Warming stimulates sporulation rates and alters community structure of litter-associated fungi in streamside channel experiment

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Warming stimulates sporulation rates and alters community structure of litter-associated fungi in streamside channel experiment

May 2021

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Submitted in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science In the HTC Honors College at Coastal Carolina University

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Abstract

Fungi, such as aquatic hyphomycetes, are important decomposers of plant litter in temperate forested headwater streams providing energy and nutrients for the higher trophic levels in these ecosystems. According to current climate change predictions, stream water temperatures are expected to rise significantly in the near future. This project addressed the effects of temperature on sporulation rates and community structure of aquatic hyphomycetes on substrates of different carbon quality (maple and rhododendron leaf litter and wood veneers). The experiment was conducted in streamside channels fed with stream water (ambient, +2°C and +4°C) and set up to mimic small streams. We found that temperature increases did cause changes to the fungal assemblages as some species became less common and others increased their relative abundances at higher temperatures. Sporulation rates of aquatic hyphomycetes were found to be affected by temperature only on rhododendron leaf litter but not on maple leaf litter or wood veneers. The temperature sensitivity of sporulation on rhododendron appeared to be very high with estimates of apparent activation energy, $E_a$, of >2.63 eV, being higher than canonical estimates for respiration (0.65 eV). Sporulation rates on rhododendron also strongly correlated with both fungal growth rate and production suggesting tight coupling of reproductive output and vegetative growth. Our findings suggest that the water temperature increases caused by climate change may have a stimulating effect on sporulation rate of aquatic hyphomycetes in temperate headwater streams during colder months when temperatures do not exceed 18°C as well as affect the fungal community structure and potentially stream ecosystem functioning.
**Introduction**

Fungi are found in all terrestrial and aquatic habitats world-wide and play a critical, yet often overlooked, role in ecosystem functioning. In aquatic ecosystems especially, fungi are key decomposers of plant matter, making the nutrients in leaves, wood, fruit, etc. accessible for the higher trophic levels (Gessner et al. 2007). Aquatic environments are particularly favorable for the growth and activity of fungi because of the constant water availability, and the often-abundant supply of dissolved nutrients (Gessner et al. 2007). Temperate forested headwater streams receive most of their energy and carbon from the microbial breakdown of terrestrial plant litter following the autumn litter fall (Ferreira et al. 2013). Decomposition of this litter often supports stream food webs throughout the year.

Plant litter is colonized by aquatic hyphomycetes through the attachment of spores. Spores are released from litter-associated fungal mycelium, dispersed by water and eventually impact the surface of another piece of plant litter (leaves or wood), becoming trapped (Gessner et al. 2007). Once spores are established, growth and maturation of fungal biomass on the litter is rapid and new spores – called conidia – can be released in as little as 6 days after colonization (Gessner et al. 2007). Fungal colonization and decomposition of litter enhances the nutritional value of the litter for the next level of consumers, such as aquatic invertebrates (Canhoto et al. 2016).

Some climate change predictions suggest global air temperature increase of up to 4°C by 2100 (Collins et al. 2013). Temperatures in streams are expected to follow the general air temperature trends. Temperature changes are predicted to be more pronounced in the higher latitudes or altitudes, such as in boreal and temperate headwater or mountain streams (Canhoto et al. 2016). Litter decomposition in such streams is a process that can be sensitive to changes in
environmental conditions, such as temperature. These changes may affect fungal communities and their activities – growth, production, sporulation rates and others (Gessner et al. 2007, Ferreira et al. 2013). Bärlocher et al. (2013) found that the projected increase in water temperature (+3-5°C) due to global climate change may significantly modulate stream fungal activity. Temperature changes can also affect the fungal assemblage structure.

Studies on the effect of temperature changes on aquatic hyphomycetes growth are sparse and the ones that exist are lab microcosm experiments which may not be representative of the responses of aquatic hyphomycetes in the field. Chauvet and Suberkropp (1998) found inconsistent responses from various species when temperatures were changed in the microcosm experiments. A few species responded to temperature increases with large increases in growth rate. Some species’ growth rates remained relatively unchanged with increased temperatures and other species’ growth rates increased to a threshold temperature before plummeting (Chauvet and Suberkropp 1998). Thus, the effects of temperature changes on growth rates of aquatic hyphomycetes can be species-specific.

Fungal production is the generation of new biomass over time and is also likely influenced by temperature increases. Increases in production rates can have both beneficial and detrimental effects on stream ecosystems (Findlay 2010). Higher production rates will generate fungal biomass faster, providing food for organisms that feed on fungi. However, increases in fungal production may also deplete the standing stock of the leaf litter the fungi are colonizing through stimulation of litter decomposition. This can reduce available food for shredders. Gessner et al. (2007) found that fungal production is seasonal and peaks in the summer, which they inferred was in response to elevated temperatures in the summer in temperate streams.
Temperature increases, whether as a result of climate change or because of other reasons, could stimulate fungal activity in forested, temperate headwater streams.

Aquatic hyphomycetes release large numbers of conidia from leaf litter, which are then transported downstream to colonize new substrates. Sporulation rate can be affected by temperature and other factors such as litter quality, alkalinity, pH and nutrient availability (Gessner et al. 2007). Many studies have found that as temperature increases sporulation rates increase as well (Gessner et al. 2007, Fernandes et al. 2009, Canhoto et al. 2016,). Additionally, Chauvet and Suberkropp (1998) found that sporulation rates responded similarly to growth rates when temperature was increased. Some species had very large increases in sporulation rates with temperature increases, while sporulation rates of other species decreased, and yet for some species, sporulation rates increased and then decreased after the optimum temperature was exceeded (Chauvet and Suberkropp 1998). However, it is known that sporulation can be stimulated by unfavorable conditions as a means of escaping to a more favorable environment (Chauvet and Suberkropp 1998).

In order to explore the possible effects of temperature on the sporulation and community structure of aquatic hyphomycetes, an experiment was conducted in heated streamside channels. The experiment was designed to assess the effects of increased water temperature (ambient to +4°C) on fungal sporulation rates and community structure on three different substrates (maple and rhododendron leaf litter and wood veneers). This experiment was performed at the Coweeta Hydrologic Laboratory site in North Carolina, where the streamside channels were set up to mimic the natural conditions of the nearby Shope Fork stream. The substrates were placed into the streamside channels, colonized by the Shope Fork stream fungal assemblages, and then periodically sampled over 99 days. In this experiment, we hypothesized that: (1) temperature
increases will affect sporulation rates and community structure of aquatic hyphomycetes and (2) the effects of temperature will differ among leaf litter species and wood.

**Methods**

*Streamside Channels*

This experiment was conducted at the Coweeta Hydrological Laboratory site in Macon County, North Carolina. The twenty streamside channels were previously set up and were available for use in this experiment. Channels were constructed out of 4m by 0.15m rain gutters and were retrofitted with 10-cm wide plastic inserts. Header tanks feeding into the channels were equipped with heaters and temperature controllers by collaborators from the University of Alabama. Stream water was pumped from Shope Fork into a primary tank and then distributed to the secondary header tanks. These header tanks heated the water from ambient temperature to +1°C, +2°C, +3°C, or +4°C. Each of the five temperature treatments (ambient included) was replicated four times for a total of 20 channels (Figure 1). In this experiment, only data from the ambient, +2°C and +4°C treatments were used. Water flow through the channels was set at 0.1L/s, which mimics the flow of small streams nearby. Water temperature for each channel was monitored throughout the experiment with HOBO temperature loggers made by Onset Computer Corp. (Bourne, MA).

*Litter Bags and Sampling*

This experiment used fine mesh litter bags made from window screening (1 mm mesh size, 21cm by 9cm bags). The autumn-shed leaves of red maple (*Acer rubrum*) and rhododendron (*Rhododendron maximum*) were collected from the Coweeta watershed. Leaf litter was dried at room temperature, weighed, and enclosed in litter bags. Maple leaf bags contained
5-6 leaves (~1.5 g), and the rhododendron bags contained 3 leaves (~2g). Additionally, there were bags of wood veneers (*Quercus alba*) which contained approximately 60 cm² of weighed material. Litter bags of each substrate type were evenly distributed between all of the different temperature treatment channels and fungal parameters were monitored for approximately three months. The experiment began on March 1st, 2019 and ended on June 8th, 2019 with a total of 4 sampling dates (days 21, 42, 63, and 99). For this thesis, data from days 21 and 63 were used for the maple and rhododendron leaf litter while wood veneer data was from days 63 and 99 (i.e. early and late stages of decomposition for both substrate types). During sampling dates litter bags were retrieved and 5-10 leaf discs (or 1-cm² wood squares) were cut out for each type of analysis (sporulation rate and community structure, fungal biomass, growth rate, production and other microbial parameters not addressed here, and a set was used to estimate litter ash-free dry mass (AFDM)). Samples were collected and analyzed by a graduate student (Kaity Ackerman) at Coastal Carolina University. She also provided fungal biomass and litter AFDM data for our estimates of sporulation per unit of fungal biomass or per unit of AFDM. The fungal growth rate and production data collected by Kaity Ackerman was also used for regression against sporulation rates that we determined in this project.

On each sampling date, subsamples of 10 leaf discs or 6 cm² of wood veneer material were incubated for 48±3 hours (time recorded precisely) in 30 mL of filtered stream water in 75-mL sterile tissue culture flasks with vented caps to induce sporulation of aquatic hyphomycetes. Incubations were performed on shakers in environmental chambers at temperatures corresponding to those in streamside channels (i.e., ambient, ±2°C and +4°C treatments). After incubation, plant material was removed while spore suspensions were preserved with 1.5 mL of formalin/Triton X-100 solution (final concentrations ca. 1.8% formalin and 0.025% Triton X-
and stored at room temperature until analysis. To determine sporulation rates of aquatic hyphomycetes and community structure based on spore identities, conidia were captured by vacuum filtration of preserved samples (Bärlocher 2005). A total of 31.5 mL was available for filtering for each sample, however, the amount filtered was not consistent and ranged from 3 mL to 25 mL per filter depending on expected concentration of conidia. The volumes were recorded, and data was standardized despite variable volumes filtered. To capture conidia, the desired volume was passed through a membrane filter, then 6-8 drops (enough to cover the entire surface of the filter) of trypan blue stain was added. The stain was allowed to sit for ~10 seconds before sucking it through the membrane filter as well. The membrane is then removed from the vacuum apparatus and placed conidia-side up on a microscope slide. A drop of stain was placed in the center of a coverslip which was then placed over the membrane filter. After waiting at least 15 minutes for the staining to develop, conidia were counted and identified.

The counting and identification of conidia is a long and tedious process (Baerlocher 2005). The 10× microscope objective was used for counting, although occasionally the 40× objective was necessary to confirm identification of conidia. Conidia were identified based on their shape and size (Gulis et al. 2020). For each membrane, at least 7 fields of view were required to be counted, and the total number of conidia counted was at least 200 to ensure statistically significant results. However, conidia densities for the wood veneers were so low that, despite counting ~50 fields per filter, often less than 100 conidia were counted per sample. Raw counts of conidia per filter (including species identity data to calculate relative abundances of fungal taxa) were used for further calculations and analyses.
Statistical analyses

The number of conidia counted, number of fields scanned, volume of conidia suspension preserved, the aliquot filtered, time of incubation and AFDM of sample were used to calculate the sporulation rate per unit of litter ash-free dry mass (no./g AFDM/d) following Gulis and Bärlocher (2017). Sporulation rates were further standardized per unit of fungal biomass (no./mg FB/d). These sporulation rates were log-transformed for analyses using general linear model (GLM). Due to differences in sampling schedule between leaf litter and wood veneers, we also ran separate GLM models for leaf litter and wood. For leaf litter, models included temperature, substrate, sampling day (each as categorical variables) and a temperature*substrate interaction term. For wood veneers, only temperature and sampling day were included as categorical variables. IBM SPSS Statistics 26.0 was used for GLM. To estimate temperature sensitivity (apparent activation energy, $E_a$) of sporulation rates, linear regressions were performed with In-transformed sporulation rates per unit of fungal biomass and a reversed temperature parameter ($1/kT$) following the conventions of standard Metabolic Theory of Ecology (MTE) plots (Brown et al. 2004, Yvon-Durocher et al. 2010, 2012). Linear regressions were also run for sporulation rates against fungal growth rates and fungal production.

Effects of temperature on fungal community structure were analyzed by entering relative abundances for each substrate and temperature into the PAST4.05 statistical software, where a 2-way PERMANOVA using the Bray-Curtis dissimilarity indices and 9999 permutations was performed with temperature and substrate as the variables. Additionally, an ordination was developed using non-metric multidimensional scaling (nMDS) (Bray-Curtis dissimilarity indices, 9999 permutations, 3 dimensions).
Results

Community Structure

Average relative abundances of aquatic hyphomycetes from different temperature treatments, substrates and sampling dates are summarized in Table 1. Analysis of the relative abundance data found that both temperature and substrate had statistically significant effects on the community structure of aquatic hyphomycetes (Figure 2, PERMANOVA, temperature: F=2.36, p=0.019, substrate: F=3.58, p=0.001). Some dominant species of aquatic hyphomycetes clearly increased their contribution to total conidia pool at higher temperatures, especially at later stages of decomposition (e.g., *Lunulospora curvula* on maple and rhododendron leaf litter), while the importance of some species declined with temperature increases (e.g., *Tricladium chaetocladium* on all substrates). Additionally, the 3D nMDS ordination based on the relative abundance data also showed clear groupings of maple and rhododendron samples by substrate type and sampling day, and to a lesser extent according to temperature (Figure 3, top panel, only axes 1 and 2 are shown). Wood veneer samples showed no clear pattern, so on the bottom panel of Figure 3, veneers were removed for clarity. Leaf litter samples were then separated according to sampling day along Axis 1 (left to right) and according to substrate along Axis 2. For later stages of colonization (d. 63 for both maple and rhododendron leaf litter clusters), fungal communities were also arranged by temperature (right to left from ambient to +4°C treatment).

Sporulation Rates

For leaf litter samples, sporulation rate of aquatic hyphomycetes per g of AFDM (Figure 4) was affected by substrate type (GLM, F_{1,41}=63.44, p<0.0001), but not temperature (F_{2,41}=1.36, p=0.268); the interaction term (temperature*substrate) was not significant (p=0.158). The same pattern was found for sporulation rate expressed per g of fungal biomass (substrate: F_{1,41}= 27.99,
p<0.0001; temperature: F_{2,41}=1.90, p=0.162); the interaction term (temperature*substrate) was also not significant (p=0.127). Interestingly, when rhododendron data was analyzed separately, the effect of temperature became statistically significant (p<0.004),

The effect of temperature on sporulation rates on wood veneers either per g of AFDM or per g of FB was not significant (GLM, F_{2,19}=1.60, p=0.228 and F_{2,19}=2.21, p=0.137, respectively).

Since sporulation rates were clearly affected by temperature only on rhododendron leaf litter (Figure 4, GLM above), we estimated temperature sensitivity of sporulation rate by linear regression of ln-transformed data per unit of FB against 1/(k*T) parameter (Figure 5). On day 21, estimate of apparent activation energy, $E_a$ (=slope of regression), was 3.73±2.74 eV (mean±95% CI, $R^2=0.479$, p=0.013), while on day 63, $E_a$ was estimated at 2.63±2.38 eV ($R^2=0.378$, p=0.033).

Sporulation Rates vs. Growth Rates and Fungal Production

We also explored relationships between sporulation rates of aquatic hyphomycetes on rhododendron leaf litter and parameters describing vegetative growth, i.e. fungal growth rate and production at early stages of decomposition (day 21). We found strong statistically significant relationships for both fungal growth rate and production (Figure 6, linear regressions, $R^2=0.783$, p=0.0001 and $R^2=0.811$, p=6.46×10^{-5}, respectively).

Discussion

In this experiment, both temperature and substrate type had significant effects on community structure of aquatic hyphomycetes. In addition to PERMANOVA results (Figure 2), mMDS ordination provided quite clear separation of samples by substrate, collection date and in
some cases by temperature, especially when wood veneer samples were removed for clarity (Figure 3, bottom panel). These clear groupings suggest that the community structure of aquatic hyphomycetes is controlled primarily by substrate type as well as duration of colonization of the substrate or the stage of decomposition suggesting successional changes. For the rhododendron and maple leaf litter, the relative abundance of *Lunulospora curvula* increased with increasing temperature, especially on day 63, whereas the relative abundance of *Articulospora tretacladia* and *Tricladium chaetocladium* decreased at higher temperatures (Figure 2). *L. curvula* is known to be a warm-water species (with a temperature optimum around 25°C) while *Flagellospora curvula*, another species observed in this thesis, was determined to be a cold-water species (with a temperature optimum around 15°C) (Ferreira et al. 2014). Duarte et al. (2013) reported similar results from the microcosm study where *L. curvula* had elevated growth rates at higher temperatures whereas the growth rate of *A. tretacladia* was depressed. Additionally, Ferreira et al. (2014) suggested that the effects of temperature can be quite different for individual species of aquatic hyphomycetes due to varying temperature optima. When a thermal optimum is exceeded, fungal activity including biomass accrual and spore production would decline. This indicates that water temperature increases as a result of climate change could result in changes to fungal assemblages as species that prefer colder water are outcompeted by species that do well in warmer water.

GLM analyses found that, for maple and rhododendron leaf litter, sporulation rates of aquatic hyphomycetes were significantly affected by substrate, as expected. However, sporulation rates were not significantly affected by temperature in our GLMs for leaf litter and wood. Only when rhododendron data was run separately, the effect of temperature became significant. The increased sporulation rates on rhododendron at higher temperatures are also
obvious by examining Figures 4 and 5 and is further confirmed by regression analysis. Our estimates of temperature sensitivity of fungal sporulation on rhododendron on day 21 and 63 with $E_a$ of at 3.73 and 2.63 eV, respectively, appear to be very high compared to canonical value for the activation energy of respiration (0.65 eV) (Brown et al. 2004, Yvon-Durocher et al. 2012), suggesting that at least on rhododendron sporulation rates can be very sensitive to elevated temperatures. Higher water temperatures were found to increase fungal activity such as growth and reproduction in some earlier studies (Ferreira and Chauvet 2011b, Ferreira et al. 2014). It was also suggested that this stimulatory effect is likely to occur regardless of nutrient availability in the water, i.e., both oligotrophic and eutrophic streams are expected to see increases in fungal growth and reproduction with increasing temperatures (Ferreira and Chauvet 2011b). Ferreira and Chauvet (2011a) also found that these increased levels of growth and reproduction would increase the rate of leaf litter decomposition. This can have serious consequences for the temperate headwater stream ecosystems because faster litter decomposition could lead to substantial food depletion for the higher trophic levels (Ferreira and Chauvet 2011a).

As fungal growth rate and production increase, it is reasonable to assume that fungal reproduction would also increase. Indeed, we observed a very strong relationship between reproductive output from rhododendron leaf litter and parameters of vegetative growth (Figure 6). Gessner and Chauvet (1997) used ergosterol as a biomarker for fungal biomass in samples from lab microcosms and from the field. They found that as ergosterol content increased, indicating an increase in litter-associated fungal biomass, sporulation rates also increased (Gessner and Chauvet 1997). Fitzgerald and Gulis (unpublished) also found a very strong relationship between sporulation rates of aquatic hyphomycetes and fungal production.
Conclusions

In this project, it was hypothesized that: (1) temperature increases will affect sporulation rates and community structure of aquatic hyphomycetes and (2) the effects of temperature will differ among leaf litter species and wood. We found that temperature increases did cause changes to the fungal assemblages in the experimental streamside channels as some species became less common and others increased their relative abundances at higher temperatures. Sporulation rates of aquatic hyphomycetes were found to be affected by temperature only on rhododendron leaf litter but not on maple leaf litter or wood veneers. The temperature sensitivity of sporulation on rhododendron appeared to be very high with estimates of apparent activation energy, $E_a$, of $>2.63$ eV, being higher than canonical estimates for respiration ($0.65$ eV). Sporulation rates on rhododendron also strongly correlated with both fungal growth rate and production suggesting tight coupling of reproductive output and vegetative growth in this case. Our findings suggest that the water temperature increases caused by climate change may have a stimulating effect on sporulation rate of aquatic hyphomycetes in temperate headwater streams during colder months when temperatures do not exceed $18^\circ$C as well as affect the fungal community structure and potentially stream ecosystem functioning.
Literature Cited


Warming alters the metabolic balance of ecosystems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365, 2117-2126.

Table 1. Average relative abundances (%) of aquatic hyphomycetes associated with decomposing plant litter in streamside channels. Column labels designate temperature treatment (0 = ambient, 2 = +2°C, 4 = +4°C), substrate type (M = maple leaf litter, R = rhododendron leaf litter, V = wood veneers) and sampling date (21 and 63 for leaf litter, 63 and 99 for wood veneers).

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**Figure 1.** *Top panel:* Experimental set up 20 streamside channels (5 temperature treatments × 4 replicates). *Bottom panel:* 10 of the streamside channels (two replicates of each temperature treatment: 0 (ambient control), +1, +2, +3, and +4°C) showing ±1°C differences in temperature (thermal imaging); photo credit: J. Benstead, University of Alabama.
Figure 2. Relative abundances of dominant species of aquatic hyphomycetes associated with decomposing maple and rhododendron leaf litter and wood veneers at ambient, +2 and +4 °C temperature treatments on days 21 and 63 (63 and 99 for veneers) of the experiment in streamside channels.
**Figure 3.** Top panel: Ordination of samples from different temperature treatments, substrates, and sampling dates by non-metric multidimensional scaling (nMDS) based on fungal community structure (relative abundances of fungal species based on spores were used to calculate Bray-Curtis dissimilarity indices). Sample codes: 0=ambient temperature, 2=+2°C treatment, 4=+4°C treatment, M=maple leaf litter, R=rhododendron leaf litter, V=wood veneers. 21, 63, and 99 denote sampling days. Bottom panel: Ordination with wood veneer data omitted for clarity.
Figure 4. Sporulation rates of aquatic hyphomycetes associated with decomposing maple and rhododendron leaf litter and wood veneers at ambient, +2 and +4 °C temperature treatments on days 21 and 63 (63 and 99 for veneers) of the experiment in streamside channels. *Left panels* show sporulation rates per unit of leaf litter ash-free dry mass (AFDM) and *right panels* are scaled per unit of fungal biomass (FB).
Figure 5. Temperature sensitivity of fungal sporulation rates associated with rhododendron leaf litter on days 21 and 63. The dotted line indicates the slope corresponding to the activation energy of respiration (0.65 eV). Rhododendron, d. 21: Sporulation rate = -3.732*(1/(k*T)) + 154.94, R²=0.479, p=0.013. Rhododendron, d. 63: Sporulation rate = -2.633*(1/(k*T)) + 112.65, R²=0.378, p=0.033.
Figure 6. Relationships between sporulation rate of aquatic hyphomycetes and litter-associated fungal growth rate (top panel) and fungal production (bottom panel) on rhododendron leaf litter on day 21. Sporulation vs. Growth rate: $\log_{10}(\text{Sporulation rate}) = 1.88 \log_{10}(\text{Growth rate}) + 4.07$, $R^2=0.783$, $p=0.0001$. Sporulation vs. Production: $\log_{10}(\text{Sporulation rate}) = 1.60 \log_{10}(\text{Production}) + 2.24$, $R^2=0.811$, $p=6.46 \times 10^{-5}$. 