Effects of litter type, origin of isolate, and temperature on decomposition of leaf litter by macrofungi

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Abstract The dependence of hyphal growth and litter decomposition on litter type and incubation temperature used as substratum was compared for litter-decomposing macrofungi (LDM) originating from subtropical (ST), cool temperate (CT), and subalpine forests (SA) in Japan. In the first series of pure culture decomposition tests using a total of 39 litter types as substrata inoculated with six fungal isolates from the three climatic regions, the fungal decomposition of litter was negatively affected by the content of AUR or extractives and positively by N content in the litter. Secondly, cross-inoculation tests were performed to examine the mass loss of leaf litter of broad-leaved trees from ST, CT, and SA, each inoculated with three *Mycena* species from the three climates and incubated at seven temperatures between 5°C and 35°C. Fungal isolate, litter type, incubation temperature, and their interactions significantly affected the mass loss of litter during the incubation. The greatest values of mass loss were found at 20°C or 25°C, and were generally consistent with the optimum temperatures of colony diameter growth rate of these isolates. Isolates from cooler regions were more sensitive to higher temperature than isolates from warmer regions. The decomposition of recalcitrant compounds (as acid-unhydrolyzable residues, AUR) by *Mycena* sp. from ST was also affected by litter type and incubation temperature, but the degree of selective decomposition of AUR relative to other components, such as cellulose, was insensitive to the range of temperature tested.

Keywords Acid-unhydrolyzable residue · Temperature · Leaves · Litter-decomposing macrofungi · Selective delignification

Introduction

Litter-decomposing macrofungi (LDM) include active ligninolytic species in Basidiomycota and Ascomycota and play central roles in leaf-litter decomposition processes (Osono 2007; Lindahl and Boberg 2008; van der Wal et al. 2013). Ligninolytic abilities of LDM have been examined with the pure culture test and compared for multiple species that were commonly found at a single site (Lindeberg 1946; Miyamoto et al. 2000; Steffen et al. 2007; Valášková et al. 2007; Boberg et al. 2011; Žifčáková et al. 2011). Few studies have been available that compared the diversity and functioning of LDM across multiple sites of different climatic regions. Recently, I reported that the species richness of LDM varied with climatic region, declining from subtropical to temperate forest, and then from temperate to subalpine forest in Japan (Osono in press b). Then, I compared the potential abilities of diverse LDM from those forests to decompose leaf litter and recalcitrant compounds, such as lignin, under constant laboratory conditions and found that the decomposing abilities were variable among LDM species within a climatic region but that the variability in decomposing ability was relatively similar among climatic regions (Osono submitted). These results led to a further hypothesis that the decomposing ability of LDM originating from different climates varies in its sensitivity to temperature and litter quality. Several studies have examined the dependence of fungal decomposition on incubation temperature and/or the quality of leaf litter (Lindeberg 1946; Mikola 1956; Miyamoto et al. 2000; Osono and Takeda 2006; Osono et al. 2011c). To the knowledge of the author, however, few studies have explored chemical components that can limit fungal decomposition and compared the dependence of decomposition on temperature and litter types between major LDM originating from regions with different climatic conditions (Osono 2011).

The purpose of the present study was to assess under pure culture conditions the dependence of fungal growth and decomposition on litter types used as substrata, incubation temperature, and the origin of the isolate. I used six isolates of LDM species, five of which belonged to *Mycena*, obtained in subtropical, cool temperate, and subalpine forests in Japan because (i) they occurred as fruiting bodies most frequently on the forest floor of the respective forests (Osono in press b) and (ii) they exhibited the greatest activity of the LDM isolates to decompose leaf litter (Osono submitted). I performed two pure culture tests that manipulated litter types and incubation temperature. First, a total of 39 litter types and six fungal isolates from the three climatic regions were used to examine possible limiting factors of fungal decomposition (denoted as the litter types test). Secondly, the pure culture decomposition tests were designed to evaluate the effects of litter type, incubation temperature, *Mycena* isolates of different origins, and the interactions of these factors on the decomposition of leaf litter and recalcitrant compounds in the litter (denoted as the cross inoculation test).

Materials and methods

Study site

Isolates of macrofungi and litter materials used in pure culture decomposition tests were collected at three sites in Japan: a subtropical (ST), a cool temperate (CT), and a subalpine (SA) forest. ST was located in an evergreen broadleaved forest in the northern part of Okinawa Island. CT was located in a deciduous broadleaved forest in Kyoto. SA was located in an evergreen coniferous forest in Gifu. The mean annual temperature was 22°C, 10°C, and 2°C in ST, CT, and SA, respectively. The study sites received similar amounts of precipitation annually (approximately 2500 mm). Further details about the location, climatic condition, vegetation, and properties of the forest floor are given in Osono (in press a, in press b).

Fungal isolates

Six isolates were used, two isolates from each of the three study sites: two unidentified *Mycena* species (denoted as *Mycena* sp.1 MAFF241586 and *Mycena* sp.2 MAFF241604) from ST, *Mycena polygramma* IFO33011 and an unidentified *Clitocybe* species from CT, and *Mycena aurantiidisca* O2_07101503b and *M. epipterygia* O9_07101508 from SA. The *Mycena* species were the macrofungal taxa most frequently encountered as fruiting bodies at each study site (Osono in press b) and were active decomposers of leaf litter (Osono submitted). *Clitobybe* sp. fruited at low frequency in CT (Osono in press b) and was a selective decomposer of recalcitrant compounds on the forest floor (Osono et al. 2011a). These macrofungal isolates were obtained from tissues of fruiting bodies collected at the study sites (Osono submitted), and maintained on slants of 1% malt extract agar medium [MEA, malt extract 1% and agar 2% (w/v)] at 20°C in darkness until the tests were performed. *Mycena* sp.1 and sp.2 from ST were analyzed for base sequences of the rRNA gene 28S D1/D2 (accession numbers: AB512383 for *Mycena* sp.1 and AB512392 for *Mycena* sp.2), but identification to species level was not successful.

Litter materials

Newly shed leaves of 12, 15, and 12 plant species without obvious fungal or faunal attack were collected from the surface of the forest floor in January to April 2008, November 2000, and October 2009 in ST, CT, and SA, respectively (see Table S1 in Electronic Supplementary Material) and used for the litter type test. For the cross inoculation test, newly shed leaves of *Castanopsis sieboldii*, *Fagus crenata*, and *Betula ermanii* without obvious fungal or faunal attack were collected from the surface of the forest floor of ST, CT, and SA at peak periods of litter fall in April 2009, October 2009, and October 2009, respectively. The litters were oven-dried at 40°C for one week and preserved in vinyl bags for one to two months until the experiments were started.

Litter type test

The effect of litter type on decomposition was examined under pure culture conditions in the litter type test. Two isolates each from ST, CT, or SA were inoculated onto 12, 15, or 12 litter types that differed in chemical composition collected at ST, CT, or SA, respectively.

An individual pure culture decomposition test consisted of one fungal isolate inoculated to one litter type. Litter (0.3 g) was sterilized by exposure to ethylene oxide gas at 60°C for 6 hours and used in the tests, according to the methods described in Osono et al. (2011c). The sterilized litters were placed on the surface of Petri dishes (9 cm diameter) containing 20 ml of 2% agar. Inocula for each assessment were cut out of the margin of previously inoculated Petri dishes on 1% MEA with a sterile cork borer (6 mm diameter) and placed on the agar adjacent to the litter, one plug per plate. The plates were incubated for 12 weeks in the dark at 20°C. The plates were sealed firmly with laboratory film during incubation so that moisture did not limit decomposition on the agar. After incubation the litters were retrieved, oven-dried at 40°C for 1 week, and weighed. The initial, undecomposed litters were also sterilized, oven-dried at 40°C for 1 week, and weighed to determine the original mass. Three to four plates were prepared for each isolate, and four uninoculated plates served as a control. Mass loss of litter was determined as a percentage of the original mass, taking the mass loss of litter in the uninoculated and incubated control treatment into account, and the mean values were calculated for each plate. Prior to the tests, the sterilized litters were placed on 1% MEA, and after 8 weeks of incubation at 20°C in darkness, no microbial colonies had developed on the plates. Thus, the effectiveness of the sterilization method used in the present study was verified. The initial litters of multiple tree species were analyzed for the contents of acid-unhydrolyzable residues (AUR), total carbohydrates, extractives, and nitrogen (N) as described below.

Cross-inoculation test

Cross-inoculation tests were carried out to examine the effects of litter type, incubation temperature, and origin of *Mycena* isolates on fungal decomposition. Three fungal isolates (*Mycena* sp.2 from ST, *M. polygramma* from CT, and *M. aurantiidisca* from SA) were inoculated onto leaves of three litter types (*Castanopsis sieboldii* from ST, *Fagus crenata* from CT, and *Betula ermanii* from SA), and each combination of the three isolates × three litter types was incubated at seven temperatures (5, 10, 15, 20, 25, 30, and 35°C).

Prior to this decomposition test, colony diameter growth rates were measured for the isolates of *Mycena* sp.2, *M. polygramma*, and *M. aurantiidisca*. Mycelial disks of the three isolates, 6 mm in diameter, were taken from the edge of cultures on Petri dishes containing 1% MEA and incubated at 20°C for 2 weeks. They were transferred to the center of another Petri dish (9 cm in diameter) containing 20 ml 1% MEA. The plates were incubated at one of the seven temperatures in the dark. Colony diameter was measured in two directions at right angles from each other 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.

The decomposition tests were performed as described above, except that the plates were incubated at one of the seven temperatures. The initial litter, the control litter, and the litter with at least 5.0% mass loss were used for the analysis of AUR as described below.

Chemical analyses

Litter materials were combined to make one sample for each test and ground in a laboratory mill (0.5 mm screen). The amount of 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 so that the concentration of sulfuric acid reached 2.5% 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 subjected to total carbohydrate analysis. The amount of carbohydrate in the filtrate was estimated by means of the phenol-sulfuric acid method (Dubois et al. 1956). One milliliter of 5% phenol (v/v) and 5 ml of 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 N content was measured by automatic gas chromatography (NC analyzer SUMIGRAPH NC-900, Sumitomo Chemical Co., Osaka, Japan).

Mass loss of AUR was determined as a percentage of the original mass, taking the mass loss of AUR in the uninoculated and incubated control treatment into account. AUR/litter mass (AUR/L) loss ratio is a useful index of the selective delignification caused by each fungal species (Osono and Hirose 2009). AUR/L loss ratio of each fungal species was calculated using the equation:

AUR/L loss ratio = mass loss of AUR (% of original AUR mass) / mass loss of litter (% of original litter mass)

Statistical analysis

In the litter type test, Pearson's correlation coefficients were calculated for linear relationships between chemical properties of litter and mass loss of the litter caused by macrofungal isolates. In the cross inoculation test, factors affecting the mass loss of litter were analyzed with generalized linear models (GLMs) with fungal species, litter type, incubation temperature, and their interactions as categorical predictors. Factors affecting the mass loss of AUR and AUR/L loss ratio were also analyzed with GLMs with litter type and incubation temperature as categorical predictors, but only the data of *Mycena* sp.2 were used because the numbers of samples of *M. polygramma* and *M. aurantiidisca* used for AUR analysis were too low. Tukey's HSD test was used for multiple comparisons. JMP 6.0 for Macintosh was used to perform these analyses.

Results

Litter mass loss in the litter type test

To examine the effect of litter types and possible effects of chemical components on fungal decomposition, six fungal isolates were inoculated onto multiple litter types that differed in chemical properties and incubated at 20°C, and the mass loss of litter during the incubation was related to the chemical composition of the litters. The initial content of AUR in leaf litter of a total of 39 litter types used in the tests ranged from 8.8% to 45.6%, that of total carbohydrates from 20.1% to 44.9%, that of extractives from 5.3% to 20.6%, and that of N from 0.41% to 1.75% (see Table S1).

Overall, the mean mass loss of litter caused by six fungal isolates ranged from -2.8% to 50.7% (see Table S1), indicating that there were large variations of decomposition between litter types caused by fungal isolates. Three significant correlation coefficients were detected out of a total of 24 combinations (six fungal isolates × four chemical properties) (Table 1). Mass loss of litter caused by *Mycena* sp.2 from ST and *M. polygramma* from CT was significantly and negatively correlated with AUR content in litter; and that caused by *M. epipterygia* from SA was significantly and negatively correlated with the content of extractives and significantly and positively correlated with N content (Fig. 1).

Colony diameter growth rate as related to temperature

The colony diameter growth rate increased linearly with temperature from 5 to 25°C for *Mycena* sp.2 from ST and from 5 to 20°C for *M. polygramma* from CT and *M. aurantiidisca* from SA (Fig. 2). The optimal growth rate occurred at 25°C for *Mycena* sp.2 and at 20°C for *M. polygramma* and *M. aurantiidisca*. The growth rate was lower at temperatures above the optimal temperature. At 25°C, *M. aurantiidisca* displayed more severely reduced growth than *M. polygramma*. *Mycena* sp.2 was the only isolate that grew at 30°C. No growth occurred at 35°C for the three isolates.

Litter mass loss in the cross inoculation test

Overall, the mean value of mass loss of litter ranged from -1.4% to 54.4% (Fig. 3, upper). Fungal isolate, litter type, incubation temperature, and their interactions significantly affected the mass loss of litter during the incubation (Table 2A). The mass loss of litter was in the order: *Mycena* sp.2 > *M. polygramma* > *M. aurantiidisca*, and *Betula*> *Castanopsis, Fagus*. The mass loss of litter increased with temperature: from 5°C to 25°C for *Mycena* sp.2 and *M. polygramma* and from 10°C to 20°C for *M. aurantiidisca*. The greatest values of mass loss were found at 25°C for *Mycena* sp.2 and at 20°C and 25°C for *M. polygramma*, and at 20°C for *M. aurantiidisca*, which were generally consistent with the optimum temperatures of colony diameter growth rate of these isolates (Fig. 2). Mass loss of litter was negligible at 5°C for all three isolates and at 35°C for *Mycena* sp.2, at 30°C to 35°C for *M. polygramma*, and at 25°C to 35°C for *M. aurantiidisca* (Fig. 3).

Incubation temperature affected the mass loss of litter caused by three *Mycena* isolates, and the interactions between temperature and fungal isolate and between temperature and litter type were also significant (Fig. 3; Table 2), indicating that the temperature effect depended on the fungal isolates and litter types. For example, the changes in the mass loss of litter in relation to temperature were larger for *Mycena* sp.2, which had greater ability to cause mass loss than *M. polygramma* and *M. aurantiidisca*, while these changes were less obvious when the fungal isolates were inoculated onto *Fagus* litter, which was more recalcitrant than *Castanopsis* and *Betula* litters.

AUR decomposition in the cross inoculation test

The litters with more than 5.0% mass loss were analyzed for the mass loss of AUR. The mean value of mass loss of AUR ranged from 2.6% to 68.9% (Fig. 3, middle). Litter type and incubation temperature significantly affected the mass loss of AUR caused by *Mycena* sp.2 during the incubation (Table 2B). Mass loss of AUR by *Mycena* sp.2 was lower at 10°C than at 15 to 30°C and was higher on *Betula* than on *Fagus* and *Castanopsis*.

The mean values of AUR/L loss ratio ranged from 0.3 to 2.6 (Fig. 3, lower). Litter type significantly affected AUR/L loss ratio for *Mycena* sp.2, whereas incubation temperature had no significant effect on AUR/L loss ratio (Table 2B).

Discussion

The decomposition of litter by *Mycena* isolates varied with litter type (Tables S1, 2), and the extent of decomposition in various litter types was correlated negatively with the contents of AUR or extractives and positively with N content (Fig. 1; Table 1). Recalcitrant compounds in leaves designated as AUR, such as lignin and tannin, have often been shown to limit the rate of decomposition in forest soils (Geng et al. 1993; Stump and Binkley 1993; Murphy et al. 1998; Osono and Takeda 2005) and of litter decomposition by ligninolytic fungi under pure culture conditions (Osono et al. 2011b, 2011c; Hagiwara et al. 2012). Extractives include soluble polyphenols, hydrocarbons, and pigments that are often released rapidly from decomposing litter (Osono et al. 2014), and can include inhibitory substances for fungal growth (Hughes et al. 2007). Nitrogen is a major essential element limiting fungal growth and enzyme production and was found to be positively related to mass loss caused by ligninolytic fungi (Osono et al. 2011b).

The findings that growth and decomposition were maximal at 20°C and 25°C (Figs. 2 and 3), respectively, indicate that the *Mycena* isolates used are mesophilic. The isolate from cooler regions was more sensitive to higher temperature than the isolate from warmer regions (Fig. 2), suggesting physiological adaptations of these fungi to the climatic conditions of the respective study sites (subtropical versus cool temperate versus subalpine). The optimal temperature of hyphal growth was generally consistent with that of decomposition, which agrees with the finding of Osono et al. (2011c) for three isolates of *Xylaria* sp. from CT.

Incubation temperature affected the mass loss of litter by the three *Mycena* isolates and the mass loss of AUR by *Mycena* sp.2 (Fig. 3; Table 2). However, AUR/L loss ratio of *Mycena* sp.2 was not affected by incubation temperatures (Table 2), suggesting that the degree of selective AUR decomposition by *Mycena* sp.2 was insensitive to the range of temperature adopted in the present study. Previously, Adaskaveg et al. (1995) and Osono et al. (2011c) reported that at temperatures above the optimum growth temperatures, the ligninolytic activity of fungi increased at the expense of cellulolytic activity, resulting in suppressed overall decomposition of leaf litter. My finding here for *Mycena* sp.2 from ST suggested that the sensitivity of selective delignification to temperature could vary with fungal species. Osono and Takeda (2006) also showed that the degree of selective delignification caused by four ligninolytic fungi did not differ between incubation at 10°C or 20°C, whereas *Gymnopus dryophilus* decomposed AUR more selectively at 10°C than at 20°C. More studies are needed to evaluate the sensitivity to temperature of ligninolytic activity of litter-decomposing macrofungi, including those from cooler regions, such as *M. polygramma* and *M. aurantiidisca*.

The three *Mycena* isolates from different climates differed significantly in their ability to decompose litter and in AUR and AUR/L loss ratio (Fig. 3, Table 2). This difference in decomposing ability was within the range of variability found for a suite of *Mycena* species encountered at these sites. That is, Osono (submitted) compared the ability of 32 *Mycena* isolates from ST, CT, and SA to decompose leaf litter and demonstrated a similar variation in decomposing ability among the climatic regions. The results of the present study were consistent with those of Osono (submitted) showing that some *Mycena* species generally are active decomposers of AUR. *Mycena polygramma* IFO33011 from CT is an outlier as this isolate has been reported to decompose cellulose selectively over AUR

(Osono and Takeda 2002; Osono et al. 2003).

The effects of litter type, origin of isolate, and temperature on the decomposition of leaf litter by ligninolytic *Mycena* and other LDM may have implications regarding the changes in fungal decomposition of leaf litter in relation to climatic conditions. First, the variation of decomposition of litter between the fungal isolates was larger than the variations with temperature and litter type (Table 2), suggesting that a shift in fungal species composition can affect the decomposition at the level of LDM assemblages more than changes in temperature and/or litter. Studying the species composition of fungal assemblages, decomposing abilities of individual fungal species, and their geographical distributions is thus crucial for predicting the response of fungal decomposition to possible climate changes. However, studies on the geographical distribution of litter-decomposing fungi are still scarce (e.g. Iwamoto and Tokumasu 2001; Tokumasu 2001; Hosoya et al. 2010). Further studies are needed to examine the geographical distribution of fungi in conjunction with their decomposing abilities.

Secondly, the ability of individual *Mycena* species to decompose litter and AUR also varied with temperature and litter type (Table 2; Fig. 1). This suggests that the functioning of LDM could change along with a 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) and/or with concomitant changes in vegetation (Tsukada 1983; Matsui et al. 2004). For example, an increase of litter temperature to optimum growth temperatures could lead to enhanced decomposition by LDM, resulting in positive feedback to the atmospheric CO_2 level. An increase in the relative abundance of tree species with low AUR content in litter might have a similar positive feedback. These predictions are obviously oversimplified, but data such as the results of the present study will provide useful insights into the possible effects of future climate changes on fungal decomposition in forest soils.

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Table 1. Pearson's correlation coefficients for linear relationship between contents of organic chemical components and nitrogen in leaf litter and mass loss of leaf litter caused by isolates of macrofungi. *** P<0.001, * P<0.05, ns not significant.

	Fungus	Number of tree species used	AUR		Total carbohydrates		Extractives		Nitrogen	
ST	*Mycena* sp.1	12	-0.51	ns	0.06	ns	0.05	ns	0.21	ns
	Mycena sp.2	12	-0.78	**	0.02	ns	0.13	ns	0.35	ns
CT	*Mycena polyramma*	15	-0.80	***	0.26	ns	0.17	ns	-0.25	ns
	Clitocybe sp.1	15	-0.36	ns	0.22	ns	-0.27	ns	-0.14	ns
SA	*Mycena aurantiidisca*	12	0.21	ns	0.14	ns	-0.52	ns	0.52	ns
	Mycena epipterygia	12	0.29	ns	0.15	ns	-0.60	*	0.57	*

Osono Table 2

Table 2. Results of generalized linear models (GLMs) examining the effect of fungal species, litter species, and temperature on litter mass loss (A), and examining the effect of litter species and temperature on AUR mass loss and AUR/litter mass (AUR/L) loss ratio for *Mycena* sp.2. *** P<0.001, ** P<0.01, * P<0.05, ns not significant.

(A) Litter mass loss	d.f.	χ^2	
Model	62	504.7	***
Fungal isolate	2	313.8	***
Litter type	2	100.5	***
Temperature	6	280.1	***
Fungal isolate × Litter type	4	25.5	***
Fungal isolate × Temperature	12	251.9	***
Litter type × Temperature	12	63.5	***
Fungal isolate × Litter type × Temperature	24	37.6	*

(B)	AUR mass loss			AUR/L loss ratio		
	d.f.	χ^2		d.f.	χ^2	
Model	6	41.2	***	6	13.1	*
Litter type	2	22.7	***	2	7.2	*
Temperature	4	37.3	***	4	8.6	ns

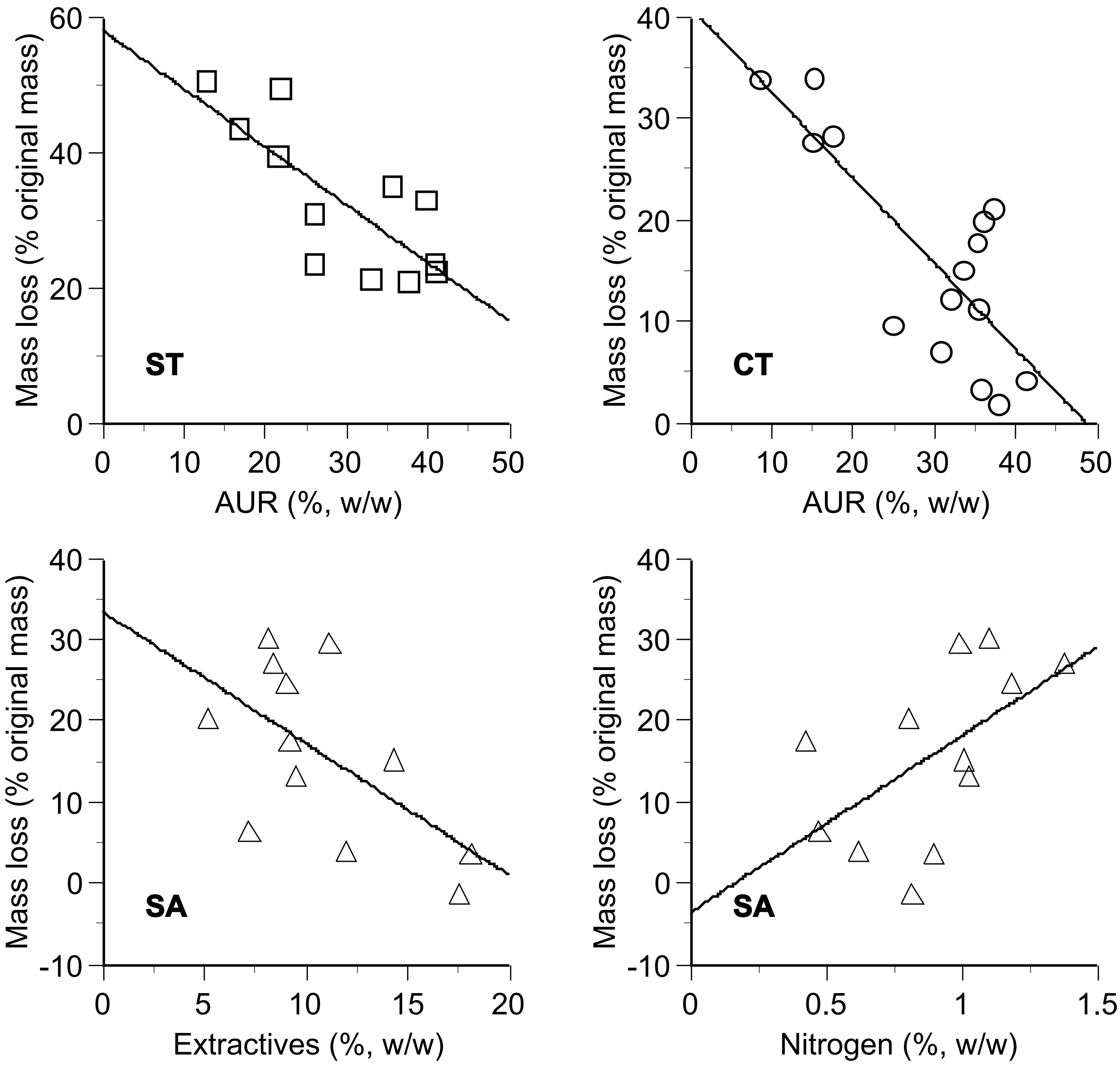
Figure legends.

Fig. 1. Mass loss of leaf litter caused by isolates of macrofungi as related to contents of organic chemical components and nitrogen. ST, mass loss caused by *Mycena* sp.2 was negatively correlated with AUR content in leaf litter of 12 tree species. CT, mass loss caused by *Mycena polygramma* was negatively correlated with AUR content in leaf litter of 15 tree species. SA, mass loss caused by *Mycena epipterygia* was negatively correlated with extractive content and positively correlated with nitrogen content in 12 litter types. Correlation coefficients for the relationships are shown in Table 1.

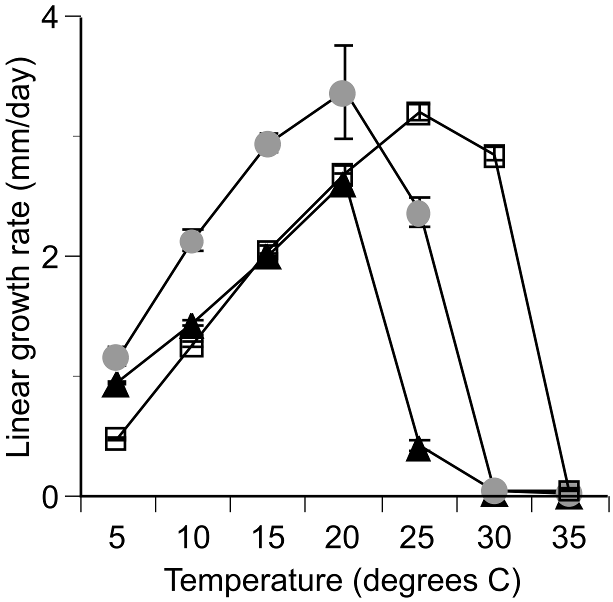
Fig. 2. Colony diameter growth rate of isolates of *Mycena* sp.2 (□, from ST), *M. polygramma* (●, from CT), and *M. aurantiidisca* (▲, from SA) as related to temperature. Bars indicate standard errors (n=4).

Fig. 3. Mass loss of leaf litter (upper) and AUR (middle) and AUR/litter (AUR/L) mass loss rate (lower) in leaf litter of *Castanopsis sieboldii* (□, from ST), *Fagus crenata* (●, from CT), and *Betula ermanii* (▲, from SA) caused *in vitro* by isolates of *Mycena* sp.2 (left, from ST), *M. polygramma* (center, from CT), and *M. aurantiidisca* (right, from SA) at seven temperatures for 12 weeks in the dark. Bars indicate standard errors (n=3 or 4).

Osono Fig. 1

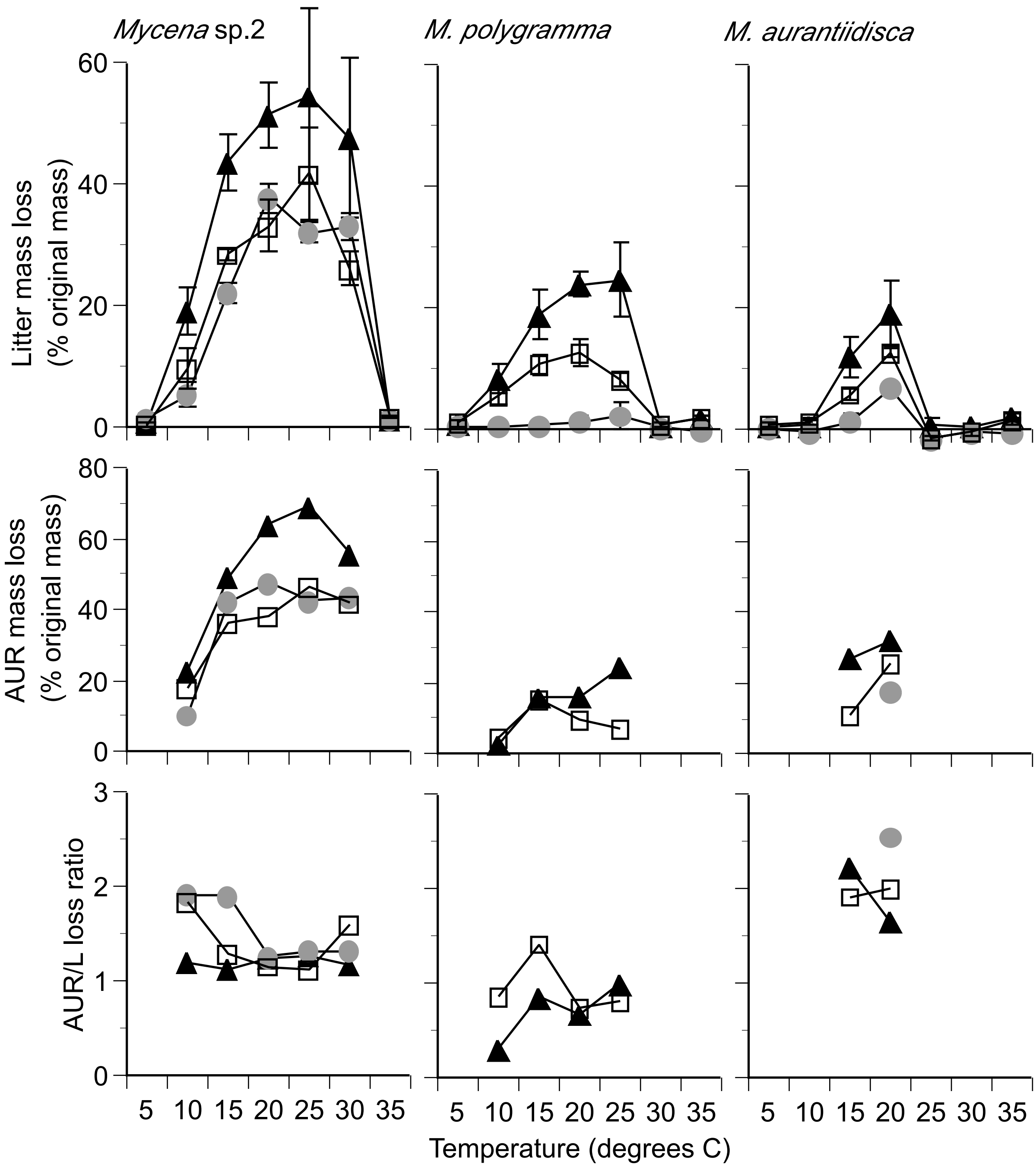

ST
CT
SA
SA
Mass loss (% original mass)
AUR (%, w/w)
Extractives (%, w/w)
Nitrogen (%, w/w)
60
40
30
20
10
0
-10
5
15
50
0.5
1
1.5

Osono Fig. 2



4
2
0
Linear growth rate (mm/day)
5
10
15
20
25
30
35
Temperature (degrees C)

1 Osono Fig. 3



Mycena sp.2
M. polygramma
M. aurantiidisca
Litter mass loss (% original mass)
AUR mass loss (% original mass)
AUR/L loss ratio
0
20
40
60
80
0
1
2
3
5 10 15 20 25 30 35
Temperature (degrees C)

Electronic Supplementary Material

Effects of litter type, origin of isolate, and temperature on decomposition of leaf litter by macrofungi

Takashi Osono

Table S1. Contents of organic chemical components and nitrogen (% w/w) and mass loss of leaf litter (% original mass) caused by isolates of macrofungi in leaf litter of multiple litter types. AUR, acid unhydrolyzable residue; TCH, total carbohydrates; Extr, extractives; N, nitrogen.

Litter type	AUR	TCH	Extr	N	Mass loss	Mass loss
Subtropical forest					***Mycena* sp.1**	***Mycena* sp.2**
Schefflera octophylla	21.7	32.0	20.6	0.69	35.6	39.3
Sapium japonicum	13.0	43.5	9.3	0.58	35.3	50.7
Dendropanax trifidus	22.0	35.6	11.3	0.55	33.1	49.6
Malotus japonicus	17.0	33.8	10.2	1.03	32.1	43.4
Heterosmilax japonica	40.0	36.7	5.9	0.72	28.5	33.0
Pinus luchuensis	41.2	44.9	6.0	0.41	26.4	22.3
Schima wallichii	35.8	29.7	10.7	0.52	26.3	34.9
Castanopsis sieboldii	26.2	34.5	11.2	0.62	23.1	30.8
Syzygium buxifolium	41.1	27.0	11.8	0.47	22.5	23.7
Podocarpus macrophyllus	33.1	43.3	6.5	0.47	19.9	21.4
Quercus miyagii	37.8	30.3	14.9	0.71	14.3	21.0
Daphniphyllum teijismannii	26.1	39.2	12.5	0.61	12.0	23.7
Cool temperate forest					*Mycena polygramma*	***Clitocybe* sp.**
Malotus japonicus	15.4	27.1	17.3	1.20	34.0	3.9
Benthamidia kousa	8.8	32.8	11.3	1.04	33.9	8.9
Carpinus laxiflora	17.8	32.6	11.5	1.25	28.3	10.2
Acer micranthum	15.3	32.3	12.5	0.78	27.7	9.6

Quercus serrata	37.4	26.3	8.0	0.94	21.1	5.4
Betula grossa	36.2	26.7	7.0	1.12	19.9	11.8
Weigela hortensis	35.5	28.6	9.9	0.95	17.8	0.4
Acer rufinerve	33.7	27.0	15.0	0.78	15.1	8.0
Castanea crenata	32.2	29.6	8.6	1.14	12.3	9.9
Quercus crispula	35.6	29.6	9.7	0.99	11.2	6.6
Malus tschonoskii	25.1	37.6	12.4	0.79	9.6	2.5
Magnolia obovata	31.0	31.4	10.4	1.04	7.1	9.5
Fagus crenata	41.5	28.9	8.8	1.68	4.2	1.1
Pterocarya rhoifolia	35.9	25.0	11.5	1.75	3.4	4.8
Aesculus turbinata	38.0	23.5	15.1	0.89	1.9	1.1
Subalpine forest					***Mycena aurantiidisca***	***Mycena epipterygia***
Betula ermanii	45.6	29.1	8.2	1.10	36.7	30.4
Sorbus commixta	30.6	33.0	11.2	1.00	35.9	29.8
Sorbus japonicum	31.9	37.0	8.5	1.38	22.2	27.2
Reynoutria japonica	44.8	31.1	5.3	0.81	20.3	20.5
Aralia cordata	23.9	25.9	9.6	1.03	16.6	13.4
Vibrnum furcatum	29.8	36.5	9.1	1.19	12.9	24.7
Sasa kurilensis	23.1	41.1	7.3	0.48	8.3	6.6
Salix sachaliensis	42.6	20.1	14.4	1.01	7.7	15.3
Pinus pentaphylla	42.1	36.1	9.3	0.43	6.3	17.6
Picea jezoensis var. *hondoensis*	34.4	36.3	17.6	0.82	1.1	-1.1
Tsuga diversiflora	38.5	25.1	18.1	0.90	0.9	3.7
Cercidiphyllum japonicum	25.7	23.9	12.0	0.62	-2.8	4.1