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SHORT COMMUNICATION

Takashi Osono · Dai Hirose

Effects of prior decomposition of *Camellia japonica* leaf litter by an endophytic fungus on the subsequent decomposition by fungal colonizers

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Abstract Effects of prior decomposition of *Camellia japonica* leaf litter by an endophytic phyllosphere fungus *Coccomyces* sp. on the subsequent decomposition of the litter by *Coccomyces* sp. and two succeeding fungi Dermateaceae sp. and *Xylaria* sp. (anamorph) were examined in a pure-culture decomposition test. The prior decomposition of litter by *Coccomyces* sp. stimulated the subsequent decomposition by the three fungi. Dermateaceae sp. caused negligible weight loss on litter previously partly decomposed by *Coccomyces* sp. and then by Dermateaceae sp. and on litter decomposed singly by Dermateaceae sp. *Xylaria* sp. (anamorph) caused greater weight loss in these litters than control, uninoculated litter.

Key words *Camellia japonica* · Carbohydrate · Decomposition · Endophyte · Lignin

Fungi play a central role in decomposition of lignin and polymer carbohydrates that are major components of plant litter in forest ecosystems (Osono 2006, 2007). The abilities of fungi to decompose lignin and polymer carbohydrates in leaf litter have been examined in pure-culture decomposition tests (reviewed in Osono and Takeda 2002, 2006; Osono et al. 2003, 2006). These studies mainly used newly dead leaves as materials for fungal decomposition. However, ecological studies of fungal succession in decomposing leaf litter have indicated that not all litter-inhabiting fungi colonize newly dead leaves and that fungi colonizing litter in more advanced stages of decomposition in particular utilize resources chemically and structurally modified by precedent fungal colonizers (Tokumasu 1996; Osono and Takeda 2001; Osono et al. 2004; Koide et al. 2005a; Osono 2005). These fungi are expected to be physiologically adapted to utilize such resources and hence to exhibit different pattern of substrate utilization in leaf litter that is previously partly decomposed by other fungi and, consequently, to have different level of lignin and polymer carbohydrates from newly dead leaves. To our knowledge, only one previous study demonstrated this, showing that pretreatment of *Fagus crenata* leaf litter by endophytic phyllosphere fungi led to the stimulation of lignin decomposition by *Mycena* spp. (Osono 2003).

The purpose of the present study was to examine the effects of prior decomposition of *Camellia japonica* leaf litter by *Coccomyces* sp. on the subsequent decomposition of the litter by three fungal colonizers including *Coccomyces* sp. itself. *Coccomyces* sp., previously denoted as Rhytismataceae sp. in Koide et al. (2005a,b), is an endophytic phyllosphere fungus of *C. japonica* leaves capable of colonizing newly dead leaves as a prior colonizer and delignifying the leaves (Koide et al. 2005b). The other two fungi, an unidentified species in Dermateaceae and *Xylaria* sp. (anamorph), are successive colonizers appearing in sequence during decomposition of the leaves (Koide et al. 2005a). In the present study, litter previously partly decomposed by *Coccomyces* sp. or Dermateaceae sp. was used as substrata in the pure-culture test, as well as uninoculated litter as a control. Moreover, the effects of successive treatment of litter by *Coccomyces* sp. and then by Dermateaceae sp. on the decomposition by Dermateaceae sp. and *Xylaria* sp. (anamorph) were evaluated to provide further insights into the role of successive exposure of leaf litter to fungal colonization on decomposition processes of the litter.

Fungal isolates of three species, one isolate per species, were used in the pure culture decomposition test: *Coccomyces* sp., Dermateaceae sp., and *Xylaria* sp. (anamorph). These species occurred frequently and successively on decomposing leaf litter of *C. japonica* and had the ability to

T. Osono (✉)[1]
Laboratory of Forest Ecology, Division of Environmental Science and Technology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

D. Hirose
Sugadaira Montane Research Center, University of Tsukuba, Sanada, Nagano, Japan

[1] *Present address:* Center for Ecological Research, Kyoto University, Shiga 520-2113, Japan
Tel. +81-77-549-8252; Fax: +81-77-549-8201
e-mail: tosono@ecology.kyoto-u.ac.jp

cause substantial mass loss of the litter under a pure culture condition (Koide et al. 2005a). *Coccomyces* sp. is an endophytic phyllosphere fungus colonizing freshly fallen leaves as a prior colonizer during the first 2 months of decomposition to delignify the leaf litter. Dermateaceae sp., previously denoted as Coelomycete sp. 1, is a cellulolytic fungus rapidly colonizing the litter at 2 months of decomposition. *Xylaria* sp. (anamorph) is a simultaneous decomposer of lignin and cellulose colonizing the litter at 12 months of decomposition. Thus, the sequence of colonization in fungal succession is in the order *Coccomyces* sp., Dermateaceae sp., and *Xylaria* sp. (anamorph). The isolate of *Coccomyces* sp. was identified based on the macromorphological characteristics of the mycelium and on the DNA sequence of 28S rRNA gene D1/D2 region using primers D1 (Peterson 2000) and NL4 (O'Donnell 1993), basically according to the method described in Hirose and Osono (2006) (DDBJ accession number AB298439). The taxon of Dermateaceae sp. was determined based on the DNA base of the same region (DDBJ accession number AB298438). Comparison of the sequence of Dermateaceae sp. with known species using BLAST searching indicated 98% similarity to *Neofabraea malicorticis* (AY544662) and 96% to *Cryptosporiopsis ericae* (AY853167). *Xylaria* sp. (anamorph) was identified by macromorphological observations according to Petrini and Petrini (1985). These isolates were obtained from surface-disinfected leaf litter of *C. japonica* collected in a temperate secondary forest in Kyoto, Japan, during the period between May 2002 and November 2003 (see Koide et al. 2005a). The isolates have been maintained on slants of 2% malt extract agar [malt extract 2%, agar 2% (w/v)]. Freshly fallen leaves of *C. japonica* were collected from the forest floor of the study site in April 2004 and oven-dried at 40°C for 1 week. The leaves were preserved in a plastic bag for 5 months at room temperature (about 20°C) until the test began.

A pure culture test was carried out to assess the effect of prior decomposition of leaves by *Coccomyces* sp., by Dermateaceae sp., or by both fungi on the subsequent decomposition by *Coccomyces* sp., Dermateaceae sp., and *Xylaria* sp. (anamorph). Four litter types with different prior treatments were prepared and used in the test (Table 1): (i) control litter without fungal inoculation (litter NI), (ii) litter previously partly decomposed singly by *Coccomyces* sp. (litter CO), (iii) litter previously partly decomposed by *Coccomyces* sp. and then by Dermateaceae sp. (litter CD), and (iv) litter previously partly decomposed singly by Dermateaceae sp. (litter DE).

The litters CO, CD, and DE were prepared for the pure culture decomposition test according to the method described in Koide et al. (2005b). Pieces (2×1 cm) of freshly fallen leaves were sterilized by exposure to ethylene gas at 60°C for 6 h. The leaves (0.5 g) were placed on the surface of Petri dishes (9 cm in diameter) containing 20 ml 2% agar. Inocula of *Coccomyces* sp. for litters CO and CD or that of Dermateaceae sp. for litter DE were cut out of the margin of the previously inoculated Petri dishes on 2% malt agar (MA) with a sterile cork borer (6 mm in diameter) and placed on the agar, one inoculum per plate. The plates were incubated for 8 weeks at 20°C in darkness. The leaves were then retrieved and oven-dried at 40°C for 1 week. To prepare litter CD, the leaves previously treated with *Coccomyces* sp. were sterilized again, inoculated with Dermateaceae sp., and incubated for another 8 weeks according to the method described above. Lignin content in the litters was analyzed with the sulfuric acid method and that of total carbohydrates with the phenol-sulfuric acid method according to the methods described in Koide et al. (2005b). Lignocellulose index (LCI) was calculated as an indicator of relative availability of carbon energy sources in the litter types according to the following equation: LCI = content of total carbohydrates/(content of total carbohydrates + content of lignin).

The ability of the isolates of *Coccomyces* sp., Dermateaceae sp., and *Xylaria* sp. (anamorph) to cause weight loss of the four litter types and lignin and total carbohydrates in these litter types was studied with the pure culture test according to the method described above. Table 2 shows the fungus–litter type combinations examined in the tests; a total of 10 combinations were examined in the present study. Five plates were prepared for each combination, and five uninoculated plates served as control for each litter type. The pretreated but subsequently undecomposed litter was also sterilized, oven-dried at 40°C for 1 week, and weighed to determine the original weight. Weight loss of decomposed materials was determined as a percentage of the original weight, taking the weight loss of litter in the uninoculated and incubated control treatment into consideration, and the mean values were calculated for each combination. The leaves from the five plates were then combined to make one sample for each combination and used for analysis of lignin and total carbohydrates as described

Table 1. Content (mg/g dry material) of lignin and total carbohydrates and lignocellulose index (LCI) in four litter types used in the decomposition test

Treatment	Litter type	Lignin	Total carbohydrates	LCI
Control, no fungal inoculation	Litter NI	276	329	0.456
Prior treatment with *Coccomyces* sp.	Litter CO	285	365	0.438
Prior treatment with *Coccomyces* sp. and Dermateaceae sp.	Litter CD	335	334	0.501
Pretreatment with Dermateaceae sp.	Litter DE	310	341	0.476

Litter NI, control litter without fungal inoculation; litter CO, litter previously partly decomposed singly by *Coccomyces* sp.; litter CD, litter previously partly decomposed by *Coccomyces* sp. and then by Dermateaceae sp.; litter DE, litter previously partly decomposed singly by Dermateaceae sp.
LCI, content of total carbohydrates/(content of total carbohydrates + content of lignin)

Table 2. Weight loss (% original weight) of leaf litter, lignin, and total carbohydrates and L/W and L/C of litters C, CO, CD, and DE decomposed by three fungal species

Fungus	Litter type	Weight loss			L/W	L/C
		Litter	Lignin	Total carbohydrates		
Coccomyces sp.	Litter NI	4.8 ± 1.4b	8.9	1.1	1.9	7.9
	Litter CO	7.2 ± 0.5a	16.9	0.0	2.3	–*
Dermateaceae sp.	Litter NI	2.5 ± 0.4b	nd	nd	nd	nd
	Litter CO	4.1 ± 0.8a	nd	nd	nd	nd
	Litter CD	0.2 ± 0.3c	nd	nd	nd	nd
	Litter DE	1.5 ± 0.4b	nd	nd	nd	nd
Xylaria sp. (anamorph)	Litter NI	10.5 ± 0.8c	2.7	14.8	0.3	0.2
	Litter CO	17.5 ± 0.9a	17.1	23.5	1.0	0.7
	Litter CD	17.0 ± 0.6a	5.5	28.6	0.3	0.2
	Litter DE	14.9 ± 1.0b	0.2	20.2	0.0	0.0

Values indicate means ± standard errors ($n = 5$)
L/W, lignin/weight loss ratio; L/C, lignin/carbohydrate loss ratio; nd, not determined
The same letters indicate not significantly different at 5% level by *t* test or LSD test
*L/C was not calculated because of no weight loss of total carbohydrates

above. Chemical analyses were performed for *Coccomyces* sp. and *Xylaria* sp. (anamorph) that caused mass loss of more than 4.5%.

Lignin/weight loss ratio (L/W) and lignin/carbohydrate loss ratio (L/C) are useful indices of substrate utilization pattern of each fungal isolates (Osono 2003). L/W and L/C were calculated according to the following equations: L/W = weight loss of lignin (% original weight of lignin)/ weight loss of litter (% original weight of litter), and L/C = weight loss of lignin (% original weight of lignin)/weight loss of total carbohydrates (% original weight of total carbohydrates).

Analysis of variance was used to determine differences among mean values of weight losses of litter NI, CO, CD, and DE by Dermateaceae sp. and *Xylaria* sp. (anamorph) (Systat 1992). The least significant difference test was used for multiple comparisons. A *t* test was used for *Coccomyces* sp. to determine differences between mean values of weight losses of litters NI and CO.

Content of lignin, total carbohydrates, and LCI varied from 276 to 335 mg/g, 329 to 365 mg/g, and 0.438 to 0.501 mg/g, respectively, among the litter types (see Table 1). Lignin content was greatest in litter CD, followed by litters DE, CO, and NI. Content of total carbohydrates was greatest in litter CO followed by litters DE, CD, and NI. The LCI was in the order of litters CO < NI < DE < CD.

Weight loss of litter differed among litter types for each fungal species (see Table 2). *Coccomyces* sp. caused significantly greater weight loss of litter CO than of litter NI, which was attributed to the enhanced decomposition of lignin in litter CO. Weight loss of litter caused by Dermateaceae sp. was significantly different among the four litter types, in the order of litters CO > NI, DE > CD. Weight loss of litter caused by *Xylaria* sp. (anamorph) was also significantly different among the litter types, in the order of litters CO, CD > DE > NI. The greater weight loss in litters CO and CD caused by *Xylaria* sp. (anamorph) was attributed to the enhanced decomposition of both lignin and total carbohydrates. *Xylaria* sp. (anamorph) decomposed lignin more selectively in litter CO and showed greater values of L/W and L/C than in litter NI, whereas L/W and L/C in litter CD was at similar levels to those in litter NI. The weight loss of litter DE was mostly the result of the selective decomposition of total carbohydrates with lower L/W and L/C values than in litter NI.

The prior decomposition of litter by *Coccomyces* sp. stimulated litter decomposition by *Coccomyces* sp. itself, Dermateaceae sp., and *Xylaria* sp. (anamorph), partly because of the increase of relative availability of total carbohydrates in litter CO (i.e., the decrease of LCI) owing to the selective delignification by *Coccomyces* sp. As non-lignified carbon energy sources such as holocellulose are necessary as growth cosubstrates for fungi to decompose lignin (Kirk et al. 1976), the more efficient decomposition of lignin in litter CO by *Coccomyces* sp. and *Xylaria* sp. (anamorph) with greater values of L/W and L/C than in litter NI could be partly ascribed to the decrease in LCI in litter CO. These results are consistent with the finding of Osono (2007) that the same isolates of *Coccomyces* sp., Dermateaceae sp., and *Xylaria* sp. (anamorph) as in the present study caused greater weight loss of leaves when inoculated to leaf portions naturally colonized and delignified by *Coccomyces* sp. than when inoculated to other portions of the same leaves uncolonized by this endophyte. Similarly, Tanaka et al. (1988) reported that pretreatment of sound wood by ligninolytic fungi enhanced subsequent decomposition of cellulose by *Trichoderma* species. However, other explanations may also be possible, which include structural disintegration caused by prior decomposition that can affect the substrate utilization patterns of the subsequent colonizers, as discussed below.

The prior decomposition of litter by Dermateaceae sp., on the other hand, resulted in the increase of LCI in litters CD and DE. Dermateaceae sp. caused negligible weight loss on litter CD, suggesting that available carbohydrates were exhausted in this litter by previous consumption by itself. In contrast, *Xylaria* sp. (anamorph) caused greater weight loss in litters CD and DE than in litter NI, despite the greater values of LCI in litters CD and DE. This result suggested that not only the relative availability of lignin and

total carbohydrates in litter but also other factors such as structural disintegration from prior decomposition affected the substrate utilization pattern of this fungus. Osono (2003) also found that *Mycena* spp. decomposed lignin effectively from litters previously partly decomposed by other fungi, regardless of the LCI values of the decomposed litters. Hence, fungal species colonizing litter in more advanced stages of decomposition may be physiologically adapted to the decomposition of litter with decreased availability of carbohydrates.

The positive effect of prior decomposition of *C. japonica* leaf litter by endophytic *Coccomyces* sp. on the subsequent decomposition by fungal colonizers provides an implication for the role of endophytic fungi in decomposition processes in a temperate forest (Koide et al. 2005a,b), as described schematically below. The colonization of *Coccomyces* sp. in the initial stage of decomposition results in the changes in leaf properties, such as the loss of lignin content and structural disintegration, within their colonies compared to the other uncolonized parts of leaves, leading to the within-leaf heterogeneity of leaf properties in leaf litter in the field (Koide et al. 2005b). In the subsequent stages, lignin and holocellulose are decomposed by successive fungal assemblages more rapidly in the areas previously colonized by the endophyte than in the uncolonized areas (Koide et al. 2005a). Thus, the results of the present study explicitly showed the positive effect of *Coccomyces* sp. on the substrate utilization by subsequent fungal colonizers and verified the role of the colonization by the endophyte in the resultant changes in decomposition processes of leaf litter.

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