Fungal succession associated with the decay of leaves of an evergreen oak, *Quercus myrsinaefolia*

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In Japan, the species component of fungal communities and fungal succession on decaying fallen leaves of broadleaf-evergreen trees has not been elucidated. In this study, we investigated the species components and structures of fungal communities inhabiting the decaying leaves of an evergreen oak, *Quercus myrsinaefolia*. Fungal succession occurs with the progressive decay of *Q. myrsinaefolia* leaves with the phyllosphere fungi, such as *Tubakia* sp. and *Colletotrichum gloeosporioides* giving way to early colonizers of fallen leaves on the ground, such as *Subramaniomyces fusisaprophyticus* and *Rhinocladiella intermedia*, and then progressing to later colonizers, such as *Trichoderma koningii* and *T. harzianum*. The whole succession pattern at this study site is characterized by the fungal succession associated with the decomposition of fallen leaves during the main leaf-fall seasons.

Key words: broadleaf-evergreen forest, fungal community, leaf decomposition, saprobic succession, warm temperature

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Introduction

Since the first systematic study of saprobic fungal succession associated with the decay of fallen pine needles by Kendrick and Burges (1962), many studies on fungal succesession have been conducted on fallen needles of conifers, such as *Pinus*, *Abies* and *Picea*, in subtropical, temperate and sub-arctic zones in the northern hemisphere (Hayes, 1965a, b; Tubaki and Saitô, 1969; Widden and Parkinson, 1973; Mitchell and Millar, 1978; Mitchell *et al*., 1978; Soma and Saitô, 1979; Aoki *et al*., 1990; Tokumasu *et al.*, 1994; Tokumasu, 1996, 1998a,b; Tokumasu and Aoki, 2002). Fungal succession on fallen leaves of broadleaf trees (Saitô, 1956; Hering, 1965; Hogg and Hudson, 1966; Macauley and Thrower, 1966; Aoki, 1987; Promputtha *et al*., 2002; Osono, 2002, 2005; Tang *et al*., 2005; Pasqualetti *et al*., 2006; Paulus *et al*., 2006; Duong *et al*., 2008) and monocotyledons (Yanna *et al*., 2002; Thongkantha *et al*., 2008) have also been studied. These studies have accumulated information on fungal succession with leaf decomposition in broadleaf trees, conifers and monocotyledons. In general, the fungal species involved in fungal succession on fallen leaves of deciduous broadleaf trees were different from those of conifers and monocotyledons, but the successional patterns were fundamentally the same (Hudson, 1968); however, information about fungal succession is still limited in the leaf litter of broadleaf-evergreen forests in warm-temperate regions in the northern hemisphere.

In southwest Japan, the original vegetation is broadleaf-evergreen forest, although the forest has been almost destroyed by human activity. It is mainly composed of evergreen trees of *Fagaceae* (*Quercus* and *Castanopsis etc*.) and *Lauraceae* (*Machilus* and *Cinnamomum etc*.). A series of pioneering studies of saprobic fungal succession on evergreen

Quercus and *Castanopsis* leaves was performed by Tubaki and Yokoyama (1971, 1973a, b), Yokoyama and Tsubaki (1973) and Yokoyama *et al.* (1977) who inserted autoclaved leaves into the surface layer of the O horizon and observed the succession occurring on the leaves. A similar experimental study using unsterilized *Cameria japonica* leaves set on litter has been attempted by Tsubaki and Yoshida (1980); however, information about saprobic fungal succession on decaying leaves of broadleaf-evergreen trees is still inadequate for Japanese warm-temperate forest litter.

In this study, we attempted to study fungal succession associated with the decay of leaves of *Quercus myrsinaefolia*, a dominant evergreen oak in the northern broadleaf-evergreen forest in Japan. To evaluate the changing fungal community on decaying leaves, we surveyed fungi colonizing the leaves at different decomposition stages by a cultural method. Obtained data were compared among decomposition stages, and fungal succession occurring on decaying leaves of *Q. myrsinaefolia* was estimated. The results were discussed by comparing with similar published data on other trees.

Materials and methods

Study site

Leaf samples were collected from forest litter in the precinct of Meiji Jingu Shrine, Shibuya, Tokyo (35° 40' N, 139° 41' E; alt. 35 m). The mean temperature and precipitation in this area from 1971 to 2004 were 16.3 °C and 1532mm, respectively, according to weather observation data from Tokyo District Meteorological Observatory (Chiyoda, Tokyo). Monthly mean temperatures and precipitations in the study period, February 2005 to January 2006, are shown in Fig. 1. The study area is covered with about 80-year-old planted trees and it is a well developed, closed broadleaf-evergreen forest mainly composed of *Quercus myrsinaefolia*, *Castanopsis sieboldii* and, *Cinnamomum camphora*. We selected a stand in the forest for leaf sampling where *Quercus myrsinaefolia* is predominant. At the sampling site, the O horizon was 3 to 6 cm in depth and composed of an intermingling of variously decayed leaves. Sub-layers corresponding to the F or H layer found at the mor site were not developed.

Measurement of litter production

To estimate the seasonal fluctuation of leaf litter production of *Quercus myrsinaefolia*, leaf litter samples were collected and weighed in April, June, August, October and December, 2005 according to the following procedure. The O horizon was cut from six plots (10 × 10 cm) at the sampling site and each sample was placed in a paper bag and brought back to the laboratory where only the leaves of *Q. myrsinaefolia* were selected from litter samples. The leaves were divided into three groups, L-type (elastic, intact and olive colored), OL-type (between L-type and F-type) and F-type (inelastic, damaged and ash-grey colored) by external appearance. These leaf samples were dried for 4 days at 40 °C, and then weighed. Mean monthly dry weights of fallen leaves at each decomposition stage were calculated.

Chemical property of fallen leaves

Litter samples at the same decomposition stages from each of four sampling seasons (April, August, October and December) were mixed to make one sample and used for chemical analyses. The dried litter samples were ground in a laboratory mill to pass through a 0.5 mm screen and used for chemical analyses. Concentrations of total N and C were measured by automatic gas chromatography (NC analyzer SUMIGRAPH NC-900, Sumitomo Chemical Co., Osaka, Japan). Soluble sugar and polyphenol were extracted with 50% methanol, and their contents were estimated with the phenol-sulphuric acid method (Dubois *et al*., 1956) and Folin-Ciocalteau method (Waterman and Mole, 1994), respectively.

The carbon to nitrogen ratio (C/N) is an useful index of litter chemical properties (Osono and Takeda, 2001) and is calculated according to the following equation:

C/N = carbon concentration (%) / nitrogen concentration (%)

Leaf sampling for fungal isolation

Fallen leaves of *Quercus myrsinaefolia* were collected from the O horizon at the

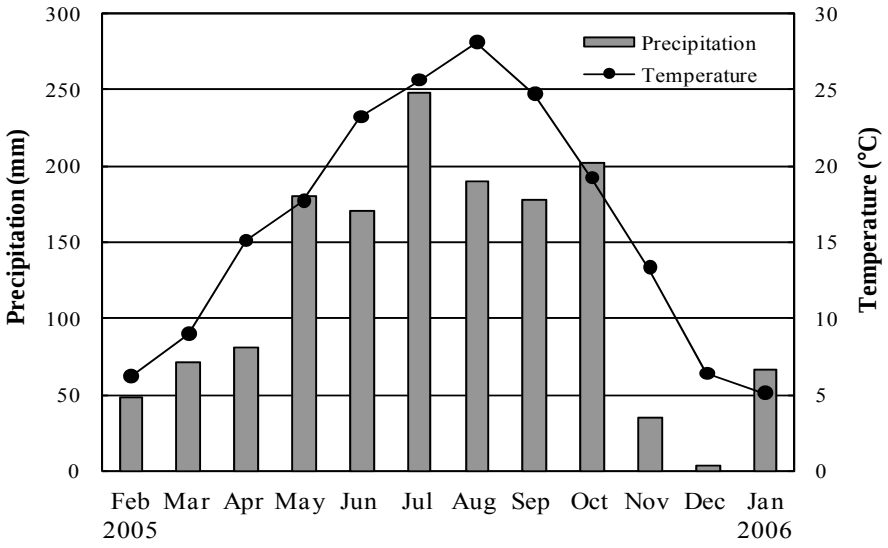

Fig. 1. Monthly average temperatures and precipitation in the study area.

sampling site on April 10, August 2, October 5, 2005 and January 17, 2006. At the same time, two-year-old symptomless green leaves on the trees (A-type leaves) were also collected from lower branches. The collected leaves were taken back to the laboratory in separate paper bags and fungi were isolated within 24 hours after collection.

For fungal isolation, ten representative leaves were selected from each leaf type and two discs per leaf were punched out of the central part of leaves except for the main veins by a 7 mm diameter corkbore. Twenty discs per leaf type, a total of 80 discs, were used for fungal isolations at each investigation time. The washing method (Tokumasu, 1980) was used for fungal isolation. Punched leaf discs of each leaf type were put in a sterile test tube with a plastic cap, and autoclaved. Then 10 ml 0.005% aero-sol OT solution (Wako, Osaka, Japan) was poured into the test tube as a washing solution using a sterile pipette. To wash the fungal diaspores off the surface of the leaf discs, the test tube was stirred for 1 min using a vortex mixer. After stirring, the washing solution was removed from the test tube using another sterile pipette, and then new washing solution was added to the test tube and stirred again. After five repeats of the washing operation, the same process was repeated three times using sterile distilled water. To inhibit the propagation of bacteria, washed and rinsed leaf discs were placed on a sterile filter paper in a 9 cm Petri dish and dried for 24 hours at room temperature (Widden and Parkinson, 1973). Dried leaf discs were adhered to 0.2% corn meal agar plates (Nissui, Tokyo, Japan) in 9 mm plastic Petri dishes with four discs per dish and cultured at room temperature.

Observation and identification of fungi

After 3 days, 10 days, 28 days and 42 days from the start of incubation, the Petri dishes were observed using both dissecting and light microscopes, and fungal species were identified directly based on the morphology of their fruit bodies formed on agar plates or leaf discs. Fungi that could not be identified by direct observation were isolated by their spores or mycelia using a sterile elgiloy wire and preserved in slant cultures. These strains were then plated out on LCA plates (Miura and Kudo, 1970; glucose 1.0 g, KH_2PO_4 1.0 g, $MgSO4{\cdot}7H_2O$ 0.2 g, KCl 0.2 g, $NaNO_3$ 2.0 g, yeast extract 0.2 g, agar 13 g, distilled water 1.0 l) to induce their sporulation. When no sporulation of fungi occurred on LCA plates, pieces of sterile *Quercus myrsinaefolia* leaf were added to the plates to induce sporulation. When spores or other propagative structures were formed on LCA plates or added leaf pieces, slides were prepared to observe them under a light microscope for identification. In this study, sterile strains were treated as unidentified fungi and excluded from the fungal species list. Fungal identification was,

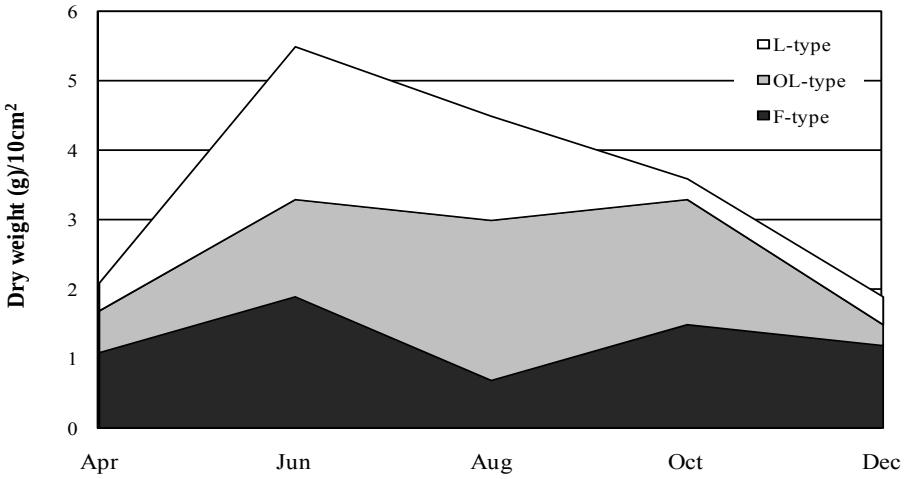

Fig. 2. Seasonal change of leaf litter production of *Q. myrsinaefolia* at the sampling site.

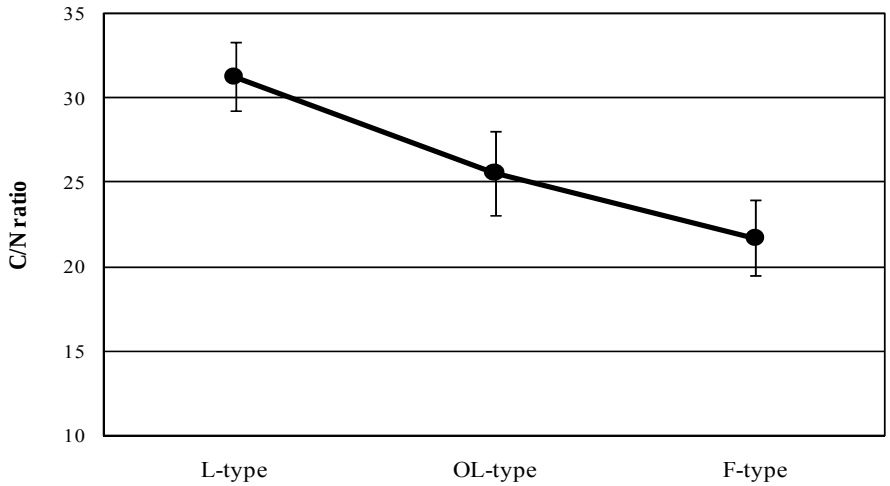

Fig. 3. C/N ratio in fallen leaves at each decomposition stage (error bars are standard deviations).

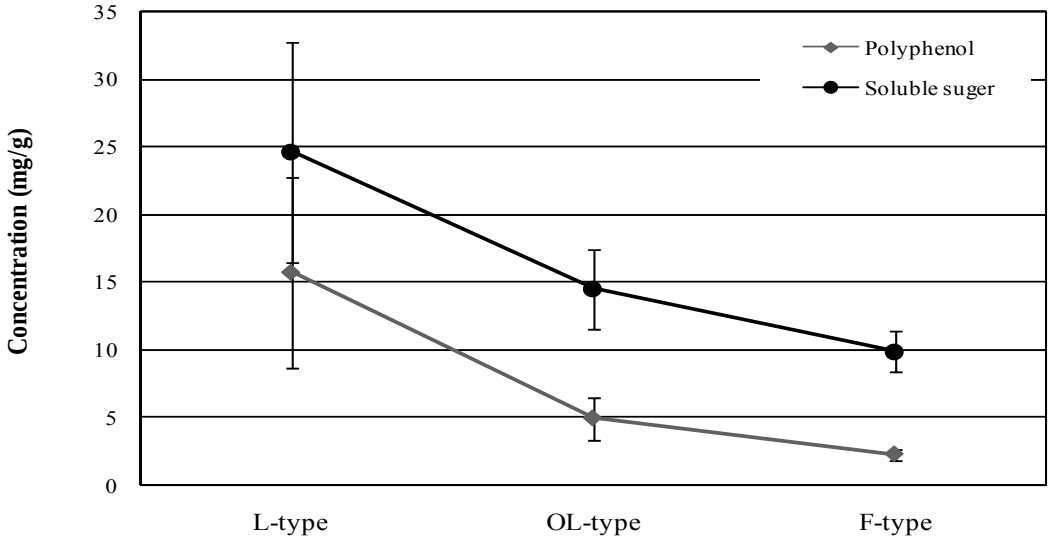

Fig. 4. Polyphenol and soluble sugar concentrations in fallen leaves at each decomposition stage (error bars are standard deviations).

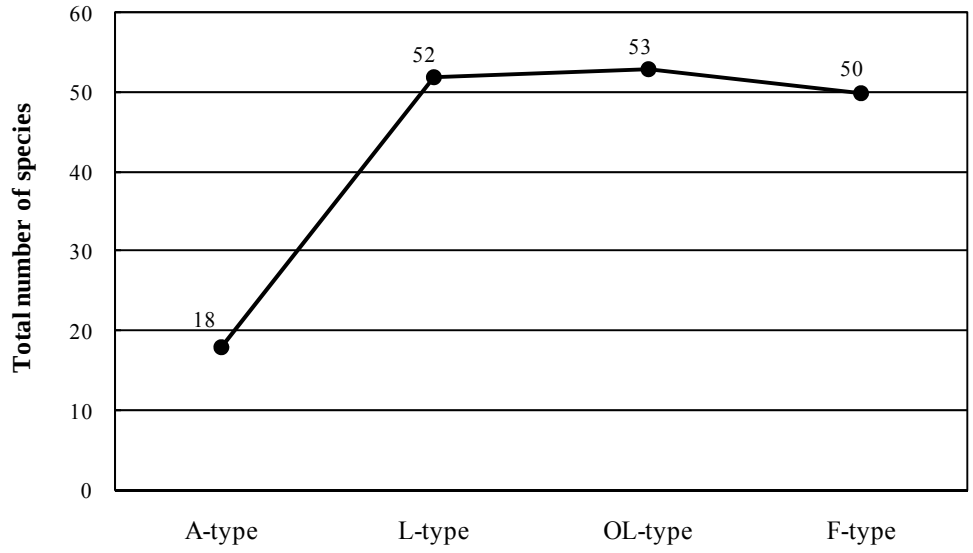

Fig. 5. Total numbers of recorded fungal species for each leaf type.

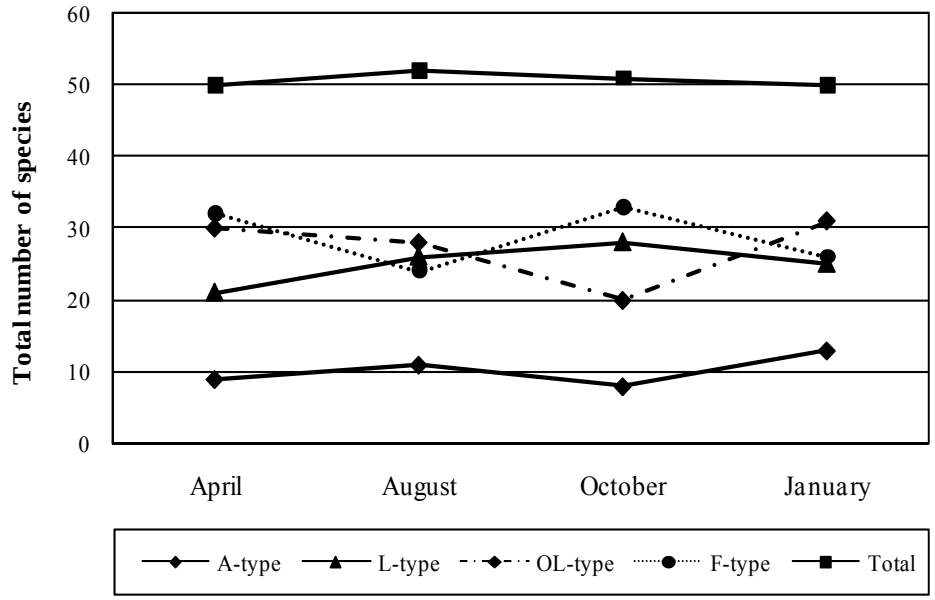

Fig. 6. Seasonal changes in numbers of recorded fungal species for each leaf type.

mainly according to studies by Ellis (1971, 1976), Matsushima (1971, 1975), Udagawa *et al*. (1978a, b), Carmichael *et al*. (1980), Domsch *et al*. (1980a, b), Sutton (1980), Nag Raj (1993), and Kiffer and Morelet (2000).

Data calculation

The frequency of occurrence and seasonality index of each fungus at each decomposition stage, and Sørensen's similarity index (Sørensen, 1948) to examine the similarity of fungal species components between leaf types were calculated according to the following expressions. Fungi which could be identified only at a generic level, such as *Fusarium* spp. and *Penicillium* spp., were calculated as one species.

Frequency of occurrence

Frequency of occurrence (%) = number of discs on which a certain species occurred / total number of discs examined (20) × 100

In this study, fungi occurring at ≥50% frequency were defined as frequent species.

Seasonality index

Seasonality index (%) = number of investigation times in which a certain species occurred / total investigation times (4) × 100

In this study, fungi recorded at 100% seasonality index were defined as constant species.

Sørensen's similarity index

Sørensen's similarity index = 2c / (a + b)

a: number of species occurring in sample A.

b: number of species occurring in sample B.

c: number of species occurring in both samples.

Results

Leaf litter production of Quercus myrsinaefolia

Seasonal change of leaf litter production of *Q. myrsinaefolia* at the sampling site is shown in Fig. 2. The maximum total dry weight of fallen leaves was recorded in June, and the second in August. Although the leaf fall of *Q. myrsinaefolia* was observed throughout the year, the dry weight of L-type leaves increased from June to August and peaked in June.

Chemical property of fallen leaves

The C/N ratio, polyphenol and soluble sugar concentrations in fallen leaves at each decomposition stage are shown in Figs. 3 and 4. These values decreased with the progress of leaf decomposition. Standard deviations of polyphenol and soluble sugar concentrations in L-type leaves were large compared with other leaf types (Fig. 4). Although it is not shown in Fig. 4, especially in December, these values in the L-type (polyphenol: 27.7 mg/g; soluble sugar: 38.8 mg/g) were higher than in other seasons (polyphenol: 10–15.8 mg/g; soluble sugar: 18.4–23 mg/g).

Numbers of species and their seasonal changes

The list of all recorded fungi is shown in Table 1. In this study, 83 species of 57 genera were recorded. Eighteen species of 17 genera were collected from living leaves and 80 species of 55 genera from fallen leaves. In A-type leaves, three fungi were recorded from the A-type only (▲ in Table 1), and the remaining 15 species of 14 genera occurred also on fallen leaves. Seven species were recorded from all leaf types (***** in Table 1), and 22 species were recorded from all fallen leaf type (♦ in Table 1).

The total numbers of species at each leaf type are shown in Fig. 5. Eighteen species of A-type leaves was the smallest for all leaf types. The numbers recorded from other leaf types were almost the same, 50 to 53 species.

Seasonal changes in the number of species recorded from each leaf type are shown in Fig. 6. The total number of species recorded from all leaf types and the number recorded from A-type leaves were relatively stable throughout the year. In L-type leaves, the species number increased gradually toward October. The species number on OL-type leaves peaked in January, with the minimum in October, while on F-type leaves it peaked in October, with the minimum in August.

Similarity indexes of species components between leaf types

The results of calculating Sørensen's similarity index for fungal species components between leaf types are shown in Table 2. Similarity indexes of L-type and OL-type (0.667), OL-type and F-type (0.757), and L-type and F-type (0.588) were higher than other combinations, and the A-type and L-type (0.423) also had a relatively high value.

Distribution patterns of fungal species in all leaf types

To understand the distribution patterns of individual fungi in all leaf types, the average occurrence frequencies of each fungus on every leaf type was calculated (Table 3). In three fungi recorded from the A-type only (▲ in Table 3), *Stenella* sp. showed a high average frequency of occurrence (84%) on A-type leaves. The average frequencies of *Tubakia* sp., *Tripospermum prolongatum* and *Colletotrichum gloeosporioides* were also high in A-type and decreased after leaf-fall.

In seven species collected from all leaf types (***** in Table 3), *Aureobasidium pullulans* and *Phoma* sp. showed high average frequencies of occurrence on L-type leaves and tended to decrease with the progress of leaf decomposition.

Table 1. Presence or absence of all recorded fungi for each leaf type (+ and - are presence and absence, respectively).

Fungi	A-type	L-type	OL-type	F-type
Zygomycetes				
Backusella circina	-	-	+	+
Gongronella butleri	-	-	+	+
Mortierella alpina	-	-	+	-
Mortierella sp.	-	-	-	+
Mucor hiemalis	-	-	+	+
Mucor sp.	-	-	+	-
Umbelopsis isabellina	-	-	-	+
Umbelopsis ramanniana	-	-	-	+
Anamorphic Fungi				
Acremonium sp.1⬩	-	+	+	+
Acremonium sp.2	-	-	+	-
Acremonium sp.3	-	+	-	-
Alternaria alternata★	+	+	+	+
Anungitea fragilis	-	-	+	-
Arachnophora sp.	-	-	-	+
Arthrinium phaeospermum▲	+	-	-	-
Apiospora montagnei	+	+	-	-
Aureobasidium pullulans★	+	+	+	+
Beltrania rhombica⬩	-	+	+	+
Beltraniella portoricensis⬩	-	+	+	+
Blastophorum truncatum	-	-	-	+
Camposporium japonicum⬩	-	+	+	+
Camposporium sp.	-	-	+	+
Centrospora gracilis	-	-	-	+
Chaetopsina fulva⬩	-	+	+	+
Chaetospermum camelliae	-	+	-	-
Chloridium sp.	-	-	+	-
Cladosporium cladosporioides★	+	+	+	+
Cladosporium herbarum⬩	-	+	+	+
Cladosporium tenuissimum⬩	-	+	+	+
Clonostachys compatiuscula	-	+	+	-
Clonostachys rosea⬩	-	+	+	+
Colletotrichum gloeosporioides	+	+	+	-
Cylindrocladium parvum⬩	-	+	+	+
Dactylaria sp.1	+	+	+	-
Dactylaria sp.2	-	+	-	-
Dactylaria spp.⬩	-	+	+	+
Dictyochaeta simplex⬩	-	+	+	+
Diplocladiella scalaroides	-	-	-	+
Discosia artocreas⬩	-	+	+	+
Epicoccum nigrum	+	+	-	-
Fusarium spp.⬩	-	+	+	+
Geotrichum sp.	-	-	+	-

▲Collected from A-type leaves only. ★Collected from all leaf types. ⬩ Collected from all fallen leaf types.

Table 1 (continued). Presence or absence of all recorded fungi for each leaf type (+ and - are presence and absence, respectively).

Fungi	A-type	L-type	OL-type	F-type
Gliocladium viride	-	+	-	-
Gonytrichum macrocladum	-	-	+	+
Hyphomycete sp.1	+	+	+	-
Hyphomycete sp.2	-	-	+	+
Idriella sp.	-	+	+	-
Leptographium sp.	-	+	-	-
Mariannaea elegans	-	-	+	+
Monacrosporium sp.	-	-	+	+
Parasympodiella longispora	-	-	-	+
Penicillifer superimpositus	-	-	-	+
Penicillium spp.★	+	+	+	+
Pestalotiopsis sp.★	+	+	+	+
Phoma sp.★	+	+	+	+
Ramichloridium sp.1	-	+	-	-
Ramichloridium sp.2	+	+	-	-
Rhinocladiella intermedia♦	-	+	+	+
Scolecobasidium cateniphorum♦	-	+	+	+
Scolecobasidium fusiforme	-	+	-	+
Scolecobasidium humicola	-	+	-	-
Scolecobasidium sp.	-	-	+	-
Solosympodiella clavata♦	-	+	+	+
Solosympodiella sp.	-	-	+	-
Sporidesmium sp.	-	-	+	+
Stenella sp.▲	+	-	-	-
Subramaniomyces fusisaprophyticus♦	-	+	+	+
Trichoconis sp.♦	-	+	+	+
Trichoderma aureoviride♦	-	+	+	+
Trichoderma hamatum♦	-	+	+	+
Trichoderma harzianum♦	-	+	+	+
Trichoderma koningii♦	-	+	+	+
Trichoderma viride	-	-	+	+
Trichoderma spp.★	+	+	+	+
Tripospermum acerinum	-	+	-	-
Tripospermum myrti	-	+	-	-
Tripospermum prolongatum	+	+	-	-
Tritirachium bulbophorum▲	+	-	-	-
Tubakia sp.	+	+	-	-
Ulocladium sp.	-	-	-	+
Volutella sp.1	-	+	+	-
Volutella sp.2	-	+	-	-
Volutella sp.3	-	+	-	-

▲Collected from A-type leaves only. ★Collected from all leaf types. ♦ Collected from all fallen leaf types.

In 22 species recorded from all fallen leaf types (♦ in Table 3), *Subramaniomyces fusisaprophyticus*, *Chaetopsina fulva* and *Beltraniella portoricensis* appeared with high average frequencies on L-type leaves and their frequencies fell on more decayed leaves. The average frequencies of *Rhinocladiella intermedia* and *Acremonium* sp.1 increased in OL-type and F-type leaves, and those of *Trichoderma harzianum* and *Fusarium* spp. increased in F-type leaves.

Seasonal change of the fungal species component of each leaf type

To understand the seasonal change of fungal species composition on individual leaf

types, the frequency values of frequent and constant species were compared between the investigated seasons.

Table 2. Sorensen's similarity indexes calculated for fungal species components between each leaf type.

	L-type	OL-type	F-type
A-type	0.423	0.278	0.203
L-type	-	0.667	0.588
OL-type	-	-	0.757

A-type

Seasonal fluctuation of frequency values of fungi occurring on A-type leaves is shown in Table 4. In this leaf type, all frequent species were also constant species. Among these species, *Tubakia* sp., *Colletotrichum gloeosporioides* and *Stenella* sp. appeared at high frequencies of 50% or more in all seasons. *Tripospermum prolongatum* was recorded at high frequency in April (60%), while the frequency was low in other seasons.

L-type

Seasonal fluctuation of frequencies of fungi occurring on L-type leaves is shown in Table 5. In this stage, 9 species were recognized as constant. In these fungi, *Subramaniomyces fusisaprophyticus* was recorded at a high frequency of 50% or more in all seasons other than winter (January). *Rhinocladiella intermedia* was recorded at high frequencies of 50% and 80% in August and October, respectively, while the frequency of these fungi was low in April and January. The frequency of *Phoma* sp. was high in April (55%) and lower in other seasons. *Aureobasidium pullulans* was recorded at a high frequency (55%) in August, but fell in other seasons. The other constant species, *Dictyochaeta simplex*, *Penicillium* spp., *Discosia artocreas*, *Beltrania rhombica* and *Cladosporium cladosporioides* were not recorded at such high frequency (lower than 50%).

Besides constant species, *Chaetopsina fulva* was recorded at high frequencies in August (70%) and October (65%) but fell dramatically in January (5%) and disappeared in April. *Tubakia* sp. was recorded at a high frequency (60%) in January, and *Beltraniella portoricensis* occurred at a rather high frequency of 55% in October. *Chaetospermum camelliae* appeared only in August at a frequency of 55%.

OL-type

Seasonal fluctuation of the frequencies of fungi collected from OL-type leaves is shown in Table 6. In this stage, 7 species were recorded as constant. *Rhinocladiella intermedia* occurred at a high frequency of 55% or more in all seasons. *Subramaniomyces fusisaprophyticus* was frequently recorded in August (60%) but fell in other seasons. *Acremonium* sp.1 was frequently recorded in October (55%). The frequencies of other constant species, *Chaetopsina fulva*, *Phoma* sp., *Discosia artocreas* and *Beltrania rhombica*, were comparatively low in all seasons.

Excluding constant species, *Trichoderma harzianum* was recorded frequently in August (55%) and January (75%). *Fusarium* spp. occurred at a high frequency in January (65%), and *Trichoderma koningii* frequently appeared in October (80%).

F-type

Seasonal fluctuation of frequencies of fungi collected from F-type leaves is shown in Table 7. In this decomposition stage, 10 species were recorded as constant. In these species, *Rhinocladiella intermedia* was recorded at a high frequency of 60% or more in all seasons. *Trichoderma harzianum* was occurred frequently in April (85%) and January (75%), and *Fusarium* spp. was recorded at 55% in April, October and January. *Subramani-omyces fusisaprophyticus* and *Trichoderma koningii* appeared at a high frequency of 55% in August. *Alternaria alternata* and, *Acremo-nium* sp.1, which is not a constant species, was recorded at 50% in April.

Discussion

Falling leaf seasons and decomposition processes of fallen leaves

Although the leaf fall of *Quercus*

Table 3. Average frequencies of occurrence of fungi for each leaf type (- indicates absence).

Fungi	A-type	L-type	OL-type	F-type
Stenella sp.▲	84	-	-	-
Arthrinium phaeospermum▲	4	-	-	-
Tritirachium bulbophorum▲	1	-	-	-
Tubakia sp.	89	31	-	-
Tripospermum prolongatum	30	10	-	-
Ramichloridium sp.2	3	3	-	-
Apiospora montagnei	1	1	-	-
Epicoccum nigrum	1	1	-	-
Colletotrichum gloeosporioides	84	13	3	-
Dactylaria sp.1	4	4	1	-
Hyphomycete sp.1	1	8	1	-
Phoma sp.★	13	33	26	20
Pestalotiopsis sp.★	10	8	5	5
Alternaria alternata★	6	10	8	19
Penicillium spp.★	4	19	14	24
Trichoderma spp.★	4	18	28	9
Cladosporium cladosporioides★	3	14	6	3
Aureobasidium pullulans★	1	26	16	8
Chaetospermum camelliae	-	14	-	-
Dactylaria sp.2	-	3	-	-
Leptographium sp.	-	3	-	-
Scolecobasidium humicola	-	3	-	-
Acremonium sp.3	-	1	-	-
Gliocladium viride	-	1	-	-
Ramichloridium sp.1	-	1	-	-
Tripospermum acerinum	-	1	-	-
Tripospermum myrti	-	1	-	-
Volutella sp.2	-	1	-	-
Volutella sp.3	-	1	-	-
Clonostachys compatiuscula	-	4	1	-
Idriella sp.	-	4	8	-
Volutella sp.1	-	3	1	-
Subramaniomyces fusisaprophyticus⬩	-	48	41	40
Rhinocladiella intermedia⬩	-	44	74	70
Chaetopsina fulva⬩	-	35	30	5
Beltraniella portoricensis⬩	-	24	4	1
Dictyochaeta simplex⬩	-	21	6	8
Discosia artocreas⬩	-	16	24	5
Beltrania rhombica⬩	-	15	8	1
Trichoderma harzianum⬩	-	14	39	56
Dactylaria spp.⬩	-	11	21	20
Trichoderma koningii⬩	-	9	29	21
Fusarium spp.⬩	-	6	29	50
Acremonium sp.1⬩	-	5	30	21
Trichoderma aureoviride⬩	-	5	6	5
Cladosporium herbarum⬩	-	4	5	8

▲Collected from A-type leaves only. ★Collected from all leaf types. ⬩ Collected from all fallen leaf types.

Table 3 (continued). Average frequencies of occurrence of fungi for each leaf type (- indicates absence).

Fungi	A-type	L-type	OL-type	F-type
Clonostachys rosea◆	-	4	6	19
Cylindrocladium parvum◆	-	4	1	5
Trichoderma hamatum◆	-	3	11	11
Camposporium japonicum◆	-	1	4	13
Cladosporium tenuissimum◆	-	1	10	4
Scolecobasidium cateniphorum◆	-	1	8	4
Solosympodiella clavata◆	-	1	4	9
Trichoconis sp.◆	-	1	11	5
Scolecobasidium fusiforme	-	1	-	5
Geotrichum sp.	-	-	6	-
Scolecobasidium sp.	-	-	4	-
Acremonium sp.2	-	-	1	-
Anungitea fragilis	-	-	1	-
Chloridium sp.	-	-	1	-
Mortierella alpina	-	-	1	-
Mucor sp.	-	-	1	-
Solosympodiella sp.	-	-	1	-
Trichoderma viride	-	-	8	18
Backusella circina	-	-	5	6
Camposporium sp.	-	-	4	3
Hyphomycete sp.2	-	-	4	3
Gongronella butleri	-	-	3	3
Mariannaea elegans	-	-	3	1
Mucor hiemalis	-	-	3	8
Gonytrichum macrocladum	-	-	1	3
Monaclosporium sp.	-	-	1	9
Sporidesmium sp.	-	-	1	5
Arachnophora sp.	-	-	-	6
Blastophorum truncatum	-	-	-	5
Centrospora gracilis	-	-	-	3
Diplocladiella scalaroides	-	-	-	3
Parasympodiella longispora	-	-	-	3
Umbelopsis ramanniana	-	-	-	3
Mortierella sp.	-	-	-	1
Penicillifer superimpositus	-	-	-	1
Ulocladium sp.	-	-	-	1
Umbelopsis isabellina	-	-	-	1

◆ Collected from all fallen leaf types.

myrsinaefolia was observed throughout the year, fallen leaves were abundant from June to August (Fig. 2). In Fig. 2, the main fallen leaf season of this evergreen oak is probably around June in the forest, because the dry weight of L-type leaves in this period was heaviest. This result almost agrees with other studies on litter production of *Q. myrsinaefolia* (Nagao and Harada, 1996; Kuramoto, 1997).

The leaves in the maximum falling leaf period appeared to decay at almost the same speed. The decay stage of these leaves at a certain sampling time can be assessed from the fluctuation of the dry weight of more decomposed leaf types; therefore, we estimate that L-type leaves that fell in June decomposed to the OL-type stage in August, the F-type in October, and the F-type or more decomposed to the OL-type stage in August, the F-type in October, and the F-type or more decomposed stages in December (Fig. 2). L-type leaves that fell in August, a later leaf-fall season, might decompose to the OL-type

Table 4. Seasonal changes in frequencies of occurrence, average frequencies and seasonal indexes of fungi occurring from A-type leaves (- indicates absence).

Fungi	Apr	Aug	Oct	Jan	Average	Seasonal index
Tubakia sp.	100	100	100	55	89	100
Colletotrichum gloeosporioides	85	95	95	60	84	100
Stenella sp.	80	95	90	70	84	100
Tripospermum prolongatum	60	30	10	20	30	100
Phoma sp.	10	25	-	15	13	75
Pestalotiopsis sp.	-	30	5	5	10	75
Penicillium sp.	-	5	5	5	4	75
Alternaria alternata	15	10	-	-	6	50
Dactylaria sp.1	5	-	-	10	4	50
Cladosporium cladosporioides	5	-	-	5	3	50
Ramichloridium sp.2	-	-	5	5	3	50
Trichoderma sp.	15	-	-	-	4	25
Arthrinium phaeospermum	-	-	15	-	4	25
Apiospora montagnei	-	5	-	-	1	25
Aureobasidium pullulans	-	5	-	-	1	25
Epicoccum nigrum	-	-	-	5	1	25
Hyphomycete sp.1	-	-	-	5	1	25
Tritirachium bulbophorum	-	-	-	5	1	25

stage in October and the F-type in December (Fig. 2).

Chemical components of each leaf type

The C/N ratio, polyphenol and soluble sugar concentration in the fallen leaves decreased with progress of leaf decomposition (Figs 3, 4). This change of chemical components is generally seen during the decomposition process of fallen leaves (Berg and McClaugherty, 2003). These results therefore support the validity of the leaf decomposition stages classified in this study.

Successional patterns of fungal species with the decay of leaves

Comparison of typical fungi characterized by each leaf type

In earlier studies, fungal succession with the decay of fallen leaves has been presumed by comparing the species components of typical fungi characterizing each decomposition stage (Kendrick and Burges, 1962; Aoki *et al*., 1990, 1992; Heredia, 1993; Tokumasu *et al*., 1994; Tokumasu, 1996). In this study, frequent and constant species were selected as typical fungi in each leaf type and fungal successsion with the decay of leaves was estimated by comparing these fungi.

The list of frequent and constant species is shown in Table 8. The frequent species in A-type leaves, *Stenella* sp., *Tubakia* sp., *Tripospermum prolongatum* and *Colletotrichum gloeosporioides*, were recorded constantly from only A-type leaves (Table 8). In particular, *Stenella* sp. did not occur on other type leaves (Table 1). On the other hand, two frequent and constant species in all fallen leaves types, *Rhinocladiella intermedia* and *Subramaniomyces fusisaprophyticus*, frequent species in the L-type such as *Beltraniella portoricensis*, *Chaetopsina fulva* and *Chaetospermum camelliae*, and frequent species in the OL-type and F-type, such as *Trichoderma harzianum* and *T. koningii*, were not recorded from A-type leaves (Table 1, 8). These results suggested the conspicuous difference of fungal species components between living leaves on the tree and fallen leaves on the ground. Indexes of similarity according to Sørensen also showed that the species composition of A-type leaves was characteristic compared with leaf types on the ground (Table 2). In contrast,

Table 5. Seasonal changes in frequencies of occurrence, average frequencies and seasonal indexes of fungi occurring from L-type leaves (- indicates absence).

Fungi	Apr	Aug	Oct	Jan	Average	Seasonal index
Subramaniomyces fusisaprophyticus	50	60	50	30	48	100
Rhinocladiella intermedia	25	50	80	20	44	100
Phoma sp.	55	30	25	20	33	100
Aureobasidium pullulans	15	55	20	15	26	100
Dictyochaeta simplex	20	35	10	20	21	100
Penicillium spp.	5	25	25	20	19	100
Discosia artocreas	40	5	10	10	16	100
Beltrania rhombica	5	20	25	10	15	100
Cladosporium cladosporioides	10	10	25	10	14	100
Chaetopsina fulva	-	70	65	5	35	75
Tubakia sp.	35	30	-	60	31	75
Beltraniella portoricensis	-	30	55	10	24	75
Dactylaria spp.	-	30	10	5	11	75
Alternaria alternata	20	10	10	-	10	75
Fusarium spp.	5	15	5	-	6	75
Trichoderma spp.	-	-	35	35	18	50
Trichoderma harzianum	25	-	30	-	14	50
Colletotrichum gloeosporioides	-	30	-	20	13	50
Tripospermum prolongatum	5	-	-	35	10	50
Pestalotiopsis sp.	-	-	20	10	8	50
Trichoderma aureoviride	5	15	-	-	5	50
Cladosporium herbarum	5	-	10	-	4	50
Cylindrocladium parvum	-	-	5	10	4	50
Scolecobasidium humicola	-	5	5	-	3	50
Chaetospermum camelliae	-	55	-	-	14	25
Hyphomycete sp.1	-	-	-	30	8	25
Trichoderma koningii	-	-	35	-	9	25
Acremonium sp.1	-	-	20	-	5	25
Dactylaria sp.1	15	-	-	-	4	25
Clonostachys rosea	-	15	-	-	4	25
Idriella sp.	-	15	-	-	4	25
Clonostachys compatiuscula	-	-	15	-	4	25
Volutella sp.1	-	10	-	-	3	25
Leptographium sp.	-	-	10	-	3	25
Dactylaria sp.2	-	-	-	10	3	25
Ramichloridium sp.2	-	-	-	10	3	25
Trichoderma hamatum	-	-	10	-	3	25
Acremonium sp.3	5	-	-	-	1	25
Apiospora montagnei	5	-	-	-	1	25
Camposporium japonicum	5	-	-	-	1	25
Ramichloridium sp.1	5	-	-	-	1	25
Scolecobasidium fusiforme	-	5	-	-	1	25
Solosympodiella clavata	-	5	-	-	1	25
Volutella sp.2	-	5	-	-	1	25
Volutella sp.3	-	5	-	-	1	25
Cladosporium tenuissimum	-	-	5	-	1	25
Epicoccum nigrum	-	-	5	-	1	25
Scolecobasidium cateniphorum	-	-	5	-	1	25
Trichoconis sp.	-	-	-	5	1	25
Tripospermum acerinum	-	-	-	5	1	25
Tripospermum myrti	-	-	-	5	1	25
Gliocladium viride	-	-	-	5	1	25

they indicated that species composition was similar among leaf types found on the ground. Moreover, the species composition of a certain decomposition stage was most similar to that of the next decomposition stage. All frequent species found on the ground, except *Chaetospermum camelliae*, were recorded from all fallen leaf types (Tables 1, 8). For example, *Subramaniomyces fusisaprophyticus* and *Rhinocladiella intermedia* appeared as frequent and constant species from all fallen leaf types (Table 8). These results suggest no remarkable differences among fallen leaf types in the composition of frequent and constant species; however, some fungi showed a tendency to occur with a rather higher frequency value in early decomposition stages such as *Aureobasidium pullulans*, *Phoma* sp., *Chaetospermum camelliae*, *Chaetopsina fulva* and *Beltraniella portoricensis*, and some showed an opposite tendency, *viz Acremonium* sp.1, *Fusarium* spp., *Trichoderma harzianum* and *T. koningii* (Table 8), suggesting the species alternation of fungi on fallen leaves of this oak. An outline of fungal succession associated with the decay of *Quercus myrsinaefolia* leaves was estimated as follows. Fungal colonizing leaves change from living leaf inhabitants, such as *Stenella* sp., *Tubakia* sp., *Tripospermum prolongatum* and *Colletotrichum gloeosporioides*, through early fallen leaf colonizers, such as *Chaetospermum camelliae*, *Chaetopsina fulva* and *Phoma* sp., to later colonizers, such as *Fusarium* spp. and *Trichoderma* spp.

Ecological features of frequent species

In four frequent species colonizing living leaves, *Stenella* sp. was a frequent and constant species on A-type leaves, and was not recorded from leaves on the ground (Tables 1, 8). *Tubakia* sp. has been isolated from living and fallen leaves of *Quercus* and *Castanopsis* (Yokoyama and Tsubaki, 1971; as *Actinopelte*)**.**

In this study, it also occurred on living and fallen leaves at an early decomposition stage (Table 1). *Tripospermum prolongatum* is known as a phylloplane fungus often seen on leaves infected with sooty mold, and *Colletotrichum gloeosporioides* is known as a multiple-host pathogen of various plants (Sato, 1996). These four species are probably phyllosphere fungi invading green leaves on the tree. According to research on *Pinus densiflora* (Tokumasu, 1996), *Abies firma* (Aoki *et al*., 1990) and *Fagus crenata* (Osono, 2002), *Phoma* spp. has been mainly reported from living and fallen leaves at early decomposition stages. Our results showed the same tendency (Tables 3, 8). *Phoma* sp. might be a phyllosphere fungus colonizing green leaves on the tree. *Aureobasidium pullulans* is known as a common primary saprophytes recorded from senescent leaves on trees and fallen leaves at an early decomposition stage (Hudson, 1968). In this study, *A. pullulans* also appeared from living and L-type leaves at high frequency and also declined with the progression of leaf decay (Tables 3, 8).

The five species mentioned below have ecological features as they quickly infect new fallen leaves. *Beltraniella portoricensis* infected newly fallen leaves and its frequency of occurrence decreased with decay (Table 3). According to Aoki *et al*. (1990) and Tokumasu (1996, 1998a), *Chaetopsina fulva* is a fungus that quickly infected freshly fallen leaves of *Pinus densiflora* and *Abies firma* in summer and autumn, and its frequency of occurrence declined sharply with leaf decomposition. Our results also showed that this fungus is an early fallen leaf colonizer in summer and autumn (Tables 3, 5). *Chaetospermum camelliae* showed a distribution pattern similar to *Chaetopsina fulva*, but occurred only in summer (Tables 3, 5). *Subramaniomyces fusisaprophyticus* was mainly found on fallen leaves in the early decomposition stage at high frequency, and then declined slowly (Table 3). *Rhinocladiella intermedia* quickly colonized freshly fallen leaves and the frequency of occurrence increased gradually as leaf decay progressed (Table 3).

Acremonium sp. 1 first occurred on freshly fallen leaves and the frequency increased in OL-type leaves (Table 3). *Trichoderma* spp. has been recorded frequently on well-decomposed leaves in other studies (Kendrick and Burges, 1962; Domsch *et al*., 1980a, b; Aoki *et al*., 1990; Tokumasu, 1996; Osono, 2002; Sadaka and Ponge, 2003). In this study, *T. harzianum* and *T. koningii* also occurred frequently and became constant species on F-type leaves (Table 8). The

Table 6. Seasonal changes in frequencies of occurrence, average frequencies and seasonal indexes of fungi occurring from OL-type leaves (- indicates absence).

Fungi	Apr	Aug	Oct	Jan	Average	Seasonal index
Rhinocladiella intermedia	55	75	100	65	74	100
Subramaniomyces fusisaprophyticus	30	60	30	45	41	100
Acremonium sp.1	5	20	55	40	30	100
Chaetopsina fulva	35	15	25	45	30	100
Phoma sp.	40	10	25	30	26	100
Discosia artocreas	20	40	20	15	24	100
Beltrania rhombica	5	5	15	5	8	100
Trichoderma harzianum	-	55	25	75	39	75
Fusarium spp.	35	-	15	65	29	75
Trichoderma koningii	-	25	80	10	29	75
Trichoderma spp.	45	-	30	35	28	75
Dactylaria spp.	15	45	-	25	21	75
Penicillium spp.	-	15	20	20	14	75
Trichoconis sp.	10	15	20	-	11	75
Scolecobasidium cateniphorum	10	10	-	10	8	75
Cladosporium cladosporioides	5	-	15	5	6	75
Dictyochaeta simplex	10	10	-	5	6	75
Aureobasidium pullulans	25	-	-	40	16	50
Trichoderma hamatum	-	25	20	-	11	50
Cladosporium tenuissimum	15	-	-	25	10	50
Alternaria alternata	15	-	-	15	8	50
Trichoderma viride	-	25	5	-	8	50
Idriella sp.	-	10	-	20	8	50
Clonostachys rosea	-	5	-	20	6	50
Pestalotiopsis sp.	10	-	10	-	5	50
Backusella circina	-	-	15	5	5	50
Camposporium japonicum	10	-	5	-	4	50
Camposporium sp.	5	10	-	-	4	50
Hyphomycete sp.2	5	10	-	-	4	50
Scolecobasidium sp.	-	5	-	10	4	50
Solosympodiella clavata	-	5	-	10	4	50
Colletotrichum gloeosporioides	5	-	-	5	3	50
Trichoderma aureoviride	25	-	-	-	6	25
Geotrichum sp.	-	-	25	-	6	25
Cladosporium herbarum	20	-	-	-	5	25
Beltraniella portoricensis	-	-	-	15	4	25
Gongronella butleri	-	10	-	-	3	25
Mariannaea elegans	-	10	-	-	3	25
Mucor hiemalis	-	-	-	10	3	25
Acremonium sp.2	-	5	-	-	1	25
Anungitea fragilis	5	-	-	-	1	25
Chloridium sp.	5	-	-	-	1	25
Clonostachys compatiuscula	5	-	-	-	1	25
Dactylaria sp.1	5	-	-	-	1	25

Table 6 (continued). Seasonal changes in frequencies of occurrence, average frequencies and seasonal indexes of fungi occurring from OL-type leaves (- indicates absence).

Fungi	Apr	Aug	Oct	Jan	Average	Seasonal index
Hyphomycete sp.1	5	-	-	-	1	25
Solosympodiella sp.	5	-	-	-	1	25
Gonytrichum macrocladum	-	5	-	-	1	25
Sporidesmium sp.	-	5	-	-	1	25
Volutella sp.1	-	5	-	-	1	25
Cylindrocladium parvum	-	-	-	5	1	25
Monacrosporium sp.	-	-	-	5	1	25
Mortierella alpina	-	-	-	5	1	25
Mucor sp.	-	-	-	5	1	25

frequencies of these *Trichoderma* species fluctuated every season (Tables 5, 6, 7). Because these fungi have rapid growth rates and various temperature preferences (Widden and Hsu, 1987), their growth rate and activity are influenced by temperature and humidity just before sampling time.

Alternaria alternata is a common primary saprophyte invading senescent leaves on trees or fallen leaves at early decomposition stages (Hudson, 1968); however, in our results, the frequency of this fungus increased in F-type leaves. Namely, the tendency of appearance was different from other studies (Table 3). The appearance directionality of this fungus was not clear (Table 3), so it is not easy to interpret the ecological features only from the data obtained in this study.

Estimation of fungal succession on Quercus myrsinaefolia leaves

The fungal succession illustrated in Fig. 7 was estimated from comparing the appearance and disappearance patterns of frequently occurring fungi with leaf decay. On leaves with phyllosphere fungi on trees, fungi such as *Tubakia* sp. and *Colletotrichum gloeosporioides* change to early colonizers of fallen leaves on the ground, such as *Subramaniomyces fusisaprophyticus* and *Rhinocladiella intermedia*, and then progress to later colonizers, such as *Trichoderma koningii* and *T. harzianum*.

Tokumasu (1998a,b) reported the seasonal change of fungi on decaying pine needles. Similarly, on fallen leaves of *Quercus myrsinaefolia*, *Chaetospermum cameliae* and *Chaetopsina fulva* are involved in fungal succession only in limited seasons of summer and autumn (Table 5, Fig. 7). The seasonal fluctuation of chemical components in fallen leaves, for example, increased polyphenol and soluble sugar in December, might be a factor in the seasonal alternation of members of the fungal community (see Results).

Furthermore, in this research, we tried to estimate fungal succession by tracing frequent species on leaves fallen in the main leaf-fall seasons throughout a whole year. Leaves of *Quercus myrsinaefolia* fell in August, later in the main leaf-fall season, were decomposed to the OL-type stage in October and reached the F-type stage in January (Fig. 2). The frequent species on each leaf type in each season are shown in Table 9. Fungal succession associated with the decomposition of fallen leaves in this period characterizes the whole succession pattern at this study site (Fig. 7, Table 9).

Characteristics of the fungal community inhabiting Quercus myrsinaefolia *leaves*

Although fungal succession accompanying the decay of *Q. myrsinaefolia* leaves was estimated as above, it is difficult to compare fungal succession directly with that on other tree leaves, especially conifers, firstly because differences in the structure of the O horizon may prevent a comparison. Although the leaf-fall of *Pinus densiflora* is seen throughout the year, the decomposition rate of *P. densiflora* leaf litter is usually very slow on mor or moder sites where a thick O horizon is formed and the horizon divides into sub-layers corresponding to different decomposition stages. Species

Table 7. Seasonal changes in frequencies of occurrence, average frequencies and seasonal indexes of fungi occurring from F-type leaves (- indicates absence).

Fungi	Apr	Aug	Oct	Jan	Average	Seasonal index
Rhinocladiella intermedia	85	65	70	60	70	100
Trichoderma harzianum	85	45	20	75	56	100
Fusarium spp.	55	35	55	55	50	100
Subramaniomyces fusisaprophyticus	40	55	45	20	40	100
Penicillium spp.	5	30	45	15	24	100
Trichoderma koningii	5	55	15	10	21	100
Dactylaria spp.	45	15	15	5	20	100
Alternaria alternata	50	10	10	5	19	100
Clonostachys rosea	5	30	25	15	19	100
Camposporium japonicum	15	20	10	5	13	100
Acremonium sp.1	50	-	30	5	21	75
Phoma sp.	45	-	25	10	20	75
Trichoderma hamatum	5	10	30	-	11	75
Solosympodiella clavata	20	-	5	10	9	75
Dictyochaeta simplex	5	-	15	10	8	75
Arachnophora sp.	10	10	5	-	6	75
Backusella circina	5	5	15	-	6	75
Chaetopsina fulva	10	5	5	-	5	75
Discosia artocreas	5	-	10	5	5	75
Trichoconis sp.	5	-	5	10	5	75
Trichoderma viride	-	30	40	-	18	50
Monaclosporium sp.	-	-	20	15	9	50
Cladosporium herbarum	10	-	20	-	8	50
Aureobasidium pullulans	5	-	-	25	8	50
Mucor hiemalis	-	25	-	5	8	50
Pestalotiopsis sp.	5	-	15	-	5	50
Scolecobasidium fusiforme	15	-	5	-	5	50
Sporidesmium sp.	-	5	15	-	5	50
Cylindrocladium parvum	-	5	-	15	5	50
Cladosporium tenuissimum	10	-	-	5	4	50
Scolecobasidium cateniphorum	10	-	-	5	4	50
Gongronella butleri	5	5	-	-	3	50
Diplocladiella scalaroides	5	-	-	5	3	50
Centrospora gracilis	-	5	5	-	3	50
Hyphomycete sp.2	-	5	5	-	3	50
Trichoderma spp.	-	-	35	-	9	25
Blastophorum truncatum	-	-	20	-	5	25
Trichoderma aureoviride	-	-	-	20	5	25
Camposporium sp.	-	-	10	-	3	25
Cladosporium cladosporioides	10	-	-	-	3	25
Umbelopsis ramanniana	-	10	-	-	3	25
Gonytrichum mactocladum	-	-	10	-	3	25
Parasympodiella longispora	-	-	-	10	3	25
Beltraniella portoricensis	5	-	-	-	1	25
Ulocladium sp.	5	-	-	-	1	25
Umbelopsis isabellina	5	-	-	-	1	25
Beltrania rhombica	-	5	-	-	1	25
Mariannaea elegans	-	5	-	-	1	25
Penicillifer superimpositus	-	-	5	-	1	25
Mortierella sp.	-	-	-	5	1	25

components on individual needles are highly homogeneous in each layer and clearly different from other layers (Tokumasu, 1980); therefore, we were able to estimate fungal succession from the differences of species composition among sub-layers. However, the O horizon under the *Quercus myrsinaefolia* forest was thin because of the fast progress of leaf decomposition. As a result, the development of sub-layers corresponding to each decomposition stage was very poor and distinction of the decay stages of individual leaves was often very difficult. As another reason, because leaves of *Quercus* have a large area compared with conifer needles, one *Quercus* leaf recognized as one decomposition stage may be composed of various parts with miscellaneous decay conditions.

As a result of comparing with earlier studies using other tree species, there are some differences in the member of frequently recorded fungi. For example, *Cladosporium cladosporioides*, which was recorded frequently from fallen leaves of *Pinus densiflora*, *Abies firma* and *Fagus crenata* (Aoki *et al*., 1990; Tokumasu, 1996; Osono, 2002), occurred only infrequently on *Quercus myrsinaefolia* leaves (Tables 5, 6, 7). In contrast, the following species have not been recorded frequently from other tree species, *viz Tubakia*, *Subramaniomyces fusisaprophyticus* and *Rhinocladiella intermedia*, which appeared on *Quercus myrsinaefolia* leaves at high frequentcies (Tables 5, 6, 7). One primary factor of this phenomenon may be the host specificity or selectivity of individual species of fungi. *Subramaniomyces fusisaprophyticus* has been reported as a fallen leaf colonizer of broadleaf-evergreen trees, especially oak trees (Matsushima, 1975; Kirk, 1982, 1983; Ellis and Ellis, 1985; Cooper, 2005). On the other hand, a saprotrophic pine leaf litter fungus has been reported, the distribution pattern of which is strongly affected by climatic factors such as annual mean air temperature or annual range (Tokumasu, 2001). The fungal species component colonizing on decaying *Quercus*

Table 8. Frequent and constant species for each leaf type.

A-type		
Colletotrichum gloeosporioides		
***Stenella* sp.**		
Tripospermum prolongatum		
***Tubakia* sp.**		
L-type		
Aureobasidium pullulans	*Beltraniella portoricensis*⬩	*Beltrania rhombica*▲
***Phoma* sp.**	*Chaetospermum camelliae*⬩	*Cladosporium cladosporioides*▲
Rhinocladiella intermedia	*Chaetopsina fulva*⬩	*Dictyochaeta simplex*▲
Subramaniomyces fusisaprophyticus	*Tubakia* sp.⬩	*Discosia artocreas*▲
		Penicillium spp.▲
OL-type		
***Acremonium* sp.1**	*Fusarium* spp.⬩	*Beltrania rhombica*▲
Rhinocladiella intermedia	*Trichoderma harzianum*⬩	*Chaetopsina fulva*▲
Subramaniomyces fusisaprophyticus	*Trichoderma koningii*⬩	*Discosia artocreas*▲
		Phoma sp.▲
F-type		
Alternaria alternata	*Acremonium* sp.1⬩	*Camposporium japonicum*▲
***Fusarium* spp.**		*Clonostachys rosea*▲
Rhinocladiella intermedia		*Dactylaria* spp.▲
Subramaniomyces fusisaprophyticus		*Penicillium* spp.▲
Trichoderma harzianum		
Trichoderma koningii		

bold type are frequent and constant species at the same time. ⬩ frequent species. ▲constant species.

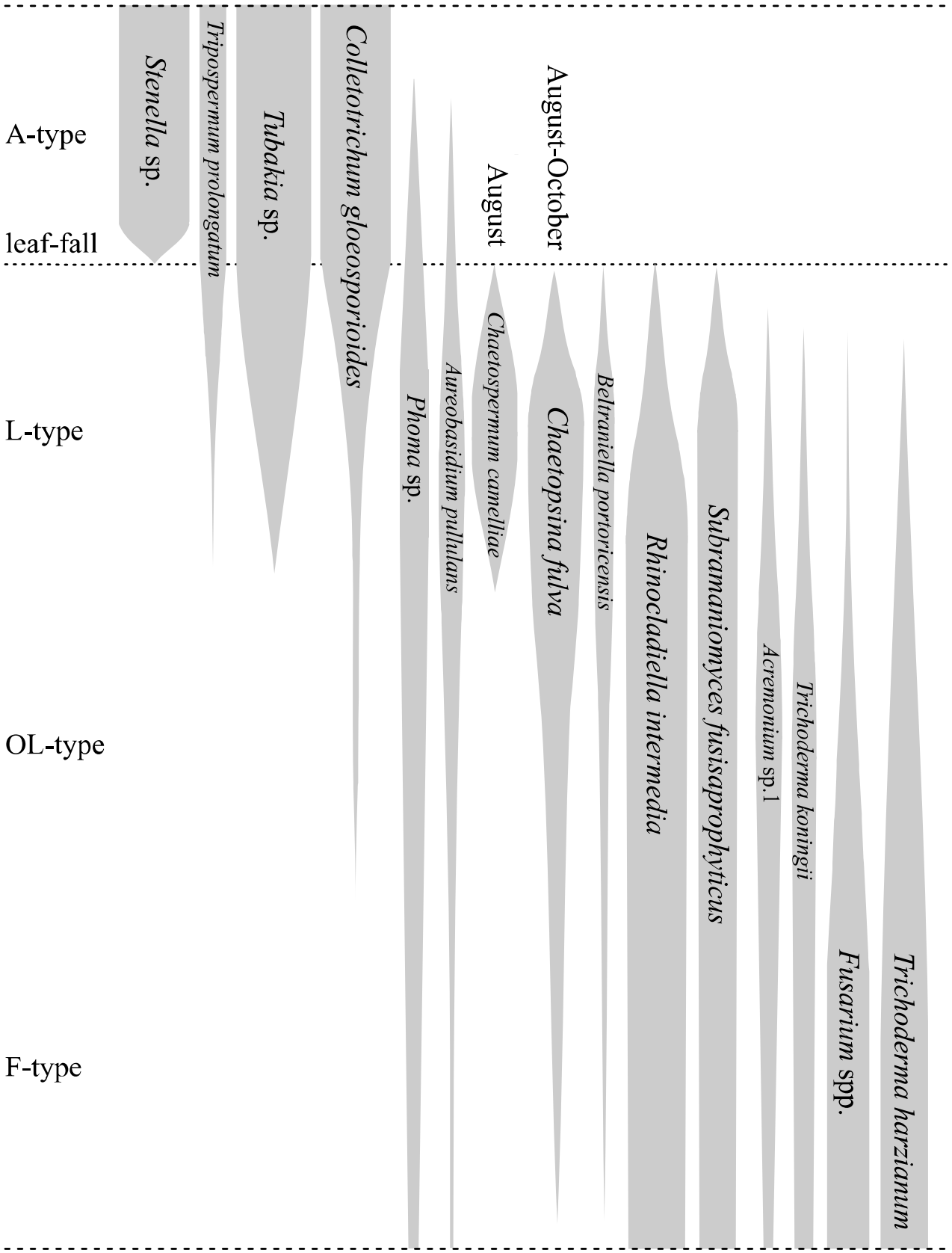

Fig. 7. Appearance and disappearance pattern of frequently occurred fungi with the decay of *Q. myrsinaefolia* leaves**.**

Table 9. Tracing the succession of frequent fungi with decomposition of fallen leaves in August (figures after fungal names are frequency of each fungus).

A-type in April	L-type in August	OL-type in October	F-type in January
Tubakia sp. (100)	*Tubakia* sp. (30)		
Colletotrichum gloeosporioides (85)	*C. gloeosporioides* (30)		
Stenella sp. (80)			
Tripospermum prolongatum (60)			
	Chaetopsina fulva (70)	*C. fulva* (25)	
	Subramaniomyces fusisaprophyticus (60)	*S. fusisaprophyticus* (30)	*S. fusisaprophyticus* (20)
	Aureobasidium pullulans (55)		*A. pullulans* (25)
	Chaetospermum camelliae (55)		
	Rhinocladiella intermedia (50)	*R. intermedia* (100)	*R. intermedia* (60)
		Trichoderma koningii (80)	*T. koningii* (10)
		Acremonium sp.1 (55)	*Acremonium* sp.1 (5)
		Trichoderma harzianum (25)	*T. harzianum* (75)
	Fusarium spp. (15)	*Fusarium* spp. (15)	*Fusarium* spp. (55)

myrsinaefolia leaves is probably decided by both the host specificity/selectivity of individual species of fungi and the climate in the study area.

Future work

In this study, fungi from leaves were isolated using traditional methodology which incorporated media. It is therefore unlikely that all of the fungi involved in leaf decay would have been detected (Hyde and Soytong, 2007). The origin of fungal saprobes on *Castanopsis diversifolia* leaf litter has discussed by Duong *et al*. (2008) that it may derive from aerially dispersed spores or ground soil. In addition, endophytes present within living leaves may participate in the decomposition process of fallen leaves (Osono, 2003; Hyde and Soytong, 2008). However, an entire fungal species component inhabiting decaying leaves has not yet been established. In future it might be wise to incorporate molecular techniques (e.g. DGGE, T-RFLP, construction of clone libraries) to detect more taxa (Duong *et al*., 2006; Seena *et al*., 2008). The fungi occurring on decaying leaves have also been shown to differ from those occurring on wood (Kodsueb *et al*., 2008a,b; Küffer *et al*., 2008) and it would be interesting to establish if this is true for Japanese broadleaf trees.

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