmer growing season. However, if an endophytic fungus were to function as a saprotroph in the Great Basin, where moisture is least limiting in the winter and early spring, it would be advantageous to be psychrotolerant. Our first hypothesis, then, is that endophytic fungi isolated from leaves of Quercus gambelii Nutt. are psychrotolerant saprotrophs, and that there are significant differences among species in their ability to function as psychrotolerant saprotrophs. Organisms frequently exhibit functional tradeoffs. For example, tradeoffs have been observed in fungi between growth and enzymatic activity (Zheng et al., 2020), between growth and reproduction [29,30] and between growth and defense [31]. Still other tradeoffs occur because organisms frequently cannot excel along the entire length of an environmental gradient. For example, plants that are active in cool climates may adapt their physiology to lower temperatures such that they do not perform well at higher temperatures, while plants that are active in warm climates adapt their physiology to higher temperatures [32]. Therefore, our second hypothesis is that there is a tradeoff among the endophytic fungi isolated from Q. gambelii such that they specialize at either winter or summer temperatures.

Materials and methods

Study sites and leaf sampling

Q. gambelii is a small tree or shrub found mainly in Utah, Arizona, New Mexico and Colorado. In Utah it is widely distributed between approximately 1,200 and 2,400 meters in elevation [33]. In order to sample leaves from across the elevational range of the species in Utah, whole, unblemished leaves were collected on 24 September 2019 at Devil’s Kitchen (39.48°12.27”N, 111.41°17.96”W), elevation 2,553 meters, near Payson, Utah and on 8 October 2019 in Slate Canyon (40.13°30.59”N, 111.37°21.9”W), elevation 1,553 meters, in Provo, Utah. Air temperatures were monitored at the Devil’s Kitchen site from 24 Jun 2019 to 24 Sep 2019 using a temperature data logger (HOBO UA-001-64, Onset Computer Corporation, Bourne, MA, USA), set to log hourly. At Devil’s Kitchen, 15 leaves were collected from each of 7 trees and, in Slate Canyon, 10 leaves were collected from each of 6 trees, totaling 165 leaves. No sampling permits were required because of the small number of samples taken. In the field, leaves were stored in plastic bags and placed in a cooler on ice. Later on the same day, the leaves were transferred to an incubator at 5°C in the laboratory.

Fungal isolation

We isolated fungi from Q. gambelii leaves in a laminar flow hood to insure sterility. External leaf surfaces were sterilized by submerging leaves in 70% ethanol for 2 seconds, immediately
submerging them in 3% sodium hypochlorite for 2 minutes, similar to Arnold et al. [7], rinsing
away the hypochlorite by submerging leaves sequentially in three beakers of sterile water, then
submerging, again, in 70% ethanol, after which they were laid to dry in a sterile petri dish.
Each surface-sterilized leaf was subsampled once using a sterilized, 6 mm diameter, paper hole
punch, taking care to re-sterilize the punch between leaves from individual trees with ethanol
that was flamed off. Each leaf disk was placed on 2% malt extract agar in its own petri dish,
sealed with parafilm and incubated initially at 10˚C, a temperature that had previously been
used to isolate psychrotolerant endophytic fungi [34]. After 3 weeks, fewer than 10 total fungal
colonies grew out from the entire collection of leaf disks. These were isolated to their own
dishes. The original dishes containing the leaf disks were then transferred to a 17˚C incubator
for an additional 3 weeks. Many new fungi growing from the leaf disks at 17˚C were thus iso-
lated. When there were multiple fungal colonies growing from a single leaf disk, each was iso-
lated separately.

Sequencing and assigning taxonomy of fungal isolates
A total of six hundred seventy-five fungal isolates were sorted into morphological groups
based on color, hyphal growth pattern and mycelial density. From these groups, 40 isolates
were chosen for study, each of which was sequenced. Each isolate was subject to direct PCR
[35–37] of the ITS region using APEX 2 Hotstart Master Mix (Genesee Scientific, El Cajon,
CA, USA) with ITS1F and ITS4 primers [38]. The thermal cycling program included activation
of the polymerase at 95˚C for 15 minutes followed by 30 cycles of denaturation (95˚C, 30 sec-
onds), annealing (55˚C, 30 seconds), and extension (72˚C, 48 seconds), then ended with a final
extension (72˚C, 7 minutes). Primers and dNTPs were eliminated using exonuclease I and
shrimp alkaline phosphatase (New England BioLabs, Ipswich, MA, USA) and sent to the Brig-
ham Young University DNA Sequencing Center (https://biology.byu.edu/dnasc) for Sanger
sequencing. Sequences were trimmed to exclude quality scores below 10 in CodonCode
Aligner (CodonCode Corporation, Centerville, MA, USA, v. 9.0.1). The average trimmed
sequence length was approximately 530 bp. Sequences were then searched against the UNITE
database (https://unite.ut.ee/index.php) using BLASTn. We used a 99% identity criterion to
match to a species and 97% identity to match to a genus. Five isolates could not be identified
to a species or genus in the database. We, therefore, constructed a phylogenetic tree to assist in
identifying these taxa. All sequences were aligned in MAFFT (https://mafft.cbrc.jp/alignment/
server/) [39,40] and cleaned in Mesquite v. 3.70 [41]. The output from Mesquite was used to
construct a tree in IQTree (http://iqtree.cibiv.univie.ac.at/) [42], which was visualized in Fig-
Tree v. 1.4.4. (http://tree.bio.ed.ac.uk/software/figtree/).

Testing hypothesis 1: Psychrotolerance
Each of the 40 isolates was grown in a 2 x 2 factorial experiment with two incubation tempera-
tures (5 and 17˚C) and two media (control and leaf litter). Our criterion for psychrotolerance
was statistically significant growth at 5˚C. See below for greater detail. The warmer tempera-
ture (17˚C) represents the temperature during the growing season for Q. gambelii. The actual
daily average temperature at Devil’s Kitchen during the majority of the growing season (24 Jun
2019 to 24 Sep 2019) was 16.9˚C (SD, 6.2). The control medium contained starter glucose. The
leaf litter medium contained starter glucose and leaf litter. There were 4 replicate 5 cm diame-
ter dishes for each medium x temperature combination, 16 total dishes per isolate, for a total
of 640 dishes. Each of the dishes was inoculated with the appropriate isolate using a 6 mm
diameter piece of mycelium taken from the outer edge of the parent culture. Each liter of con-
trol medium contained 2.0 g glucose, 8.92 g agar, 0.46 g peptone, 1.0 ml of 300 g CaCl₂ L⁻¹,
10.0 ml of 30 g KH₂PO₄ L⁻¹, and 1.0 ml of a solution containing 5 g MgSO₄ L⁻¹, 3.7 g FeSO₄ L⁻¹, 1.4 g MnSO₄ L⁻¹ and 3.7 g ZnSO₄ L⁻¹. Each liter of leaf litter medium additionally contained 7.18 g of Q. gambelii leaf litter, ground to pass a 2 mm screen in a Cyclone Mill (Retsch USA, Verder Scientific, Newtown, PA, USA). All media components were added prior to autoclaving. The litter had been collected from the forest floor of the Slate Canyon site in the summer of 2020.

The experiment was carried out in sets of 4 to 6 isolates. There were 8 sets total. Biomass growth rates were determined after 49–53 d of growth, depending on the set. To determine fungal biomass, the agar within each dish was melted within a stainless-steel tea ball (0.6 mm mesh) in boiling water for 10 min., the freed mycelium was dried on the lab bench at room temperature for 24 hours and weighed. It is unlikely that any of the fungal biomass was lost through the stainless-steel mesh of the tea ball because each mycelium consisted of a solid fungal mat. The biomass growth rate was calculated using the following formula: (DW₂ – DW₁) / elapsed time, for which we assumed DW₁ = 0. In reality, the mycelium dry weight for any replicate at the beginning of the experiment was not zero, but was less than 0.0001 g, the lower limit of our balance.

To test hypothesis 1, we defined psychrotolerance as significant saprotrophic capacity at 5˚C. We defined saprotrophic capacity as the difference between the biomass growth rate on leaf litter medium (containing starter glucose and leaf litter), and the biomass growth rate on control medium (containing starter glucose). At 5˚C, there were 4 replicates for each of the media, but leaf litter replicates were not paired with corresponding control replicates for the purpose of calculating differences in growth rate (saprotrophic capacity). Therefore, we calculated the error associated with the difference by bootstrapping. First, differences were determined for the 16 possible combinations of leaf medium (4 replicates) and control medium (4 replicates). Then, 4 of the 16 differences were randomly sampled 1000 times with replacement in order to bootstrap a frequency distribution of the difference, and the mean, standard deviation and 95% confidence intervals were determined in R [43]. If this difference was significantly greater than zero for isolates grown at 5˚C, according to the 95% confidence interval, we considered the isolate to be a psychrotolerant saprotroph. The R script for this procedure is given in the supporting information (S1 File).

We analyzed the variation in psychrotolerant saprotrophy (saprotrophic capacity at 5˚C) among isolates. Again, because leaf litter replicates were not paired with corresponding control replicates for the purpose of calculating differences in growth rate (= saprotrophic capacity), it was not possible to perform a standard analysis of variance of the difference in growth rate. Therefore, the analysis of variance was performed in R (see https://acetabulum.dk/) using the means and standard deviations of the differences in growth rate at 5˚C obtained from the bootstrapping R script to analyze the variation among isolates in psychrotolerant saprotrophy (saprotrophic capacity at 5˚C). To determine whether psychrotolerant saprotrophy differed among species, a conventional analysis of variance was performed with isolates as replicates within the four species comprising multiple isolates using Minitab v. 18 [44].

Testing hypothesis 2: Temperature specialization

To test hypothesis 2, we performed a linear correlation of saprotrophic capacities of isolates at 17˚C and at 5˚C. The P and r values were obtained through SigmaPlot for Windows, Version 11.0 [45].

Results

Based on the UNITE database, 35 of our 40 isolates were identified to the following taxa: Apiognomonia errabunda (Roberge ex Desm.) Höhn, Cladosporium herbarum (Pers.) Link Ex
S. F. Gray, *Ophiognomonia setacea* (Pers.) Sogonov, *Ophiognomonia* sp., *Pyronema omphalodes* Bull. (Fuckel), *Venturia* sp., *Coniochaeta polymorpha* Z.U. Khan, J.P. Guarro & S. Ahmad, Antonie van Leeuwenhoek, *Cladosporium allicinum* (Fr.) Bensch, U. Braun & Crous, 2012, *Tricharina cretea* (Cooke) K.S. Thind & Waraitch, *Parafenestella* sp. The remaining 5 isolates were identified using the constructed phylogenetic tree: one isolate each in the Dothideales, Diaporthales, Coniochaetales, and two isolates in the Pezizomycetes.

Thirty-six of the 40 isolates had significant saprotrophic capacity at 5˚C or, in other words, were psychrotolerant saprotrophs (Fig 1). In contrast, 4 isolates (QGsun2.2.A, *Ophiognomonia setacea*; QGsun5.7.A.4, *Coniochaetales*.; QGsun4.9.A.2.1, *Pyronema omphalodes*; QG6.6.A.1, *Ophiognomonia* sp.) did not exhibit significant psychrotolerant saprotrophy.

There was significant variation in psychrotolerant saprotrophy among fungal isolates (Fig 1, Table 1). Isolate was a significant factor with respect to psychrotolerant saprotrophy (p = 0.007), with species was a significant factor with respect to psychrotolerant saprotrophy (p = 0.007), with

![Graph showing saprotrophic capacity at 5˚C among the 40 isolates.](https://doi.org/10.1371/journal.pone.0275845.g001)

**Table 1.** ANOVA table for psychrotolerant saprotrophy (saprotrophic capacity at 5˚C) among the 40 isolates.

| Source        | df | SS     | MS     | F       | P      |
|---------------|----|--------|--------|---------|--------|
| Among isolates| 39 | 8.8673 | 0.22737| 54.6571 | 6.42e-60|
| Within isolates| 120| 0.4992 | 0.00416|         |        |
| Total         | 159| 9.3664 |        |         |        |

https://doi.org/10.1371/journal.pone.0275845.t001
among species variation accounting for 39% of the total variability (Table 2). *Ophiognomonia* sp. had a saprotrophic psychrotolerance significantly lower than that of *Apiognomonia errabunda* and *Ophiognomonia setacea* (Table 3). However, within species variability was also large, accounting for 61% of total variability (Table 2).

The correlation between the saprotrophic capacity of isolates at 17°C and 5°C was positive and significant (p = 0.0003, r = 0.5434, Fig 3).

### Discussion

Because much of the microbially-mediated decomposition of *Q. gambelii* leaf litter must occur in the colder months of the year when moisture is available, we hypothesized that endophytic fungi colonizing *Q. gambelii* leaves were capable of saprotrophy at 5°C. Of the endophytic

### Table 2. ANOVA table for psychrotolerant saprotrophy (saprotrophic capacity at 5°C) among the four species each comprising multiple isolates.

| Source          | df | SS      | MS      | F       | P      |
|-----------------|----|---------|---------|---------|--------|
| Among species   | 3  | 0.4306  | 0.14353 | 5.2     | 0.007  |
| Within species  | 24 | 0.6628  | 0.02762 |         |        |
| Total           | 27 | 1.0933  |         |         |        |

https://doi.org/10.1371/journal.pone.0275845.t002
fungi we isolated, saprotrophic psychrotolerance was common but not universal, occurring in all but 4 of the 40 tested isolates. Others have reported psychrotolerant growth of endophytic fungi on glucose-based media [34,46]. However, this constitutes the first report of psychrotolerant saprotrophy of endophytic fungi using leaf litter as the carbon source.

We also hypothesized that there was significant variation among species in saprotrophic psychrotolerance. While the species designated *Ophiognomonia sp.* had a significantly lower saprotrophic psychrotolerance than the other species, there was a surprising level of variation within species as was seen for *Aphiognomonia errabunda*, *Cladosporium herbarum* and *Ophiognomonia setacea*. This result was unexpected because members of a given species are assumed to occupy the same niche and, therefore, to be functionally similar [47]. We note, however, that the unexpectedly high degree of variation in psychrotolerance within a species was expressed under artificial experimental conditions, including controlled temperatures and

| Species                  | n  | Mean (SEM)       |
|--------------------------|----|------------------|
| *Aphiognomonia errabunda*| 4  | 0.3549 (0.0831) a|
| *Ophiognomonia setacea*  | 6  | 0.3194 (0.0678) a|
| *Cladosporium herbarum*  | 3  | 0.1637 (0.0959) ab|
| *Ophiognomonia sp.*      | 15 | 0.0668 (0.0429) b|

https://doi.org/10.1371/journal.pone.0275845.t003

![Fig 3. Plot of saprotrophic capacity of the 40 isolates at 17°C vs. 5°C. Blue is *Aphiognomonia errabunda*, red is *Cladosporium herbarum*, light green is *Ophiognomonia setacea*, dark green is *Ophiognomonia sp.*, and white are taxa that were either not identified to species or species comprising a single isolate each.](https://doi.org/10.1371/journal.pone.0275845.g003)
non-limiting availabilities of water and mineral nutrients. Under field conditions, where cold or insufficiency of water or mineral nutrients could limit saprotrophic activity, the ability to express variation in psychrotolerance may be more limited.

Among the 40 fungal isolates, we found no evidence of a tradeoff in saprotrophic capacity at 17˚C with saprotrophic capacity at 5˚C. In fact, the significant correlation between saprotrophic capacity at 17˚C and at 5˚C was positive. Therefore, these fungi appear not to specialize with respect to temperature. This result was somewhat unexpected given the existence of performance tradeoffs at different temperatures for important physiological processes such as photosynthesis [32]. The capacity to obtain nutrition saprotrophically under a wide range of temperatures, from 5˚C to 17˚C, could potentially allow endophytic fungi to obtain resources from leaf litter both during the colder months of late autumn, winter, and early spring, when moisture is most abundant, as well as during the late summer monsoon when limited rain falls [2]. We have not assessed the ability of endophytic fungi to compete against non-endophytic fungi in litter, and it is possible that endophytic fungi are generally not highly competitive. However, we previously showed that endophytic fungi do at least persist in leaf litter of *Q. gambelii* for weeks following leaf senescence [21]. The persistence of endophytic fungi in decomposing litter is not particularly surprising given that when leaves senesce and enter the litter pool, endophytic fungi are already present, have no need to colonize from the environment, and thus have priority [25] over non-endophytic fungi in capturing resources from leaf litter. Indeed, others have found that endophytic fungi can exert priority over decomposer fungi in litter [28].

As with all laboratory studies, the ecological relevance of our results should be carefully weighed. This study was carried out under controlled temperatures (constant 17˚C vs. constant 5˚C), presumably without moisture or mineral nutrient limitation, and with every isolate grown separately, all conditions that are not likely to occur under field conditions. Because we do not know how these factors influence either saprotrophic capacity or psychrotolerance of a given isolate, the extent to which our results are relevant in the field is not clear. Nevertheless, our results suggest that a number of endophytic fungi of *Q. gambelii* leaves are potentially capable of saprotrophic growth under both cold and warm conditions and thus have the potential to influence litter decomposition and nutrient cycling in this arid ecosystem.

**Supporting information**

S1 Table. Isolate designation and species identity. (DOCX)

S1 File. Bootstrapping R script. (DOCX)

S2 File. Data set. (XLSX)

**Author Contributions**

**Conceptualization:** Roger T. Koide.

**Data curation:** Emily Weatherhead, Emily Lorine Davis, Roger T. Koide.

**Formal analysis:** Emily Weatherhead, Emily Lorine Davis.

**Funding acquisition:** Roger T. Koide.

**Investigation:** Emily Weatherhead, Emily Lorine Davis.
Methodology: Roger T. Koide.

Project administration: Roger T. Koide.

Resources: Roger T. Koide.

Supervision: Roger T. Koide.

Visualization: Emily Weatherhead, Emily Lorine Davis.

Writing – original draft: Emily Weatherhead, Emily Lorine Davis.

Writing – review & editing: Roger T. Koide.

References

1. Campos X, Germino M, de Graaff M-A. Enhanced precipitation promotes decomposition and soil stabilization in semiarid ecosystems, but seasonal timing of wetting matters. Plant Soil. 2017; 416: 427–436.

2. Cook J, Irwin L. Climate-vegetation relationships between the Great Plains and Great Basin. Am Midl Nat. 1992; 127: 316–326.

3. Brooks PD, Williams MW, Schmidt SK. Microbial activity under alpine snowpacks, Niwot Ridge, Colorado. Biogeochemistry. 1996; 32: 93–113.

4. Williams MW, Brooks PD, Seastedt T. Nitrogen and carbon soil dynamics in response to climate change in a high-elevation ecosystem in the Rocky Mountains, U.S.A. Arct Alp Res. 1998; 30: 26–30. https://doi.org/10.2307/1551742

5. Morita RY. Psychrophilic bacteria. Bacteriol Rev. 1975; 39: 144–167. https://doi.org/10.1128/br.39.2.144-167.1975 PMID: 1095004

6. Moyer CL, Morita RY. Psychrophiles and psychrotrophs. Encycl Life Sci. 2007; 1–6. https://doi.org/10.1002/9780470015902.a0000402.pub2

7. Arnold A, Maynard Z, Gilbert G, Coley P, Kursar T. Are tropical fungal endophytes hyperdiverse? Ecol Lett. 2000; 3: 267–274. https://doi.org/10.1046/j.1461-0248.2000.00159.x

8. Shearin ZRCC, Filipek M, Desai R, Bickford WA, Kowalski KP, Clay K. Fungal endophytes from seeds of invasive, non-native Phragmites australis and their potential role in germination and seedling growth. Plant Soil. 2018; 422: 183–194. https://doi.org/10.1007/s11104-017-3241-x

9. Redman RS, Dunigan DD, Rodriguez RJ. Fungal symbiosis from mutualism to parasitism: Who controls the outcome, host or invader? New Phytol. 2001; 151: 705–716. https://doi.org/10.1046/j.0028-646x.2001.00210.x PMID: 11104-017-3241-x

10. Malinowski D, Belesky D. Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. Crop Sci. 2000; 40: 923–940.

11. Schardl CL, Leuchtmann A, Spiering MJ. Symbioses of grasses with seedborne fungal endophytes. Annu Rev Plant Biol. 2004; 55: 315–340. https://doi.org/10.1146/annurev.arplant.55.031903.141735 PMID: 15377223

12. Redman RS, Sheehan KB, Stout RG, Rodriguez RJ, Henson JM. Thermostolerance generated by plant/fungal symbiosis. Science (80-). 2002; 298: 1581. https://doi.org/10.1126/science.1072191 PMID: 12446900

13. Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F, et al. Stress tolerance in plants via habitat-adapted symbiosis. ISME J. 2008; 2: 404–416. https://doi.org/10.1038/ismej.2007.106 PMID: 18256707

14. Redman RS, Kim YO, Woodward CJDADA, Greer C, Espino L, Doty SL, et al. Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: A strategy for mitigating impacts of climate change. PLoS One. 2011; 6: 1–10. https://doi.org/10.1371/journal.pone.0014823 PMID: 21750695

15. Bilal S, Shahzad R, Imran M, Jan R, Kim KM, Lee IJ. Synergistic association of endophytic fungi enhances Glycine max L. resilience to combined abiotic stresses: Heavy metals, high temperature and drought stress. Ind Crops Prod. 2020; 143: 111931. https://doi.org/10.1016/j.indcrop.2019.111931

16. Rodriguez RJ, White J, Arnold A, Redman R. Fungal endophytes: diversity and functional roles. New Phytol. 2009; 182: 314–330. https://doi.org/10.1111/j.1469-8137.2009.02773.x PMID: 19236579

17. Lyons PC, Plattner RD, Bacon CW. Occurrence of peptide and clavine ergot alkaloids in tall fescue grass. Science (80-). 1986; 232: 487–489. https://doi.org/10.1126/science.3008328 PMID: 3008328
18. Parfitt D, Hunt J, Dockrell D, Rogers HJ, Boddy L. Do all trees carry the seeds of their own destruction? PCR reveals numerous wood decay fungi latently present in sapwood of a wide range of angiosperm trees. Fungal Ecol. 2010; 3: 338–346. https://doi.org/10.1016/j.fungec.2010.02.001

19. Prompuththa I, Hyde KD, McKenzie E, Peberdy J, Lumsong S. Can leaf degrading enzymes provide evidence that endophytic fungi becoming saprobes? Fungal Divers. 2010; 41: 89–99. https://doi.org/10.1007/s13225-010-0024-6

20. Müller MM, Valjakka R, Suokko A, Hantula J. Diversity of endophytic fungi of single Norway spruce needles and their role as pioneer decomposers. Mol Ecol. 2001; 10: 1801–1810. https://doi.org/10.1046/j.1365-294x.2001.01304.x PMID: 11472547

21. Szink I, Davis E, Ricky KD, Koide R. New evidence for broad trophic status of leaf endophytic fungi of Quercus gambelii. Fungal Ecol. 2016; 22: 2–9. https://doi.org/10.1016/j.bmcfl.2008.01.042

22. Osono T, Hirose D. Effects of prior decomposition of Camellia japonica leaf litter by an endophytic fungus on the subsequent decomposition by fungal colonizers. Mycossience. 2009; 50: 52–55. https://doi.org/10.1007/s10267-008-0442-4

23. Sun X, Guo LD, Hyde KD. Community composition of endophytic fungi in Acer truncatum and their role in decomposition. Fungal Divers. 2011; 47: 85–95. https://doi.org/10.1007/s13225-010-0086-5

24. Koide RT. Endophytic Fungi. eLS. Chichester, John Wiley & Sons, Ltd.; 2019. pp. 1–6. https://doi.org/10.1002/9780470015902.a0027206

25. Fukami T. Historical contingency in community assembly: integrating niches, species pools, and priority effects. Annu Rev Ecol Evol Syst. 2015; 46: 1–23. https://doi.org/10.1146/annurev-ecolsys-110411-160340

26. Hiscox J, Savoury M, Müller CT, Lindahl BD, Rogers HJ, Boddy L. Priority effects during fungal community establishment in beech wood. ISME J. 2015; 9: 2246–2260. https://doi.org/10.1038/ismej.2015.38 PMID: 25797554

27. Kennedy PG, Bruns TD. Priority effects determine the outcome of ectomycorrhizal competition between two Rhizopogon species colonizing Pinus muricata seedlings. New Phytol. 2005; 166: 631–638. Available: https://doi.org/10.1111/j.1469-8137.2005.01355.x PMID: 15819925

28. Lin Y, He X, Ma T, Han G, Xiang C. Priority colonization of Cinnamomum camphora litter by endophytes affects decomposition rate, fungal community and microbial activities under field conditions. Pedobiologia (Jena). 2015; 58: 177–185. https://doi.org/10.1016/j.pedobi.2015.09.001

29. Chan JY, Bonser SP, Powell JR, Cornwell WK. When to cut your losses: Dispersal allocation in an asexual filamentous fungus in response to competition. Ecol Evol. 2019; 9: 4129–4137. https://doi.org/10.1002/eco.3.5041 PMID: 31015993

30. Zheng Y, Xie Y, Xie Y, Yu S. Asexual reproduction and vegetative growth of Bionectria ochroleuca in response to temperature and photoperiod. Ecol Evol. 2021; 11: 10515–10525. https://doi.org/10.1002/eco.3.7856 PMID: 34367593

31. Siletti CE, Zeiner CA, Bhatnagar JM. Distributions of fungal melanin across species and soils. Soil Biol Biochem. 2017; 113: 285–293. https://doi.org/10.1016/j.soilbio.2017.05.030

32. Berry J, Bjorkman O. Photosynthetic response and adaptation to temperature in higher plants. Annu Rev Plant Physiol. 1980; 31: 491–543. https://doi.org/10.1146/annurev.pp.31.060180.002423

33. Range plants of Utah: Gambel Oak. In: Utah State University Extension [Internet]. 2017 [cited 25 Aug 2021]. Available: https://extension.usu.edu/rangeplants/shrubs-and-trees/GambelOak

34. Li HY, Shen M, Zhou ZP, Li T, Wei YL, Lin LB. Diversity and cold adaptation of endophytic fungi from five dominant plant species collected from the Baima Snow Mountain, Southwest China. Fungal Divers. 2012; 54: 79–86. https://doi.org/10.1007/s13225-012-0153-1

35. Walsh G, Knapp M, Rainer G, Peintner U. Colony-P CR is a rapid method for DNA amplification of hyphomycetes. J Fungi. 2016; 2. https://doi.org/10.3390/jf2020012 PMID: 29378929

36. Varga I, Poczai P, Cerna I, Hynonen J. Application of direct PCR in rapid rDNA ITS haplotype determination of the hyperparasitic fungus Sphaeropsis visci (Botryosphaeriaceae). J Korean Physc. 2014; 5: 1–9. https://doi.org/10.1111/j.1469-8137.2013-03569 PMID: 25332869

37. AlShahni MM, Makimura K, Yamada T, Satoh K, Ishihara Y, Takatori K, et al. Direct colony PCR of several medically important fungi using Amplidirect® Plus. Jpn J Infect Dis. 2009; 62: 164–167. PMID: 19305063

38. White TJ, Bruns TD, Lee SB, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR Protocols—a Guide to Methods and Applications. San Diego: Academic Press; 1990. pp. 315–322.

39. Kuraku S, Zmasek CM, Nishimura O, Katoh K. eLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. Nucleic Acids Res. 2013; 41: 22–28. https://doi.org/10.1093/nar/gkt388 PMID: 23677614
40. Katoh K, Rozewicki J, Yamada KD. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform. 2018; 20: 1160–1166. https://doi.org/10.1093/bib/bbx108 PMID: 28968734

41. Maddison WP, Maddison DR. Mesquite: a modular system for evolutionary analysis. Version 3.70. 2021. Available: http://www.mesquiteproject.org.

42. Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res. 2016; 44: W232–W235. https://doi.org/10.1093/nar/gkw256 PMID: 27084950

43. R Core Team. R: A language and environment for statistical computing. Vienna, Austria, Austria: R Foundation for Statistical Computing; 2020. https://doi.org/10.1007/978-3-540-74686-7

44. Minitab L. Minitab v. 18. State College, PA, USA: Minitab, LLC; 2017.

45. Systat Software. SigmaPlot for Windows, Version 11.0. Palo Alto, CA, USA: Systat Software; 2008.

46. Seas C, Chaverri P. Response of psychrophilic plant endosymbionts to experimental temperature increase: Psychrophilic symbiotic fungi. R Soc Open Sci. 2020; 7. https://doi.org/10.1098/rsos.201405 PMID: 33489283

47. Hutchinson G. Concluding remarks. Cold Spring Harbor Symposium Quantitative Biology vol 22. 1957. pp. 415–427.