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FULL PAPER

Effects of temperature and litter type on fungal growth and decomposition of leaf litter

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Abstract The dependence of fungal decomposition of leaf litter on incubation temperature and litter types used as substrata was assessed under pure culture conditions. Isolates of *Xylaria* sp., a major ligninolytic fungus in cool temperate forests in Japan, were used as the fungal material. *Xylaria* sp. is mesophilic; maximum growth and decomposition occurred at 25°C. In the temperature test, the decomposition pattern of beech leaf litter by three isolates of *Xylaria* sp. changed at a threshold at 25°C. Cellulolytic activity increased with temperature from 5 to 25°C, whereas above 25°C ligninolytic activity increased at the expense of cellulolytic activity, leading to suppressed overall decomposition as a result of the higher temperature. The mass loss of leaf litter caused at 20°C by an isolate of *Xylaria* sp. was variable among 15 litter types and was correlated negatively with acid-unhydrolyzable residue (AUR) content and positively with total carbohydrate content for the 15 litter types. The effects of temperature and litter type on the growth and decomposition of leaf litter by *Xylaria* sp. may have implications for changes in fungal decomposition of leaf litter that would be predicted in response to future environmental changes.

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Introduction

Global climate change has potential impacts on the structure and functioning of forest soils through modification of the activity of fungi that play central roles in decomposition processes (Osono 2007). Changes in temperature and moisture, for example, affect fungal growth and decomposition directly (Widden et al. 1989; Adaskaveg et al. 1995; Thormann et al. 2004; Osono and Takeda 2006). Global climate change can alter the composition of tree species within a forest stand and that of litter type supplied to the forest floor, which can lead to changes in the quality of litter available to fungal decomposers and thus influence their activity (Osono and Takeda 2001, 2006). Quantitative analyses of potential factors affecting fungal decomposition are thus crucial for predicting its response to a changing climate. However, few studies have examined temperature and litter quality controls on the growth and activity of fungi associated with leaf litter decomposition.

The purpose of the present study was to assess the dependence of fungal growth and decomposition on incubation temperature and litter types used as substrata under pure culture conditions. *Xylaria* sp., a major ligninolytic fungus in cool temperate forests (Osono and Takeda 2001, 2002; Osono and Masuya, unpublished data) was used as the fungal material. This fungus is distributed widely in cool temperate forests in Japan and colonizes leaf litter of more than 50 tree species (i.e., has low host specificity) (Masuya et al., unpublished data). Therefore, we hypothesized that temperature affects the

ability of this fungus to decompose cellulose and recalcitrant compounds such as lignin and tannin and that litter types with different chemical quality also affect the ability of this fungus to decompose the litter. We thus performed two pure culture tests that manipulated incubation temperature (denoted as temperature test) and litter types used as substrata (the litter type test) to test these hypotheses.

Materials and methods

Source of fungi and leaves

Three isolates of *Xylaria* sp. (coded Qs1-1, Bc2-2, and Cr1-2) were used in the temperature test. The three isolates were isolated with a surface-disinfection method (described in Osono and Takeda 2001) from leaf litter of *Quercus serrata*, *Benthamidia cousa*, and *Cryptomeria japonica*, respectively, collected in November 2008 from a cool temperate forest in the Ashiu Experimental Forest of Kyoto University (35°18′N, 135°43′E) located in northern Kyoto, Japan. During the past 29 years the mean annual temperature was 11.7°C and the mean annual precipitation was 2,353 mm at the office of Ashiu Experimental Forest about 5 km from the sampling site. One isolate of *Xylaria* sp. coded Gs19-1 was isolated with the surface-disinfection method from leaves of *Fagus crenata* collected in October 1996 from the study site and was used in the litter type test. Isolates were maintained on slants of 1% malt extract agar [MA; malt extract 1% and agar 2% (w/v)] at 20°C in darkness until the tests were performed.

Leaf litter of *F. crenata* used in the temperature test was collected from the forest floor of the study site in May 2009. Leaf litter of 15 tree species (Table 1) was collected in the study site in November 2000 and used in the litter type test. Leaves were cut into pieces (~1.5 × 1 cm), oven-dried at 40°C for 1 week, and preserved in vinyl bags until the experiment was started.

Colony diameter growth rate

Mycelial disks of the three isolates for the temperature test, of 6 mm in diameter, were taken from the edge of cultures on Petri dishes containing 1% MA and incubated at 20°C for 2 weeks. They were transferred to the center of another Petri dish (diameter, 9 cm) containing 20 ml 1% MA. The plates were incubated at 5, 10, 15, 20, 25, 30, or 35°C in the dark. Colony diameter was measured in two directions at right angles three to five times at given intervals during a 3-month incubation, and colony diameter growth rate was calculated by regressing the colony diameter against the days after inoculation. Four plates were prepared for each isolate.

Table 1 Chemical composition (mg/g) and mass loss (% original mass) caused by *Xylaria* sp. Gs19-1 of leaf litter of 15 tree species used in the litter type test

Litter type	AUR	Total carbohydrates	Nitrogen	Mass loss
Fagus crenata	415	289	17	9.2 ± 0.1
Aesculus turbinata	380	235	9	9.8 ± 0.6
Quercus serrata	374	263	9	16.4 ± 0.5
Betula grossa	362	267	11	13.9 ± 0.5
Pterocarya rhoifolia	359	250	18	9.0 ± 1.5
Quercus crispula	356	296	10	6.7 ± 0.4
Weigela hortensis	355	286	10	20.6 ± 1.2
Acer micranthum	337	270	8	19.2 ± 0.6
Castanea crenata	322	296	11	12.4 ± 1.1
Magnolia obovata	310	314	10	15.7 ± 1.1
Malus tschonoskii	251	376	8	23.6 ± 0.9
Carpinus laxiflora	178	326	13	25.2 ± 2.3
Malotus japonicus	154	271	12	21.3 ± 2.0
Acer micranthum	153	323	8	17.6 ± 4.7
Benthamidia kousa	88	328	10	26.4 ± 3.1

Values are mean ± standard errors ($n = 4$)

AUR acid-unhydrolyzable residue

Decomposition tests

Leaves (0.3 g) were sterilized by exposure to ethylene oxide gas at 60°C for 6 h and used in the temperature and litter type tests according to the pure culture methods described in Osono and Hirose (2009). The sterilized leaves were placed on the surface of Petri dishes (diameter, 9 cm) containing 20 ml 2% agar. Inocula for each assessment were cut with a sterile cork borer (6-mm diameter) from the edge of cultures on Petri dishes containing 1% MA, incubated at 20°C, and placed on the agar adjacent to the leaves, one plug per plate. The plates were incubated for 12 weeks in the dark at 5, 10, 15, 20, 25, 30, or 35°C in the temperature test or at 20°C in the litter type test. The plates were sealed firmly with laboratory film during incubation so that moisture did not limit decomposition on the agar. After incubation the leaves were retrieved, oven-dried at 40°C for 1 week, and weighed. The initial, undecomposed leaves were also sterilized, oven-dried at 40°C for 1 week, and weighed to determine the original mass. Four plates were prepared for each isolate, and four uninoculated plates served as a control. Mass loss of leaves was determined as a percentage of the original mass, taking the mass loss of litter in the uninoculated and incubated control treatment into consideration, and the mean values were

calculated for each isolate, each incubation temperature, and each litter type. In the temperature test, the initial leaves, the control leaves, and the leaves incubated at 20, 25, and 30°C and exhibiting mass loss of more than 3.0% were used for chemical analyses as described next. The initial leaves of the 15 tree species used in the litter type test were also used for chemical analyses.

Chemical analysis

Leaf materials were combined to make one sample for each treatment and ground in a laboratory mill (0.5-mm screen). The amount of acid-unhydrolyzable residue (AUR) in the samples was estimated by means of gravimetry as acid-insoluble residue, using hot sulfuric acid digestion (King and Heath 1967). Samples were extracted with alcohol–benzene at room temperature (15–20°C), and the residue was treated with 72% sulfuric acid (v/v) for 2 h at room temperature with occasional stirring. The mixture was diluted with distilled water to make a 2.5% sulfuric acid solution and autoclaved at 120°C for 60 min. After cooling, the residue was filtered and washed with water through a porous crucible (G4), dried at 105°C, and weighed as acid-insoluble residue. The filtrate (autoclaved sulfuric acid solution) was used for total carbohydrate analysis. The amount of carbohydrates in the filtrate was estimated by means of the phenol–sulfuric acid method (Dubois et al. 1956). Then, 1 ml 5% phenol (v/v) and 5 ml 98% sulfuric acid (v/v) were added to the filtrate. The optical density of the solution was measured using a spectrophotometer at 490 nm, using known concentrations of D-glucose as standards. Total nitrogen content was measured by automatic gas chromatography (NC analyzer SUMIGRAPH NC-900; Sumitomo Chemical, Osaka, Japan).

Mass loss of AUR and total carbohydrates in the temperature test was determined as a percentage of the original mass, taking the mass loss of AUR and total carbohydrates in the uninoculated and incubated control treatment into consideration. The AUR/leaf weight loss ratio (AUR/W) is a useful index of the substrate utilization pattern of each fungal species (Osono 2010). AUR/W of each fungal species was calculated according to the equation:

AUR/W = mass loss of AUR (% of original AUR mass)/mass loss of leaves (% of original leaf mass).

Statistical analysis

Pearson's correlation coefficients were calculated for linear relationships between the initial contents of AUR, total carbohydrates, and nitrogen and mass loss of leaf litter for the 15 tree species in the litter type test.

Results

Colony diameter growth rate as related to temperature

The colony diameter growth rate of three isolates of *Xylaria* sp. increased exponentially with temperature from 5 to 25°C (Fig. 1). The optimal growth rate was recorded at 25°C (Qs1-1 and Bc2-2) and at 30°C (Cr1-2). No growth occurred at 35°C for the three isolates.

Mass loss of leaf litter as related to temperature

The initial content of AUR in *Fagus crenata* leaf litter used in the temperature test was 454 mg/g and that of total carbohydrates was 286 mg/g. The mass loss of leaf litter caused by the three isolates varied with temperature (Fig. 2). The mass loss of leaf litter caused by Bc2-2 and Cr1-2 increased linearly with temperature from 5 to 25°C, whereas that caused by Qs1-1 was relatively constant from 5 to 15°C and increased linearly with temperature from 15 to 25°C. The optimal mass loss was recorded at 25°C for the three isolates, and mass loss decreased at 30°C. Mass loss was negligible at 35°C because there was no hyphal growth for the three isolates.

Analysis of organic chemical components showed that the three isolates decomposed total carbohydrates more rapidly than AUR (Table 2). The mass loss of AUR was negligible at 20 and 25°C for the three isolates, ranging from –1.1 to 1.9% of the original AUR mass. In contrast, the mass loss of AUR caused by Qs1-1 and Cr1-2 was enhanced at 30°C to reach 3.3 and 4.6%, respectively. The mass loss of AUR was not determined for Bc2-2 because the mass loss of leaf litter was too low (i.e., 2.8%) to obtain a reasonable result of chemical analysis. The mass loss of

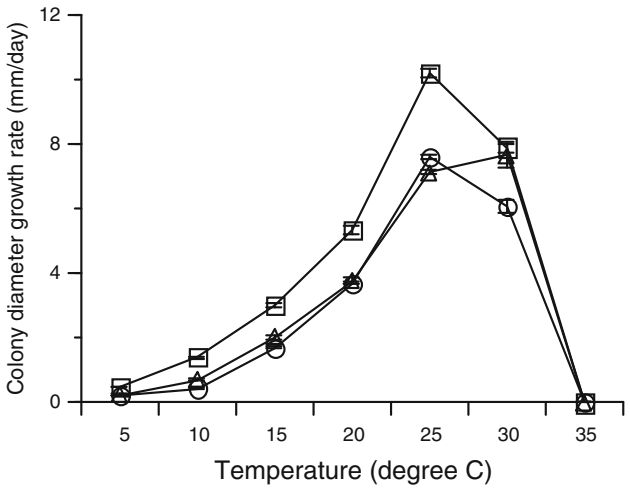

Fig. 1 Colony diameter growth rate of three isolates of *Xylaria* sp. as related to temperature. *Bars* indicate standard errors ($n = 4$). *Squares*, Qs1-1; *circles*, Bc2-2; *triangles*, Cr1-2

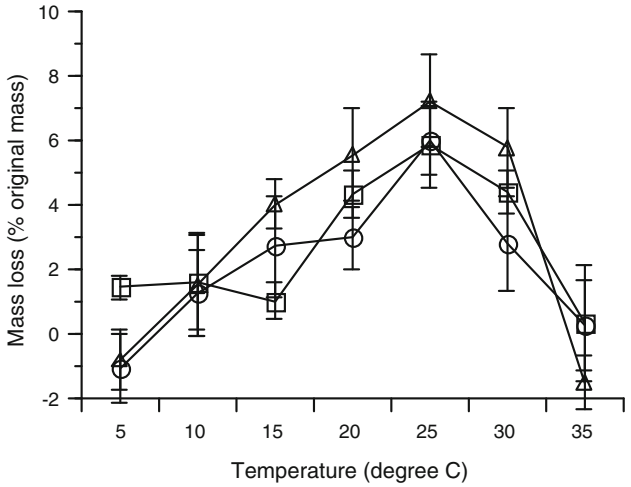

Fig. 2 Mass loss of leaf litter of *Fagus crenata* caused by three isolates of *Xylaria* sp. as related to temperature. *Bars* indicate standard errors ($n = 4$). *Squares* Qs1-1, *circles* Bc2-2, *triangles* Cr1-2

Table 2 Mass loss (% original mass) of leaf litter, AUR, and total carbohydrates after incubation at 20, 25, and 30°C for 12 weeks, and AUR/litter weight loss ratio (AUR/W) for three *Xylaria* isolates in the temperature test

Isolate	Temperature (°C)	Leaf litter	AUR	Total carbohydrates	AUR/W
Qs1-1	20	4.4 ± 0.7	−1.1	15.3	−0.24
	25	5.9 ± 1.4	0.4	20.7	0.06
	30	4.4 ± 0.7	3.3	14.4	0.75
Bc2-2	20	3.0 ± 1.0	0.2	21.6	0.06
	25	6.1 ± 1.1	1.9	25.6	0.31
	30	2.8 ± 1.5	nd	nd	nd
Cr1-2	20	5.6 ± 1.5	1.0	23.5	0.18
	25	7.3 ± 1.4	−1.1	21.6	−0.15
	30	5.8 ± 1.3	4.6	16.4	0.79

Values indicate mean ± standard errors ($n = 4$)

nd not determined because mass loss of leaf litter was less than 3% (see text)

total carbohydrates caused by the three isolates ranged from 15.3 to 25.6% at 20 and 25°C, whereas the mass loss of total carbohydrates caused at 30°C by Qs1-1 and Cr1-2 was lower than that at 20 and 25°C. Accordingly, AUR/W was higher at 30°C (0.75–0.79) than at 20 and 25°C (–0.24 to 0.31), indicating more selective decomposition of AUR at 30°C.

Mass loss of leaf litter of 15 tree species

The initial content of AUR in leaf litter of the 15 tree species used in the litter type test ranged from 88 to 415 mg/g, that of total carbohydrates from 235 to 376 mg/g, and that of nitrogen from 8 to 18 mg/g (see Table 1). The mass loss of leaf litter caused by Gs19-1 at 20°C ranged from 6.7 to 26.4% of the original mass. The mass loss of the 15 litter types was correlated significantly and negatively with AUR content ($R = –0.75$, $P < 0.01$) and significantly and positively with total carbohydrate content ($R = 0.57$, $P < 0.05$) (Fig. 3a, b). No significant correlation was found between mass loss of leaf litter and nitrogen content ($R = 0.38$, $P > 0.05$) (Fig. 3c).

Discussion

The fact that growth and decomposition were maximal at 25 and 30°C (Figs. 1, 2) indicates that *Xylaria* sp. is mesophilic. The optimal temperature of hyphal growth was consistent with that of decomposition for Qs1-1 and Bc2-2, but not so for Cr1-2, which supports the finding of Loman (1962) regarding wood-decomposing fungi that the optimum temperature for growth was not necessarily equal to that for decomposition.

The substrate utilization of *Xylaria* sp. changed at the threshold of 25°C (see Table 2): cellulolytic activity increased with temperature below 25°C. The stimulation of cellulose decomposition below the optimum growth temperature was consistent with previous studies (Widden et al. 1989; Donnelly et al. 1990; Adaskaveg et al. 1995; Osono and Takeda 2006). In contrast, above 25°C the ligninolytic activity of *Xylaria* sp. increased at the expense of cellulolytic activity, leading to the suppressed overall decomposition at higher temperatures. A wood-decomposing basidiomycete, *Ganoderma colossum*, also showed an increase of lignin decomposition, concomitant suppression of cellulose decomposition, and suppression of the overall mass loss of wood at temperatures above the optimum growth temperature (45°C) (Adaskaveg et al. 1995). It is unknown, however, whether such temperature-dependent shifts in the relative decomposition of lignin and carbohydrates are common to other ligninolytic fungi, and this question merits further investigation.

The variation of decomposition among the 15 litter types was correlated negatively with AUR content and positively with total carbohydrate content of the litter (see Fig. 3). Recalcitrant compounds in tree leaves designated as AUR, such as lignin and tannin, have often been shown to limit the rate of decomposition in forest soils (summarized in Osono and Takeda 2005) and of fungal decomposition in pure culture conditions (Hagiwara et al. unpublished data). Fungal decomposition of lignin depends on the availability of other carbon energy sources such as carbohydrates (Eriksson et al. 1990), which can account for the faster mass loss in litter types enriched in carbohydrates relative to lignin.

The effects of temperature and litter type on the growth and decomposition of leaf litter by *Xylaria* sp. may have

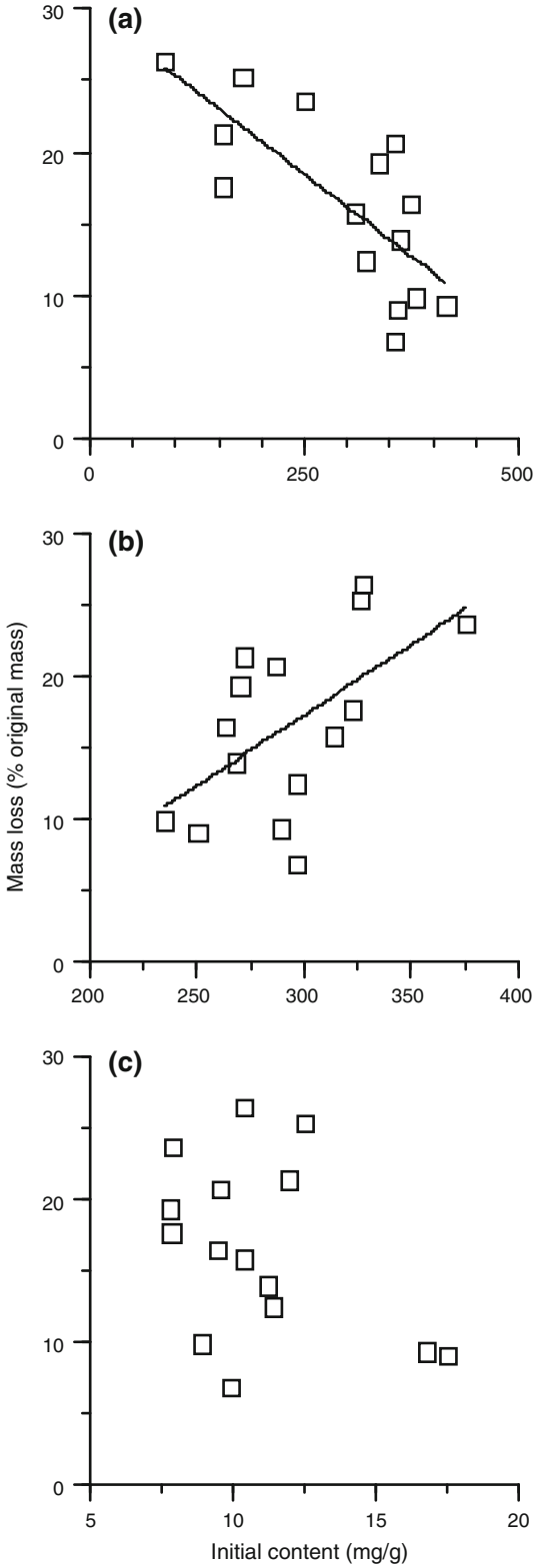

Fig. 3 Mass loss of leaf litter of 15 tree species as related to initial content of acid-unhydrolyzable residue (AUR) (a), total carbohydrates (b), and nitrogen (c). *Bars* indicate standard errors ($n = 4$)

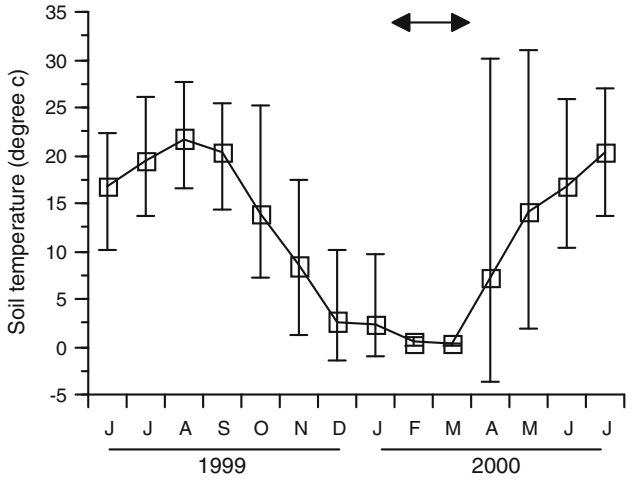

Fig. 4 Seasonal changes in temperature of the litter layer at the study site in Kyoto, Japan. Temperature was recorded every hour for a 13-month period between June 1999 and July 2000. Values are means; *bars* represent the maximum and the minimum temperature of each month. *Double-headed arrow* indicates period of snow

implications regarding the changes in fungal decomposition of leaf litter predicted to occur as a consequence of future environmental changes in the study site. Seasonal changes in temperature in the litter layer indicate that the temperature at the study site rarely exceeds 25°C (Fig. 4), suggesting that *Xylaria* sp. generally functions as a cellulolytic fungus. However, the possible future increase in temperature worldwide, which in temperate regions is expected to be an increase of 2–3°C (Manabe et al. 1991; Boer et al. 1992; Russell et al. 1995), may lead to an overall suppression of fungal decomposition and enhanced decomposition of AUR relative to cellulose. These changes are especially likely during the period before leaf expansion in the canopy in May, when the temperature reaches as high as 30°C because of direct irradiation to the forest floor, and during the midsummer period in July and August. Similarly, an increase in the relative abundance of rapid-growing tree species with low AUR content in litter, for example, can lead to enhanced decomposition by *Xylaria* sp., resulting in positive feedback to the atmospheric CO_2 level. These predictions are obviously oversimplified, but data such as the results of the present study will provide useful insights into the effects of future environmental changes on fungal decomposition in forest soils.

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