Fungal Diversity

A new approach to studying microfungal succession on decaying pine needles in an oceanic subtropical region in Japan

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The sequence of fungal succession on decaying pine needles was evaluated using a novel approach. Twenty-two microfungal community data sets were obtained from individual surveys in an oceanic subtropical region and were rearranged and modified. The constancy and the abundance values for individual species were calculated by selecting the dominant species among the 122 species recorded. Three succession stages were recognized and characteristic species at each stage are considered. The results of this novel approach are compared with the orthodox method based on determining the vertical distribution of microfungal communities at a single site.

Key words: fallen pine needles, fungal succession, new approach, subtropical fungi.

Introduction

The mor type O horizons found in pine forests or under pine stands distributed in temperate or cooler climatic regions are ideal for succession studies of fungi associated with the decay of conifer needles (Kendrick, 1959). In the O horizons, the L, F, and H sub-layers are well developed and each layer is composed of pine needles at an almost equal stage of degradation. In O horizons, we can investigate microfungal communities of individual sub-layers and establish a sequence of fungal succession associated with the decay of needles. This is achieved by arranging the fungi in order from the L to the H layer at the study site. Many investigators have studied fungal succession on decaying pine needles on the forest floor at a single site utilizing this method (Kendrick and Burges, 1962; Hayes, 1965a,b; Parkinson and Balassoria, 1967; Tubaki and Saito, 1969; Widden and Parkinson, 1973; Soma and Saitô, 1979; Ponge, 1991; Tokumasu *et al.*, 1994; Tokumasu, 1996). In most cases, the

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vertical distribution of microfungal communities in the sub-layers at the sites has been investigated several times in a year. This is because pine needles usually fall throughout all seasons of the year and the dominant early colonisers of freshly fallen needles may differ between seasons (Tokumasu, 1998a,b). A general pattern of fungal succession corresponding to the progress of pine needle decomposition has been established from these studies.

Most studies on the fungal succession on fallen pine needles have demonstrated that a few initial colonisers are present on the tree, and these are replaced by a larger number of secondary saprobic species that colonise litter on the ground after needle fall. Hayes (1965b), however, pointed out that the majority of dominant species at each stage in succession, at a given site, often differs from those found by other workers studying neighbouring pine forests. For example, *Sympodeiella acicola* and *Troposporella monospora* (= *Helicoma monospora*), which are dominant species in the F_1 needle layer of *Pinus sylvestris* in a pine forest of England (Kendrick and Burges, 1962), have been reported rather infrequently in similar subsequent studies in various British Islands and Western Europe (Parkinson and Balasooria, 1967; Tokumasu *et al.*, 1994). This is despite the fact the these are dematiaceous fungi that are easy to observe because of their large characteristic conidia when present on decaying pine needles.

In studies on the geographical distribution of *Parasympodiella longispora*, a hyphomycete found on decaying pine needles, Tokumasu and Tubaki (1983) and Tokumasu (1987) demonstrated that the occurrence of the species at a certain site was limited by the climatic conditions of the site, especially temperature. Recently, more detailed studies on the geographical distribution of conifer litter inhabiting fungi have been published by van Maanen and Gourbriére (1997), van Maanen *et al.* (2000) and Tokumasu (2001). These workers suggest that the abundance of a selected fungus at a given site reflects the results of inter-specific interactions that are greatly influenced by the climate conditions at the site. They conclude that where pine forests occur in the same climatic region, then the same fungal species would be recurrent at the same stage in fungal succession on decaying pine needles. However, this appears to contradict the fact that different dominant species are found in fungal successions in the O horizons of pine forests at single sites.

In this paper, we established a sequence of fungal succession using a novel approach. The approach integrates the survey results from many sites, where fungal succession on pine needles was studied on separate occasions. Three islands distributed in an oceanic subtropical climate were selected for this study, because there is relatively little information on fungal succession on decaying pine needles in subtropical regions (Tokumasu, 1985).

Table 1. Brief descriptions of individual samples examined.

Sample no.	Island	Altitude (*ca.* m)	Lat. (°.'N)	Lon. (°.'E)	AM[a] (C)	Ar[b] (C)	Pre (mm)	*Pinus* species	Number of needles / decay stage	Data of collection
2	Iriomote	25	24.24	123.51	23.5	10.8	2223.5	*P. luchuensis*	20	Jun. 05 1984
3	Iriomote	10	24.19	123.54	23.5	10.8	2223.5	*P. luchuensis*	20	Jun. 03 1985
4	Ishigaki	50	24.26	124.08	24.2	10.8	2307.0	*P. luchuensis*	20	Oct. 10 1986
12	Iriomote	50	24.22	123.45	23.4	10.3	2384.6	*P. luchuensis*	10	Mar. 17 1989
13	Iriomote	50	24.22	123.45	23.4	10.3	2384.6	*P. luchuensis*	10	Mar. 17 1989
14	Iriomote	25	24.16	123.53	23.5	10.8	2223.5	*P. luchuensis*	10	Mar. 18 1989
15	Iriomote	25	24.24	123.51	23.5	10.8	2223.5	*P. luchuensis*	10	Sep. 20 1989
16	Ishigaki	50	24.26	124.08	24.2	10.8	2307.0	*P. luchuensis*	10	Sep. 19 1989
17	Iriomote	10	24.23	123.51	23.5	10.8	2223.5	*P. luchuensis*	10	Jan. 30 1991
18	Ishigaki	75	24.22	124.11	23.8	10.8	2108.0	*P. luchuensis*	10	Jan. 27 1991
19	Yonaguni	100	24.27	123.00	23.7	10.4	2392.2	*P. luchuensis*	10	Mar. 19 1992
20	Iriomote	75	24.17	123.52	23.5	10.8	2223.5	*P. luchuensis*	10	Jan. 26 1994
21	Yonaguni	100	24.27	123.00	23.7	10.4	2392.2	*P. luchuensis*	10	May 14 1994
22	Iriomote	10	24.23	123.54	23.5	10.8	2223.5	*P. luchuensis*	10	Mar. 11 1996
24	Ishigaki	25	24.29	124.17	23.7	10.5	2187.9	*P. luchuensis*	10	Mar. 12 1996
25	Ishigaki	50	24.26	124.08	24.2	10.8	2307.0	*P. luchuensis*	10	Mar. 12 1996
26	Ishigaki	50	24.35	124.09	24.2	10.8	2108.0	*P. luchuensis*	10	Mar. 13 1996
27	Ishigaki	10	24.35	124.18	23.7	10.5	2187.9	*P. luchuensis*	10	Mar. 12 1996
28	Yonaguni	100	24.27	123.00	23.7	10.4	2392.2	*P. luchuensis*	10	Mar. 31 1998
29	Yonaguni	100	24.27	123.00	23.7	10.4	2392.2	*P. luchuensis*	10	Apr. 02 1998
30	Iriomote	25	24.23	123.52	23.5	10.8	2223.5	*P. luchuensis*	10	Feb. 02 1999
31	Ishigaki	50	24.26	124.08	24.2	10.8	2108.0	*P. luchuensis*	10	Feb. 08 2000

[a] Annual mean air temperature; [b] Annual range; [c] Annual precipitation.

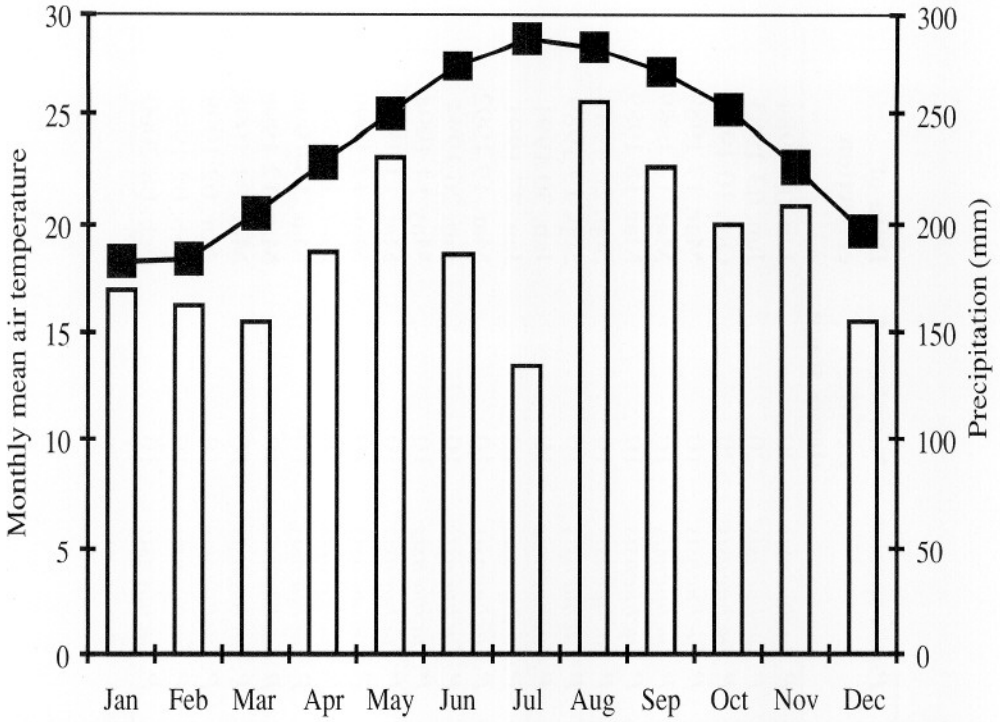

Fig. 1. Monthly mean temperature and precipitation in the Yaeyama Islands. The graph was made using the mean value of 5 weather stations distributed in the area.

Materials and methods

Study area

Ishigaki, Iriomote, and Yonaguni Islands of the Yaeyama island group, Okinawa Prefecture, Japan are distributed between latitude 23° and 24°N and longitude 123° and 125°E. The climate of these islands is oceanic subtropical and the annual range in the air temperature is very small. Precipitation occurs during all seasons (Fig. 1). Iriomote Island, the largest island in the Yaeyama Islands is about 290 km^2 in area, located in the west of Ishigaki Island. Most of the island is mountainous, over 90% of which is covered with subtropical pristine forests. Ishigaki Island (about 229 km^2 in area) and Yonaguni Island, the most western small island (about 28.9 km^2 in area) are well-developed and local farming and cattle breeding are common on both islands.

Collection of pine leaf litter

A total 22 *Pinus luchuensis* litter samples were collected between 1984 and 2000 (Table 1). This is an endemic pine in the Nansei Islands. The sampling sites varied in vegetation type and the degree of disturbance. Macroclimatic conditions of the collection sites were almost uniform. Samples were collected throughout various seasons (Table 1).

In most of the sampling sites, the O horizons were thin and did not stratify. Consequently, a mixture of needles of various degradation stages was

collected from the ground surface of the site. The collected samples were quickly air-dried and preserved at room temperature until they could be processed.

Mycological observations

In the laboratory the needles in a litter sample were sorted into three categories: freshly fallen, brown needles (indicated as L-type needles in this text); partly decomposed, faded needles (as OL-type needles); and decomposed, blackish or grayish, uncollapsed needles (as F11-type needles) that correspond to the upper layer needles of the F_1 layer in the mor type O horizon. The dead needles attached to branches were also collected from 3 sites.

A washing method (Tokumasu, 1980) was adopted for the pre-treatment of all samples in order to record fungal species. A set of ten or twenty (in 3 samples) needles sampled from individual needle types was washed. A set of two washed needles was placed on the surface of a weak strength cornmeal agar plate. The plates were incubated on the laboratory bench where light and temperature conditions were not precisely controlled. They were kept for one month and observed using a light microscope at least four times at equal intervals and the fungi sporulating on and around each of the needles were recorded.

To distinguish between surface and internal colonisers of decaying needles, samples were also surface sterilized as described in Kendrick and Burges (1962).

Processes of crude data

In processing the crude data, we followed Mueller-Dombois and Ellenberg (1974).

At the end of observation period, all species recorded on a given sample were listed and the percentage frequencies of occurrence calculated for individual needle types (primary table). A total 22 primary tables were prepared and used as basic data for the analysis.

A constancy table was constructed in the following manner. Species names in the sample of the minimum number of the recorded species were listed in the far left column and the sample number was written at the top of the second column. The number of the needle type from which a listed species was recorded was written in the blank of the second column. The data for the second sample was transferred into the next column and the species names, not yet recorded in the first transfer, were added, and the numeral of recorded needle types was written in the third column for every species listed. The data

from other samples was transferred in the following columns successively in the same manner.

The constancy for individual species were calculated using the constancy table as follows:

Species constancy (%) = number of the samples on which the species was recorded / total number of samples examined (22) × 100.

Species abundance (sense van Maanen *et al.*, 2000) was the proportion of the needles colonised by the species / the number of needles examined. This was calculated by a modified constancy table listing the number of colonised needles instead of the number of needle types from which a given species occurred. It was calculated as follows:

Species abundance (%) = number of needles colonised by a species / total number of needles examined (750) × 100.

The values were used as a dominancy index of a species in the fungal community involved the decay of pine needles in the study area.

Statistical analysis

To estimate the general sequence of developmental stages in the fungal succession on decaying pine needles in the study area, the means of frequency values of the species with a high and moderately high constancy in three needle types were compared. The significance level of difference in paired mean values derived from two successive needle types was calculated using the t-test.

Analyses were performed using Microsoft Excel 2000 software. $\alpha = 0.05$ was considered to be statistically significant level.

Results

One hundred and twenty-two fungal taxa were recorded from 22 samples examined between 1984 to 2000 (Table 2). The number of species per sample ranged from 20 to 42 (average 29 ± 5.7 SD.). The species were divided into five groups according to their constancy i.e., high, moderately high, intermediate, low and rare. About 62.3% (76 spp.) of all species belonged to the rare species group. The species with a high or a moderately high constancy were about 10% (12 spp.).

It appears that the species with a high constancy are more or less characteristic of the microfungal community (local population) involved in the decay of pine needles in the study area, while those with a low constancy may be considered as possible accidental occurrences, as Mueller-Dombois and Ellenberg (1974) stated concerning plant communities.

Table 2. Twenty-two microfungal communities in three islands of the Yaeyama island group, arranged according to increasing sample number. Numerals 1,2 and 3 indicate the numbers of needle types from which a given species ocuurred.

Fungus	Constancy (%)	Abundance (%)	Sample number																					
			2	3	4	12	13	14	15	16	17	18	19	20	21	22	24	25	26	27	28	29	30	31
High constancy species (8 spp.)																								
Dactylaria fusiformis	100.00	60.93	2	3	3	3	3	3	3	3	2	3	3	2	3	3	2	3	1	3	2	3	2	3
Thozetella cristata	90.91	22.53	3	3	3	3	2	2	2	2	3	1	2		2	2	3	2	3	2		2	3	3
Dactylaria naviculiformis	90.91	20.53	3		2	3	1	3	2	2		1	3	1	3	1	3	1	1	2	2	3	2	2
Dictyochaeta simplex	90.91	19.33	2	1	3	1	3	2	2	2	2	1	2	1		3	2	3	1	2	3		2	2
Cladosporium spp.	86.36	19.07	2	3	2	3	1	2	1	3	2	1			3	2	3	2	3	3	1		2	1
Penicillium spp.	86.36	17.33	3	3	2	3	3	1	3	3		2	3	2		2	1	3	1	3	3		1	2
Pestalotiopsis glandicola	81.82	28.27	2	3	1		1		2	3	1	2	2	3	1	2	3	3	2	3			1	3
Stenella cf. *variabilis*	81.82	12.00	2	2	2	1	1	2	1	1	1	2		2	1	1	1	1	2		1			1
Moderately high constancy species (4 spp.)																								
Acremonium sp. 1	77.27	7.20	1		1	1		1	1		1	1	1		1	2		2	1	1	1	1	1	2
Phaeoramularia hachijoensis	72.73	9.07	3				1		2		1	1	1	1	2	2	2	2	2	2	1	3		1
Rhizomorph forming basidiomycetes	68.18	8.67		1	3		1		2	2	2	1		2			2	1	1	1	2		2	1
Cladosporium tenuissimum	63.64	13.60					3	3	2	2	1	2	3	1	1	2	2	3	2	1				
Intermediate constancy species (12 spp.)																								
Trichoderma harzianum	54.55	21.20	3	3	2						2	3	3	2	2						2	1	3	3
Trichoderma spp.	54.55	16.93	1			3	3	2	3	3						3	3	3	2	3				2
Monacrosorium sp. 1	54.55	4.13		1	1	1	2	2		1		1				1	2		1	1		1		
Mortierella isabellina	50.00	7.73	3	3	2							1	2	2	2	3			1			2		1
Beltraniella pini	50.00	3.60		2	1	1	1	1				1		1	1				1	1		1		
Chaetopsina fulva	50.00	3.07	1	1		1					1	1	1		1			1	3		1			2
Aspergillus japonicus	45.45	3.60		1	1				2	1			2	2	1	1		1		2				
Dactylaria breviphora	45.45	3.07	2	1	1						1			1	1		1	1	2		1			
Trichoderma tomentosum	40.91	6.40	2	1	3			1				3	1	1	2				1					
Aureobasidium spp.	40.91	3.33	1			3	2	1		1						1			1		2	1		

Table 2 continued.

Fungus	Constancy (%)	Abundance (%)	Sample number 2	3	4	12	13	14	15	16	17	18	19	20	21	22	24	25	26	27	28	29	30	31
Acremonium spp.	40.91	2.80	2		2			1		1		2								1		1	1	2
Volutella minima	40.91	2.67			3	1	1				1						3	2	3	1				1
Low contancy species (22 spp.)																								
Mortierella ramanniana	36.36	6.67	2	1			1							3	2	2			1		3			
Parasympodiella longispora	36.36	4.93	1			1	1		1			2						2	3			3		
Monacrosporium spp.	36.36	2.53										1	2			1			1		2	1	1	2
Cryptophiale udagawae	31.82	4.93		2		2	1	1	2			2			3									
Fusarium semitectum	31.82	4.67		3		1					1	2	3	1			1							
Chalara cf. *affinis*	31.82	3.73		3			3				1				1			3					3	1
Trichoderma crassum	27.27	4.93	3		1	2	1								1						1			
Olpitrichum sphaerospermum	27.27	2.93		1						1		1			2			1	2					
Mirandina cf. *aunardii*	27.27	2.40							1		2	1						1	1					1
Clonostachys compactiuscula	27.27	2.27	1								1	2	2						2			1		
Mariannaea elegans	27.27	2.27	1		1									1	1				1	1				
Mucor spp.	27.27	2.13		2	1	1						1		1	2									
Beltraniella portoricensis	27.27	1.73	1								1							1	2				2	2
Sporothrix sp.	27.27	1.47	1	1	1							1		1			1							
Fusarium spp.	22.73	3.60													3			1	3	3			2	
Pestalotiopsis foedans	22.73	3.60												3	1			1	1			1		
Henicospora cylindrocalava	22.73	2.27	3	2	1										1							2		
Chloridium virescens	22.73	2.00		3			1		2												1			1
Phoma spp.	22.73	1.33		1									1				2			1			1	
Scolecobasidum humicola	22.73	1.33	3	1	1				1										1					
Alternaria alternata	22.73	1.07		2			1						1		1		2							
Polyscytalum sp.	22.73	0.93	2		1			1	1										1					
Rare species (76 spp.)																								
Penicillium cf. *brevicompactum*	18.18	2.40															2	2	2	2				

Table 2 continued.

Fungus	Constancy (%)	Abundance (%)	Sample number																					
			2	3	4	12	13	14	15	16	17	18	19	20	21	22	24	25	26	27	28	29	30	31
Chaetendophragmia triangulata	18.18	2.00				2							2		1							2		
Dactylaria sp. 1	18.18	1.73								2		1	1											2
Endophragmiella bouwei	18.18	1.73						2								1	2			1				
Verticillium balanoides	18.18	1.47						2							1				1		2			
Colletotrichum sp.	18.18	1.33											2		2		1		1					
Myrothecium verrucaria	18.18	1.20	1					1	1											2				
Sporidesmium goidanichii	18.18	1.20					2		1		1										1			
Catenosubulispora honiaraensis	18.18	0.67			1				1			1					1							
Gyrothrix podosperma	18.18	0.67														1			2	1			1	
Idriella fertina	18.18	0.67	1																1	1				1
Nigrospora sphaerica	18.18	0.67	1										1					1						1
Trichoderma koningii	13.64	2.80			1									2										1
Alysidiopsis sp.	13.64	1.60								1				2						1				
Selenosporella sp.	13.64	1.60								1												3		1
Verticicladium trifidum	13.64	1.47	1						2		1													
Monocillium tenue	13.64	1.20		1							1							2						
Ramichloridium schulzeri	13.64	0.93	2											1			1							
Myrothecium roridum	13.64	0.80								1	1												1	
Stachybotrys parvispora	13.64	0.80	1				2		1															
Acremonium sp. 2.	13.64	0.53	2		1								1											
Curvularia lunata	13.64	0.53																	1			1		2
Trichothecium campaniforme	13.64	0.53				1														1			1	
Verticillium spp.	13.64	0.53		1								1				1								
Mirandina coticola	9.09	0.80													1							2		
Pestalotiopsis neglecta	9.09	0.80																			1		1	
Unidentified species 5	9.09	0.67																			2	1		

Table 2 continued.

Fungus	Constancy (%)	Abundance (%)	Sample number 2	3	4	12	13	14	15	16	17	18	19	20	21	22	24	25	26	27	28	29	30	31
Mucor genevensis	9.09	0.53					1							2										
Pseudobotrytis terrestris	9.09	0.53																				1		1
Acrodontium simplex	9.09	0.40		2																			1	
Curvularia ergostidis	9.09	0.40												1		1								
Gliocladium viride	9.09	0.40															1						1	
Paecilomyces sp.	9.09	0.40	2								1													
Trichoderma aureoviride	9.09	0.40											1								1			
Volutella ramkamarii	9.09	0.40			1												1							
Absidia cylindrospora	9.09	0.27																1	1					
Cordana sp.	9.09	0.27										1						1						
Geniculisporium anamorph of *Hypoxylon serpens*	9.09	0.27													1						1			
Gyrothrix circinata	9.09	0.27											1											1
Phialomyces macrosporus	9.09	0.27	1											1										
Pithomyces mydicus	9.09	0.27				1							1											
Rhinocladiella atrovirens	9.09	0.27							1					1										
Zygosporium gibbum	9.09	0.27																1	1					
Fusarium solani	4.55	0.80															2							
Gonatobotrys sp.	4.55	0.67																				3		
Rhamichloridium cf. *musae*	4.55	0.53															1							
Chalara cf. *stipitata*	4.55	0.40										1												
Clonostachys rosea	4.55	0.40		1																				
Stenella plectroniae	4.55	0.40																					1	
Unidentified species 3	4.55	0.40																						2
Anungitea sp.	4.55	0.27											1											
Arthrinium phaeospermum	4.55	0.27			1																			
Chloridium sp.	4.55	0.27																				1		
Mortierella exigua	4.55	0.27															1							

Table 2 continued.

Fungus	Constancy (%)	Abundance (%)	Sample number																					
			2	3	4	12	13	14	15	16	17	18	19	20	21	22	24	25	26	27	28	29	30	31
Scolecobasidium constrictum	4.55	0.27									2													
Sesquicillium sp.	4.55	0.27	1																					
Unidentified species 1	4.55	0.27			1																			
Unidentified species 4	4.55	0.27																					2	
Cunninghamella elegans	4.55	0.13							1															
Curvularia pallescens	4.55	0.13														1								
Dactylaria cf. *ampuliformis*	4.55	0.13		1																				
Drechsrella hawaiensis	4.55	0.13			1																			
Gonytrichum macrocladium	4.55	0.13					1																	
Hyalodendron sp.	4.55	0.13																						1
Idriella lunata	4.55	0.13								1														
Kramasamuha sibika	4.55	0.13	1																					
Mariannaea sp.	4.55	0.13																	1					
Nigrospora oryzae	4.55	0.13																			1			
Phaeodactylium sp.	4.55	0.13		1																				
Phialocephala bactrospora	4.55	0.13																						1
Scolecobasdium tricladiatum	4.55	0.13			1																			
Sporidemium sp.	4.55	0.13																			1			
Tripospermum sp.	4.55	0.13										1												
Veronaea sp.	4.55	0.13							1															
Unidentified species 2	4.55	0.13										1												
Unidentified species 6	4.55	0.13				1																		
Total number of species	122		39	34	34	23	27	20	28	21	25	35	28	28	32	23	29	30	42	27	25	24	24	33

Table 3. Major microfungi on fresh dead needles attached to long branches on trees (figures indicate percentage abundance).

Fungus	Sample number			Average
	02	04	30	
Pestalotiopsis glandicola	100	10	100	70
Stenella cf. *variabilis*	100	100	0	66.7
Cladosporium cladosporioides	55	20	10	28.3
Stenella plectroniae	0	0	50	16.7
Trichoderma harzianum	45	0	0	15
Beltraniella pini	20	10	0	10
Myrothecium verrucaria	30	0	0	10
Total number of species	29 spp.			

Table 4. Mean frequencies (%) of high and moderately high constancy species in each needle type.

Fungus	Average frequency in each needle type			Observed significance level of t-test between needle types ($\alpha = 0.05$)		
	L	OL	F11	L-OL	OL-F11	L-F11
Colonisers on the tree						
Stenella cf. *variabilis*	32.27	5.45	0	0.0004	0.012	
Pestalotiopsis glandicola	36.82	26.59	15	0.338	0.1626	0.0296
Cladosporium spp.	22.27	18.64	12.73	0.612	0.3056	0.162
Colionzers on the ground						
Acremonium sp. 1	18.18	2.73	1.82	0.0059	0.6771	0.003
Cladosporium tenuissimum	27.27	14.55	8.64	0.1306	0.2824	0.025
Phaeoramularia hachijoensis	14.55	8.18	5.91	0.2125	0.5585	0.0811
Dictyochaeta simplex	21.82	32.05	5.23	0.2035	0.0001	0.0048
Thozetella cristata	23.86	27.05	17.73	0.6593	0.2164	0.3804
Dactylaria fusiformis	28.41	72.5	66.14	0	0.5236	0.0002
Dactylaria naviculiformis	8.18	22.73	32.05	0.012	0.2019	0.0009
Penicillium sp.	6.36	22.5	19.32	0.0033	0.6299	0.0112
Rhizomorph forming basidiomycetes	2.05	12.73	13.18	0.0216	0.9404	0.0201

The correlation coefficient between constancy and abundance was 0.8030, which shows that there was a strong positive correlation between both indices. Thus, the species with a high or moderately high constancy were also abundant species.

The number of species recorded from fresh dead needles on the tree at 3 sampling sites was 29 and those with a frequency of 10 or more are shown in Table 3. Three species, *Pestalotiopsis glandicola*, *Stenella* cf. *variabilis* and *Cladosporium cladosporioides* would be the initial colonisers of fresh dead needles on the tree.

Means of frequency of the species with a high or moderately high constancy are shown in Table 4. Most isolates of *Cladosporium* were *C. cladosporioides* so that mean frequencies of the top three colonisers on the tree were the highest in the L-type needle and decreased in order the OL and F11-type needle. *Stenella* cf. *variabilis* was never recorded from the F11-type needles

The distribution pattern of *Acremonium* sp. 1 between three needle types was very similar to that of *Stenella* cf. *variabilis*. The former species however, was not recorded from dead needles on the tree and appeared to colonise fallen needles on the ground.

Cladosporium tenuissimum and *Phaeoramularia hachijoensis* were infrequently recorded from the dead needles on the tree, but became prominent on fallen needles. Although no significant differences in mean frequencies between two successive needle-types, their frequency values also decreased with the progress of needle decay.

Dictyochaeta simplex and *Thozetella cristata* had a characteristic distribution pattern among dominant or subdominant species, respectively. From the results of statistical analyses, *Dictyochaeta simplex* appeared to be more dominant in both the L and OL-type needles than the F_{11}-type needles, and *T. cristata* inhabited decaying needles at almost equal proportion in three needle types. The latter species appeared to be a major interior coloniser of fallen pine needles judging from the results of surface sterilization examinations.

The other four entities appeared to colonise mostly fallen needles on the ground, though they also occurred sporadically on dead needles on the tree. *Penicillium* spp. were composed of plural species and the distribution pattern of individual species was not clear. This was also applied to rhizomorph forming basidiomycetes.

Two *Dactylaria* species appeared to invade freshly fallen needles from litter quickly and to become dominant in the OL and F_{11}-type needles. *Dactylaria fusiformis* occurred at all sites examined and had the greatest abundance (60.93%) among all fungal species recorded (Table 2). Both species were surface colonisers and appeared to change the colour of decaying needles to dark by dark-coloured mycelia developed on the needle surface.

Discussion

Preamble to fungal succession

It appears that the species with a high and moderately high constancy are more or less characteristic for the microfungal community (population)

involved the decay of pine needles in the study area, while those with a low constancy occur more or less accidentally. Mueller-Dombois and Ellenberg (1974) has found this to be the case in plant communities. It would therefore, be appropriate to assume that fungal succession in this climatic region is based on the distribution of these characteristic species.

Three stages of fungal succession were recognizable when comparing the paired values of constancy and abundance for the dominant fungi in the three needle types. Saprobic species colonising fresh dead needles on the tree (refer to Canopy group) form the first stage on freshly fallen needles in the O horizon. The freshly fallen needles harbouring the Canopy group would be invaded rapidly by many saprobic fungi originally inhabiting the O horizon and the latter fungi would gradually replace the Canopy group. The new colonisers were distinguishable as two sub-groups. One was composed of the species that would immediately reproduce and rapidly decline, and the other was of those persisting for a rather long period on / in decaying needles. The former species may comprise the *Dictyochaeta* group and the latter the *Dactylaria* group.

The representative species of the Canopy group were *Cladosporium cladosporioides*, *Stenella* cf. *variabilis* and *Pestalotiopsis glandicola*. The former two species produce dry and wind-dispersed conidia, while the latter form wet conidia that may be insect-dispersed, judging from their long appendages and mucilaginous sheaths. *Cladosporium cladosporioides* is one of the most common primary saprobes *sensu* Hudson (1968) in temperate regions of Japan (Tokumasu, 1978, 1996). The other two species rarely occur in the same habitat in temperate regions and appears to be characteristic of the climate of the study area.

The second succession stage was characterized by the abundant occurrence of *Acremonium* sp. 1 and *Dictyochaeta simplex*. Both species produce wet phialoconidial heads. They mainly colonise on the surface of needles and have a relatively short sporulation period. Their occurrence and abundance may be different during different seasons. The distribution of *D. simplex* is somewhat similar to that of *Thysanophora penicillioides* in a moder type O horizon (Tokumasu, 1996, 1998b) in having the highest average frequency value in the OL-type needle. *Acremonium* sp. 1 appeared to have a shorter sporulation period than *Dictyochaeta simplex*. These species are also probably surface colonisers. *Cladosporium tenuissimum* and *Phaeoramularia hachijoensis* are tentatively included in this succession stage as indicated by their distribution pattern among the three needle types. These species are different from the other species in producing abundant wind-dispersed blastoconidia. Members of *Dictyochaeta* group were also recorded in an oceanic island in the Pacific Ocean where there was also an oceanic subtropical

climate (Tokumasu, 1985). Thus, they are characteristic species in this climatic region. It should also be noted that the pine species examined was also *Pinus luchuensis* introduced from the Nansei Island.

The third succession stage recognized was dominated by the surface coloniser *Dactylaria fusiformis* and probably occupied the same ecological niche as *Sporidesmium goidanichii* in the warm temperate regions of Japan (Tokumasu, 2001), This species grows very slowly and forms small blackish colonies in culture despite of its very high abundance on pine needles. It may also be an ecological equivalent to *Sympodiella acicola* or *Troposporella monospora* in England (Kendrick and Burges, 1962). *Dactylaria naviculiformis* was also a surface coloniser with a high constancy and abundance. This species may not contribute greatly for the darkening of the colour of needles, since it grows moderately and forms a pale-coloured, diffused colony in the culture media tested. The relationship between the two *Dactylaria* species could not be clarified in this study.

Thozetella cristata, a large synnematous fungus, was a major interior coloniser of fallen needles and is included in the *Dactylaria* group. The species is probably ecologically equivalent to *Verticicladium trifidum* which is widely recognized as the dominant interior coloniser in the similar fungal successions in European pine forests (see van Maanen and Gourbiere, 1997: van Maanen *et al*., 2000) and *Selenosporella curvispora* in a fungal succession in Sugadaira, Japan (Tokumasu, 1996, 1998a). The species produces a large amount of wet conidia of two types, while both *Selenosporella curvispora* and *Verticicladium trifidum* produce non-germinative dry conidia (Tokumasu, 1998a).

The majority of dominant species involved in the decay of fallen pine needles in the Yaeyama island group differed from those found in other regions with different macro-climates. Thus, the composition of fungal species involved in the decay of fallen pine needles appears to be largely influenced by the climatic conditions in the study area.

Evaluation of study method

In this study, a novel approach was used to establish a sequence of fungal succession on fallen pine needles. In this comparatively simple method, data from the microfungal community on decaying pine needles at a sampling site obtained from a single survey were arranged in Table 2, then further tables were integrated into a constancy table. Using the table and additional tables modified from it, the values of constancy and abundance were calculated for individual species. The average frequency values of individual species were also calculated using similar tables for individual needle types. The dominant species in the study area were established in comparison with these values, and

the sequence of dominant species at each succession stage was estimated by comparing average frequencies of occurrence of the dominant species between different needle types.

The results of surveys of 22 sites from three adjacent islands distributed in the same climatic region were analysed using this approach. Through the analyses, we were able to recognize three stages in the succession of fungi and establish the characteristic species at each stage. The second stage recognized by this approach was composed of the saprobic species with a relatively short sporulation period. In such species, the abundance on the needle appears to fluctuate greatly over short periods. The discovery of this stage by this approach appears to be more certain than the orthodox method in which the vertical distribution of microfungi at a single site are repeatedly surveyed several times during one year. Therefore, we consider that the present approach may be useful for the studies on the fungal succession on decaying pine needles in other climatic regions. The minimum number of samples in an objective area necessary for applying this approach remains uncertain.

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