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FULL PAPER

Takashi Osono

Effects of prior decomposition of beech leaf litter by phyllosphere fungi on substrate utilization by fungal decomposers

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Abstract Changes in litter quality resulting from pretreatment of leaf litter by phyllosphere fungi may affect its subsequent decomposition by succeeding fungi. The purpose of this study is to clarify the effect of prior decomposition of leaf litter by two phyllosphere fungi of beech, *Xylaria* sp. and *Ascochyta* sp., on substrate utilization of 12 fungal species in the Basidiomycota, the Ascomycota, and the Zygomycota, in a laboratory experiment. *Mycena* sp. caused significantly higher weight loss in litter previously partly decomposed by *Xylaria* sp. than in control litter without fungal inoculation and litter previously partly decomposed by *Ascochyta* sp., whereas prior decomposition retarded litter decomposition or had no significant effect on 11 other species. Prior decomposition by phyllosphere fungi affected the substrate utilization patterns of two *Mycena* species in the Basidiomycota, shifting from simultaneous removal of lignin and carbohydrates to selective delignification.

Key words Basidiomycota · Carbohydrate · *Fagus crenata* · Lignin · Phyllosphere fungi

Introduction

Phyllosphere fungi include those fungi that colonize the interior and surface of living leaves (Petrini 1991). Mycological studies have found that phyllosphere fungi also occur on fallen leaves of various plant species at the initial stage of decomposition (reviewed in Hudson 1968; Osono 2002). Functionally, some phyllosphere fungi, called primary saprophytes by Hudson (1968), utilize readily available carbohydrates, and others such as xylariaceous endophytes degrade cellulose more selectively than lignin (Osono and Takeda 2002). According to the resource utilization by phyllosphere fungi, litter decomposition progressed along with fungal succession on the litter (Hudson 1968; Osono and Takeda 2001b). The successive change from phyllosphere species to litter and soil mycobiota during decomposition suggests that the changes in litter quality resulting from prior colonization and consumption of available resources by phyllosphere fungi affected the growth, substrate utilization, and litter decomposition by succeeding fungi. White-rot species in the Basidiomycota, vigorous decomposers of lignocellulose, may be especially adapted physiologically to the effective removal of lignin and related recalcitrant substances remaining in litter previously partly decomposed by phyllosphere fungi. The effects of pretreatment of wood by wood-inhabiting fungi on its subsequent decomposition by another fungus have been reported (Tanaka et al. 1988). However, such effects have not been investigated for litter-inhabiting fungi.

The purpose of the present study is to clarify the effect of prior decomposition of leaf litter by phyllosphere fungi on substrate utilization by saprophytic fungi in the Basidiomycota, the Ascomycota, and the Zygomycota, in a laboratory experiment. Japanese beech was chosen for the study because it has already been used in studies evaluating phyllosphere mycobiota (Osono 2002) and in studies of fungal succession and litter decomposition (Osono and Takeda 2001b, 2002).

Materials and methods

Source of fungi and litters

Twelve fungal species were used in this experiment: *Mycena* sp., *M. polygramma* (Bull.: Fr.) S.F. Gray (Basidiomycota), *Xylaria* sp. (anamorph) (xylariaceous Ascomycota), *Alternaria alternata* (Fr.) Keissler, *Ascochyta* sp., *Cladosporium cladosporioides* (Fres.) de Vries, *Epicoccum nigrum* Link

T. Osono (✉)
Laboratory of Forest Ecology, Division of Environmental Science and Technology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
Tel. +81-75-753-6079; Fax +81-75-753-6080
e-mail: fujijun@kais.kyoto-u.ac.jp

ex Link, *Gliocladium roseum* Bain., *Trichoderma koningii* Oudem., *Penicillium citrinum* Thom (Ascomycota other than Xylariaceae), *Mucor hiemalis* Wehmer, and *Mortierella ramanniana* (Moller) Linnem. var. *ramanniana* (Zygomycota). *Xylaria* sp. and *Ascochyta* sp. were endophytic phyllosphere species on Japanese beech (*Fagus crenata* Blume). *A. alternata*, *C. cladosporioides*, *E. nigrum*, and *G. roseum* were epiphytic phyllosphere fungi. *Mycena* sp., *M. polygramma*, *T. koningii*, *P. citrinum*, *M. hiemalis*, and *M. ramanniana* were litter fungi (Osono 2002). These fungal species were collected from October 1996 to November 1997 from a cool temperate deciduous forest dominated by Japanese beech in the Ashiu Experimental Forest of Kyoto University, Kyoto, Japan. Isolations were made from mass basidiospores, or from green leaves or leaf litter of beech by the surface sterilization method or the washing method. These fungi have been maintained on slants of a modified malt-yeast-soytone agar [malt extract 0.35%, soytone 0.05%, yeast extract 0.025%, and agar 1.5% (w/v)] (Kinugawa 1988). Details of sources and method of isolation were described in Osono and Takeda (2002).

Beech litters used in the decomposition tests were collected by litter-traps in autumn 1997 at the Ashiu Experimental Forest. Disks including the primary vein were punched out from these leaf litters with a cork borer (20 mm diameter) to use in the tests.

Decomposition test

An in vitro decomposition test was carried out to assess the effect of prior decomposition by two phyllosphere fungi on substrate utilization by the 12 fungal species (Osono and Takeda 2002). *Xylaria* sp. and *Ascochyta* sp. were used as prior decomposers. Three litter types were used in the test: (i) control litter without fungal inoculation (litter C), (ii) litter previously partly decomposed by *Xylaria* sp. (litter X), and (iii) litter previously partly decomposed by *Ascochyta* sp. (litter A).

In the decomposition test of litter C, the disks were exposed to each of 12 fungi for 8 weeks without pretreatment by the phyllosphere fungi. Disks were autoclaved at 120°C for 20 min and placed on the surface of Petri dishes (9 cm diameter) containing 20 ml 2% plain agar. Inocula for each assessment were cut from the margin of the growing colonies on 2% malt extract agar with a sterile cork borer (5.5 mm diameter) and placed on the center of the plates, including seven disks around the inoculum. The plates were incubated at 20°C for 8 weeks in darkness. After incubation, leaf disks were collected, oven-dried at 40°C for 4 days, and weighed. Ten plates were prepared for each species. The disks were then combined and used for chemical analyses.

In the decomposition test of litters X and A, the disks were exposed to each of the phyllosphere fungi for 8 weeks, then autoclaved and transferred to the plates inoculated with each of 12 fungi and incubated for another 8 weeks, according to the method described above.

Weight loss of the leaf disks was determined as a percentage of the original weight. When analyzing the weight loss of leaf litter, the arcsin transformation was used because the data were in the form of proportions. Analysis of variance (Systat 1992) was used to determine differences among mean values of weight losses of litters C, X, and A. Tukey's honestly significant difference (HSD) test was used for multiple comparisons. A *t* test was used for *M. polygramma* to determine differences between mean values of weight losses of litters C and X, instead of ANOVA, because no data were obtained from the experiment with *M. polygramma* on litter A due to contamination. Part of the results have already been presented by Osono and Takeda (1999, 2002).

Chemical analyses

Leaf samples were ground in a laboratory mill (0.5-mm screen) for chemical analyses. The amount of lignin in samples was estimated by gravimetry using hot sulfuric acid digestion (King and Heath 1967). Samples were extracted with alcohol-benzene at room temperature, and the residue was treated with 72% sulfuric acid (v/v) for 2 h at room temperature with occasional stirring. The mixture was then diluted with distilled water to make a 2.5% sulfuric acid solution and autoclaved at 120°C for 60 min. After cooling, the residue was filtered and washed with water through a porous crucible (G4), dried at 105°C, and weighed as acid-insoluble residue. The filtrate (autoclaved sulfuric acid solution) was used for total carbohydrate analysis. The amount of total carbohydrate in the filtrate was estimated by the phenol-sulfuric acid method (Dubois et al. 1956). Five percent phenol (v/v) and 98% sulfuric acid (v/v) were added to the filtrate. The optical density of the solution was then measured by a spectrophotometer at 490 nm using known concentrations of D-glucose as standards.

The term lignin is commonly used for the material as determined by the sulfuric acid digestion method. Although the lignin fraction contains not only true lignin but also lignin-like materials (secondary compounds and humic substances) produced during fungal decomposition, in this study the term lignin is used for the suite of lignin and lignin-like materials for the sake of simplicity.

Lignin/weight loss ratio (L/W) and lignin/carbohydrate loss ratio (L/C) are useful indices of substrate utilization pattern of each fungal species (Osono and Takeda 2002). L/W and L/C of each fungal species are calculated according to the following equations:

L/W = weight loss of lignin (% of original lignin weight)/ weight loss of litter (% of original litter weight)

L/C = weight loss of lignin (% of original ignin weight)/ weight loss of carbohydrate (% of original carbohydrate weight)

Results

Table 1 shows the initial chemical composition of litters C, X, and A. Nitrogen concentration was similar among

Table 1. Concentrations (%) of nitrogen, soluble carbohydrate, lignin, and total carbohydrate in three litter types used in the decomposition test

Treatment	Code	Nitrogen	Soluble carbohydrate	Lignin	Total carbohydrate
Control, no fungal inoculation	Litter C	1.30	2.9	39.6	34.2
Xylaria sp. inoculation[a]	Litter X	1.26	1.1	39.2	35.3
Ascochyta sp. inoculation[a]	Litter A	1.25	0.9	44.9	27.0

[a] Litters were incubated for 8 weeks at 20°C with each fungus

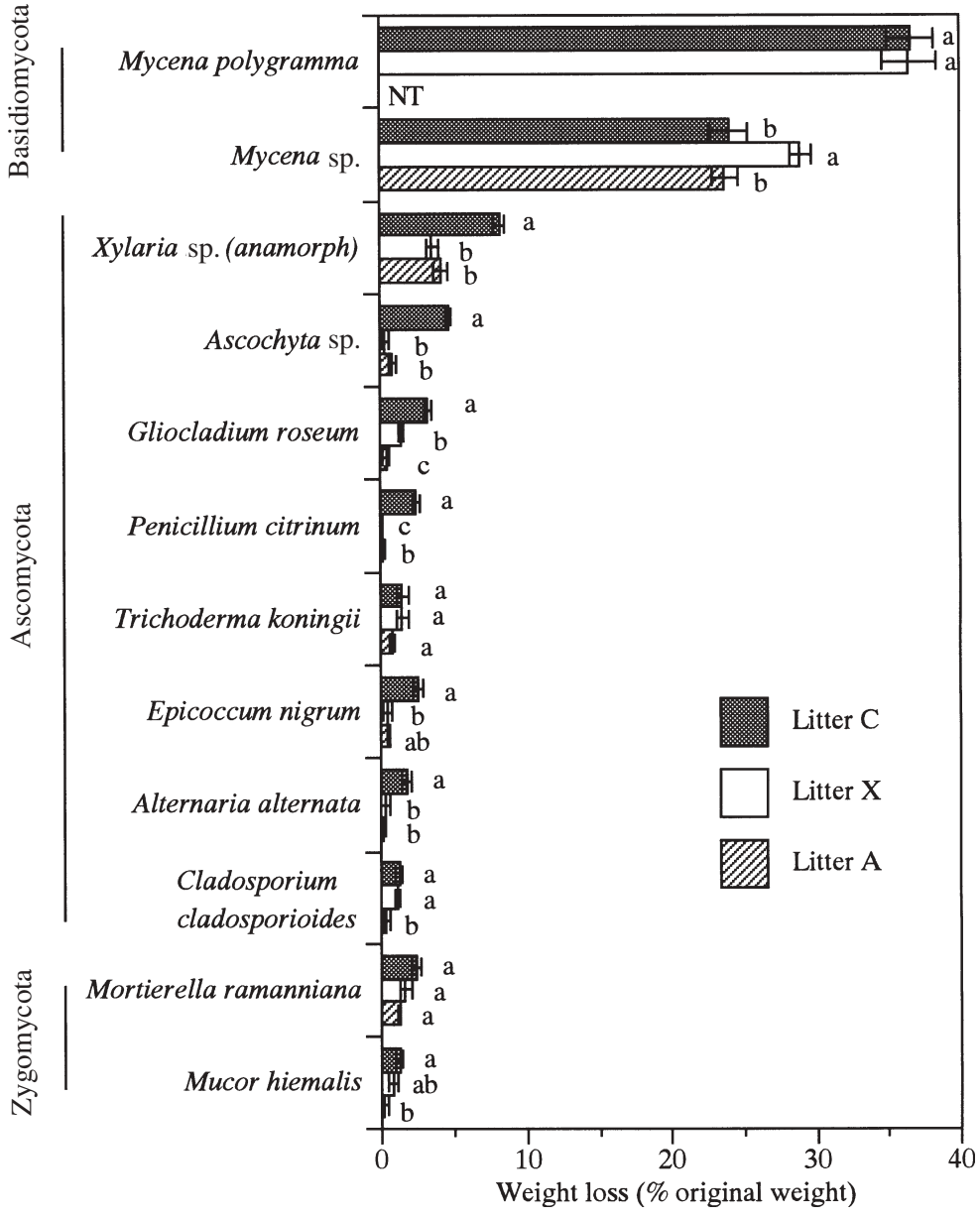

Fig. 1. Weight loss (% original weight) of litters C, X, and A decomposed by 12 fungal species. *Bars* indicate standard errors. The same letters are not significantly different at the 5% level by Tukey's HSD test. *NT*, not tested

the litters. Concentration of soluble carbohydrate was lower in litters X and A than in litter C. Concentrations of lignin and total carbohydrate were similar between litters C and X, and concentration of lignin was higher and that of total carbohydrate was lower in litter A than in litters C and X. Weight losses (% original weight) of litters X and A during the prior decomposition period were 8.2% and 4.7%, respectively.

Figure 1 shows the weight loss (% original weight) of litters C, X, and A decomposed by the 12 fungal species. Weight loss was higher in the 2 species of the Basidiomycota, *Mycena polygramma* and *Mycena* sp., than

Table 2. Weight loss (% original weight) of leaf litter, lignin, and carbohydrate and L/W and L/C of litters C, X, and A decomposed by *Mycena polygramma* and *Mycena* sp.

Fungus	Weight loss (% original weight)				L/W	L/C
	Litter type	Litter	Lignin	Total carbohydrate		
Mycena polygramma	Litter C	36.6	33.2	47.8	0.9	0.7
Mycena polygramma	Litter X	36.5	56.1	23.6	1.5	2.4
Mycena sp.	Litter C	24.0	27.0	30.2	1.1	0.9
Mycena sp.	Litter X	29.0	48.4	13.2	1.7	3.7
Mycena sp.	Litter A	23.8	34.8	22.1	1.5	1.6

L/W, Lignin/weight loss ratio; L/C, lignin/carbohydrate loss ratio

in the other 10 species. *Mycena* sp. caused significantly higher weight loss in litter X than in litters C and A. *Xylaria* sp., *Ascochyta* sp., *Gliocladium roseum*, *Penicillium citrinum*, *Epicoccum nigrum*, *Alternaria alternata*, *Cladosporium cladosporioides*, and *Mucor hiemalis* caused significantly lower weight losses in litter X and/or litter A than in litter C. *Trichoderma koningii* and *Mortierella ramanniana* caused weight losses that were not significantly different among litters C, X, and A.

Table 2 shows the weight loss (% original weight) of leaf litter, lignin, and total carbohydrate and L/W and L/C of litters decomposed by *Mycena polygramma* and *Mycena* sp. For both *Mycena* species, weight loss of lignin was higher and that of total carbohydrate was lower in litter X than in litter C. L/W and L/C were higher in litter X than in litter C. For litter A in *Mycena* sp., weight loss of lignin, L/W, and L/C were higher than litter C but not as high as for litter X.

Discussion

The present study shows that marked decomposition was caused by two *Mycena* species in the Basidiomycota. *Xylaria* sp. was next to these two species. The other nine fungi in Ascomycota and the Zygomycota caused low weight losses. The difference in litter-decomposing ability among taxonomic groups has been already shown by Osono and Takeda (2002). Osono and Takeda (2002) found that *Mycena* sp., *Mycena polygramma*, and *Xylaria* sp. were vigorous decomposers of lignocellulose and that the other nine species, common to the present study, were considered as cellulose decomposers or sugar fungi that may rely for their growth on readily available energy sources such as nonlignified holocellulose or soluble carbohydrates.

The prior decomposition by two phyllosphere fungi retarded litter decomposition or had no significant effect on subsequent decomposition by *Xylaria* sp. and the nine other species in the Ascomycota and the Zygomycota. The decrease in weight losses in litters X and A compared to litter C is probably ascribable to the prior consumption of nonlignified holocellulose and soluble carbohydrates by *Xylaria* sp. and *Ascochyta* sp., as soluble carbohydrate content in litters X and A was lower than in litter C. Furthermore, the weight losses decreased when *Xylaria* sp. and *Ascochyta* sp. were inoculated to litters previously decomposed by themselves, indicating that litter decomposition by phyllosphere species made the substratum unsuitable for these species. Therefore, by consuming the readily available resources in litter, phyllosphere fungi may affect the colonization of competitors that have a similar requirement for these resources. This idea is consistent with the suggestion of Osono and Takeda (2001b) that the competitive interactions between fungal colonizers may be one of the important factors causing fungal succession on decomposing litter.

The prior decomposition by two phyllosphere fungi affected the substrate utilization patterns of two *Mycena* species, shifting from simultaneous removal of lignin and carbohydrates (L/W and L/C nearly equal to 1) to selective delignification (L/W and L/C more than 1). This observation suggests that some litter-inhabiting species in the Basidiomycota may be physiologically adapted to the selective removal of lignin and related recalcitrant substances remaining in litters previously partly decomposed by phyllosphere fungi. It is unclear whether the result can be applied to other basidiomycetous species, as only two species in *Mycena* were used in this experiment. However, a similar situation was found in the results of Osono and Takeda (2001b) and Osono et al. (unpublished data) in which some fungi in the Basidiomycota (*Mycena* spp. and *Clitocybe* sp.) preferentially colonized the litter in later stages of decomposition when lignin and related recalcitrant substances had accumulated in the litter. Saito (1957) and Hintikka (1970) related the vigorous ligninolytic activity of the Basidiomycota to the production of "white-rot humus" on forest soils.

The change of the substrate utilization pattern of *Mycena* spp. on the previously decomposed litters is difficult to explain. A possible explanation is the regulatory effects of carbon and nitrogen nutrition on the degree of selective delignification, which have been reported in some cases. For example, lignin decomposition by wood decay fungi depends on the availability of nonlignified carbohydrates (Kirk et al. 1976); *Xylaria* sp. decomposed lignin more selectively in litter that had a higher lignin to carbohydrate ratio (Osono and Takeda 2001a). This explanation can be applied to litter A in which the lignin to carbohydrate ratio was higher than in litter C, but seems to be inappropriate for litter X, which has a lignin to carbohydrate ratio similar to that of litter C. Further experiments are required to evaluate the effect of carbon and nitrogen

nutrition on lignin decomposition by litter-inhabiting species in the Basidiomycota.

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