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Short Communication

Factors affecting microfungal diversity

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Species diversity of microfungal communities in decaying oak leaves was analyzed based on the hyphal growth rates of the component species. Dominant species of a low-diversity community had faster hyphal growth rates, while dominant species of a high-diversity community had slower growth rates. These results implies that when the faster growing species became dominant, these species exhausted resources rapidly during the initial stage of fungal colonization. This exclusive utilization of resources made it impossible for other species to invade the substratum and led to a low diversity. Hyphal growth rates played an important role in determining the diversity of microfungal communities.

Key Words——decaying oak leaf; hyphal growth rate; microfungal community; species diversity.

Species diversity is an important ecological characteristic of biotic communities. Therefore, many researchers have extensively studied the diversity of various communities. Intensive studies of soil microfungal communities in Wisconsin showed that high diversities of soil microfungi were associated with high diversities of higher plant vegetation (Tresner et al., 1954; Orpurt and Curtis, 1957; Christensen et al., 1962; Christensen and Whittingham, 1965; Christensen, 1969). Furthermore, species diversities have been shown to be higher in the litter-humus layer than in the underlying soil (Novak and Whittingham, 1968; Wicklow and Whittingham, 1974).

On the basis of the heterogeneity of environmental conditions, Christensen (1984) explained the differences in diversities among fungal communities. She suggested that complex vegetation provides various kinds of substrata, thereby allowing different fungal species to coexist. She also proposed that complex conditions of the litter-humus layer support various kinds of microfungal species, because this layer consists of a mixture of decaying leaves in various stages and is exposed to air and spore vectors.

However, even relatively homogeneous plant communities support complex microfungal flora, the number of species of which often exceeds that of higher plant species (Novak and Whittingham, 1968; Apinis, 1972). This implies that various fungal species could share the space of a single substratum.

When we consider the mechanisms controlling microfungal diversity, therefore, it is important to clarify how the fungal species colonize the space of a substratum. If each fungal species occupies a smaller portion of the entire space, a larger number of species can coexist in that space, thereby causing a higher level of species diversity. On the other hand, if each fungal species occupies a larger portion of the entire space, a fewer species can grow in that space, thereby reducing species diversity.

The purpose of this study is to explain the differences in microfungal diversities based on the pattern of fungal colonization of substrata. First, we illustrate two cases of microfungal diversity as examples to be analyzed. Then we take into account distributions of nutrients in the substrata and hyphal growth rates of the component species to explain differences in diversity among the fungal communities.

Fallen leaves of *Quercus serrata* Murr. and *Castanopsis* sp. were used to analyze fungal diversity. Fallen leaves of *Q. serrata* were collected from the surface of the organic horizon in a deciduous oak forest at Ashikawa, Yamanashi Pref., Japan on 22 Sep. 1993. Sample leaves of *Castanopsis* sp. were collected from the same layer in an evergreen oak forest at Yakushima Island, Kagoshima Pref., Japan on 7 Nov. 1993. To determine the species compositions in each sample leaf, we obtained twenty disks from each sample leaf using a puncher with a 6 mm diam. Using the method described by Tokumasu (1978, 1980), the disks were washed and rinsed three times. The disks were dried for 1 d on sterile filter paper in 9-cm diam Petri dishes to suppress bacterial growth after plating (Widden and Parkinson, 1973). Ten dried disks were laid onto Miura's agar plates (LCA; Miura and Kudo, 1970) and incubated at 25°C for 4 wk. During the incubation, we isolated and identified fungi appearing on and around the disks. For each species, the frequencies of occurrence were calculated by the following equation:

$$\text{Frequency of occurrence} = \frac{\text{the number of disks from which the fungus was detected}}{\text{the total number of disks in each examination.}}$$

Ten other dried disks were used to examine distributions of constituents in each sample leaf. The disks were completely dried in a vacuum desiccator. The carbon and nitrogen contents of each disk were determined with an organic elemental analyzer (Perkin Elmer 2400 II CHN/O).

To estimate hyphal growth rates of the isolated species, each was inoculated onto a LCA plate and incubated at 25°C. The hyphal growth was recorded every day, and the average growth rates per day were calculated. The cultures used for this experiment are preserved in the Laboratory of Biology, College of Pharmacy, Nihon University, Funabashi, Japan.

Table 1 shows the fungi isolated from a decaying leaf of *Q. serrata*. In total, 20 species were isolated. The dominant species were *Mirandina cylindrospora* Matsushima, *Dactylaria naviculiformis* Matsushima and *Helicosporium* sp., which occurred with frequencies of occurrence of 0.8, 0.7 and 0.7, respectively. Another 17 species occasionally occurred on the disks. *Phaeoramularia* sp., *Idriella lunata* P. E. Nelson & Wilhelm and *Monochaetia* sp. appeared on 5 disks, and *Dactylaria irregularis* de Hoog on 4 disks. Thirteen species appeared on fewer than 3 disks.

We isolated 12 species from the *Castanopsis* sp. leaf (Table 2). The three dominant species, *Trichoderma harzianum* Rifai aggr., *T. hamatum* (Bonord.) Bain. aggr. and *I. lunata*, occurred with frequencies of occurrence of 0.7. Additionally, we isolated 9 occasional species, most of which occurred on fewer than 3 disks.

Microfungal diversity in the *Q. serrata* leaf was higher than that in the *Castanopsis* sp. leaf, in terms of both richness and evenness of distribution of species. On average, 2 dominant species and 4 occasional ones colonized each disk of the *Q. serrata* leaf, whereas 2 dominant species and 2 occasional ones colonized each disk of the *Castanopsis* sp. leaf. Furthermore, low frequencies of occasional species in the *Castanopsis* sp. leaf implies that the dominant species colonized a large space

Table 1. Fungi isolated from a decaying leaf of *Quercus serrata*.

Species	Disk number										
	1	3	7	6	10	2	8	4	9	5	Fr[a]
Dominant species											
Mirandina cylindrospora		*[b]	*	*	*	*	*		*	*	.8
Dactylaria naviculiformis		*	*		*		*	*	*	*	.7
Helicosporium sp.			*		*	*	*	*	*	*	.7
Occasional species											
Phaeoramularia sp.	*				*			*	*	*	.5
Idriella lunata				*			*	*	*	*	.5
Monochaetia sp.	*				*	*		*	*		.5
Dactylaria irregularis				*			*		*	*	.4
Dactylaria sp.			*	*			*				.3
Penicillium sp. 1				*	*						.2
Rhinocladiella sp.									*	*	.2
Cladosporium cladosporioides						*		*			.2
Trichoderma hamatum		*						*			.2
Fusarium sp.						*					.1
Tripospermum sp.						*					.1
Verticillium sp.								*			.1
Gliocladium roseum										*	.1
Tritirachum sp.										*	.1
Alternaria alternata							*				.1
Mucor sp.	*										.1
Unidentified sp.1						*					.1
Number of dominant species detected on each disk	0	2	3	1	3	2	3	2	3	3	
	(ave. 2.2 dominant species/disk)										
Number of occasional species detected on each disk	3	1	1	4	3	5	4	6	5	6	
	(ave. 3.8 occasional species/disk)										
Number of all species detected on each disk	3	3	4	5	6	7	7	8	8	9	
	(ave. 6.0 species/disk)										
Total number of detected species on all disks	20										

a) Frequency of occurrence.

b) Species that occurred.

Table 2. Fungi isolated from a decaying leaf of *Castanopsis* sp.

Species	Disk number 2	6	4	7	8	10	3	9	5	1	Fr[a]
Dominant species											
Trichoderma harzianum		*[b]	*		*		*	*	*	*	.7
Trichoderma hamatum	*	*		*	*	*	*	*			.7
Idriella lunata			*	*	*		*	*	*	*	.7
Occassional species											
Dactylaria obstriangularia							*	*	*	*	.4
Cladosporium cladosporioides					*	*			*		.3
Dactylaria naviculiformis						*		*		*	.3
Pestalotia sp.									*	*	.2
Penicillium sp.1						*				*	.2
Penicillium sp.2			*				*				.2
Mucor sp.				*							.1
Gliocladium roseum									*		.1
Speiropsis pedatospora				*							.1
Number of dominant species detected on each disk	1	2	2	2	3	1	3	3	2	2	
	(ave. 2.1 dominant species/disk)										
Number of occasional species detected on each disk	0	0	1	2	1	3	2	2	4	4	
	(ave. 1.9 occasional species/disk)										
Number of all species detected on each disk	1	2	3	4	4	4	5	5	6	6	
	(ave. 4.0 species/disk)										
Total number of detected species on all disks	12										

a) Frequency of occurrence.
b) Species that occurred.

in that leaf.

Carbon and nitrogen contents of the sample disks are summarized in Table 3. The carbon contents of the disks of the *Q. serrata* leaf (0.47 mg C/mg dry wt.) were slightly lower than those of the disks of the *Castanopsis* sp. leaf (0.50 mg C/mg dry wt.), whereas the nitrogen contents of the former samples (0.023 mg N/mg dry wt.) were higher than those of the latter samples (0.018 mg N/mg dry wt.). The standard deviations of both nutrients were less for the *Q. serrata* leaf than for the *Castanopsis* sp. leaf. This meant that the nutrients were distributed more heterogeneously in the *Castanopsis* sp. leaf than the *Q. serrata* leaf.

In this study, the nutrient distributions in the substrata cannot explain the differences in diversities. Since heterogeneously distributed nutrients should provide various habitats for species with different optimal growth conditions, the standard deviation should be greater for the *Q. serrata* leaf than the *Castanopsis* sp. leaf. However, the opposite result was obtained.

Figure 1 shows hyphal growth rates of the fungal species isolated from the *Q. serrata* and *Castanopsis* sp. leaves. The dominant species of the high-diversity fungal community in the *Q. serrata* leaf grew at slower rates than those of the low-diversity fungal community in the *Castanopsis* sp. leaf. The respective growth rates of *M. cylindrospora* (PCNB 131), *D. naviculiformis* (PCNB 152) and *Helicosporium* sp.(PCNB 127) were 3.1, 0.3, and 0.5 mm day^{-1}; and those of *T. harzianum* (PCNB 154) and *T. hamatum* (PCNB 153) were 14.3 and 12.9 mm day^{-1}.

These results imply that the hyphal growth rates played an important role in determining the diversity of microfungal communities. When slower growing species became dominant, they do not exhaust such resources as space and nutrients at the initial stage of invasion. This permissive utilization of resources allows other species to coexist, giving rise to a fungal community of high diversity. On the other hand, when faster growing species became dominant, they exhaust the resources rapidly at the initial stage. This exclusive utilization of resources makes it impossible for other species to invade the substratum, resulting in a low species diversity.

The occurrence of faster growing species such as *Trichoderma* may hide the occurrence of other slower growing species. However, we isolated 7 species other than *Trichoderma* from a leaf disk of *Q. serrata* (disk number 4). This means that the methods used in this

Table 3. Carbon and nitrogen contents of the sample disks.

	Quercus serrata	*Castanopsis* sp.
C content (mg C/mg dry wt.)	0.47 (±0.005)[a]	0.50 (±0.01)
N content (mg N/mg dry wt.)	0.023 (±0.002)	0.018 (±0.003)

a) Standard deviation.

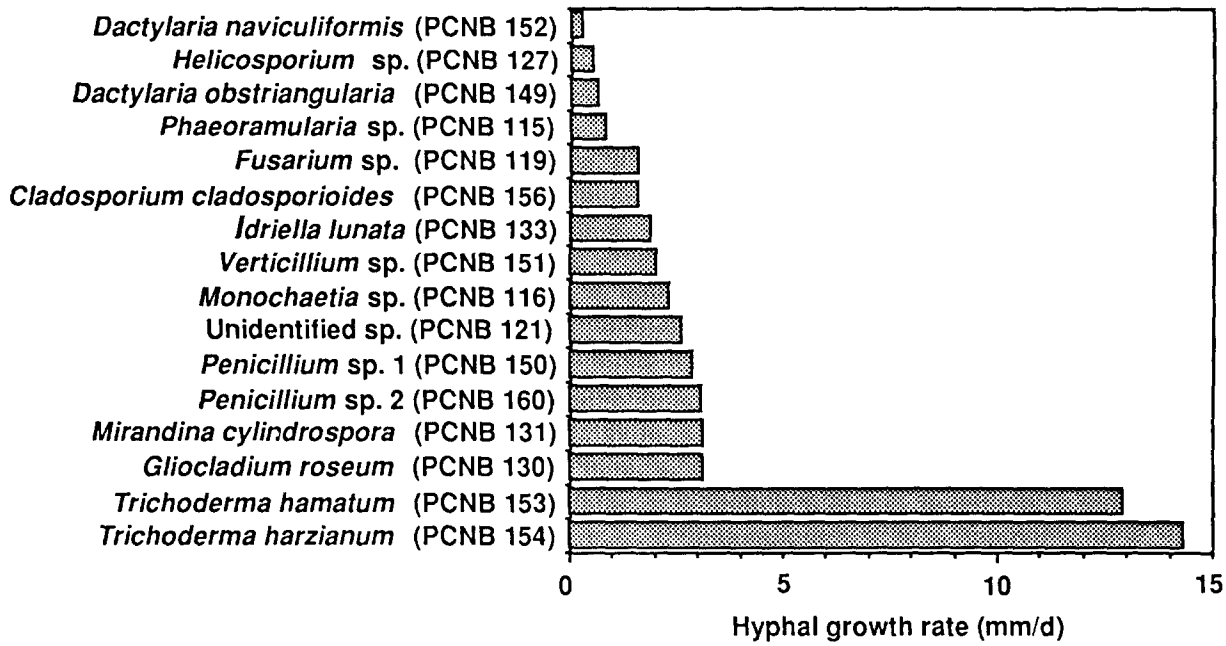

Fig. 1. Hyphal growth rates of the fungal species isolated from the *Quercus serrata* and *Castanopsis* sp. leaves. The growth rates were measured at 25°C on LcA plates. ( ): Strain number.

study could detect slower growing species even in the presence of *Trichoderma* species.

As a first step in a physiological approach to the fungal diversity, it seems rational to analyze community structures on the basis of the hyphal growth rates, because growth is the overall outcome of various metabolic activities such as enzyme production, nutrient uptake, and biosynthesis of cellular compounds. Of course, the estimated growth rates (Fig. 1) are not necessarily realized on natural substrata, but these rates should reflect the differences in colonization ability among the component species.

In this study, fewer species were observed than in other studies. Tubaki (1973) scrutinized microfungal flora on leaves of evergreen oaks in various stages of decay in three localities in Japan. For deuteromycetes, he reported 11-12 high-frequency species and 44-45 low-frequency species. The reason we observed fewer species in our study may be that we examined the microfungi in a particular stage of decay.

Although we examined microfungi on only 10 disks of decaying leaves, the data presented in Tables 1 and 2 should reflect the community structures of the two microfungal communities. By increasing the sample size, we should be able to find more low-frequency species. At the same time, we should find high-frequency species on many disks and their dominancy should be unchanged. Thus, the community structure should not be significantly influenced by further increases of sample size.

Literature cited

Apinis, A. E. 1972. Facts and problems. Mycopathol. Mycol. Appl. **48**: 93-109.

Christensen, M. 1969. Soil microfungi of dry to mesic conifer-hardwood forests in northern Wisconsin. Ecology **50**: 9-27.

Christensen, M. 1984. Species diversity and dominance in fungal communities. In: The fungal community, (ed. by Caroll, G. C and Wicklow, D. T.), pp. 201-232. Marcel Dekker, New York.

Christensen, M. and Whittingham, W. F. 1965. The soil microfungi of open bogs and conifer swamps in Wisconsin. Mycologia **57**: 882-896.

Christensen, M., Whittingham, W. F. and Novak, R. O. 1962. The soil microfungi of wet-mesic forests in southern Wisconsin. Mycologia **54**: 372-388.

Miura, K. and Kudo, M. Y. 1970. An agar medium for aquatic hyphomycetes. Trans. Mycol. Soc. Japan **11**: 116-118.

Novak, R. O. and Whittingham, W. F. 1968. Soil and litter microfungi of a maple-elm-ash flood plain community. Mycologia **60**: 776-787.

Orpurt, P. A. and Curtis, J. T. 1957. Soil microfungi in relation to the prairie continuum in Wisconsin. Ecology **38**: 626-637.

Tokumasu, S. 1978. Leaf litter fungi of forests of *Pinus densiflora* and four introduced pines at Sugadaira, central Japan. Trans. Mycol. Soc. Japan **19**: 383-390.

Tokumasu, S. 1980. Observations on the fungal flora on pine leaf litter. In: Biseibutsu no seitai, vol. 7, pp. 129-144. Gakkai Shuppan Center, Tokyo. (In Japanese.)

Tresner, H. D., Backus, M. P. and Curtis, J. T. 1954. Soil microfungi in relation to the hardwood forest continuum in southern Wisconsin. Mycologia **46**: 314-333.

Tubaki, K. 1973. Some aspects of geographical distribution of leaf litter fungi in Japan. Shokubutsu Byogai Kenkyu (Forsch. Gebiet Pflanzenkrankh.), Kyoto **8**: 61-69.

Wicklow, D. T. and Whittingham, W. F. 1974. Soil microfungal changes among the profiles of disturbed conifer-hardwood forests. Ecology **55**: 3-16.

Widden, P. and Parkinson, D. 1973. Fungi from Canadian coniferus forest soils. Can. J. Bot. **51**: 2275-2290.