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

2 litter by macrofungi

3

4 Takashi Osono

5

6 T. Osono

7 Center for Ecological Research, Kyoto University, Otsu, Shiga 520-2113 Japan

8 e-mail: tosono@ecology.kyoto-u.ac.jp

9

10 **Abstract**The dependence of hyphal growth and litter decomposition on litter type

11 and incubation temperature used as substratum was compared for

12 litter-decomposing macrofungi (LDM) originating from subtropical (ST), cool

13 temperate (CT), and subalpine forests (SA) in Japan. In the first series of pure

14 culture decomposition tests using a total of 39 litter types as substrata inoculated

15 with six fungal isolates from the three climatic regions, the fungal decomposition

16 of litter was negatively affected by the content of AUR or extractives and

17 positively by N content in the litter. Secondly, cross-inoculation tests were

18 performed to examine the mass loss of leaf litter of broad-leaved trees from ST, CT,
19 and SA, each inoculated with three *Mycena* species from the three climates and
20 incubated at seven temperatures between 5°C and 35°C. Fungal isolate, litter
21 type, incubation temperature, and their interactions significantly affected the
22 mass loss of litter during the incubation. The greatest values of mass loss were
23 found at 20°C or 25°C, and were generally consistent with the optimum
24 temperatures of colony diameter growth rate of these isolates. Isolates from cooler
25 regions were more sensitive to higher temperature than isolates from warmer
26 regions. The decomposition of recalcitrant compounds (as acid-unhydrolyzable
27 residues, AUR) by *Mycena* sp. from ST was also affected by litter type and
28 incubation temperature, but the degree of selective decomposition of AUR relative
29 to other components, such as cellulose, was insensitive to the range of
30 temperature tested.

31

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

34

35 **Introduction**

36

37 Litter-decomposing macrofungi (LDM) include active ligninolytic species in
38 Basidiomycota and Ascomycota and play central roles in leaf-litter decomposition
39 processes (Osono 2007; Lindahl and Boberg 2008; van der Wal et al. 2013).

40 Ligninolytic abilities of LDM have been examined with the pure culture test and
41 compared for multiple species that were commonly found at a single site
42 (Lindeberg 1946; Miyamoto et al. 2000; Steffen et al. 2007; Valášková et al. 2007;

43 Boberg et al. 2011; Žifčáková et al. 2011). Few studies have been available that
44 compared the diversity and functioning of LDM across multiple sites of different
45 climatic regions. Recently, I reported that the species richness of LDM varied with

46 climatic region, declining from subtropical to temperate forest, and then from
47 temperate to subalpine forest in Japan (Osono in press b). Then, I compared the
48 potential abilities of diverse LDM from those forests to decompose leaf litter and

49 recalcitrant compounds, such as lignin, under constant laboratory conditions and
50 found that the decomposing abilities were variable among LDM species within a
51 climatic region but that the variability in decomposing ability was relatively

52 similar among climatic regions (Osono submitted). These results led to a further
53 hypothesis that the decomposing ability of LDM originating from different
54 climates varies in its sensitivity to temperature and litter quality. Several studies
55 have examined the dependence of fungal decomposition on incubation
56 temperature and/or the quality of leaf litter (Lindeberg 1946; Mikola 1956;
57 Miyamoto et al. 2000; Osono and Takeda 2006; Osono et al. 2011c). To the
58 knowledge of the author, however, few studies have explored chemical components
59 that can limit fungal decomposition and compared the dependence of
60 decomposition on temperature and litter types between major LDM originating
61 from regions with different climatic conditions (Osono 2011).

62 The purpose of the present study was to assess under pure culture
63 conditions the dependence of fungal growth and decomposition on litter types
64 used as substrata, incubation temperature, and the origin of the isolate. I used six
65 isolates of LDM species, five of which belonged to *Mycena*, obtained in subtropical,
66 cool temperate, and subalpine forests in Japan because (i) they occurred as
67 fruiting bodies most frequently on the forest floor of the respective forests (Osono
68 in press b) and (ii) they exhibited the greatest activity of the LDM isolates to

69 decompose leaf litter (Osono submitted). I performed two pure culture tests that
70 manipulated litter types and incubation temperature. First, a total of 39 litter
71 types and six fungal isolates from the three climatic regions were used to examine
72 possible limiting factors of fungal decomposition (denoted as the litter types test).
73 Secondly, the pure culture decomposition tests were designed to evaluate the
74 effects of litter type, incubation temperature, *Mycena* isolates of different origins,
75 and the interactions of these factors on the decomposition of leaf litter and
76 recalcitrant compounds in the litter (denoted as the cross inoculation test).

77

78 **Materials and methods**

79

80 Study site

81

82 Isolates of macrofungi and litter materials used in pure culture decomposition
83 tests were collected at three sites in Japan: a subtropical (ST), a cool temperate
84 (CT), and a subalpine (SA) forest. ST was located in an evergreen broadleaved
85 forest in the northern part of Okinawa Island. CT was located in a deciduous

86 broadleaved forest in Kyoto. SA was located in an evergreen coniferous forest in
87 Gifu. The mean annual temperature was 22°C, 10°C, and 2°C in ST, CT, and SA,
88 respectively. The study sites received similar amounts of precipitation annually
89 (approximately 2500 mm). Further details about the location, climatic condition,
90 vegetation, and properties of the forest floor are given in Osono (in press a, in
91 press b).

92

93 Fungal isolates

94

95 Six isolates were used, two isolates from each of the three study sites: two
96 unidentified *Mycena* species (denoted as *Mycena* sp.1 MAFF241586 and *Mycena*
97 sp.2 MAFF241604) from ST, *Mycena polygramma* IFO33011 and an unidentified
98 *Clitocybe* species from CT, and *Mycena aurantiidisca* O2_07101503b and *M.*
99 *epipterygia* O9_07101508 from SA. The *Mycena* species were the macrofungal
100 taxa most frequently encountered as fruiting bodies at each study site (Osono in
101 press b) and were active decomposers of leaf litter (Osono submitted). *Clitocybe* sp.
102 fruited at low frequency in CT (Osono in press b) and was a selective decomposer

103 of recalcitrant compounds on the forest floor (Osono et al. 2011a). These
104 macrofungal isolates were obtained from tissues of fruiting bodies collected at the
105 study sites (Osono submitted), and maintained on slants of 1% malt extract agar
106 medium [MEA, malt extract 1% and agar 2% (w/v)] at 20°C in darkness until the
107 tests were performed. *Mycena* sp.1 and sp.2 from ST were analyzed for base
108 sequences of the rRNA gene 28S D1/D2 (accession numbers: AB512383 for
109 *Mycena* sp.1 and AB512392 for *Mycena* sp.2), but identification to species level
110 was not successful.

111

112 Litter materials

113

114 Newly shed leaves of 12, 15, and 12 plant species without obvious fungal or faunal
115 attack were collected from the surface of the forest floor in January to April 2008,
116 November 2000, and October 2009 in ST, CT, and SA, respectively (see Table S1 in
117 Electronic Supplementary Material) and used for the litter type test. For the cross
118 inoculation test, newly shed leaves of *Castanopsis sieboldii*, *Fagus crenata*, and
119 *Betula ermanii* without obvious fungal or faunal attack were collected from the

120 surface of the forest floor of ST, CT, and SA at peak periods of litter fall in April
121 2009, October 2009, and October 2009, respectively. The litters were oven-dried at
122 40°C for one week and preserved in vinyl bags for one to two months until the
123 experiments were started.

124

125 Litter type test

126

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

131 An individual pure culture decomposition test consisted of one fungal
132 isolate inoculated to one litter type. Litter (0.3 g) was sterilized by exposure to
133 ethylene oxide gas at 60°C for 6 hours and used in the tests, according to the
134 methods described in Osono et al. (2011c). The sterilized litters were placed on the
135 surface of Petri dishes (9 cm diameter) containing 20 ml of 2% agar. Inocula for
136 each assessment were cut out of the margin of previously inoculated Petri dishes

137 on 1% MEA with a sterile cork borer (6 mm diameter) and placed on the agar
138 adjacent to the litter, one plug per plate. The plates were incubated for 12 weeks
139 in the dark at 20°C. The plates were sealed firmly with laboratory film during
140 incubation so that moisture did not limit decomposition on the agar. After
141 incubation the litters were retrieved, oven-dried at 40°C for 1 week, and weighed.
142 The initial, undecomposed litters were also sterilized, oven-dried at 40°C for 1
143 week, and weighed to determine the original mass. Three to four plates were
144 prepared for each isolate, and four uninoculated plates served as a control. Mass
145 loss of litter was determined as a percentage of the original mass, taking the mass
146 loss of litter in the uninoculated and incubated control treatment into account,
147 and the mean values were calculated for each plate. Prior to the tests, the
148 sterilized litters were placed on 1% MEA, and after 8 weeks of incubation at 20°C
149 in darkness, no microbial colonies had developed on the plates. Thus, the
150 effectiveness of the sterilization method used in the present study was verified.
151 The initial litters of multiple tree species were analyzed for the contents of
152 acid-unhydrolyzable residues (AUR), total carbohydrates, extractives, and
153 nitrogen (N) as described below.

154

155 Cross-inoculation test

156

157 Cross-inoculation tests were carried out to examine the effects of litter type,
158 incubation temperature, and origin of *Mycena* isolates on fungal decomposition.

159 Three fungal isolates (*Mycena* sp.2 from ST, *M. polygramma* from CT, and *M.*
160 *aurantiidisca* from SA) were inoculated onto leaves of three litter types
161 (*Castanopsis sieboldii* from ST, *Fagus crenata* from CT, and *Betula ermanii* from
162 SA), and each combination of the three isolates × three litter types was incubated
163 at seven temperatures (5, 10, 15, 20, 25, 30, and 35°C).

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

171 right angles from each other three to five times at given intervals during a
172 3-month incubation, and colony diameter growth rate was calculated by
173 regressing the colony diameter against the days after inoculation. Four plates
174 were prepared for each isolate.

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

179

180 Chemical analyses

181

182 Litter materials were combined to make one sample for each test and ground in a
183 laboratory mill (0.5 mm screen). The amount of AUR in the samples was
184 estimated by means of gravimetry as acid-insoluble residue, using hot sulfuric
185 acid digestion (King and Heath 1967). Samples were extracted with
186 alcohol-benzene at room temperature (15-20°C), and the residue was treated with
187 72% sulfuric acid (v/v) for 2 h at room temperature with occasional stirring. The

188 mixture was diluted with distilled water so that the concentration of sulfuric acid
189 reached 2.5% and autoclaved at 120°C for 60 min. After cooling, the residue was
190 filtered and washed with water through a porous crucible (G4), dried at 105°C and
191 weighed as acid-insoluble residue. The filtrate (autoclaved sulfuric acid solution)
192 was subjected to total carbohydrate analysis. The amount of carbohydrate in the
193 filtrate was estimated by means of the phenol-sulfuric acid method (Dubois et al.
194 1956). One milliliter of 5% phenol (v/v) and 5 ml of 98% sulfuric acid (v/v) were
195 added to the filtrate. The optical density of the solution was measured using a
196 spectrophotometer at 490 nm, using known concentrations of D-glucose as
197 standards. Total N content was measured by automatic gas chromatography (NC
198 analyzer SUMIGRAPH NC-900, Sumitomo Chemical Co., Osaka, Japan).

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

204
$$\text{AUR/L loss ratio} = \text{mass loss of AUR (\% of original AUR mass)} / \text{mass loss}$$

205 of litter (% of original litter mass)

206

207 Statistical analysis

208

209 In the litter type test, Pearson's correlation coefficients were calculated for linear
210 relationships between chemical properties of litter and mass loss of the litter
211 caused by macrofungal isolates. In the cross inoculation test, factors affecting the
212 mass loss of litter were analyzed with generalized linear models (GLMs) with
213 fungal species, litter type, incubation temperature, and their interactions as
214 categorical predictors. Factors affecting the mass loss of AUR and AUR/L loss
215 ratio were also analyzed with GLMs with litter type and incubation temperature
216 as categorical predictors, but only the data of *Mycena* sp.2 were used because the
217 numbers of samples of *M. polygramma* and *M. aurantiidisca* used for AUR
218 analysis were too low. Tukey's HSD test was used for multiple comparisons. JMP
219 6.0 for Macintosh was used to perform these analyses.

220

221 **Results**

222

223 Litter mass loss in the litter type test

224

225 To examine the effect of litter types and possible effects of chemical components
226 on fungal decomposition, six fungal isolates were inoculated onto multiple litter
227 types that differed in chemical properties and incubated at 20°C, and the mass
228 loss of litter during the incubation was related to the chemical composition of the
229 litters. The initial content of AUR in leaf litter of a total of 39 litter types used in
230 the tests ranged from 8.8% to 45.6%, that of total carbohydrates from 20.1% to
231 44.9%, that of extractives from 5.3% to 20.6%, and that of N from 0.41% to 1.75%
232 (see Table S1).

233 Overall, the mean mass loss of litter caused by six fungal isolates ranged
234 from -2.8% to 50.7% (see Table S1), indicating that there were large variations of
235 decomposition between litter types caused by fungal isolates. Three significant
236 correlation coefficients were detected out of a total of 24 combinations (six fungal
237 isolates × four chemical properties) (Table 1). Mass loss of litter caused by *Mycena*
238 sp.2 from ST and *M. polygramma* from CT was significantly and negatively

239 correlated with AUR content in litter; and that caused by *M. epipterygia* from SA
240 was significantly and negatively correlated with the content of extractives and
241 significantly and positively correlated with N content (Fig. 1).

242

243 Colony diameter growth rate as related to temperature

244

245 The colony diameter growth rate increased linearly with temperature from 5 to
246 25°C for *Mycena* sp.2 from ST and from 5 to 20°C for *M. polygramma* from CT and
247 *M. aurantiidisca* from SA (Fig. 2). The optimal growth rate occurred at 25°C for
248 *Mycena* sp.2 and at 20°C for *M. polygramma* and *M. aurantiidisca*. The growth
249 rate was lower at temperatures above the optimal temperature. At 25°C, *M.*
250 *aurantiidisca* displayed more severely reduced growth than *M. polygramma*.
251 *Mycena* sp.2 was the only isolate that grew at 30°C. No growth occurred at 35°C
252 for the three isolates.

253

254 Litter mass loss in the cross inoculation test

255

256 Overall, the mean value of mass loss of litter ranged from -1.4% to 54.4% (Fig. 3,
257 upper). Fungal isolate, litter type, incubation temperature, and their interactions
258 significantly affected the mass loss of litter during the incubation (Table 2A). The
259 mass loss of litter was in the order: *Mycena* sp.2 > *M. polygramma* > *M.*
260 *aurantiidisca*, and *Betula* > *Castanopsis*, *Fagus*. The mass loss of litter increased
261 with temperature: from 5°C to 25°C for *Mycena* sp.2 and *M. polygramma* and
262 from 10°C to 20°C for *M. aurantiidisca*. The greatest values of mass loss were
263 found at 25°C for *Mycena* sp.2 and at 20°C and 25°C for *M. polygramma*, and at
264 20°C for *M. aurantiidisca*, which were generally consistent with the optimum
265 temperatures of colony diameter growth rate of these isolates (Fig. 2). Mass loss of
266 litter was negligible at 5°C for all three isolates and at 35°C for *Mycena* sp.2, at
267 30°C to 35°C for *M. polygramma*, and at 25°C to 35°C for *M. aurantiidisca* (Fig. 3).

268 Incubation temperature affected the mass loss of litter caused by three
269 *Mycena* isolates, and the interactions between temperature and fungal isolate and
270 between temperature and litter type were also significant (Fig. 3; Table 2),
271 indicating that the temperature effect depended on the fungal isolates and litter
272 types. For example, the changes in the mass loss of litter in relation to

273 temperature were larger for *Mycena* sp.2, which had greater ability to cause mass
274 loss than *M. polygramma* and *M. aurantiidisca*, while these changes were less
275 obvious when the fungal isolates were inoculated onto *Fagus* litter, which was
276 more recalcitrant than *Castanopsis* and *Betula* litters.

277

278 AUR decomposition in the cross inoculation test

279

280 The litters with more than 5.0% mass loss were analyzed for the mass loss of AUR.

281 The mean value of mass loss of AUR ranged from 2.6% to 68.9% (Fig. 3, middle).

282 Litter type and incubation temperature significantly affected the mass loss of

283 AUR caused by *Mycena* sp.2 during the incubation (Table 2B). Mass loss of AUR

284 by *Mycena* sp.2 was lower at 10°C than at 15 to 30°C and was higher on *Betula*

285 than on *Fagus* and *Castanopsis*.

286 The mean values of AUR/L loss ratio ranged from 0.3 to 2.6 (Fig. 3, lower).

287 Litter type significantly affected AUR/L loss ratio for *Mycena* sp.2, whereas

288 incubation temperature had no significant effect on AUR/L loss ratio (Table 2B).

289

290 **Discussion**

291

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

305 The findings that growth and decomposition were maximal at 20°C and
306 25°C (Figs. 2 and 3), respectively, indicate that the *Mycena* isolates used are

307 mesophilic. The isolate from cooler regions was more sensitive to higher
308 temperature than the isolate from warmer regions (Fig. 2), suggesting
309 physiological adaptations of these fungi to the climatic conditions of the respective
310 study sites (subtropical versus cool temperate versus subalpine). The optimal
311 temperature of hyphal growth was generally consistent with that of
312 decomposition, which agrees with the finding of Osono et al. (2011c) for three
313 isolates of *Xylaria* sp. from CT.

314 Incubation temperature affected the mass loss of litter by the three
315 *Mycena* isolates and the mass loss of AUR by *Mycena* sp.2 (Fig. 3; Table 2).
316 However, AUR/L loss ratio of *Mycena* sp.2 was not affected by incubation
317 temperatures (Table 2), suggesting that the degree of selective AUR
318 decomposition by *Mycena* sp.2 was insensitive to the range of temperature
319 adopted in the present study. Previously, Adaskaveg et al. (1995) and Osono et al.
320 (2011c) reported that at temperatures above the optimum growth temperatures,
321 the ligninolytic activity of fungi increased at the expense of cellulolytic activity,
322 resulting in suppressed overall decomposition of leaf litter. My finding here for
323 *Mycena* sp.2 from ST suggested that the sensitivity of selective delignification to

324 temperature could vary with fungal species. Osono and Takeda (2006) also
325 showed that the degree of selective delignification caused by four ligninolytic
326 fungi did not differ between incubation at 10°C or 20°C, whereas *Gymnopus*
327 *dryophilus* decomposed AUR more selectively at 10°C than at 20°C. More studies
328 are needed to evaluate the sensitivity to temperature of ligninolytic activity of
329 litter-decomposing macrofungi, including those from cooler regions, such as *M.*
330 *polygramma* and *M. aurantiidisca*.

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

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

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

356 Secondly, the ability of individual *Mycena* species to decompose litter and
357 AUR also varied with temperature and litter type (Table 2; Fig. 1). This suggests

358 that the functioning of LDM could change along with a possible future increase in
359 temperature worldwide, which in temperate regions is expected to be an increase
360 of 2-3°C (Manabe et al. 1991; Boer et al. 1992; Russell et al. 1995) and/or with
361 concomitant changes in vegetation (Tsukada 1983; Matsui et al. 2004). For
362 example, an increase of litter temperature to optimum growth temperatures could
363 lead to enhanced decomposition by LDM, resulting in positive feedback to the
364 atmospheric CO₂ level. An increase in the relative abundance of tree species with
365 low AUR content in litter might have a similar positive feedback. These
366 predictions are obviously oversimplified, but data such as the results of the
367 present study will provide useful insights into the possible effects of future
368 climate changes on fungal decomposition in forest soils.

369

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376

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Osono Table 1

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 | <i>Mycena</i> sp.1 | 12 | -0.51 ns | 0.06 ns | 0.05 ns | 0.21 ns |
| | <i>Mycena</i> sp.2 | 12 | -0.78 ** | 0.02 ns | 0.13 ns | 0.35 ns |
| CT | <i>Mycena polyramma</i> | 15 | -0.80 *** | 0.26 ns | 0.17 ns | -0.25 ns |
| | <i>Clitocybe</i> sp.1 | 15 | -0.36 ns | 0.22 ns | -0.27 ns | -0.14 ns |
| SA | <i>Mycena aurantiidisca</i> | 12 | 0.21 ns | 0.14 ns | -0.52 ns | 0.52 ns |
| | <i>Mycena eipterygia</i> | 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 \times Litter type | 4 | 25.5 | *** |
| Fungal isolate \times Temperature | 12 | 251.9 | *** |
| Litter type \times Temperature | 12 | 63.5 | *** |
| Fungal isolate \times Litter type \times 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 |

1 Figure legends.

2

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

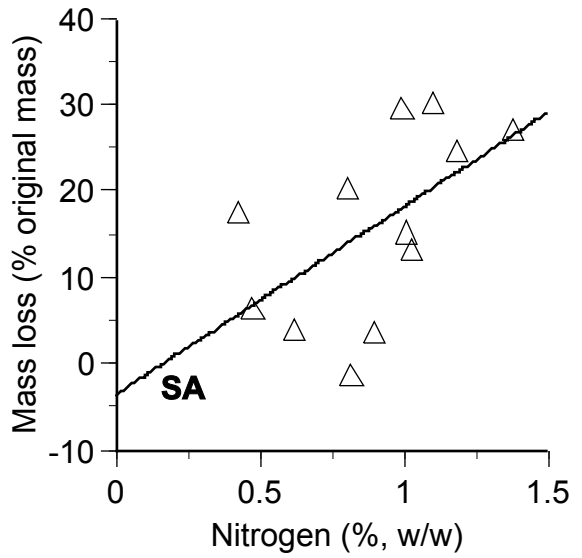
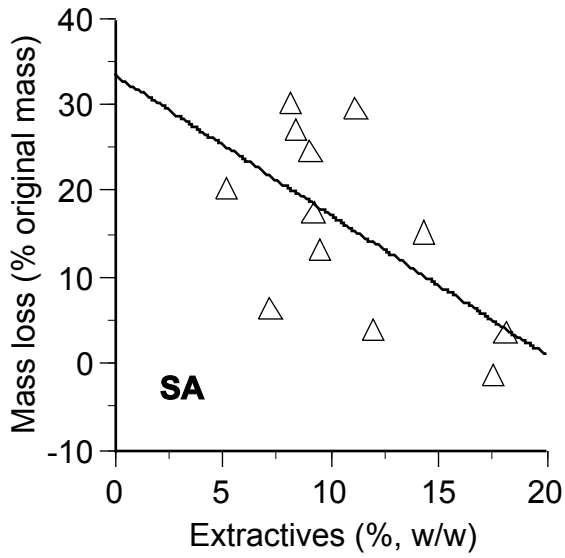
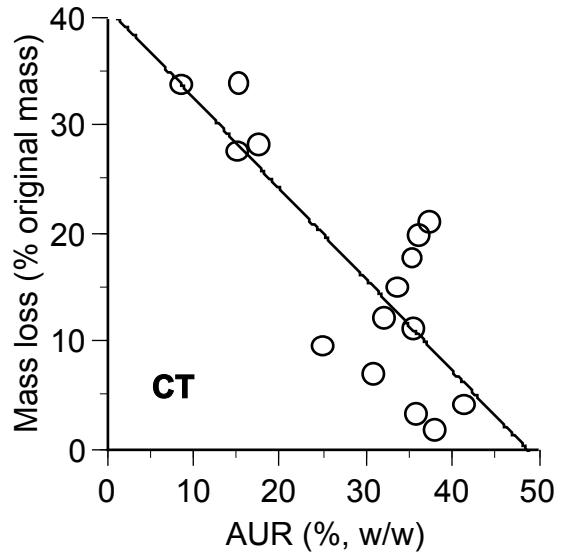
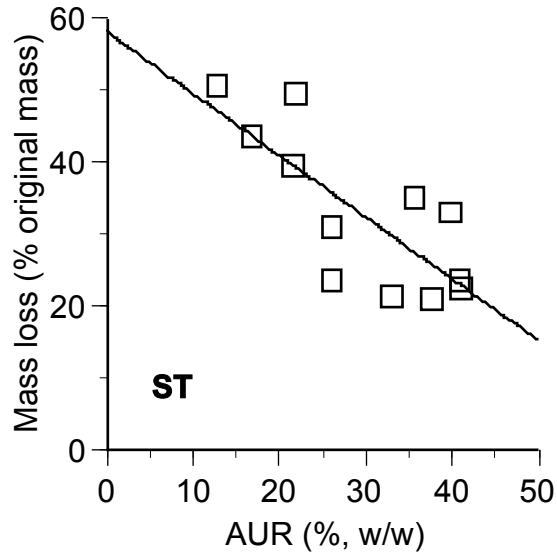
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12 Fig. 2. Colony diameter growth rate of isolates of *Mycena* sp.2 (□, from ST), *M.*
13 *polygramma* (●, from CT), and *M. aurantiidisca* (▲, from SA) as related to
14 temperature. Bars indicate standard errors (n=4).

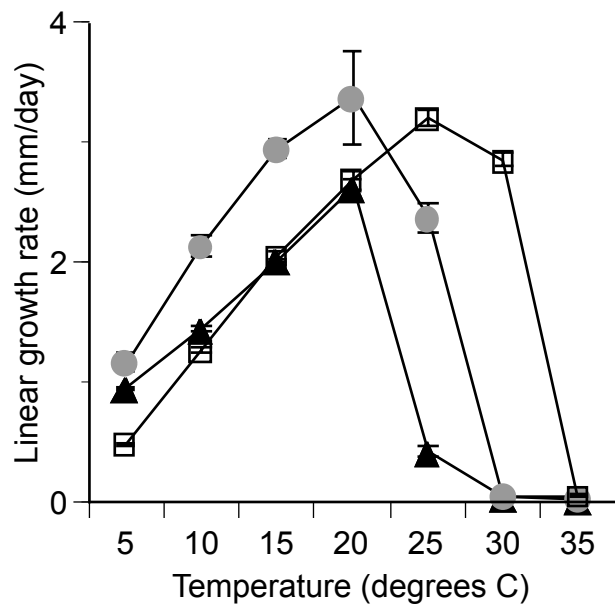
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16 Fig. 3. Mass loss of leaf litter (upper) and AUR (middle) and AUR/litter (AUR/L)
17 mass loss rate (lower) in leaf litter of *Castanopsis sieboldii* (□, from ST), *Fagus*
18 *crenata* (●, from CT), and *Betula ermanii* (▲, from SA) caused *in vitro*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

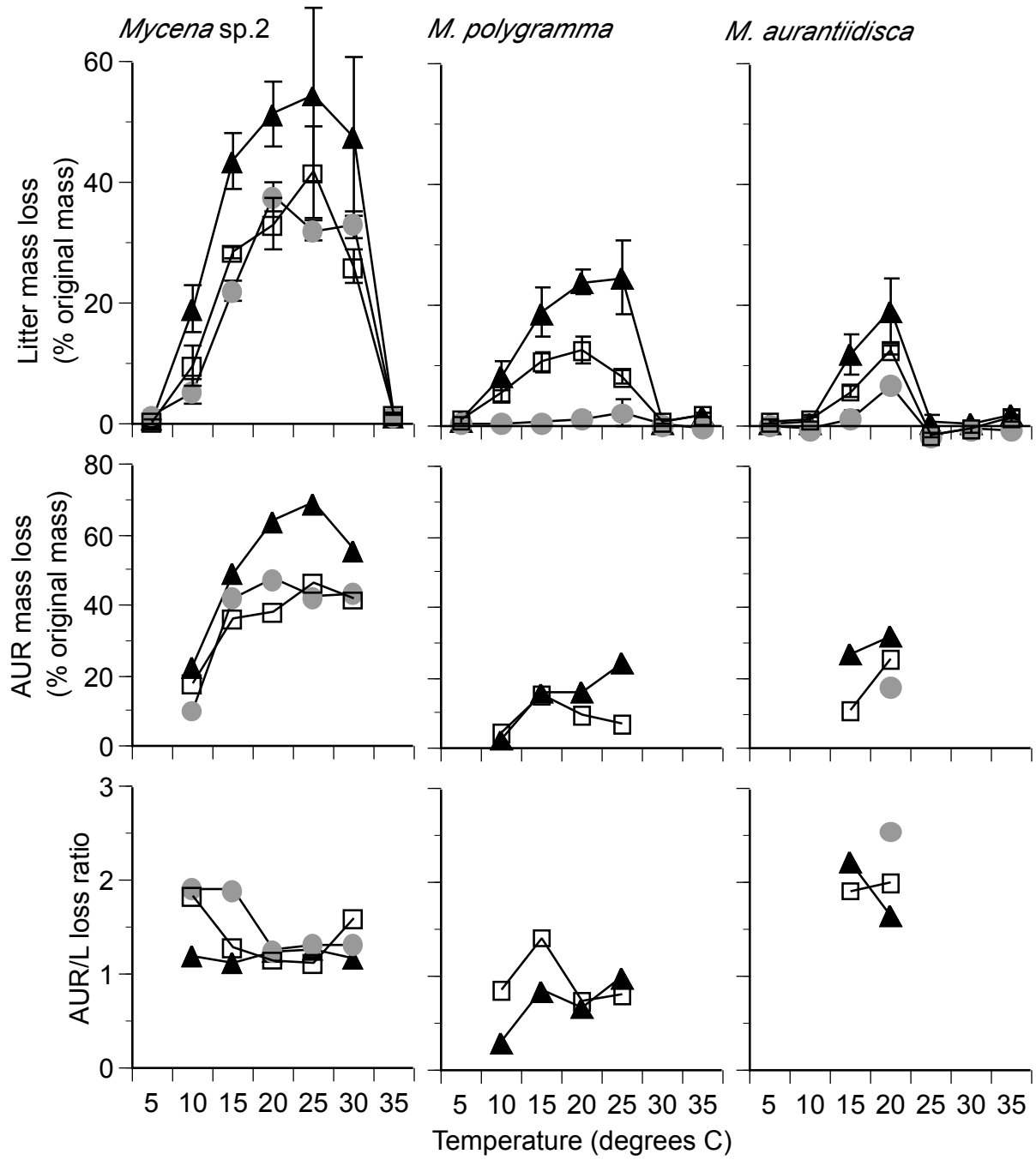
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1 Osono Fig. 2
3



1 Osono Fig. 3



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

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