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Keywords: Acid-unhydrolyzable residue (AUR), beech, holocellulose, humus accumulation, twig decay

Received 7 December 2012; accepted 12 February 2013

Abstract

The effects of variations in environmental moisture on fungal decomposition of the chemical components in Japanese beech (*Fagus crenata*) twigs were examined using a pure culture test with two ascomycetes (*Phomopsis* sp. and *Xylaria* sp.) and two basidiomycetes (*Mycena polygramma* and *Phanerochaete filamentosa*). Moisture did not significantly affect the weight loss of the twigs after a 6-month incubation, but it significantly altered the decay preferences of each fungus for different wood components. *Phanerochaete filamentosa* shifted from being a selective decomposer of acid-unhydrolyzable residue (AUR) with high moisture to being a selective decomposer of holocellulose with low moisture. *Xylaria* sp. shifted from being a simultaneous decomposer of AUR and holocellulose with high moisture to being a selective decomposer of holocellulose with low moisture. Thus, our findings indicate that dry conditions stimulate AUR accumulation in twig litter.

Introduction

The decomposition of woody litter is an important factor controlling soil humus formation in forest ecosystems (Swift et al. 1979). Woody litters are mainly composed of acid-unhydrolyzable residues (AUR, formerly referred to as lignin) and holocellulose (Eriksson et al. 1990). AUR is the main precursor of humus (Stevenson 1982), so the decay ratio of AUR to holocellulose is crucial in determining the accumulation of soil organic matter and humus formation in forests (Osono 2007).

Fungi have a central role in the process of decomposition of woody litter (Boddy 1991); abiotic conditions such as moisture are primary agents that indirectly affect litter decomposition processes mediated by microbial activity (Prescott 2010). Fine woody litters such as twigs and branches are particularly affected by environmental moisture because moisture conditions differ depending on weather they are attached, hanging in the air, or lying on the ground (Boddy and Swift 1983). Drier conditions usually delay the litter decay rate by reducing mycelial activity (Swift et al. 1976; Erickson et al. 1985; Osono et al. 2003b), but the appropriate moisture range for mycelial growth and decay activity varies among fungal species (Griffin 1972). For example, fungi living in dry places, such as attached dead twigs, usually have a lower optimal moisture level (Boddy et al. 1985; Chapela and Boddy 1988a) whereas soil-borne fungi have a higher optimal moisture level (Griffin 1972). Thus, it is hypothesized that the effect of moisture on fungal decay of AUR and holocellulose will differ among fungal species. However, little is known about the effects of moisture on fungal preferences for litter chemical components during decay processes.

In this study, we focused on the effect of moisture on the abilities of fungi to decay beech twig litter and its chemical components. Fungi associated with beech twigs include *Phomopsis* sp. (anamorph of *Di-*

aporthe) and *Xylaria* sp. (anamorph) as the dominant endophyte ascomycetes on healthy Japanese beech twigs (Sahashi et al. 1999; Osono and Mori 2003) and they act as primary colonizers of twig litter (Griffith and Boddy 1990). *Mycena polygramma* (Bull. Fr.) S.F. Gray and *Phanerochaete filamentosa* (Berk. et Curt.) Burdsall are basidiomycetes that are found on beech forest floors in Japan (Osono 2002; Fukasawa et al. 2009a). These four fungal species were used in pure culture decay experiments with two different environmental moisture levels, i.e., low (50%) and high (100%), which replicated the moisture conditions in an attached dead twig (Griffith and Boddy 1991) and the soil at our study site (Hishi et al. 2004), respectively. The results for the high moisture level have already been published (Fukasawa et al. 2009b) according to which *P. filamentosa* is AUR-selective, *Phomopsis* sp. is holocellulose-selective, and *Xylaria* sp. and *M. polygramma* are simultaneous decomposers of AUR and holocellulose.

Materials and methods

Source of fungi and twig litters

Four isolates in four fungal species were used in this experiment. *Phomopsis* sp. (Code Phom397) and *Xylaria* sp. (Code Xyl470) were isolated from surface-sterilized healthy twigs (diameter < 0.5 cm) of Japanese beech collected from a crown of recently-fallen beech tree in a cool temperate deciduous forest in the Ashiu Experimental Forest of Kyoto University, Kyoto, Japan in October 2004. *Mycena polygramma* (Code NBRC33011) was obtained from the culture collection (NBRC, Tokyo, Japan). *Phanerochaete filamentosa* (Code 1070506) was isolated from a basidiocarp collected at the Ashiu Experimental Forest in July 2001. These two basidiomycetes were used in *in vitro* decomposition tests to determine their ability to decompose leaf litter (Osono and Takeda 2002; Osono et al. 2003a) and wood block (Fukasawa et al. 2005). The fungi were maintained on 2% (w/v) malt extract agar medium [MA: malt extract (Nacalai Tesque, Kyoto, Japan) 20 g, agar 15 g, and distilled water 1000 ml] until the tests started.

Beech twigs (diameter < 0.5 cm) used in the decomposition tests were collected from recently fallen beech trees in October 2004 at the Ashiu Experimental Forest. Twigs were oven-dried for 1 week at 40 °C and cut into short lengths of 500 mg each (approximately 5 cm in length), and sterilized with ethylene oxide gas at 60 °C for 3 h. The sterilized twigs were used for decomposition experiments.

Twig decomposition experiment

Experimental set up followed Fukasawa et al. (2009b). Glass jars (110 ml) were filled with 10 g air-dried perlite (a naturally occurring, processed volcanic glass; Koyoen, Japan) and sterilized for 1 h at 180 °C. Sterilized distilled water (5 ml or 10 ml) was added to each jar to achieve a 50% moisture reproducing the conditions of attached dead twigs that is the habitat of endophytic ascomycetes (Griffith and Boddy 1991) or 100% moisture reproducing the conditions of the soil of the Ashiu Experimental Forest (Hishi et al. 2004) where saprobic basidiomycetes inhabit, respectively. Each of these two levels of water contents was referred to as low and high moisture level, respectively.

One twig sterilized with ethylene oxide gas was buried in each jar using sterilized forceps. An inoculum of one of the four fungal species was cut out from the actively growing margin of 2-week-old cultures on 2% MA plate using sterilized cork borer (5 mm in diameter) and inoculated into each jar. The jars were capped and sealed by parafilm to prevent evaporation, and incubated in the dark at 20 °C for 3 or 6 months. Sterilized twigs inoculated with agar plugs without fungi were set as control. Three replicate jars were prepared for each treatment. After the incubation, the twigs were retrieve, dried to a constant mass at 40 °C and weighed.

Chemical analysis

The dried twigs were ground in a laboratory mill (0.5 mm screen). The amount of AUR in the sample was estimated gravimetrically using hot sulfuric acid digestion (King and Heath 1967). Total carbohydrate was analyzed with the phenol-sulfuric acid method (Dubois et al. 1956). Details of chemical analyses were described in Fukasawa et al. (2009b). The mass of holocellulose (insoluble carbohydrate) was calculated as the difference between total carbohydrates and soluble carbohydrates. Initial mass of AUR and holocellulose within 500 mg healthy beech twig were 171.0 and 221.3 mg, respectively (Fukasawa et al. 2009b).

The AUR/weight loss ratio (AUR/W) is a useful index of the substrate utilization pattern of each fungal species (L/W in Osono and Takeda 2002). The AUR/

W was calculated according to the following equations:

AUR/W = weight loss of AUR (% of original AUR weight) / weight loss of twig (% original weight)

Weight loss of the twigs and AUR were determined as the difference from the weight loss of control, expressed as a percentage of the original weight.

Data presentation and statistical analysis

Data are presented with the standard error of the mean (SE). We referred to Fukasawa et al. (2009b) for the weight loss data in high moisture level to compare with the data in low moisture level obtained in this study. Weight losses of twig, AUR and holocellulose, and AUR/W were compared among fungal species and between moisture levels, using two-way ANOVA. Where indicated, Tukey-Kramer's honestly significant difference (HSD) test was performed. Percentage weight loss data were all arcsine transformed before the analysis. All statistical analyses were performed with JMP version 5.1.1 (SAS Institute 2004).

Results

Weight loss of the twigs ranged from 0.6% to 4.6% at 3 month, and from 8.0% to 12.0% at 6 months (Fig. 1). Neither moisture level nor fungal species significantly affected weight loss of the twigs (2-way ANOVA) for both incubation periods. After 6 months incubation, the chemical components of the twigs were analyzed.

Weight loss of AUR and AUR/W were significantly affected by both moisture and fungal species (Table 1). The weight loss of holocellulose was affected by fungal species but not by moisture. Significant interactions between the effects of moisture and fungal species were observed for weight losses of AUR and holocellulose, and AUR/W.

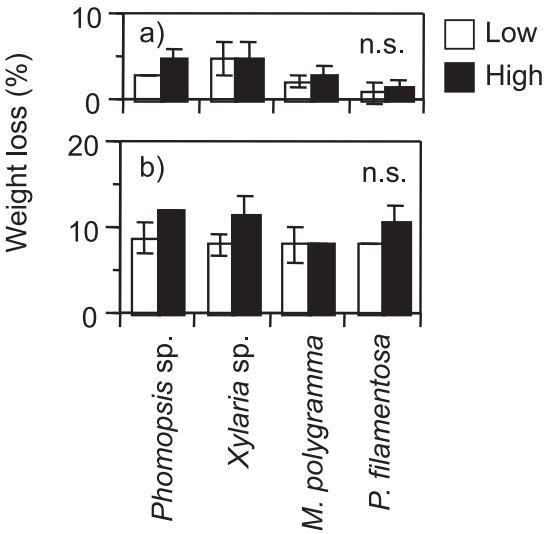

Fig. 1. Wight loss of beech twigs after a) 3 months and b) 6 months incubation period. Open bars, low moisture level (50% water content); Closed bars, high moisture level (100% water content). Error bars, SE. Data of high moisture were from Fukasawa et al. (2009b)

The weight loss of AUR ranged from –0.4% to 19.7% (Fig. 2), and was highest for *P. filamentosa* at high moisture level. Weight losses of AUR for *Xylaria* sp. and *P. filamentosa* were significantly lower at low moisture level compared to high moisture level. The weight loss of holocellulose ranged from 3.3% to 24.3%, and was highest for *Phomopsis* sp. at high moisture level. Weight loss of holocellulose for *P. filamentosa* was significantly higher at low moisture level compared to high moisture level.

AUR/W ranged from –0.1 to 1.9 (Fig. 3), highest in *P. filamentosa* at high moisture level and lowest in Phomopsis sp. at low moisture level. L/W for *Xylaria* sp. and *P. filamentosa* were significantly lower at low moisture level compared to high moisture level.

Table 1. Results of two-way ANOVA comparing weight losses (%) of AUR and holocellulose, and AUR/W after 6 months incubation period

	Moisture level			Fungi			Moisture × Fungi		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
AUR	1	27.08	0.0001	3	20.18	< 0.0001	3	11.91	0.0003
Holocellulose	1	1.17	0.2962	3	17.70	< 0.0001	3	14.38	0.0001
AUR/W	1	28.91	< 0.0001	3	36.65	< 0.0001	3	15.39	< 0.0001

AUR, acid unhydrolyzable residue.
AUR/W = weight loss of AUR / weight loss of twig.

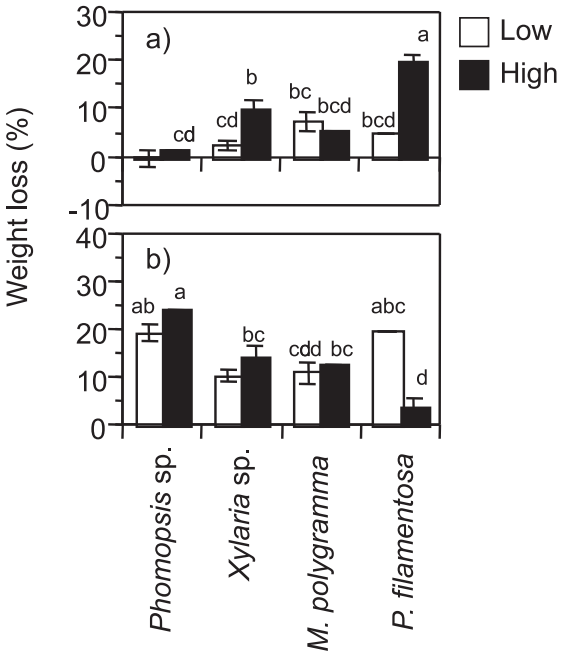

Fig. 2. Weight losses of chemical components of beech twigs after 6 months incubation period. a) acid-unhydrolyzable residue (AUR), b) holocellulose. Bars as for Fig. 1. Same letters above bars indicate no significant difference. Data of high moisture level were from Fukasawa et al. (2009b)

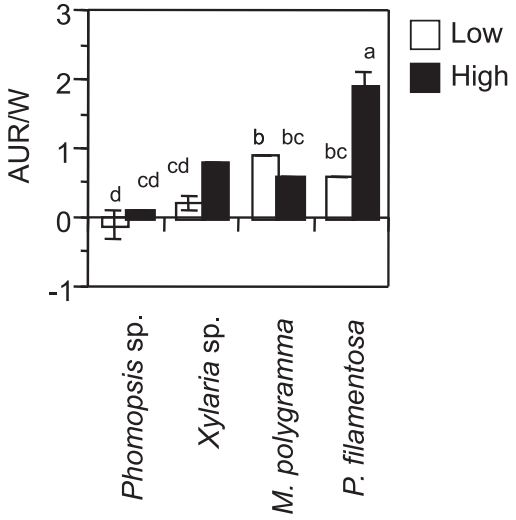

Fig. 3. AUR/W of beech twigs after 6 months incubation period. Bars as for Fig. 1. Same letters above bars indicate no significant difference. Data of high moisture level were from Fukasawa et al. (2009b)

Discussion

The present study demonstrates the effects of variations in environmental moisture on twig decomposition by fungi. The weight loss of twigs did not differ between the low and high moisture levels, but significant effects of moisture were observed on the fungal preference for decomposition of different chemical components of the twigs. With the high moisture level, *Phomopsis* sp. selectively decayed holocellulose, *Xylaria* sp. and *M. polygramma* simultaneously decayed AUR and holocellulose, whereas *P. filamentosa* selectively decayed AUR as described in Fukasawa et al. (2009b). In contrast, with the low moisture level, the AUR/W for the three fungal species were less than 1, with the exception of *M. polygramma*, suggesting their preference for holocellulose. It was surprising that the preference of *P. filamentosa*, known to be a highly selective decomposer of AUR, shifted to holocellulose-selective in dry conditions. *Xylaria* sp. also shifted to holocellulose selective decomposition under dry conditions. In contrast, the AUR/W for *Phomopsis* sp. and *M. polygramma* did not vary with moisture levels. These results suggest that the effect of moisture conditions on the decay preferences of fungi for different twig components depends on the fungal species, although there were no similarities in the moisture responses within each fungal ecological strategy such as endophytes (*Phomopsis* sp. and *Xylaria* sp.) and saprophytes (*M. polygramma* and *P. filamentosa*)

There have been few studies on the implications of moisture on AUR decomposition (e.g., ligninase activities, Bastos and Magan 2009), but high water potential may facilitate AUR decomposition when the reaction occur as hydrolysis (Thomsen et al. 2007). The reason why holocellulose decomposition by *P. filamentosa* was more stimulated at the low moisture level compared to the high moisture level is unclear. Previous reports indicate a higher decomposition rate of holocellulose under high water potential conditions (Summerell and Burgess 1989; Thomsen et al. 2007). Nevertheless, these results suggest that dry conditions could stimulate AUR accumulation in twig litter, which may retard subsequent decay processes (Berg and McClaugherty 2003). Further researches are essential to determine the effects of moisture on the litter decay abilities of a greater variety of fungi, and its subsequent decay processes.

Acknowledgements

We thank Dr. Chisato Kobayashi and Mr. Yasunori Baba for invaluable comments. Financial support for this study was provided in part by the Research Fellowship Division, Japan Society for the Promotion of Science for the first author.

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