Diversity, resource utilization, and phenology of fruiting bodies of litter-decomposing macrofungi in subtropical, temperate, and subalpine forests

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AbstractThe diversity, vegetative and reproductive characteristics, and phenology of litter decomposing macrofungi (LDM) were compared between humus forms and climatic regions. Fruiting bodies of LDM were examined for the forest floor of subtropical (ST), cool temperate (CT), and subalpine (SA) forests in Japan. Field surveys during one growing season yielded 35, 32, and 18 species in ST, CT, and SA, respectively. Species richness was generally higher in mull than in moder humus and in warmer than in cooler climate. A total of 10 fungal families were observed, and species in the Mycenaceae dominated in the LDM assemblages at

all study sites. A larger number of species fruited on deeper F layers of the forest floor in SA than in ST, where 74% of species fruited directly on leaf litter. This observation was consistent with the analysis of radiocarbon content in fruiting bodies, implying that LDM tended to utilize older carbon accumulated at deeper layers of the forest floor in cooler climates. Seasonal changes in the fruiting frequency over a growing season exhibited similar two-peak patterns for all the study sites, coinciding with the periods of rainfall and increasing and decreasing air temperatures in early summer and autumn, respectively, but the fruiting period extended longer in warmer than in cooler climate.

Keywords Climate · Decomposition · *Mycena* · Radiocarbon · Seasonal changes

Introduction

Litter-decomposing macrofungi (LDM) are major components of the diversity of soil organisms in terrestrial ecosystems and play central roles in the

35 decomposition of structural and soluble components in litter that often limit
36 carbon and nutrient cycling in soil (Osono 2007; van der Wal et al. 2013). A suite of
37 LDM with the ability to decompose lignin and other recalcitrant compounds are of
38 particular importance because the colonization of litter materials by these fungi
39 often stimulates the turnover of organic matter and nutrients in soil (Steffen et al.
40 2007; Valášková et al. 2007; Osono et al. 2011a). Fruiting bodies of LDM provide
41 reliable and useful information about their taxonomy, diversity, and reproduction
42 and have been surveyed for their diversity (e.g. Schmit et al. 1999; Mueller et al.
43 2007) and seasonal patterns (Murakami 1989; Yamashita and Hijii 2004) and for
44 the effects on them of vegetation (Hansen and Tyler 1992; Lange 1993; Såstad
45 1995), soil conditions (Tyler 1985; Rastin et al. 1990), and elevational gradient
46 (Gómez-Hernández et al. 2012). Moreover, the observation of vegetative mycelia
47 at the base of fruiting bodies can often yield insights into the substrate utilization
48 and decomposing ability of LDM (Osono et al. 2011a). Currently, however, few
49 studies have investigated the diversity, vegetative and reproductive
50 characteristics, and phenology of LDM simultaneously and compared these
51 between humus forms and climatic regions. It is hypothesized that the pattern of

52 diversity, substrate utilization, and phenology of fruiting bodies of LDM change
53 along gradients of soil conditions and climate.
54 The purpose of the present study was to investigate fruiting bodies of
55 LDM emerging from the forest floor of subtropical, cool temperate, and subalpine
56 forests in Japan. Field sampling of fruiting bodies over growing seasons yielded
57 information about the structure, diversity, and species composition of LDM
58 assemblages and seasonal patterns of occurrence. Each LDM species was recorded
59 for the soil layer from which its fruiting body emerged to examine the substrate
60 its vegetative mycelia utilized. Radiocarbon (^{14}C) contents of fruiting bodies were
61 measured for major LDM species to estimate the age of carbon (i.e. time since
62 death of plant litter) utilized by these species. The diameter of the pileus and the
63 length of the stipe were measured for fruiting bodies found in the three forest soils,
64 and possible roles of the size variation of fruiting bodies in the seasonal patterns
65 of fruiting bodies were discussed.
66
67 **Materials and methods**
68

69 Study site

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71 Samples were collected from three sites in Japan: a subtropical forest (ST), a cool
72 temperate forest (CT), and a subalpine forest (SA). ST was located in Okinawa,
73 southern Japan. CT was located in Kyoto, Japan. In CT, two study plots were
74 established on the upper and lower parts of a northwest-facing slope
75 (approximately 200 m long). SA was located on Mt. Ontake, Gifu, Japan. Details
76 of the location, climatic conditions, and vegetation are given in Osono (submitted).
77 In summary, the three sites differed in mean annual temperature (22°C, 9°C, and
78 2°C in ST, CT, and SA, respectively), seasonal patterns of change in air
79 temperature, and the duration of the growing season, but they received similar
80 amounts of precipitation annually. The study sites experience a rainy season from
81 May to June in ST and from June to July in CT and SA. Snow covers the forest
82 floor of CT from December to April and that of SA from mid-November to early
83 June. Table 1 shows properties of the forest floor of the study sites. The
84 accumulation of forest floor material, in terms of the depth and the mass, was in
85 the order: ST, CT (lower) < CT (upper) < SA, whereas the order was generally

86 reversed for the leaf fall mass. Consequently, the turnover time of the forest floor
87 was lower in SA and CT (lower) (less than two years) than in CT (upper) (10.4
88 years) and in SA (29.1 years).
89
90 Study plot and field survey
91
92 A study plot of 50 × 10 m (500 m^2) was laid out in each of ST, CT (upper), CT
93 (lower), and SA sites and was divided into 125 grids of 2 × 2 m. The study area of
94 500 m^2 was found to be large enough to describe species richness of macrofungi in
95 CT sites, according to Okabe (1986).
96 Fruiting bodies of LDM were collected in the study plots, seven times at
97 1- to 2-month intervals from March 2007 to January 2008 in ST, nine times at 2-
98 to 4-week intervals from May to November 2001 in CT, and five times at 1-month
99 intervals from June to October 2008 in SA. On each sampling occasion, all fruiting
100 bodies encountered on the surface of the forest floor were recorded, excepting
101 obviously immature or rotting ones. Records were kept of taxa and of grid number
102 and soil horizons (L layer, the border between L and F layers, F layer, or A layer)

103 from which the fruiting bodies emerged (see Table S1 in Electronic
104 Supplementary Material). Fruiting bodies occurring on logs, twigs, or roots that
105 were fallen or buried were not recorded. Ascomycetes were omitted, but the
106 Xylariaceae on leaf litter were included because of their ligninolytic activity
107 (Osono et al. 2011b). In October and November 2011, fruiting bodies were
108 measured for the diameter of their pileus and length of their stipe at the three
109 sites.

110 Identification was primarily made macroscopically after Imazeki et al.
111 (1988), Imazeki and Hongo (1987, 1989), and Hongo (1994). Small fruiting bodies
112 of *Mycena* and *Marasmius* that were difficult to distinguish and identify at the
113 species level in the field were classified at the genus or section level, which was
114 referred to as species in the present study for the sake of simplicity (but see
115 Discussion). Tissues of some fruiting bodies were further analyzed for the DNA
116 sequence of amplicons of rDNA ITS region obtained using primers ITS5 and ITS4
117 (White et al. 1990) and of the 28S rRNA gene D1/D2 region using primers D1
118 (Peterson 2000) and NL4 (O'Donnell 1993), according to the method of Hirose and
119 Osono (2006). The sequences determined were compared with the available rDNA

120 sequences in the GenBank database by means of BLAST+ (Camacho et al. 2009)
121 and assigned taxonomically. The data of molecular analyses will be given in a
122 future paper.
123 The frequency of occurrence of LDM was calculated as a percentage of
124 incidences based on the number of grids in which the fruiting body was
125 encountered relative to the total number of grids (125) at each study site. Relative
126 frequency of an individual species was calculated as the percentage of its
127 frequency of occurrence with respect to the grand sum of the frequency of
128 occurrence of all species at each study site. Data of fruiting bodies of mycorrhizal
129 fungi were excluded from the following analyses.
130
131 Radiocarbon analysis
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133 Samples of fruiting bodies were ground in a laboratory mill to make particles that
134 would pass through a 0.5-mm screen and sent to the Institute of Accelerator
135 Analysis Ltd, Kanagawa, Japan, for accelerator mass spectrometry
136 measurements of radiocarbon. The methods are described in Hyodo et al. (2006).

137 Radiocarbon values were reported as $\Delta^{14}C$ (‰), which is the part per-thousand
138 deviation from the activity of nineteenth century wood, and corrected for the
139 fractionation using stable carbon (C) isotope ratios of the samples.

140 The method to estimate the carbon age of fruiting bodies of LDM followed
141 Hyodo et al. (2006). The carbon age of fruiting bodies of fungi was defined as the
142 time elapsed since C in their substrates was fixed from atmospheric CO_2 by
143 primary producers. $\Delta^{14}C$ values of samples were compared with those of
144 atmospheric CO_2 recorded at Schauinsland, Germany, for 1976-97 (Levin and
145 Kromer 1997). I estimated the $\Delta^{14}C$ values of atmospheric CO_2 after 1997 by
146 extrapolation of the exponential function: $\Delta^{14}C(t) = 417 \times \exp(-t/16.0)$, where t is
147 the year after 1974 (Levin and Kromer 1997). This method yielded two estimates
148 of the year of C fixation for the measured $\Delta^{14}C$ values of fruiting bodies, one before
149 and another after the peak of bomb-$\Delta^{14}CO_2$ in mid-1960s, and hence two carbon
150 ages (Hyodo et al. 2006). I adopted the carbon ages estimated from the year of C
151 fixation after the peak bomb-$\Delta^{14}C$, because these estimated carbon ages were
152 compatible with the turnover rates of the forest floor (1.5 to 29.1 years in the
153 study sites, Table 1).

154

155 Statistical analysis

156

157 The observed number of LDM species at each study site was denoted as S_{obs}. It
158 was possible that S_{obs} be underestimated when the abundance of fruiting bodies of
159 LDM encountered (i.e., the sampling effort) was low at any study site, compared
160 to other sites. To avoid this, I used an individual-based Coleman rarefaction curve
161 (Colwell and Coddington 1994) to depict the cumulative number of species versus
162 the observation of fruiting bodies (Fig. S2 in Electronic Supplementary Material).
163 In the present study, the number of observation of fruiting bodies was variable
164 among the study sites, ranging from 77 at CT (upper) to 620 observations at ST.
165 Thus, the study sites were compared for the estimated numbers of LDM species at
166 77 observations (denoted as S_{est}). Calculations were performed with R version
167 3.0.2 for Mac (R Development Core Team 2009) and its vegan package (Oksanen
168 2013).

169 Simpson's diversity index (D) and equitability (E) were calculated in the
170 following equations (Osono et al. 2002): $D = 1 / \Sigma P_i^2$, $E = D / S_{obs}$, where P_i was a

proportion of the frequency of occurrence of *i*th species to the sum of frequency of all species.

Generalized linear models (GLMs) were used with a Gaussian distribution to compare the size of fruiting bodies of LDM between the study sites. The GLMs were performed with the *glm* function and with the *glht* function of the R multcomp package for multiple comparisons with Tukey's test.

Results

Species richness and taxonomic composition

A total of 35, 32, and 18 species of LDM were observed (S_{obs}) in ST, CT, and SA, respectively; and in CT, 25 and 11 species were observed at lower and upper slopes, respectively (see ESM; summarized in Table 2). The number of singleton species (i.e. species encountered in only one grid) accounted for 17% to 49% of the total number of species, in the order: ST > CT (lower) > CT (upper) > SA (Table 2). Simpson's diversity index was in the order: ST > SA > CT (lower) > CT (upper),

188 and equitability was in the order: CT (upper) > SA > ST > CT (lower) (Table 2).
189 Rarefaction analysis showed that the estimated number of LDM species (S_{est})
190 were higher in mull [ST and CT (lower)] than in moder humus [CT (upper) and
191 SA] (Table 2).

192 A total of 10 fungal families were observed: five, seven, five, and five
193 families in ST, CT (lower), CT (upper), and SB, respectively (Table 2). *Mycena*
194 species in the Mycenaceae dominated in the LDM assemblages at each study site
195 in terms of the number of species (27% to 50% of the total number of species; Table
196 2) and the relative frequency (Fig. 1). The frequencies of occurrence of two major
197 *Mycena* species reached more than 90% (i.e. the fruiting bodies of these species
198 occurred in more than 90% of the 125 grids) in ST and between 13.6% to 60.8% in
199 CT and SB (see ESM). These major *Mycena* species were followed by species in
200 Marasmiaceae in ST and CT (upper), by species in Agaricaceae in CT (lower), and
201 by species in Hymenogasteraceae in SA (Table 2; Fig. 1).

202

203 Soil layer from which macrofungi fruited

204

Soil layer from which LDM fruited differed among the study sites: more number of LDM species that fruited from deeper layers of the forest floor at cooler climate. That is, 74% (26/35) of species in ST fruited on the surface L layer (i.e. emerging directly on leaf litter), whereas 73% to 92% from the border between L and F layers in CT, and 78% from the F layer in SA (Table 2) did so. Those that fruited on L layer were 'component-restricted' sensu Osono (2007) in that individual mycelia were limited in extent by the physical boundaries of the litter component they occupied. Conversely, those that fruited on the L-F border and F layer were 'component-non-restricted' in that the entire forest floor, rather than an individual litter component, provided a habitat for the fungi.

Radiocarbon content

The mean $\Delta^{14}C$ values of fruiting bodies ranged between 48.2‰ and 139.7‰ (Table 3), indicative of the fungal uptake of bomb-$\Delta^{14}C$ that was primarily derived from the litter that these LDM utilized (Hyodo et al. 2006). The carbon age of fruiting bodies from ST ranged from 2.8 to 9.0 years. These values suggested that these

LDM utilized leaves that died at least 1.8 to 8.0 years previously, because tree leaves in ST were mostly evergreen and had leaf longevity of more than one year. In contrast, the carbon age of fruiting bodies from CT ranged from 3.6 to 11.4 years, suggestive of the utilization of deciduous leaves that died as long as 11.4 years before. The carbon age of fruiting bodies from SA reached as old as 20.3 years, suggestive of the utilization of evergreen leaves (maximum ages of 6 to 11 years, Mori and Takeda 2004) that had died at least 10 years before. These results, together with the results of direct observation of fruiting bodies, suggested that LDM tended to utilize older carbon accumulated at deeper layers of the forest floor in cooler climates.

Size and phenology of fruiting bodies

The mean size of the fruiting bodies, measured as the diameter of pileus and length of stipe, was significantly different among the study sites (ANOVA, $p<0.05$), in the order: CT > SA > ST (Table 4). This was accounted for by the difference in size of the major *Mycena* species in these study sites (Table 4).

239 The frequency of occurrence of fruiting bodies was generally higher in ST
240 than in CT or SA (Fig. 2). Seasonal changes in the frequency over a growing
241 season exhibited similar two-peak patterns for all the study sites: a peak during
242 the rainy season in early summer and another in autumn (Fig. 2). That is, the
243 peaks were found in June and in September to January at ST, in June and in
244 September to November at CT, and in July and in September-October at SA. The
245 number of LDM species followed similar seasonal patterns as the frequency of
246 occurrence of fruiting bodies, except that there was a rapid increase in the
247 number of species in June in ST.

248 The major LDM species differed in the seasonal patterns of their
249 frequency of occurrence over a growing season (Fig. 3). In ST, fruiting bodies of
250 some major species, such as *Mycena* sp.ST1 and *Marasmius* sp.ST1, occurred
251 relatively constantly over the growing season, whereas *Mycena* sp.ST2, *Xylaria*
252 sp.ST1, and *Crinipellis* sp.ST1 displayed fruiting peaks in June and/or in
253 September to January. In CT and SA, the frequencies of major species increased
254 once in autumn (*My. polygramma* in CT and *My. epipterygia*, *G. atkinsoniana*,
255 and *Mycena* sp.SA1 in SA) or twice (in early summer and in autumn) (*My. filopes*

256 in CT and *My. aurantiidisca* in SA) over the growing season.

257

258 **Discussion**

259

260 The numbers of species of LDM observed in CT and SA (Table 2) were within the
261 range reported previously in temperate and boreal forests (Tyler 1985; Hintikka
262 1988; Villeneuve et al. 1989; Brunner et al. 1992; Miyamoto et al. 2000;
263 Outerbridge 2002; Richard et al. 2004; Gates et al. 2011; O'Hanlon and
264 Harrington 2012), despite the short survey period (one growing season) at each
265 study site. The dominance of *Mycena* in terms of the number of species and the
266 frequency of occurrence is consistent with these previous reports. López-Quintero
267 et al. (2012) also observed the occurrence of *Mycena* and *Marasmius* species in
268 Amazon tropical rainforests, but comparative studies on the diversity of LDM in
269 tropical and subtropical forests have been relatively scarce, especially in Asian
270 tropical regions (Mueller et al. 2007).

271 The number of observed and estimated species and Simpson's diversity
272 index of LDM were generally higher in mull than in moder humus (Table 2). This

was evident in CT, where the richness of LDM was higher at the lower (mull) than the upper (moder) slope. The two most frequent species were common to the two sites, so that infrequent species accounted for the low similarity of species composition (Table 2, Fig. 1). Rastin et al. (1990) also compared LDM between the lower and upper slope of a spruce forest in Germany and reported that the species composition was generally similar between those parts, in contrast to the results of the present study. This discrepancy may be partly due to the slope length [30 m in Rastin et al. (1990) versus 200 m in the present study]. The causal factors for the higher LDM richness in mull of CT remain unclear, but the relatively favorable moisture condition at the lower slope could possibly favor the fruiting and co-occurrence of more LDM species on the forest floor.

The LDM diversity was generally higher in warmer than in cooler climates, suggesting a climatic gradient of diversity. Similar climatic gradients of fungi have been found for litter decomposing microfungi (Osono 2011) and foliar endophytic fungi (Arnold and Lutzoni 2007; Ikeda et al. 2014). At least two explanations are possible for the putative higher diversity of fruiting bodies of LDM in warmer locations. First, the warmer condition throughout the year and

290 lack of snow cover period in winter of ST can favor the fruiting (and possibly, the
291 co-existence) of multiple LDM. This is illustrated in the fruiting phenology of
292 major LDM species (Fig. 3): fruiting bodies occurred throughout the year or with
293 multiple peaks in ST, whereas in CT and SA they peaked once or twice over the
294 growing season. Such differences may be partly due to suitability of the climatic
295 conditions for establishment, growth, and fruiting of more LDM species in ST.

296 Secondly, differences in the quality of resources can also affect the
297 diversity of LDM. I found that LDM from a cooler climate produced fruiting bodies
298 that originated from deeper soil layers than those from a warmer climate (Table 2).
299 In accordance with this, LDM from a cooler climate appeared to utilize more aged
300 dead carbon than those from a warmer climate (Table 3). Given that more
301 decomposed materials in deeper layers contain less readily available organic
302 carbon sources, such as non-lignified holocellulose and soluble carbohydrates
303 (Berg 1986; Osono et al. 2003), the utilization of resources at deeper layers in a
304 cooler climate may be unfavorable for the growth and fruiting of LDM. Osono
305 (2011) demonstrated that non-ligninolytic microfungi are major components of
306 fungal assemblages on recently fallen litter in a cooler climate, suggesting that

307 ligninolytic LDM are less competitive for readily available resources in the
308 surface litter in a cooler climate (Osono 2007). The dominance in SA of conifers,
309 whose needles are rich in secondary compounds that inhibit the growth of LDM
310 (Bağci and Diğrak 1996), can also affect the colonization of the L layer by LDM.
311 Two peaks were found for the occurrence of fruiting bodies over a growing
312 season at all three climates, but the fruiting period extended longer at warmer
313 than at cooler climates (Fig. 2). The two peaks coincided with the period of rainfall
314 and with the increasing and decreasing air temperatures in early summer and
315 autumn, respectively, at the three forest sites. Similar one- or two-peak patterns
316 of fruiting of LDM have commonly been found in temperate forests (Okabe 1983;
317 Straatsma et al. 2001; Yamashita and Hijii 2004; Gates et al. 2011).
318 The fruiting phenology of major *Mycena* species may also be associated
319 with the size of fruiting bodies. For example, *Mycena* species with smaller fruiting
320 bodies fruited more frequently over a growing season than those with larger ones;
321 *M. filopes* in CT and *M. aurantiidisca* in SA with smaller fruiting bodies showed
322 two peaks, whereas *M. polygramma* in CT and *M. epipterygia* in SA with larger
323 fruiting bodies showed only one peak in autumn (Fig. 3, Table 4), and even

smaller *Mycena* sp. ST1 and ST2 in ST fruited throughout the growing season. Because the production of larger fruiting bodies should require more resources to be utilized, the size of fruiting bodies can set a limit on the reproduction. This discussion is obviously speculative, however, as few data have been available regarding the population structure and reproductive biology of individual LDM species. More studies are needed to examine the life history strategy of LDM, especially in tropical and subtropical regions.

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Osono Table 1

Table 1. Forest floor and field survey of fruiting bodies in the study sites.

Site	ST	CT	SA
Humus type	Mull	Mull (lower), Moder (upper)[a]	Moder[b]
Depth of L layer (cm)[c]	1.1 ± 0.1	1.2 ± 0.1	2.7 ± 0.3
Depth of FH layer (cm) [c]	1.0 ± 0.2	4.0 ± 0.4	13.4 ± 1.2
Forest floor mass (Mg/ha)	12.0[d]	7.7 (lower) 33.3 (upper)[e]	104.6[f]
Leaf fall mass (Mg/ha/yr)	7.95[d]	4.10 (lower) 3.20 (upper)[e]	3.59[f]
Turnover time (yr)[g]	1.5	1.9 (lower) 10.4 (upper)	29.1

[a]Takeda and Kaneko (1988). [b]Tian et al. (1997). [c]Values are means ± standard errors (n=20). Measurement was carried out in the three study sites in October 2012. Values of CT were from the lower slope. [d]Xu et al. (1998a, 1998b). [e]Tsukamoto (1996). [f]Fukasawa et al. (2014). [g]Turnover time = forest floor mass / annual leaf fall mass.

Osono Table 2

Table 2. Assemblage structure and family composition of macrofungi and the soil layer from which fruiting bodies occurred. Numbers of macrofungal species are indicated for fungal families and the litter layers. Numbers in parentheses indicate the proportion relative to the observed number of species. Values of S_{est} indicate means ± standard deviations. See text for S_{est}.

	ST	CT (lower)	CT (upper)	SA
Diversity				
Observed number of species (S_{obs})	35	25	11	18
Singleton species	17 (49)	11 (44)	4 (36)	3 (17)
Simpson's D	7.68	4.47	3.40	4.86
Equitability	0.22	0.18	0.31	0.27
Estimated number of species (S_{est})	14.2±2.1	17.2±2.0	11.0±0.0	11.9±1.7
Family composition				
Mycenaceae	13 (37)	10 (40)	3 (27)	9 (50)
Marasmiaceae	12 (34)	3 (12)	3 (27)	2 (11)
Agaricaceae	3 (9)	6 (24)	2 (18)	1 (6)
Tricholomataceae	5 (14)	2 (8)	0 (0)	3 (17)
Strophariaceae	0 (0)	1 (4)	2 (18)	0 (0)
Psathyrellaceae	0 (0)	2 (8)	0 (0)	0 (0)
Pluteaceae	0 (0)	1 (4)	0 (0)	0 (0)
Hygrophoraceae	0 (0)	0 (0)	1 (9)	0 (0)
Hymenogasteraceae	0 (0)	0 (0)	0 (0)	1 (6)
Xylariaceae	1 (3)	0 (0)	0 (0)	0 (0)
Unidentified	1 (3)	0 (0)	0 (0)	2 (11)
Soil layer				
L layer	26 (74)	0 (0)	0 (0)	0 (0)
L-F border	9 (26)	23 (92)	8 (73)	3 (17)
F layer	0 (0)	0 (0)	1 (9)	14 (78)
A layer	0 (0)	2 (8)	2 (18)	1 (6)

Table 3. Radiocarbon content in fruiting bodies of macrofungi. Samples from CT were from the lower slope.

	Species	Soil layer	Collection date	Laboratory code	$\delta^{13}C$	$\Delta^{14}C$	Carbon age (yr)
ST	*Mycena* sp. ST1	L layer	Apr 11	IAAA-111556	-27.3 ± 0.5	48.2 ± 2.8	2.8
	Gymnopus sp. ST1	L layer	Oct 11	IAAA-111557	-30.4 ± 0.5	52.0 ± 3.0	4.5
	Marasmius sp. ST4	L-F border	Oct 11	IAAA-111558	-22.9 ± 0.4	68.6 ± 2.7	9.0
CT	*Mycena polygramma*	L-F border	Oct 01	IAAA-81685	-25.5 ± 0.3	132.6 ± 3.7	9.5
	Mycena amygdalina	L-F border	Nov 11	IAAA-111554	-20.2 ± 0.4	79.6 ± 2.8	11.4
	Gymnopus peronatus	L-F border	Nov 01	IAAA-111555	-24.3 ± 0.4	91.0 ± 2.7	3.6
SA	*Mycena aurantiidisca*	L-F border	Oct 08	IAAA-81687	-24.9 ± 0.4	96.0 ± 3.8	11.3
	Mycena epipterygia	L-F border	Oct 11	IAAA-111552	-23.0 ± 0.4	139.7 ± 2.8	20.3
	Galerina atkinsoniana	L-F border	Oct 11	IAAA-111553	-28.9 ± 0.4	56.7 ± 2.7	5.9

Table 4. Size of fruiting bodies of macrofungi. DP, diameter of pileus; LS, length of stipe. Values are means ± standard errors in cm. Numbers in parentheses indicate the number of samples. The same letters indicate that the values are not significantly different at 5% level by Tukey's HSD test. Data from CT are from the lower slope.

	Total		Major species 1		Major species 2		Major species 3	
	DP	LS	DP	LS	DP	LS	DP	LS
ST	3.7 ± 1.3 b	13.9 ± 2.3 c	1.6 ± 0.3	11.3 ± 1.1	4.6 ± 0.7	12.0 ± 0.8	22.5	46.0
	(16)		*Mycena* sp. ST1 and ST2 (10)		*Gymnopus* sp. ST1 (4)		*Marasmius* sp. ST4 (1)	
CT	12.0 ± 1.2 a	63.8 ± 5.7 a	3.8	30.0	14.0	116.5	15.8 ± 2.3	73.5 ± 7.0
	(23)		*Mycena filopes* (2)		*Mycena polygramma* (2)		*Mycena crocata* (6)	
SA	5.8 ± 0.5 b	35.1 ± 2.1 b	4.1 ± 0.3	30.9 ± 2.3	6.9 ± 0.5	46.9 ± 5.6	4.6 ± 0.7	25.5 ± 3.3
	(38)		*Mycena aurantiidisca* (15)		*Mycena epipterygia* (6)		*Galerina atkinsoniana* (8)	

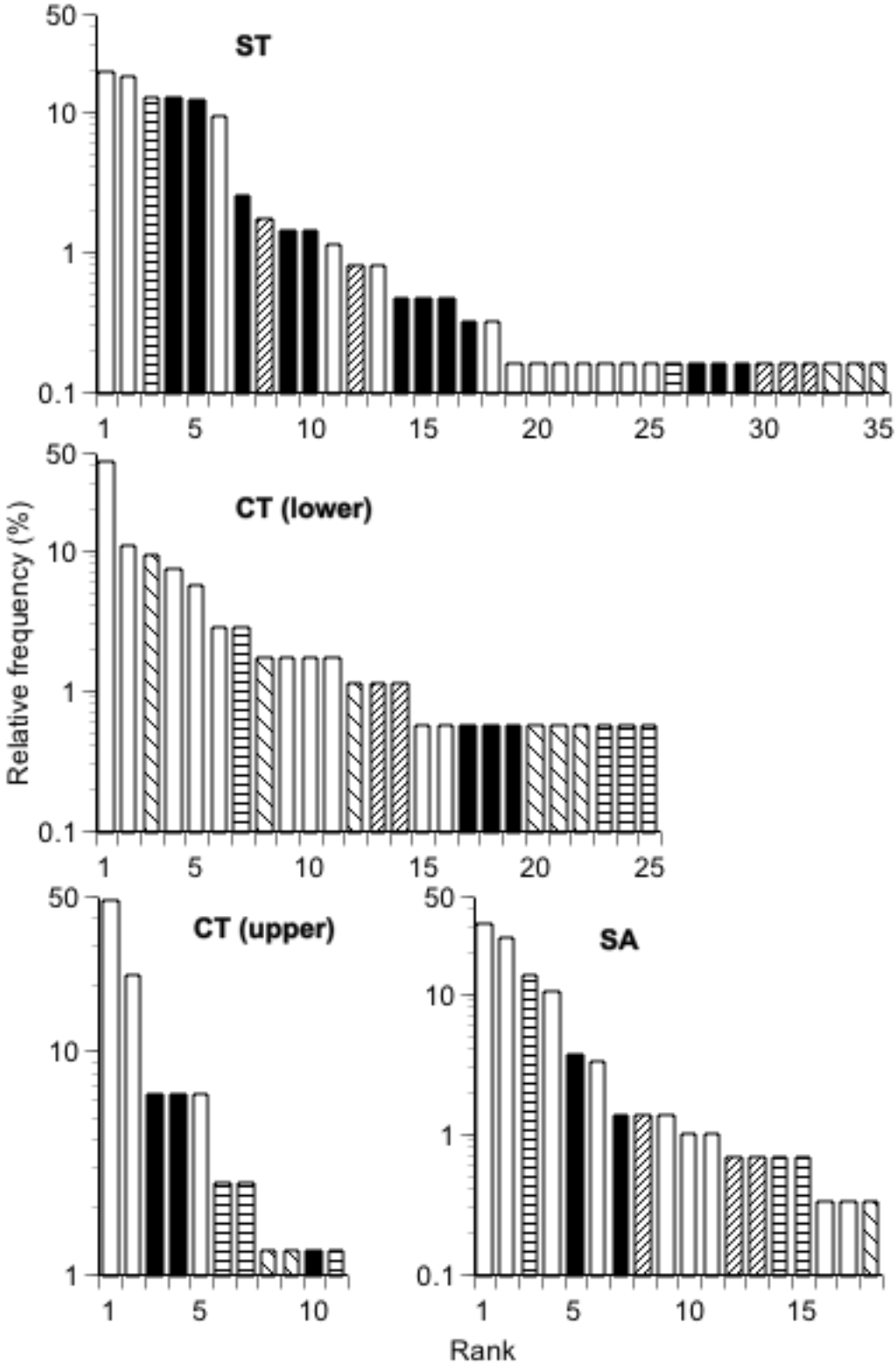
Figure legends

Fig. 1. Rank-relative frequency distribution of macrofungal assemblages in subtropical (ST), cool temperate (CT), and subalpine forests (SA). Open, Mycenaceae; filled, Marasmiaceae; coarse oblique mesh, Agaricaceae; fine oblique mesh, Tricholomataceae; horizontal mesh, others (Hygrophoraceae, Hymenogasteraceae, Pluteaceae, Psathyrellaceae, Strophariaceae, Xylariaceae, and unidentified). The survey in CT was performed at lower and upper parts of a forest slope.

Fig. 2. Seasonal changes in the frequency of occurrence (upper) and the number of species (lower) of fruiting bodies of macrofungi. □, subtropical forest (ST); ○, lower part of a slope in cool temperate forest, [CT (lower)]; ●, upper part of a slope in cool temperate forest [CT (upper)]; ▲, subalpine forest (SA).

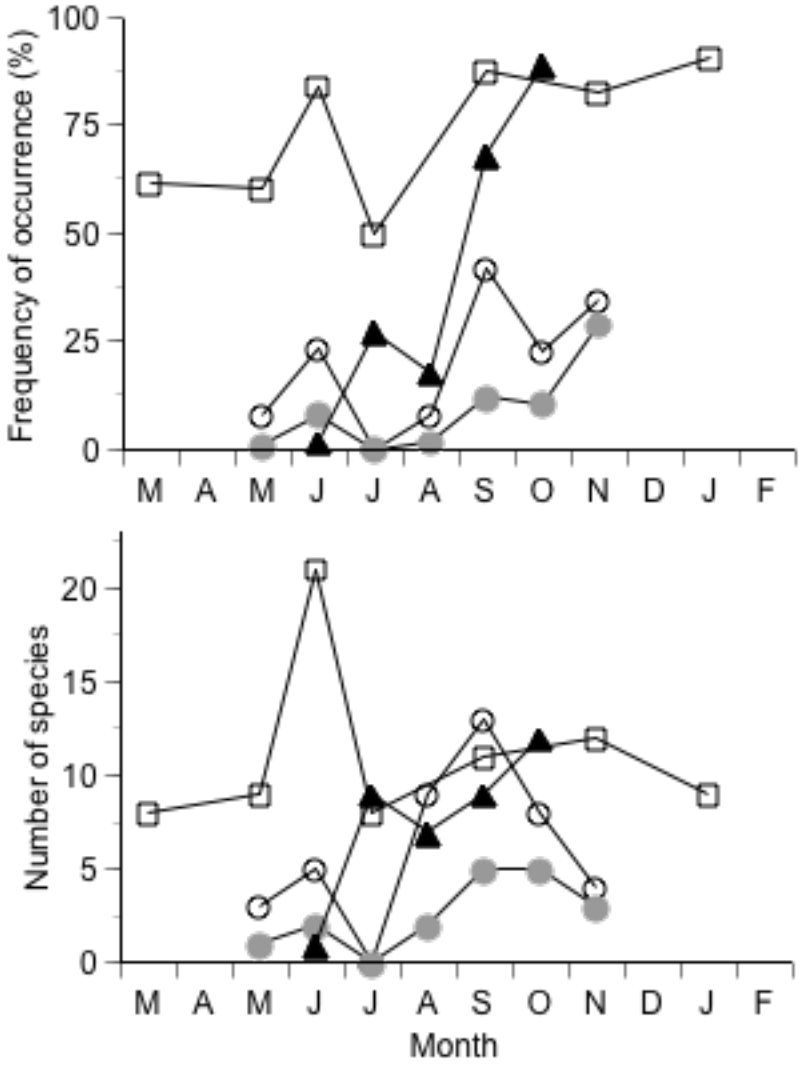
Fig. 3. Seasonal changes in the frequency of occurrence of major macrofungal species in subtropical forest (ST), cool temperate forest (CT), and subalpine forest (SA). For CT, blank and shaded bars indicate lower and upper slopes, respectively.

1 Osono Fig. 1

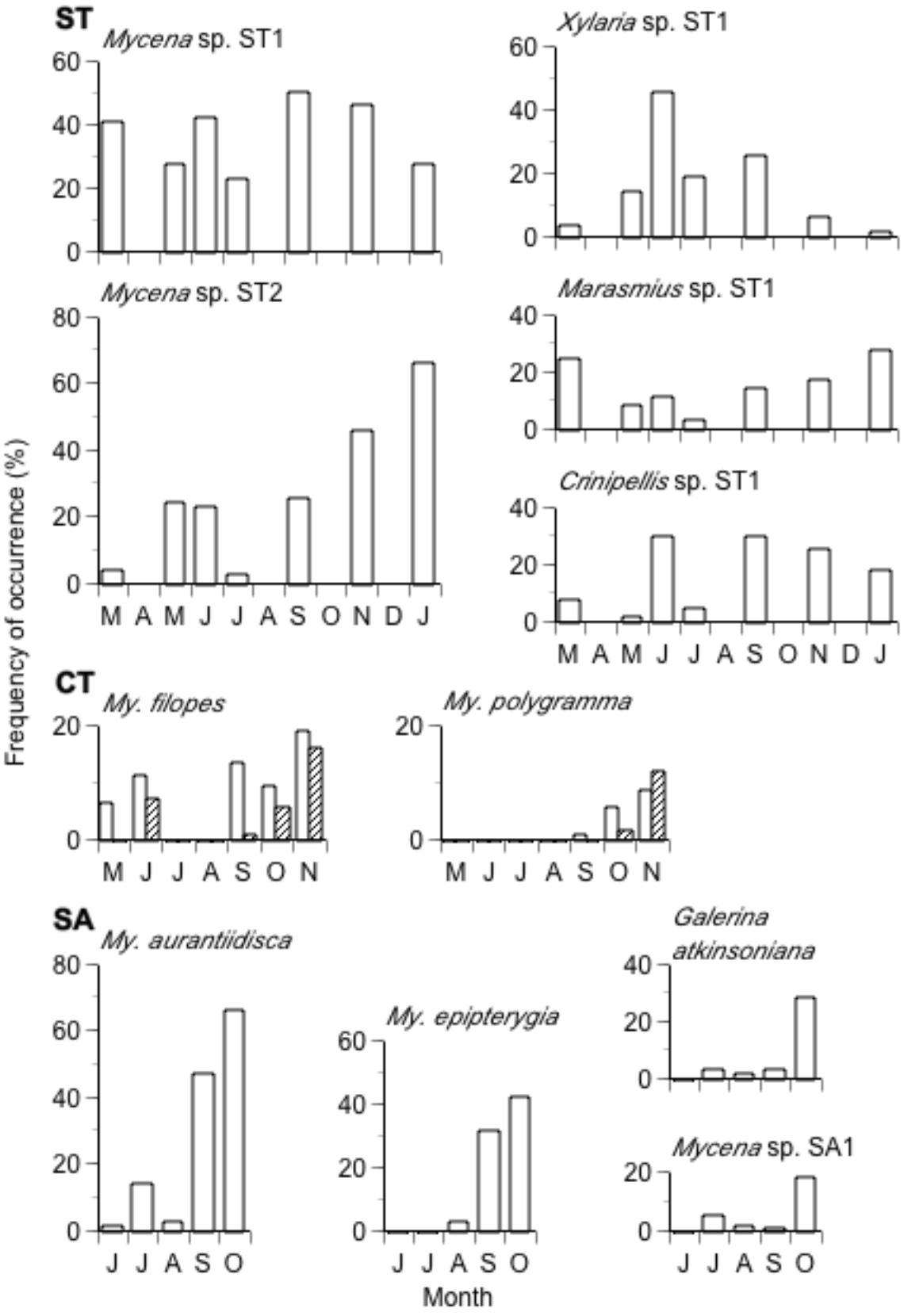

ST
CT (lower)
CT (upper)
SA
Relative frequency (%)
Rank
50
10
1
0.1
1
5
10
15
20
25
30
35

1 Osono Fig. 2

2



1 Osono Fig. 3


ST
Mycena sp. ST1
Xylaria sp. ST1
Mycena sp. ST2
Marasmius sp. ST1
Crinipellis sp. ST1
CT
My. filopes
My. polygramma
SA
My. aurantiidisca
My. epipterygia
Galerina atkinsoniana
Mycena sp. SA1
Frequency of occurrence (%)
M A M J J A S O N D J
J J A S O N
Month

Electronic Supplementary Material

Patterns in diversity, resource utilization, and phenology of fruiting bodies of litter-decomposing macrofungi in subtropical, temperate, and subalpine forests

Takashi Osono

Table S1: Frequency of occurrence of fruiting bodies of macrofungi and the soil layer from which the fruiting bodies occurred. Ag, Agaricaceae; Hg, Hygrophoraceae; Hm, Hymenogasteraceae; Mr, Marasmiaceae; My, Mycenaceae; Tr, Tricholomataceae; Pl, Pluteaceae; Ps, Psathyrellaceae; St, Strophariaceae; Xy, Xylariaceae; and Un, unidentified. L/F, L-F border.

Taxa	Family	Soil layer	Frequency (%)
Subtropical forest			
Mycena section Basipedes 'sp. ST1'	My	L	96.8
Mycena section Roridae 'sp. ST2'	My	L	90.4
Xylaria spp. ST1	Xy	L	64.8
Marasmius spp. ST1	Mr	L	63.2
Crinipellis sp. ST1	Mr	L	60.8
Mycena sp. ST3	My	L	46.4
Gymnopus sp. ST1	Mr	L	12.8
Tricholomataceae sp. ST1	Tr	A	8.8
Gymnopus sp. ST2	Mr	L	7.2
Marasmiellus sp. ST1	Mr	L	7.2
Mycena sp. ST4	My	L	5.6
Tricholomataceae sp. ST2	Tr	A	4.0
Mycena sp. ST5	My	L	4.0
Crinipellis sp. ST2	Mr	L	2.4
Gymnopus sp. ST3	Mr	L	2.4
cf. *Calyptella* sp. ST1	Mr	L	2.4

Marasmius sp. ST2	Mr	L	1.6	
Mycena sp. ST6	My	L	1.6	
Agaricaceae sp. ST1	Ag	A	0.8	
Agaricaceae sp. ST2	Ag	A	0.8	
Gymnopus sp. ST4	Mr	L	0.8	
Mycena sp. ST7	My	L	0.8	
Leucocorpinus sp. ST1	Ag	A	0.8	
Marasmiellus sp. ST2	Mr	A	0.8	
Marasmiellus sp. ST3	Mr	L	0.8	
Mycena sp. ST8	My	L	0.8	
Mycena sp. ST9	My	L	0.8	
Mycena sp. ST10	My	L	0.8	
Unidentified ST1	Un	L	0.8	
Mycena sp. ST11	My	A	0.8	
Mycena sp. ST12	My	L	0.8	
Tricholomataceae sp. ST3	Tr	A	0.8	
Tricholomataceae sp. ST4	Tr	A	0.8	
Tricholomataceae sp. ST5	Tr	L	0.8	
Xeromphalina sp. ST1	My	L	0.8	
Cool temperate forest			**Upper**	**Lower**
Mycena amygdalina	My	L/F	29.6	60
Mycena polygramma	My	L/F	13.6	15.2
Gymnopus peronatus	Mr	L/F	4.0	0.8
Gymnopus sp. CT1	Mr	L/F	4.0	0.0
Mycena sp. CT2	My	L/F	4.0	0.0
Stropharia aeruginosa	St	L/F	1.6	0.8
Hygrocybe cantharellus	Hy	L/F	1.6	0.0
Agaricaceae sp. CT1	Ag	L/F	0.8	0.0
Lepiota fusciceps	Ag	F	0.8	0.0
Marasmius sp. CT2	Mr	A	0.8	0.0
Naematoloma sublateritium	St	A	0.8	0.0
Lycoperdon perlatum	Ag	A	0.0	12.8

Mycena pura	My	L/F	0.0	10.4
Mycena sp. CT1	My	L/F	0.0	8.0
Mycena amicta	My	L/F	0.0	4.0
Psathyrella candolleana	Ps	L/F	0.0	4.0
Agariaceae sp.CT2	Ag	L/F	0.0	2.4
Mycena crocata	My	L/F	0.0	2.4
Mycena luteopallens	My	L/F	0.0	2.4
Mycena sp. CT3	My	L/F	0.0	2.4
Agaricus praeclaresquamosus	Ag	L/F	0.0	1.6
Clitocybe sp. CT1	Mr	L/F	0.0	1.6
Pseudoclitocybe cyathiformis	Tr	L/F	0.0	1.6
Lepiota cf. *pseudogranulosa*	Ag	L/F	0.0	0.8
Lepiota cygnea	Ag	L/F	0.0	0.8
Lepiota sp. CT1	Ag	A	0.0	0.8
Marasmius pulcheriipes	Mr	L/F	0.0	0.8
Marasmius sp. CT1	Mr	L/F	0.0	0.8
Mycena cf. *osmundicola*	My	L/F	0.0	0.8
Mycena sp. CT4	My	L/F	0.0	0.8
Psathyrella piluliformis	Ps	L/F	0.0	0.8
Volvariella speciosa var. *gloiocephala*	Pl	L/F	0.0	0.8
Subalpine forest				
Mycena aurantiidisca	My	F	75.2	
Mycena epipterygia	My	F	60.8	
Galerina atkinsoniana	Hm	F	32.8	
Mycena cf. *filopes*	My	L/F	24.8	
Clitocybe sp. SA1	Mr	F	8.8	
Mycena sp. SA2	My	L/F	8.0	
Marasmius androsaceus	Mr	L/F	3.2	
Tricholomataceae sp. SA1	Tr	F	3.2	
Mycena cf. *stipata*	My	F	3.2	
Mycena sp. SA3	My	F	2.4	
Mycena cf. *pura*	My	F	2.4	

Collybia cookei	Tr	F	1.6
Tricholomataceae sp. SA2	Tr	F	1.6
Unidentified SA1	Un	F	1.6
Unidentified SA2	Un	F	1.6
Mycena sp. SA5	My	F	0.8
Mycena sp. SA4	My	F	0.8
Lycoperdon perlatum	Ag	A	0.8

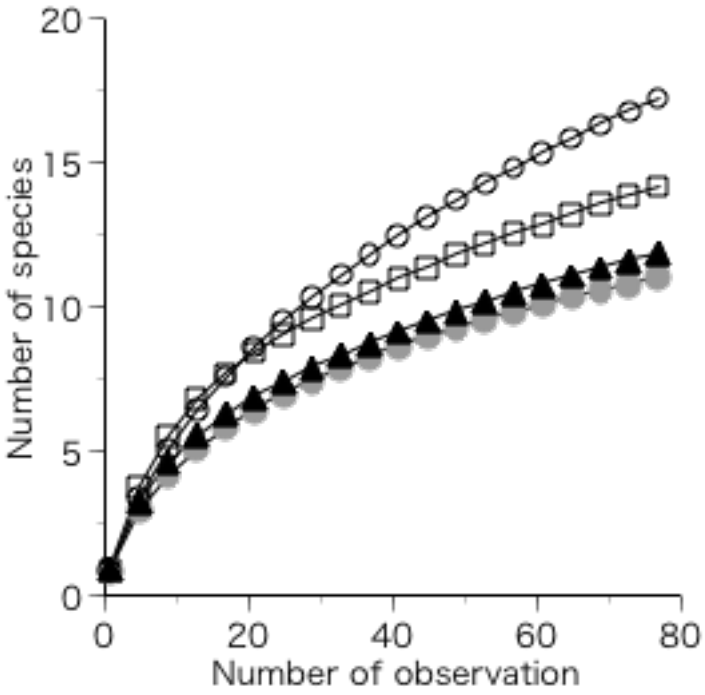


Fig. S2. Rarefaction curves for litter-decomposing macrofungal (LDM) assemblages. □, subtropical forest (ST); ○, lower part of a slope in cool temperate forest, [CT (lower)]; ●, upper part of a slope in cool temperate forest [CT (upper)]; ▲, subalpine forest (SA).