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Effects of Temperature on Activity of Aquatic Hyphomycetes: A Microcosm Study

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Abstract

Predicted increases in temperature under climate change scenarios are expected to affect not only atmospheric and water temperatures, but also the rate of heterotrophic activity and carbon dynamics and retention in ecosystems. The magnitude of the increase in metabolic activity of living organisms with increased temperature can be predicted by the Metabolic Theory of Ecology, however, multiple factors can cause deviations from simple predictions. The goal of this study is to assess the temperature sensitivity of aquatic hyphomycetes and fungi-mediated leaf litter decomposition by following responses to temperature of fungal biomass accrual, respiration and decomposition rates in laboratory microcosms simulating stream conditions. We found that fungal parameters and leaf litter decomposition rates often do not follow simple predictions of the MTE. We observed much greater temperature sensitivity of microbial parameters at low temperatures (5-10° C interval) and more moderate sensitivity at higher temperatures (15-20° C interval). These trends may have important implications for stream ecosystems under climate change scenarios since the bulk leaf litter input and the highest microbial activity coincide with the coldest season (autumn-winter) in temperate streams.

Introduction

A 3-5° C increase in temperature is predicted over the next 100 years (Yvon-Durocher et al., 2010). Predicted increases in temperature are expected to affect not only atmospheric and water temperatures, but also the rate of heterotrophic activity and carbon dynamics and retention in ecosystems. A very large amount of carbon is stored within the world's soils, and the processes that balance the inputs and output are temperature

dependent. This means as a result of an increase in temperature, a shift will occur releasing more carbon dioxide into the atmosphere (Davidson and Janssens, 2006) since decomposition of organic matter is more sensitive to changes in temperature than the primary production (Kirschbaum, 1995). The result of this increased release of CO_2 is dependent upon the mean annual temperature of the area in which the warming is occurring. There are greater amounts of carbon stored in cooler soils in comparison to warmer soils, in terms of absolute amounts on a global scale (Kirschbaum, 1995). An additional 10% of organic soil carbon would be lost with a 1° C increase in temperature in areas with 5° C mean annual temperature compared to only 3% additional C loss with 1° C increase at 30° C (Kirschbaum, 1995).

Not only respiration but also gross primary production (GPP) and microbially mediated nutrient cycling can be affected by temperature in aquatic ecosystems including streams. When whole-stream metabolism and nutrient cycling were studied in streams with different temperatures, GPP, ecosystem respiration (ER) and nutrient uptake were significantly higher in warm streams in comparison to cold streams (Demars et al., 2011). It was also suggested that an increase in temperature could reduce the downstream transport and availability of nutrients in warmer months with the concurrent increased release of carbon dioxide into the atmosphere due to stimulated ER (Demars et al., 2011). The availability of nutrients within an ecosystem is essential for all biological processes. Whole-stream nutrient addition experiments demonstrated stimulation of fungal biomass accrual, fungal production and litter-associated microbial respiration (Gulis and Suberkropp, 2004; Suberkropp et al., 2010). The quality (including N and P content) of leaf litter also affects the rate of microbial activity, however, to a lesser extent than water

temperature (Ferreira and Chauvet, 2011). Metabolic activity at elevated temperatures is generally more nutrient limited than at lower temperatures (Ferreira and Chauvet, 2011). The effects of temperature may be greater than the effects of nutrients, with decomposition rates increasing by more than 30% with a 5° C increase in temperature (Ferreira and Chauvet, 2011).

Fungi, such as aquatic hyphomycetes, are key players in stream ecosystems that decompose organic matter (e.g. leaf litter and wood) and emit carbon dioxide into the atmosphere as a result of their respiratory activities (Suberkropp et al., 2010). However, the effects of temperature on these fungi are poorly understood. In general, the magnitude of the increase in metabolic activity of living organisms with increased temperature can be predicted by the Metabolic Theory of Ecology (MTE, Brown et al., 2004). While the MTE can make predictions of the rate of future metabolic activity in response to warming in theory, there are factors that may cause actual results to differ. In both terrestrial and aquatic ecosystems microorganisms may be able to acclimate to temperature changes, leading to lower rates of activity than predicted (Crowther and Bradford, 2013). The magnitude and rate at which organisms can thermally acclimate can be species-specific and can result in shifts in microbial community structure. Thermal acclimation may also result in a lower growth efficiency and decreased fungal decomposition of organic matter, resulting in a lower carbon dioxide output than expected (Crowther and Bradford, 2013). Regardless of acclimation, temperature changes can result in shifts in community structure since fungal species may have different temperature optima. Significant differences in community structure were observed at varied temperatures (Dang et al., 2009). Daily temperature oscillations also had unpredicted effects on microbial activity

(Dang et al., 2009). Thus, when studying microorganisms and how they respond to changes in temperature it is necessary to study individual species as well as multispecies assemblages, as communities may reassemble and change predicted outcomes (Chauvet and Suberkropp, 1998).

Even though fungi dominate microbial communities associated with decaying plant litter in streams, bacteria may be important in sediments and associated with fine particulate organic matter (FPOM). In streams, Sand-Jensen et al. (2007) found that temperature stimulated respiration by heterotrophic bacteria to a greater extent than primary productivity. With the metabolism of most bacteria increasing with increasing temperature, the growth efficiency actually decreased with increasing temperature (Sand-Jensen et-al., 2007). Both bacterial and fungal metabolism increase with increasing temperature, however, they are probably affected in a similar way (Suberkropp and Weyers, 1996). Nevertheless, fungal carbon production in most aquatic ecosystems is much greater than bacterial carbon production, with recorded values of fungal production being up to 1 or 2 orders of magnitude greater than bacterial production (Sand-Jensen et al., 2007, Suberkropp et al., 2010).

Along with respiratory CO_2 losses, carbon cycling in aquatic ecosystems also involves the breakdown of coarse particulate organic matter (CPOM) and the production of FPOM (Tant et al., 2015). Aquatic hyphomycetes are known as the primary drivers of this transformation, which is stimulated by increased nutrients and potentially temperature (Tant et al., 2015). The litter quality, which is affected by carbon dioxide concentration during the plant growth, also affects microbial activity associated with submerged decaying plant litter (Tuchman et-al., 2002). Leaves that were produced under

elevated CO_2 concentrations were not able to support high bacterial activity, while fungal activity was unaffected (Tuchman et-al., 2002). Thus, it is challenging to predict the outcomes of the interplay between litter quality changes and temperature increases under climate change scenarios.

The goal of this study is to assess the temperature sensitivity of aquatic hyphomycetes and fungi-mediated plant litter decomposition by following responses to temperature of fungal biomass accrual, respiration and decomposition rates in laboratory microcosms simulating stream conditions.

Materials and Methods

Fungal Cultures

Several isolates of aquatic hyphomycetes were used for experiments in laboratory microcosms (Table 1). These fungi were originally isolated from streams with different temperature regimes. *Tricladium alaskense* (a cold water species) was isolated from a stream in the vicinity of Toolik Lake Field Station, AK; *Aquanectria penicilliodes* (a warm water species) was isolated from Firehole River at Biscuit Basin, Yellowstone NP, WY; and the temperate species used for the 6-species assemblage treatment were all isolated from streams at Coweeta Hydrologic Lab, NC. Cultures of single-spore isolates of aquatic hyphomycetes were sub-cultured and grown on 1% malt extract agar and LCA medium at 15° C in Petri dishes. After 3-wk incubation, strips of agar taken from the leading edge of the growing colonies were placed in 25 mL of sterile nutrient solution and placed on a shaker to induce sporulation. Nutrient solution contained inorganic N and P added as sterile stocks of $NaNO_3$ and KH_2PO_4 at final concentrations of 2.0 mg/L of

NO_3-N and 0.275 mg/L of PO_4-P (N:P molar ratio 16:1), as well as 0.25 g/L of 3-(N-morpholino) propanesulfonic acid (MOPS), pH 6.5. The same nutrient solutions were used for leaf litter incubations in microcosms (see below). After four days, subsamples of 0.5 to1.5mL of conidia suspensions were obtained and filtered through 0.2-μm membrane filters, conidia were stained and counted with a compound microscope to determine conidia concentrations. Appropriate aliquots of conidia suspensions were then used to inoculate microcosms with sterile leaf disks (see below) keeping conidia load at 5,000 conidia per microcosm for monocultures (*T. alaskense* and *A. penicillioides*) and ~833 conidia of each species per microcosm for 6-species treatment.

Experimental microcosms and sampling

Tissue culture flasks (250mL, with membrane-filter caps) filled with 100 mL of sterile nutrient solution (see above) and stocked with leaf disks were used as microcosms to simulate stream conditions. The leaf disks used in this experiment (red maple, *Acer rubrum*) were leached, dried at 60° C for at least 24 h, weighed in sets, and then sterilized in the autoclave at 121° C for 20 minutes. Each set of 45 disks was added aseptically to a tissue culture flask, filled with a sterile nutrient solution and allowed to pre-soak for one day. Microcosms were then inoculated with appropriate aliquots of fungal spore suspensions as described above. Thus, we had microcosms with three fungal treatments (*T. alaskense, A. penicillioides* and the 6-species assemblage) that were incubated at 5, 10, 15, and 20° C on a shaker to provide turbulence and aeration and then harvested on d. 12, 27 and 31. All treatments were set in triplicate. Every three days, the nutrient solutions in the experimental flasks were aseptically evacuated and replaced with fresh sterile nutrient solutions to maintain nutrient concentrations. In addition, prior to each

solution change, aliquots of solutions with fungal spores were preserved for analyses (not discussed in this thesis). On each of the sampling days (12, 27 and 31), sets of leaf disks were sampled for microbiological analyses (see below) and to determine their dry mass.

Fungal Respiration

Respiration measurements were performed with an Orion 5-Star Plus multimeter equipped with a BOD probe to measure dissolved oxygen concentration. Leaf disks to be used for respiration were removed from the experimental flasks (8-18 disks depending on sampling date and temperature) and placed in the screw-cap vials (26 or 30 mL) filled with fresh sterile nutrient solution of the respective temperature, sealed and incubated for 2-8 h at 5, 10 15 or 20° C. Blanks of the same temperatures were also filled and initial O_2 concentrations (mg O_2/L) and temperatures measured. Final oxygen concentration and temperature were recorded after several hours of incubation. Fungal oxygen uptake rate (mg O_2 g^{-1} leaf DM h^{-1}) in sealed vials was estimated based on the change in O_2 concentration, vial volume, mass of leaf disks and incubation time; values were blank-corrected.

Fungal Biomass

Fungal biomass was determined from ergosterol concentrations of leaf disks, obtained when samples were sacrificed on days 12, 27 and 31, by lipid extraction and quantification with HPLC. Leaf disks to be used for ergosterol extraction were stored in 5 mL of methanol in the freezer until the day of extraction. The ergosterol associated with leaf disks was extracted using the liquid-to-liquid extraction technique (Gulis and Suberkropp 2006). One mL of methanol was used to redissolve final lipid extracts in a sonication bath and filtered with a 0.2-μm syringe filter (PTFE, National Scientific,

Rockwood, TN) into HPLC vials. A Shimadzu 10-VP HPLC system equipped with a reverse phase C18 column (Kinetex 2.6 μm core-shell, 4.6 x 150 mm, Phenomenex, Torrance, CA) was run isocratically at 0.75 mL min^{-1} of methanol at 35° C; ergosterol in 20-μL injections of lipid extracts was detected at 282 nm and eluted at 5.0 min. HPLC areas were converted to ergosterol concentrations using external ergosterol standards (Acros Organics, Morris Planes, NJ) and a conversion factor of 5.5 mg ergosterol per g of fungal dry mass.

Leaf Mass Loss and Decomposition Rates

Mass of leaf disks on d. 12 were obtained by drying the samples in an oven (60° C for at least 24 h) and weighing to 0.0001 g. On d. 27 and 31, subsamples of leaf disks were freeze-dried and then weighed. Mass loss of leaf litter and percentage of mass remaining were calculated by taking into account the number of disks weighed and the initial and final mass of disk sets. Decomposition rates (k, d^{-1}) were estimated using the negative exponential model (Webster and Benfield 1986).

Results

Fungal biomass accrual was generally stimulated by increasing incubation temperatures for a given species/assemblage (Figure 1). A noteworthy exception is a decrease in fungal biomass from 15 to 20° C seen in almost all samples on all days. The 6-species assemblage fungal biomass values were similar between days, with the decrease between 15 to 20° C becoming more prominent at later stages of decomposition (Figure 1). Fungal biomass was generally lower in monocultures. It was slightly higher in *T. alaskense* than *A. penicillioides* on d. 27 and 31.

Respiration rate per gram of dry mass generally increased with increased incubation temperatures within a species/assemblage for each sample day (d. 12, 27, 31), with the notable exception of the 6-species assemblage respiration rates on d. 27 and 31 (Figure 2). The highest respiration rates occurred for the 6-speecies assemblage on d. 12 at 20° C, with measurements from 10 and 15° C being the next highest. After these peaks in respiration rate of the 6-species assemblage occurring on d. 12, subsequent sampling days showed decreasing respiration rates with increasing incubation temperature (Figure 2). Overall trends exhibited by *T. alaskense* and *A. penicillioides* were similar for all sampling days, with minimal respiration rates at 5° C and increasing rates with higher incubation temperatures (Figure 2). Similar to patterns of fungal biomass, respiration rates were higher in *T. alaskense* than *A. penicillioides* on d. 27 and 31. In general, the greatest differences in respiration rates were observed between 5 and 10° C, as they occurred in the 6-species assemblage on d. 12 and in *T. alaskense* and *A. penicillioides* on d. 27 and 31 (Figure 2).

Fungal biomass and respiration rates data were combined to examine the respiration rate per gram of fungal biomass for each species/assemblage and sample day (Figure 3). The highest rates occurred in *T. alaskense* and *A. penicillioides* at 10° C on d. 12. In comparison to the respiration rates per gram of dry mass, respiration rates per gram of fungal biomass showed higher variability and followed a less clear pattern. In general, values were higher on d. 12 indicating higher levels of physiological activity and decreased at later stages of decomposition for all species/assemblage (Figure 3).

Leaf litter mass loss by d. 31 followed patterns similar to other measured parameters being higher at elevated temperatures, with the 6-species assemblage at 10, 15

and 20° C causing >50% leaf mass loss (Figure 4, top panel). *T. alaskense* caused higher leaf mass loss than *A. penicillioides* by the end of the experiment, with the exception of the 5° C treatment. There were no difference in leaf litter mass loss between 15 and 20° C treatments for each monoculture and the 6-species assemblage (Figure 4, top panel).

Leaf litter decomposition rates (k) calculated based on leaf mass loss data from all 3 sampling dates, presented very similar trends (Figure 4, bottom panel). The fastest decomposition rate was observed for the 6-species assemblage at 20° C, with *T. alaskense* closely following. A sharp increase in decomposition rates is present for the 6-species assemblage between 5 and 10° C and between 5 and 15° C for *T. alaskense*. Decomposition rates were generally low and did not vary greatly between temperatures in *A. penicillioides* (Figure 4, bottom panel).

Leaf litter mass remaining was also plotted for each species/assemblage across sampling dates. Overall, each species/assemblage exhibited the expected decreasing trend in leaf mass remaining throughout the duration of the experiment (Figure 5). For each sampling date, the decreased leaf mass remaining with the increased temperature is also evident for all species, especially for later stages of decomposition. The lowest values were observed for the 6-species assemblage on d. 31, with 10, 15, and 20° C treatments being very similar. Minimal mass loss was observed between the start of the experiment and d. 12, with the exception of higher temperatures of the 6-species assemblage. The greatest differences in leaf mass remaining among temperature treatments occurred in *T. alaskense* (Figure 5).

Discussion

Throughout the experiment, the nutrient solution remained standardized with respect to N and P concentrations, which were kept relatively high to prevent nutrient limitation of microbial activity. Dissolved nutrients are known to exert strong control on the rates of microbial activity, however, to a lesser extent than temperature (Ferreira and Chauvet, 2011). It should be noted that aquatic fungi are more likely to be nutrient limited with increasing temperatures (Ferreira and Chauvet, 2011), however, given high N and P concentrations in our microcosms, it is unlikely that nutrient limitation occurred.

Aquatic hyphomycetes are major fungal decomposers of organic matter in stream ecosystems that convert plant litter carbon into their own biomass and emit carbon dioxide to the atmosphere as a result of their respiratory activities (Suberkropp et al., 2010). In our study, fungal biomass accrual was considerably faster for the 6-species assemblage than for monocultures across all temperatures throughout the experiment. This could be explained by the complementarity effect or simply by the possibility that the individual species chosen for the experiment have a slow growth rate or are not very effective leaf decomposers. However, the interspecific comparisons were not the major goal of this study. Interestingly, all fungal treatments showed large increases in fungal biomass accrual between 5 and 10° C treatments, while the differences in fungal biomass were small or non-existent at higher temperatures, demonstrating considerably lower temperature dependence of fungal growth and biomass accrual at these higher temperatures. Thus, it appears that the response of fungal growth to temperature is non-linear within the relatively narrow temperature interval (5-20° C) as suggested by Dang et al. (2009).

Fungal respiration rates observed in this study are comparable to the rates observed for leaf litter associated microbial communities dominated by fungi at respective temperatures (Manning et al., unpublished). The increase in metabolic activity of microorganisms with increased temperature is predicted by the MTE, however, potential deviations may complicate the prediction (Brown et al., 2004). In our experiment, very large increases in fungal respiration rates occurred with temperatures increasing from 5 to 10° C, with the magnitude of the increase greatly exceeding predictions by the MTE. On the other hand, at the highest temperature interval (15 to 20° C), in many cases we found slight or no increase at all in the rate of fungal respiration, contrary to the simple predictions of the MTE that suggest monotonous exponential increase in respiration rates across the range. Clearly, the pattern does not hold true for relatively cold-adapted aquatic hyphomycetes (stream temperatures rarely exceed 20° C in temperate climates). Similar non-linear, non-exponential, but rather sigmoid response of fungal growth rate to temperature with a much greater response at lower (5 to 10° C) than at higher temperatures (15 to 20° C) has been observed by Dang et al. (2009) in their experiments with pure fungal cultures.

Our estimates of respiration rates per unit of fungal biomass that were supposed to give a more direct insight into the effects of temperature on fungal physiology did not follow an expected pattern. We observed 2.5-3x increase in respiration rates per unit of fungal biomass for 5 to 10° C interval at early stages of decomposition (d. 12) when fungal mycelium is expected to show the highest physiological activity. This increase is considerably higher than the MTE predictions. However, respiration rates per unit of fungal biomass actually decreased for the 10-20° C interval, in contrast to any

expectations or predictions. In field experiments, nutrient limitation at higher temperatures could have explained the observed pattern, however, it is highly unlikely in our microcosms with high nutrient concentrations and still relatively low fungal biomass at d. 12.

Trends of leaf litter mass loss or leaf decomposition rates were somewhat similar to those of fungal biomass and respiration. We found about 2x increases for the 5-10° C interval for 6-species assemblage and similar increases for both 5-10° C and 10-15° C intervals for *T. alaskense*. Again, as with other parameters, no increases in decomposition rates were found at higher temperatures (15-20° C). These data can be compared to findings from terrestrial ecosystems. The rate of decomposition of organic matter in soils is temperature dependent and was found to be determined by the mean annual temperature of the environment. Cooler soils also store more carbon in comparison to warmer soils (Kirschbaum, 1995). Estimates suggest that around 10% of organic soil carbon would be lost additionally with 1° C increase in temperature in areas with 5° C mean annual temperature while only 3% of additional C would be lost with 1° C increase at 30° C (Kirschbaum, 1995). Clearly, we found similar patterns for fungal activity and decomposition rates in our experiment, with much greater temperature sensitivity of microbial parameters at low temperatures (5-10° C interval) and more moderate sensitivity at higher temperatures (15-20° C interval). Ferreira and Chauvet (2011) reported that decomposition rates of leaf litter colonized by aquatic fungi increased by ~30% with a 5° C increase in temperature, while our findings suggest that decomposition responses to temperature can be highly variable and potentially much higher than that

during the coldest season (autumn-winter) when the substrate availability in streams and microbial activity on areal basis are the highest (Suberkropp et al. 2010).

With the biological processes controlling the carbon dynamics in both terrestrial and aquatic ecosystems, temperature increases may result in more carbon dioxide released into the atmosphere as well as decreased carbon storage in many ecosystems. While a 3-5° C increase in temperature is predicted over the next 100 years (Yvon-Durocher et al., 2010), our study provides unique new insights into the effects of temperature on activity of aquatic hyphomycetes and fungi-mediated leaf litter decomposition utilizing laboratory temperature increments of 5° C. We found that fungal biomass, respiration and leaf litter decomposition rates often do not follow simple predictions of the MTE. Specifically, we observed much greater temperature sensitivity of microbial parameters at low temperatures (5-10° C interval) and more moderate sensitivity at higher temperatures (15-20° C interval). These trends may have important implications for stream ecosystems under climate change scenarios since the bulk leaf litter input and the highest microbial activity coincide with the coldest season (autumn-winter) in temperate streams.

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Table 1. List of isolates of aquatic hyphomycetes used in microcosm experiment. The isolates used in the 6-species assemblage treatment are denoted in the column to the right by the word 'Mix'.

Species	**Isolate**	
Aquanectria penicillioides	230-7	
Tricladium alaskense	69-2	
Tricladium chaetocladium	27-1	Mix
Heliscus lugdunensis	62-1	Mix
Anguillospora filiformis	23-4	Mix
Articulospora tetracladia	25-1	Mix
Dimorphospora foliicola	62-4	Mix
Flagellospora minuta	31-4	Mix

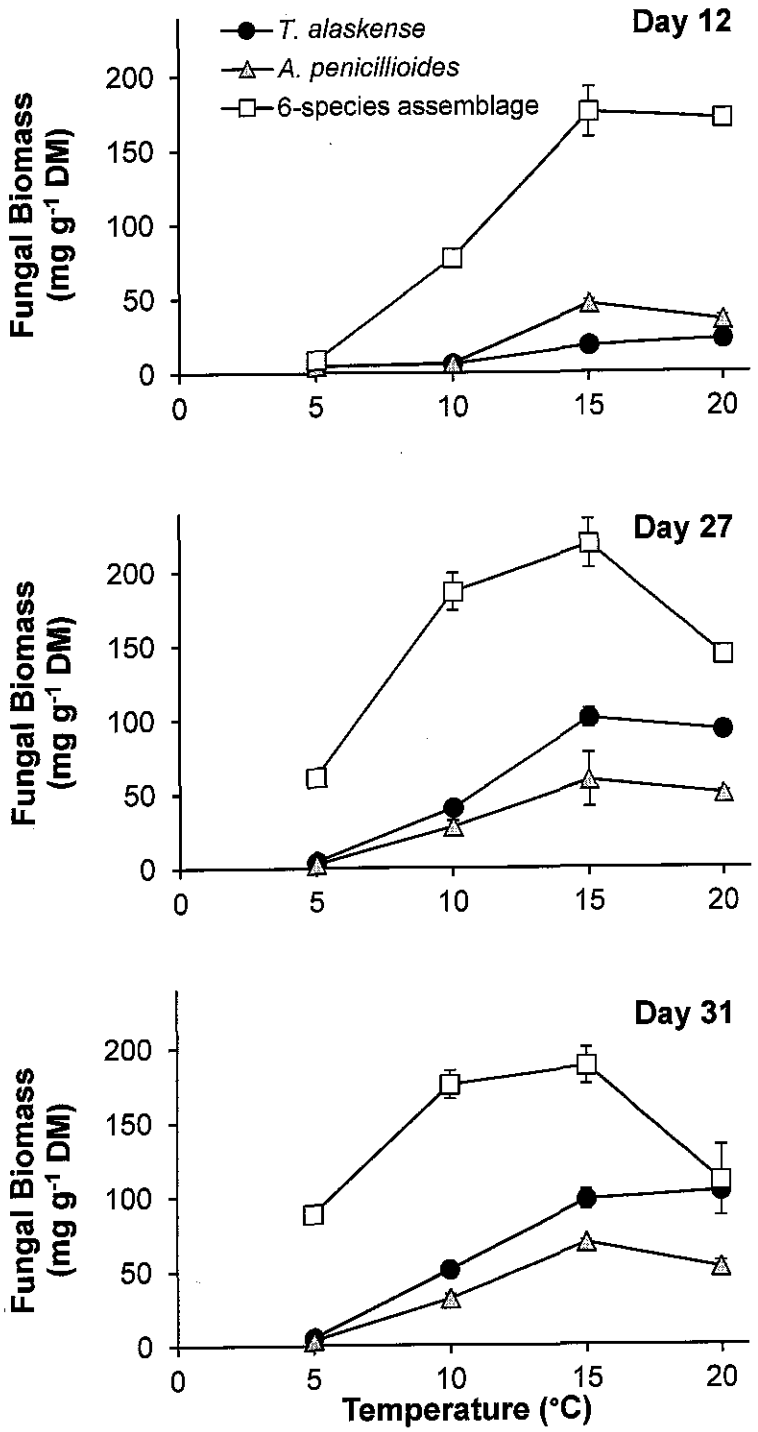

Figure 1. Fungal biomass associated with decomposing leaf litter on d. 12, 27 and 31 at different temperatures. Means ± 1 SE are shown.

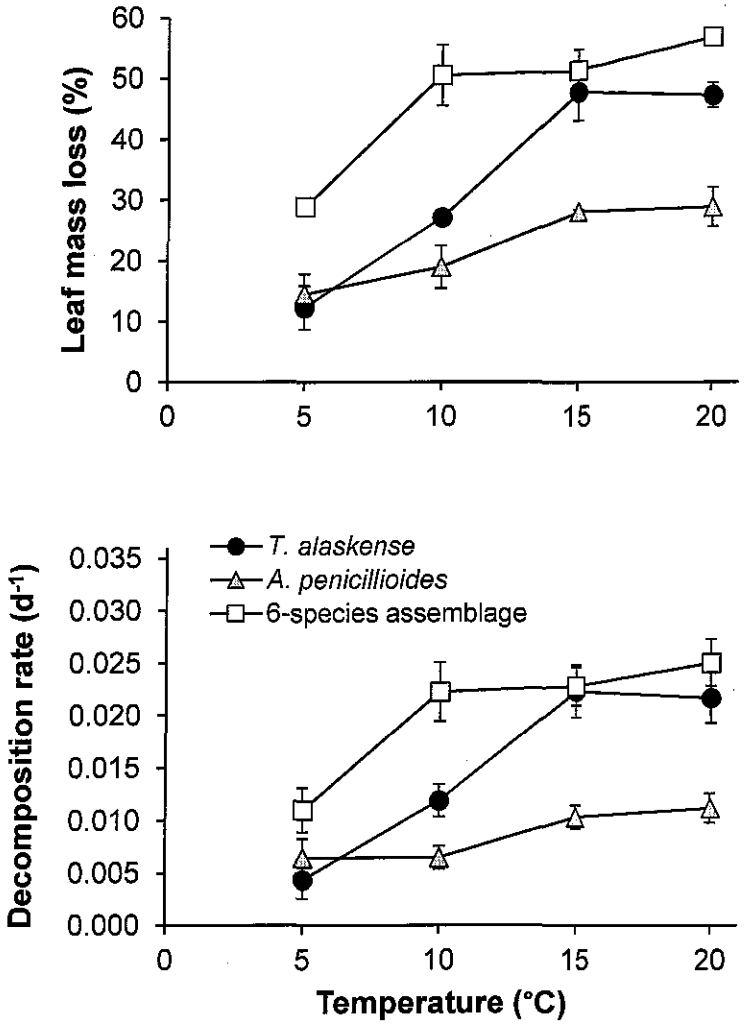

Figure 4. Leaf mass loss (top panel) and decomposition rate (k) (bottom panel) of leaf litter after 31 d. incubation in laboratory microcosms at different temperatures. Means ± 1 SE are shown.

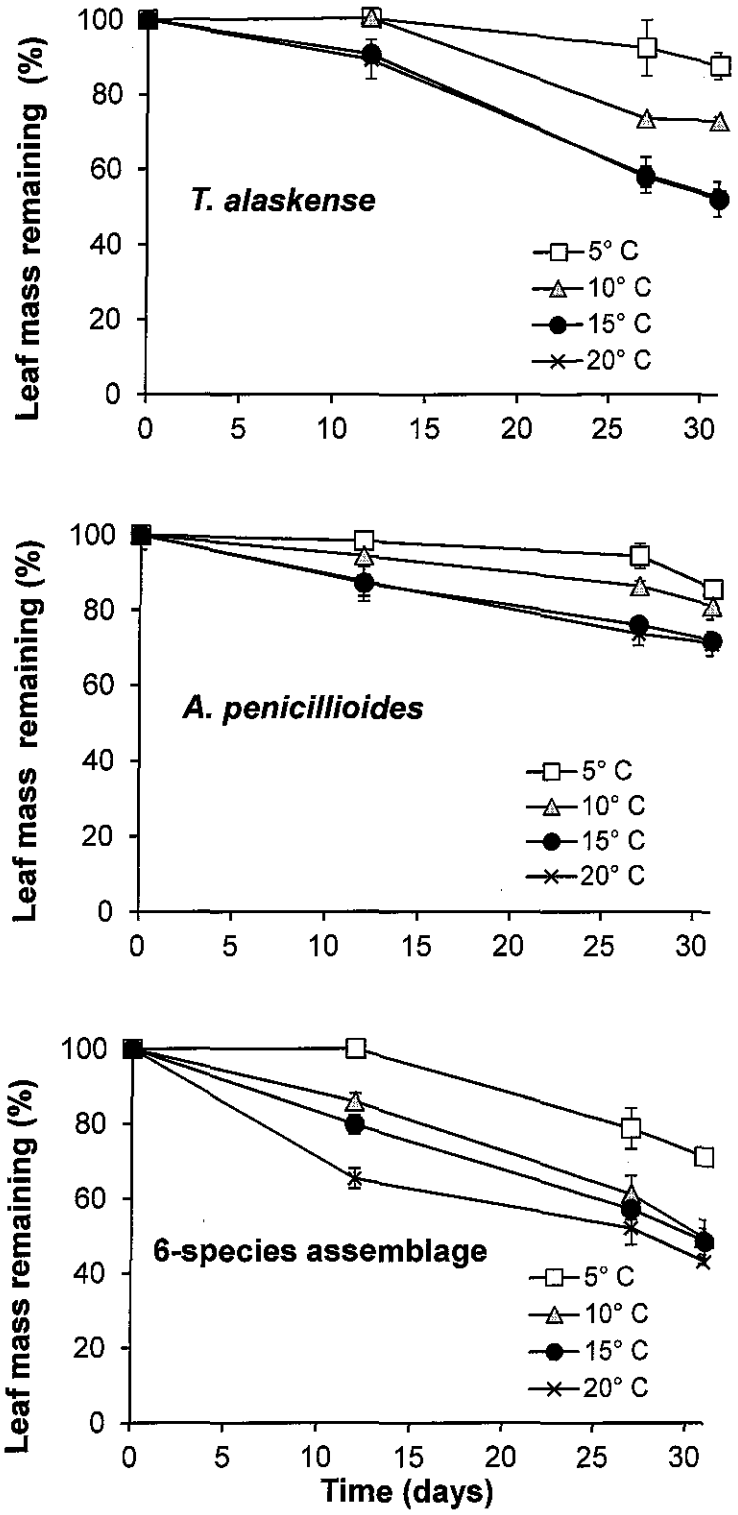

Figure 5. The remaining mass of leaf disks decomposing in microcosms at different temperature treatments. Means ± 1 SE are shown.