Mycoscience (2005) 46:209–214
DOI 10.1007/s10267-005-0236-x

FULL PAPER

Yu Fukasawa · Takashi Osono · Hiroshi Takeda

Small-scale variation in chemical property within logs of Japanese beech in relation to spatial distribution and decay ability of fungi

Received: November 10, 2003 / Accepted: February 3, 2005

Abstract Fungal communities within a naturally fallen bough of Japanese beech (*Fagus crenata*) were investigated with reference to chemical properties of decay columns. Five logs were cut out from the fallen bough, which ranged from 10.7 to 20.5 cm in diameter. Nine fungal species and one sterile fungus were isolated from decay columns that elongated along a longitudinal axis and were delimited by black zone lines and wood discoloration. *Lampteromyces japonicus* and *Trichoderma* spp. were isolated from all five logs. *Lampteromyces japonicus* and *Antrodiella albocinnamomea* occupied the largest volume in the logs. Lignin and carbohydrate contents, lignocellulose index (LCI), nitrogen content, and water content were different among decay columns colonized by different fungal species in each log. In *L. japonicus,* LCI of decay column was correlated to that of wood blocks decayed under pure culture condition by the fungi isolated from the decay columns. These results suggest that the small-scale variation in chemical properties within fallen logs of Japanese beech reflects the distribution and the decay ability of colonized fungi.

Key words Decomposition · *Fagus crenata* · Lignocellulose · Nitrogen · Water content

Introduction

Fungi play a central role in wood decomposition in forest ecosystems. Wood decay by fungi is broadly grouped into three types: white-rot, brown-rot, and soft-rot (Rayner and Boddy 1988). These differences occur as a result of enzymatic activities of fungi that utilize chemical components of wood in various proportions (Eriksson et al. 1990). Decay ability and decay type of fungi have been studied with pure culture decay test under laboratory conditions (Otjen et al. 1987; Tanesaka et al. 1993; Worrall et al. 1997; Fukasawa et al. 2005). On the other hand, there have been fewer studies on the chemical changes of wood decomposed by fungi under natural conditions.

Kawase (1962) showed that the chemical properties of wood decayed under natural conditions depended on decay types of fungi as well as on tree species. Kawase (1962) regarded each log as to be colonized by a single fungal colony. Recent studies, however, indicate that complex mosaic-like distribution of fungal colonies exist even within a single log, forming decay columns that elongate along a longitudinal axis and are delimited by black zone lines (Rayner and Todd 1979; Rayner and Boddy 1988). Boddy et al. (1989) showed that mass loss rate and instantaneous decay rate of wood were variable among decay columns within logs of European beech, *Fagus sylvatica* L., 1 year after felling. These results suggest that the small-scale variation in chemical properties within a single log reflects community structure, decay ability, and decay type of fungi.

European beech has been studied intensively in the fungal community development within logs and branches (Rayner 1977a,b; Coates and Rayner 1985a,b,c; Chapela and Boddy 1988a,b,c; Chapela et al. 1988; Boddy et al. 1989; Griffith and Boddy 1990) and concerning the occurrence of fruiting bodies of macrofungi on the log surfaces (Lange 1992; Fenwick 1996; Heilmann-Clausen 2001). In Japanese beech, *F. crenata* Bl., fruiting bodies of macrofungi (Ueyama 1966) and microfungal succession (Okada et al. 1984; Tokumasu et al. 1987) on wood have been studied. Our preliminary study suggested the relationship between fungal communities and the small-scale variation in chemical properties within small-diameter logs of Japanese beech (Fukasawa et al. 2002).

In the present study, we described the fungal communities inside the five larger-diameter logs derived from a single bough. A single log was regarded as an independent resource for the fungal community development. Chemical properties of individual decay columns within the log were

Y. Fukasawa · T. Osono (✉) · H. Takeda
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

investigated and compared among decay columns colonized by different fungal species. The chemical properties of decay columns were also compared with those of beech wood decayed under pure culture conditions by fungi isolated from the decay columns (Fukasawa et al. 2005) to determine whether the chemical property was derived from fungal decay ability.

Materials and methods

Study area

The study was carried out in a cool temperate deciduous forest dominated by *Fagus crenata* and *Quercus crispula* in the Ashiu experimental forest of Kyoto University (35°18′ N, 135°43′ E) about 40 km north from Kyoto, Japan. Mean annual temperature was 9.7°C. The mean annual precipitation was 2495 mm. The study area was covered with snow from December to April.

Sampling of logs

A naturally fallen large bough of *F. crenata* was used in the present study as in Fukasawa et al. (2002). The diameter at the base of the bough was 76.7 cm. The exact year after the fall was unknown. Decay class of the bough was 3 according to the criterion of Heilmann-Clausen (2001). The bough that included the main stem and several branches was divided into a total of 82 segments, each of which was 100 cm in length. The diameter at the center of each segment ranged from 4.5 to 76.7 cm. Five of 82 segments were randomly chosen, and one log (30 cm long) was cut from the center of each five segments by a handsaw in October and November 2001. The logs were numbered as logs 1, 2, 3, 4, and 5, put into vinyl bags, and brought back to the laboratory.

Sound wood samples were collected from three standing trees to measure the initial chemical property of beech wood as undecayed substrate. The diameters at breast height (DBH) of the trees were 20.6, 21.5, and 22.5 cm, respectively. Three radial wood plugs (6 mm diameter) were sampled at breast height of each bole using an increment borer. Bark was removed, and each plug was radially separated into three segments (rim, middle, and central parts), ranging from 3.4 to 3.7 cm in length.

Analysis of fungal community within logs

The locations of fungal fruit bodies and rhizomorphs on log surfaces were recorded. Each log was then cut into ten successive disks (3 cm thickness) by a handsaw and numbered. One cut surface in each disk was observed for the fungal colonies, each of which was distinguished by zone lines and wood discoloration. The cut surfaces observed were photocopied and the area of each fungal colony was measured using a planimeter (Planix 5000; Tamaya Digitizing area-line meter). Volume of each fungal colony was calculated by multiplying disk thickness to area.

Fungi were isolated from each colony in each disk. The disk surface was first removed with a flame-sterilized chisel and then small wood chips were cut from the inside. A chip was transferred aseptically onto a plate of 2% malt extracted agar (MA; 20 g malt extract, 15 g agar, 1 L distilled water). Plates were incubated at 25°C in darkness for more than 1 month. Fungi growing out from the wood chips were identified based on the connection between the decay column and the fruit bodies on log surface and (or) the comparison of macro- and micromorphological characteristics of the mycelium on 2% MA with those of strains isolated from fruit bodies. The classification of fungal taxa followed Hawksworth et al. (1995).

Chemical analyses

Five disks in each log were used for chemical analyses. Wood chips (about 1 g) were cut from each fungal colony, oven dried at 40°C for 1 week, and ground in a laboratory mill to pass a 0.5-mm screen. Other wood chips (about 0.5 g) were dried at 105°C for 1 week to determine water content: water content (%) = weight of water (g)/weight of water plus wood chip (g) × 100.

The content of lignin in the samples was estimated by gravimetry according to a standardized method using hot sulfuric acid digestion (King and Heath 1967). The sample (about 50 mg) was extracted with alcohol benzene at room temperature and the residue 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 for 1 h, and weighed as acid-insoluble lignin. The filtrate (autoclaved sulfuric acid solution) was used for total carbohydrate analysis as described below.

Total carbohydrate content was estimated by the phenol-sulfuric acid method (Dubois et al. 1956) according to Fukui (1969). Five percent phenol (v/v) and 98% sulfuric acid (v/v) were added to the sulfuric acid solution derived from the lignin analysis. The optical density of the solution was then measured by the spectrometer at 490 nm using the known concentration of D-glucose as the standard. Total nitrogen contents in the samples (about 50 mg) were measured by automatic gas chromatography (NC analyzer Sumigraph NC-900; Sumitomo Chemical, Osaka, Japan).

Lignocellulose index (LCI) that is a useful index of litter chemical quality (Berg et al. 1984) was calculated according to the following equation:

$$\text{LCI} = \text{carbohydrate conc. (\%)} / [\text{lignin conc. (\%)} + \text{carbohydrate conc. (\%)}]$$

Sound wood samples were also analyzed for contents of lignin, carbohydrates, and nitrogen as already described.

Statistical analysis

A one-way analysis of variance (ANOVA) (Systat version 5.2; Systat, Evanston, IL, USA) was performed to evaluate differences in chemical properties (contents of lignin and carbohydrates, LCI, and nitrogen content) and water content among decay column in each log. Pearson's correlation coefficients (Systat version 5.2) were calculated for LCI and nitrogen content of *Lampteromyces japonicus* between decay columns and wood blocks decayed under pure culture condition (Fukasawa et al. 2005).

Results and discussion

Community structure

Fungal colonies were evident on all cut surfaces, which were delimitated by black zone lines and wood discoloration (Fig. 1). Each colony was continuous for the ten successive disks from each log, thus forming decay column that elongated along a longitudinal axis. A total of nine species and one sterile fungus were isolated from the logs (Table 1). In most cases, a single fungal species was isolated from each colony. However, *Trichoderma* species were often isolated from colonies of another fungal species. Number of isolated species in each log ranged from two to six, and species composition and their relative volume of decay columns were different among logs. These spatial patterns of fungal community were similar to those reported in decayed wood of European beech and other angiospermous tree species (Boddy and Griffith 1989; Boddy 1992).

Lampteromyces japonicus and *Trichoderma* spp. were isolated from all five logs. *Lampteromyces japonicus* and *Antrodiella albocinnamomea* occupied the largest volume in the logs. Fruit bodies of *L. japonicus* were observed on logs and snags of various decay phases and diameters in the present study site (Fukasawa et al. 2005), suggesting the fungus as one of the representative fungi in beech wood decomposition. Several species in *Antrodiella* were reported as white-rot fungi (Imazeki and Hongo 1989) or as secondary colonizers of wood previously colonized by another fungus (Niemelä et al. 1995). Species in *Tricho-*

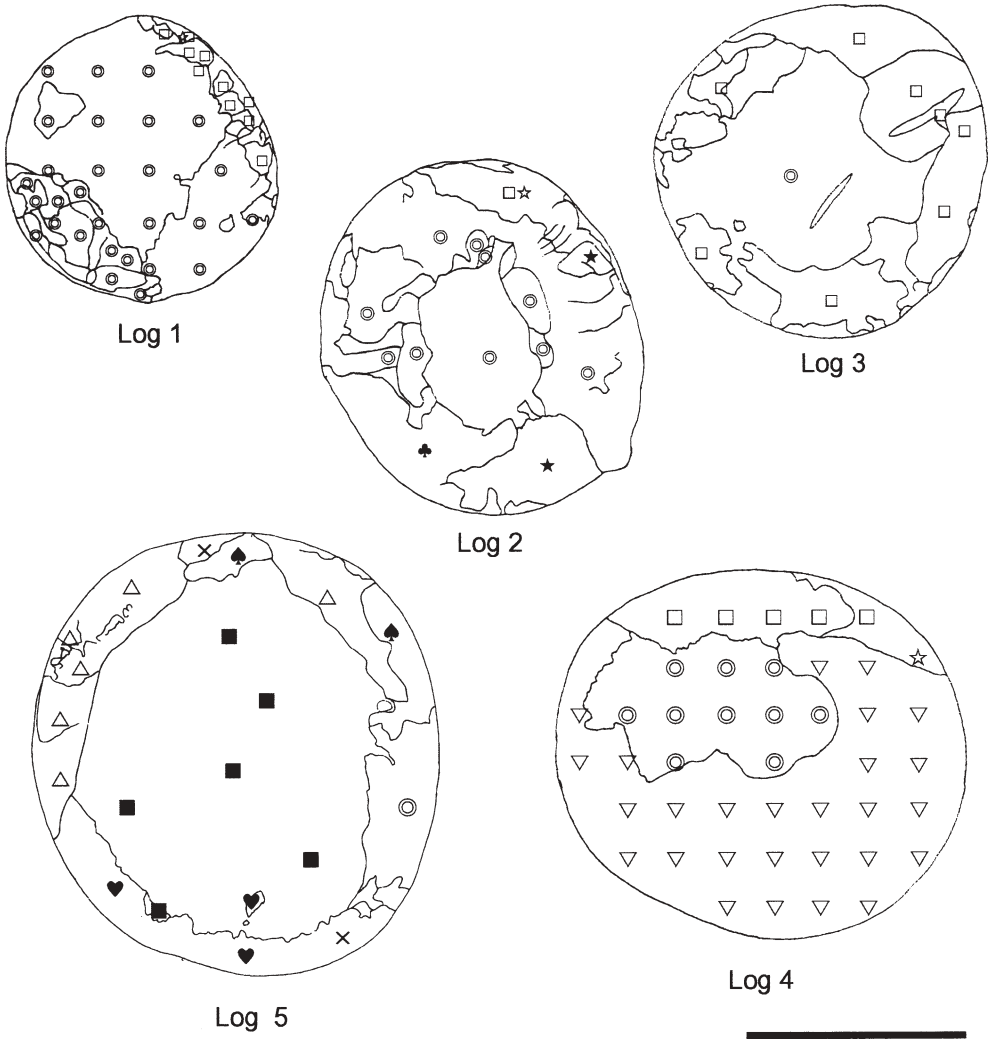

Fig. 1. The cut surface of five beech logs showing the spatial distribution of fungal decay columns surrounded by zone lines. *Symbols* indicate the point of isolation. ◎, *Lampteromyces japonicus*; ■, *Antrodiella albocinnamomea*; □, *Trichoderma* spp.; ▽, basidiomycete 2; ♣, basidiomycete 1; ♠, basidiomycete 3; ♥, *Armillaria* sp.; ×, unknown; △, *Psathyrella candolleana*; ★, sterile mycelium; ☆, *Steccherinum rhois*. *Bar* 10 cm

Table 1. Fungi isolated from decay columns in beech logs and the chemical properties of the decay columns

Fungus	Code	Decay column in each log			Lignin (%)	Carbohydrates (%)	Lignocellulose index (LCI)	Nitrogen (%)	Water (%)
		Volume (cm^3)	Proportion (%) to total volume	Position occupied					
Sound wood (rim part)					25.2 ± 0.6a	55.8 ± 0.8a	0.69 ± 0.01a	0.08 ± 0.00a	nd
Sound wood (middle part)					25.5 ± 0.7a	53.8 ± 1.1a	0.68 ± 0.01a	0.07 ± 0.00b	nd
Sound wood (central part)					26.2 ± 0.8a	53.5 ± 1.3a	0.67 ± 0.01a	0.07 ± 0.00b	nd
Log 1 (D = 10.7 cm)									
Lampteromyces japonicus	LJ1	2517	92.7	Almost all	21.9 ± 0.8a	62.0 ± 0.8a	0.74 ± 0.01a	0.20 ± 0.01a	37.8 ± 1.5a
Trichoderma sp.	TR1	110	4.0	Rim	24.6 ± 0.8a	60.0 ± 1.5a	0.71 ± 0.01b	0.21 ± 0.02a	33.4 ± 2.7a
Steccherinum rhois	SR1	10	0.4	Rim	nd	nd	nd	nd	nd
Log 2 (D = 14.0 cm)									
L. japonicus	LJ2	2932	62.6	Center	26.6 ± 0.6b	71.7 ± 1.0a	0.73 ± 0.00ab	0.16 ± 0.01bc	38.6 ± 1.3c
Basidiomycete 1	B12	634	13.5	Rim	27.7 ± 0.6ab	70.4 ± 1.5a	0.72 ± 0.01b	0.21 ± 0.01a	44.1 ± 0.9b
Trichoderma sp.	TR2	321	6.9	Rim	26.3 ± 0.7b	72.7 ± 1.2a	0.73 ± 0.00a	0.19 ± 0.0ab	51.5 ± 1.1a
Sterile mycelium	SM2	293	6.3	Rim	28.7 ± 0.4a	72.7 ± 0.9a	0.72 ± 0.00b	0.14 ± 0.01c	32.4 ± 0.9d
Log 3 (D = 14.1 cm)									
Trichoderma sp.	TR3	2806	62.3	Rim	33.1 ± 1.6a	63.7 ± 1.4b	0.66 ± 0.01b	0.24 ± 0.01a	64.8 ± 3.7a
L. japonicus	LJ3	1186	26.3	Center	25.0 ± 1.3b	72.3 ± 1.4a	0.74 ± 0.01a	0.15 ± 0.01b	50.2 ± 1.7b
Log 4 (D = 16.8 cm)									
Basidiomycete 2	B24	3685	57.3	Rim	24.0 ± 0.2a	71.1 ± 2.1a	0.75 ± 0.01a	0.20 ± 0.01a	42.2 ± 1.1ab
L. japonicus	LJ4	1188	18.5	Center	23.4 ± 0.4a	74.5 ± 0.8a	0.76 ± 0.00a	0.19 ± 0.00ab	40.1 ± 0.6b
Trichoderma sp.	TR4	774	12.0	Rim	24.9 ± 0.8a	74.1 ± 1.7a	0.75 ± 0.01a	0.16 ± 0.01b	42.8 ± 0.9a
S. rhois	SR4	38	0.6	Rim	nd	nd	nd	nd	nd
Log 5 (D = 20.5 cm)									
Antrodiella albocinnamomea	AA5	6619	70.0	Center	24.5 ± 0.7c	73.0 ± 0.9a	0.75 ± 0.01a	0.15 ± 0.01d	39.2 ± 0.8d
Psathyrella candolleana	PC5	918	9.7	Rim	30.5 ± 1.1a	66.4 ± 2.1b	0.69 ± 0.01c	0.26 ± 0.01b	44.4 ± 2.5c
Basidiomycete 3	B35	702	7.4	Rim	26.0 ± 0.4bc	69.1 ± 0.7b	0.73 ± 0.00b	0.20 ± 0.01c	55.4 ± 0.7b
Armillaria sp.	AR5	495	5.2	Rim	24.7 ± 0.5c	69.1 ± 1.4b	0.74 ± 0.00ab	0.22 ± 0.01c	67.6 ± 1.9a
L. japonicus	LJ5	290	3.1	Rim	28.1 ± 1.2ab	60.3 ± 1.9c	0.68 ± 0.00c	0.35 ± 0.02a	65.3
Trichoderma sp.	TR5	240	2.5	Rim	28.4	59.5	0.68	0.31	81.6

Values indicate means ± standard errors (n = 9 for sound wood, n = 2–23 for decay column)
nd, not determined
The same letters indicate values that are not significantly different at the 5% level by the least significant difference test

derma, which were often isolated from colonies of another fungus, seemed to be mycoparasites of wood decay fungi or secondary colonizers as reported previously (Lumsden 1981; Tanaka et al. 1988). In fact, *Trichoderma* spp. isolated in the present study showed negligible decay ability of sound beech wood (Fukasawa et al. 2005). Tanaka et al. (1988) reported that *Trichoderma* spp. were incapable of degrading sound wood, but degraded cellulose component of wood that was previously delignified by ligninolytic basidiomycetes.

Chemical property of decay column

Contents of lignin and carbohydrates and LCI were not significantly different among the rim, the middle, and the central parts of sound wood (see Table 1). Some of these indices in each log were significantly different among decay columns. These differences may be attributable to the decay ability of colonized fungal species in each log. LCI in *L. japonicus* varied among logs (Fig. 2) and was positively correlated to that of wood blocks decayed by the fungus under pure culture condition ($n = 5$, $R = 0.96$, $P < 0.01$).

Nitrogen content was significantly different among the rim, the middle, and the central part of sound wood (see Table 1). Radial gradient in nitrogen content has already been reported in sound wood of other trees (Merrill and Cowling 1966; Penninckx et al. 2001). The mean nitrogen content of all decay columns was higher than that of sound wood and varied among decay column. The variation may be attributable to the fungal species colonized. In *L. japonicus,* nitrogen content of decay columns was not significantly correlated to that of wood blocks decayed under pure culture condition ($n = 5$, $R = -0.60$).

Water content was significantly different among decay columns in each log (see Table 1). Boddy et al. (1989) reported a similar result to that of the present study that water contents were different among decay columns and found that decay column colonized by Xylariaceae were drier than those that colonized by other fungi, probably because of the formation of impermeable pseudosclerotial plates.

The present study demonstrated that the small-scale variation in chemical properties within logs of Japanese beech reflected the spatial distribution and the decay ability of colonized fungi. Further studies are needed to follow the relationship between fungal succession and chemical changes of wood during the decomposition processes.

Acknowledgments We are grateful to Dr. T. Hattori, Dr. C. Tanaka, Mr. S. Morimoto, Dr. Y. Shimono, Mr. D. Sakuma, and Mr. T. Ueda for their guidance to identification of fungi. We thank Dr. S. Tokumasu, Mr. D. Hirose, Ms. K. Mori, and Ms. K. Koide for their useful discussion; and members of the Laboratory of Forest Ecology, Kyoto University for their suggestions and help in field and laboratory work.

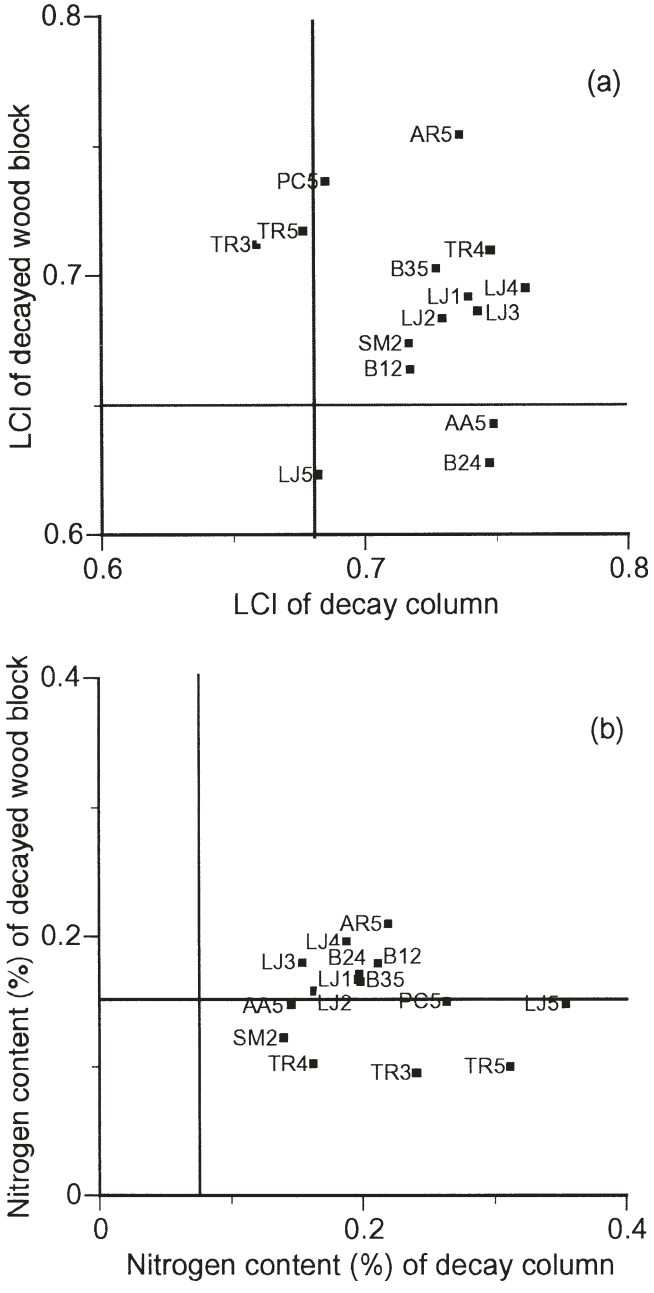

Fig. 2. Comparison of lignocellulose index (*LCI*) (**a**) and nitrogen content (**b**) between decay columns within beech logs and wood block decayed by fungi under pure culture condition. *Lines* indicate LCI or nitrogen content of sound wood and control wood block. The wood block data were derived from the original data in Fukasawa et al. (2005). Abbreviations (codes) are indicated in Table 1

References

Berg B, Ekbohm G, McClaugherty CA (1984) Lignin and holocellulose relations during long term decomposition of some forest litters. Long-term decomposition in Scots pine forest. IV. Can J Bot 62:2540–2550

Boddy L (1992) Development and function of fungal communities in decomposing wood. In: Carroll GC, Wicklow DT (eds) The fungal community, 2nd edn. Dekker, New York, pp 749–782

Boddy L, Griffith GS (1989) Role of endophytes and latent invasion in the development of decay communities in sapwood of angiospermous trees. Sydowia 41:41–73

Boddy L, Owens EM, Chapela IH (1989) Small scale variation in decay rate within logs one year after felling: effect of fungal community structure and moisture content. FEMS Microbiol Ecol 62:173–184

Chapela IH, Boddy L (1988a) Fungal colonization of attached beech branches. I. Early stages of development of fungal communities. New Phytol 110:39–45

Chapela IH, Boddy L (1988b) Fungal colonization of attached beech branches. II. Spatial and temporal organization of communities arising from latent invaders in bark and functional sapwood, under different moisture regimes. New Phytol 110:47–57

Chapela IH, Boddy L (1988c) The fate of early fungal colonizers in beech branches decomposing on the forest floor. FEMS Microbiol Ecol 53:273–284

Chapela IH, Boddy L, Rayner ADM (1988) Structure and development of fungal communities in beech logs four and a half years after felling. FEMS Microbiol Ecol 53:59–70

Coates D, Rayner ADM (1985a) Fungal population and community development in cut beech logs. I. Establishment via the aerial cut surface. New Phytol 101:153–171

Coates D, Rayner ADM (1985b) Fungal population and community development in cut beech logs. II. Establishment via the buried cut surface. New Phytol 101:173–181

Coates D, Rayner ADM (1985c) Fungal population and community development in cut beech logs. III. Spatial dynamics, interactions and strategies. New Phytol 101:183–198

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Eriksson KEL, Blanchette RA, Ander P (1990) Microbial and enzymatic degradation of wood and wood components. Springer-Verlag, Tokyo

Fenwick GA (1996) The breakdown of a beech tree – the first five years. Mycologist 10:26–28

Fukasawa Y, Osono T, Takeda H (2002) Fungal community structure and physical and chemical properties of decaying beech logs in a cool temperate natural forest (in Japanese with English abstract). Appl For Sci 11:7–14

Fukasawa Y, Osono T, Takeda H (2005) Decomposition of Japanese beech wood by diverse fungi isolated from a cool temperate deciduous forest. Mycoscience 46:97–101

Fukui S (1969) Analysis of reducing sugars. Tokyo University Press, Tokyo

Griffith GS, Boddy L (1990) Fungal decomposition of attached angiosperm twigs. I. Decay community development in ash, beech and oak. New Phytol 116:407–415

Hawksworth DL, Kirk PM, Sutton BC, Pegler DN (1995) Ainsworth & Bisby's dictionary of the fungi, 8th edn. CAB International, Wallingford

Heilmann-Clausen J (2001) A gradient analysis of communities of macrofungi and slime moulds on decaying beech logs. Mycol Res 105:575–596

Imazeki R, Hongo T (1989) Colored Illustrations of mushrooms of Japan, vol 2 (in Japanese). Hoikusha, Osaka

Kawase K (1962) Chemical components of wood decayed under natural condition and their properties. J Fac Agric Hokkaido Univ Sapporo 52:186–245

King HGC, Heath GW (1967) The chemical analysis of small samples of leaf material and the relationship between the disappearance and composition of leaves. Pedobiologia 7:192–197

Lange M (1992) Sequence of macromycetes on decaying beech logs. Persoonia 14:449–456

Lumsden RD (1981) Ecology of mycoparasitism. In: Wicklow DH, Carroll GC (eds) The fungal community. Dekker, New York, pp 295–318

Merrill W, Cowling E (1966) Role of nitrogen in wood deterioration: amounts and distribution of nitrogen in tree stems. Can J Bot 44:1555–1580

Niemelä T, Renvall P, Penttilä R (1995) Interactions of fungi at late stages of wood decomposition. Ann Bot Fenn 32:141–152

Okada G, Tokumasu S, Haraguchi T, Tubaki K (1984) Early stages of fungal succession on small beech sapwood strips in a beech forest litter. Trans Mycol Soc Jpn 25:461–474

Otjen L, Blanchette R, Effland M, Leatham G (1987) Assessment of 30 white rot basidiomycetes for selective lignin degradation. Holzforschung 41:343–349

Penninckx V, Glineur S, Gruber W, Herbauts J, Meerts P (2001) Radial variations in wood mineral element concentrations: a comparison of beech and pedunculate oak from the Belgian Ardennes. Ann For Sci 58:253–260

Rayner ADM (1977a) Fungal colonization of hardwood stumps from natural sources. I. Non-basidiomycetes. Trans Br Mycol Soc 69:291–302

Rayner ADM (1977b) Fungal colonization of hardwood stumps from natural sources. II. Basidiomycetes. Trans Br Mycol Soc 69:303–312

Rayner ADM, Boddy L (1988) Fungal decomposition of wood: its biology and ecology. Wiley, Chichester

Rayner ADM, Todd NK (1979) Population and community structure and dynamics of fungi in decaying wood. Adv Bot Res 7: 333–420

Tanaka H, Enoki A, Fuse G, Nishimoto K (1988) Interactions in successive exposure of wood to varying wood-inhabiting fungi. Holzforschung 42:29–35

Tanesaka E, Masuda H, Kinugawa K (1993) Wood degrading ability of basidiomycetes that are wood decomposers, litter decomposers, or mycorrhizal symbionts. Mycologia 85:347–354

Tokumasu S, Okada G, Tubaki K (1987) Late stages of fungal succession on small beech sapwood strips in a beech forest litter. Bull Sugadaira Montane Res Cent 8:9–15

Ueyama A (1966) Studies on the succession of higher fungi on felled beech logs (*Fagus crenata*) in Japan. Mat Org 1:325–332

Worrall JJ, Anagnost SE, Zabel RA (1997) Comparison of wood decay among diverse lignicolous fungi. Mycologia 89:199–219