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FULL PAPER

Toshizumi Miyamoto · Tsuneo Igarashi

Spatial distribution of *Collybia pinastris* sporophores in a *Picea abies* forest floor over a 5-year period

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Abstract *Collybia pinastris* is a relatively common litter-decomposing basidiomycete in spruce forests in Hokkaido, Japan. The spatial distribution of sporophores of *C. pinastris* was investigated for 5 years within a plot of 3 m × 10 m in size (subdivided into 0.5 m × 0.5 m subplots) in a pure stand of *Picea abies*. There were significant differences in the total numbers of sporophores during the sampling years. The total number of subplots in which sporophores occurred were also significantly different during the sampling years. However, the spatial distribution of the subplots with sporophores showed agreement with the distributions in subsequent years. There was no significant correlation between the number of sporophores and the thickness of the litter layer in the subplots, whereas the litter layers in the subplots with sporophores were significantly thinner than those without sporophores. These results suggested that perennial or renewable mycelia of *C. pinastris* occupied the same or close locations year to year for at least 5 years and that the spatial distribution in this plot was restricted not by a shortage of substrates but by other factors.

Key words Basidiomycete · Decomposer · Litter · Rhizomorphs · Sporophores

Introduction

Fungi that inhabit the forest floor are important for both nutrient dynamics and the spatial heterogeneity of nutrients in the soil, as has been demonstrated by field studies of nutrient conditions in soils that were and were not colonized by fungi (Hintikka 1970; Entry et al. 1992; Aguilera et al. 1993; Terashima and Mizoguchi 1995). However, the magnitude of the contribution of fungi to nutrient cycling in the forests cannot be closely estimated without information about their distribution patterns in both time and space. The contribution of fungi may be small if the fungi are ephemeral or restricted to small areas.

Because it is difficult to observe mycelia in the soil directly and identify them to species, many studies have judged mycelium existence by the appearance of the sporophores using the mapping or grid method. These studies have investigated correlations between the distribution of fungi and host trees (Ford et al. 1980; Mason et al. 1982; Tyler 1992; Matsuda and Hijii 1998), phenomena suggesting mutual extinction among different species (Ogawa 1977; Murakami 1987; Tyler 1994), and specific spatial distribution patterns using mathematical techniques (Okabe 1981; Jansen 1984; Fukiharu and Kato 1997). These studies elucidated the mycelium existence of the fungi in the soil without destroying the mycelium system, and provided useful information for understanding the life styles of the fungi, although the information was not complete because the methods cannot prove the absence of mycelium. However, very few studies, especially long-term studies, have been made on litter-decomposing fungi in the forest, in spite of the recognition that the litter-decomposing fungi perform important functions in nutrient cycling of forest ecosystems.

Saprotrophic, decomposer fungi grow "freely" in the substrate, and mycorrhizal fungi grow "tethered" to the host roots. To understand the spatial distribution and dynamics of fungi, it is necessary to investigate the distribution of substrate or host roots at the same time. However, previous studies have not always examined the environmental conditions of the habitat of the fungi. In litter-decomposing fungi, some species are known to have a preference for the litter of particular plant species (Smith 1947; Antonín and Noordeloos 1993), and the distribution of fungi may be affected by the heterogeneity of the substrate. This complication can be avoided by making observations of the distribution of fungi in areas where the substrate is

T. Miyamoto (✉)
Division of Environmental Resources, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan
Tel. +81-11-706-2536; Fax +81-11-706-2536
e-mail: tmiyamot@for.agr.hokudai.ac.jp

T. Igarashi
Kitahiroshima, Hokkaido, Japan

homogeneous. Several of the above-cited studies either investigated plots that contained different kinds of plants or did not examine the quality or quantity of the substrate.

To understand the ecological functions of fungi, especially the litter-decomposing fungi, on nutrient cycling in the forests, one would like to have information on the spatial distribution of the fungi and the stability of the distribution based on long-term investigations. *Collybia pinastris* (Kauffman) Mitchel and Smith is a relatively common litter-decomposing basidiomycete in spruce forests in Hokkaido, Japan. In this study, we investigated the spatial and temporal distribution of *C. pinastris* sporophores in a *Picea abies* (L.) Karsten plantation over a 5-year period. In addition, to understand the effects of resources and habitat conditions, the spatial distribution of the sporophores was studied in relation to the thickness of the litter layer on the forest floor.

Materials and methods

Study sites

The study is conducted at a plantation of ca. 40-year-old *P. abies* at Uryu Experimental Forest of Hokkaido University, Uryu-gun, Horokanai-cho, Hokkaido. This site was situated at an elevation of 300 m. Mean annual precipitation and air temperature based on records for the period 1956–1989 are 1540 mm and 3.0°C respectively. Snow cover persists from November to April. The canopy of *P. abies* was closed. Ground flora consisted of only a few species and was sparse. The litter layer consisted of needles and small twigs of *P. abies*. The soil type is Dystrochrept, and the parent material consists of andesite of Tertiary age. The soil is slightly acidic, and pH (H_2O) of soil (10–80 cm) ranges from 3.9 to 4.5 (Ozawa et al. 2001). In this site, a permanent 3 m × 10 m plot was established and was subdivided into 0.5 m × 0.5 m subplots. In the last year of this investigation, the plot was expanded to 4 m × 10 m to obtain a more precise examinination of the relationship between sporophores and litter layer distribution. The forest floor in this plot was almost level, but there were some mounds that were formed by the topography of the mineral soil and ~30 cm higher than the surrounding floor. Daily mean air temperature and monthly rainfall during the study period (1991–1995) (Fig. 1) were taken at the weather station of Uryu Experimental Forest of Hokkaido University (Hokkaido University Forests Database (unpublished data; http://www.agr.hokudai.ac.jp/exfor/index-e.html). The weather station was 1 km away from the study site.

Fungus studied

C. pinastris is known to occur only in coniferous needle litter (Halling 1983). This species lives in the litter layer of the forest floor and decomposes the needle litter of *P. abies* well in vitro (Miyamoto et al. 2000). In the present study, the base of sporophores stipes were observed originating from 1 to 11 mm soil depth. Furthermore, it forms rhizomorph-like, thickened mycelial threads on needles of the L layer (not humus) near the sporophores (Miyamoto et al. 1998). This species seems to be common in spruce forests in Hokkaido.

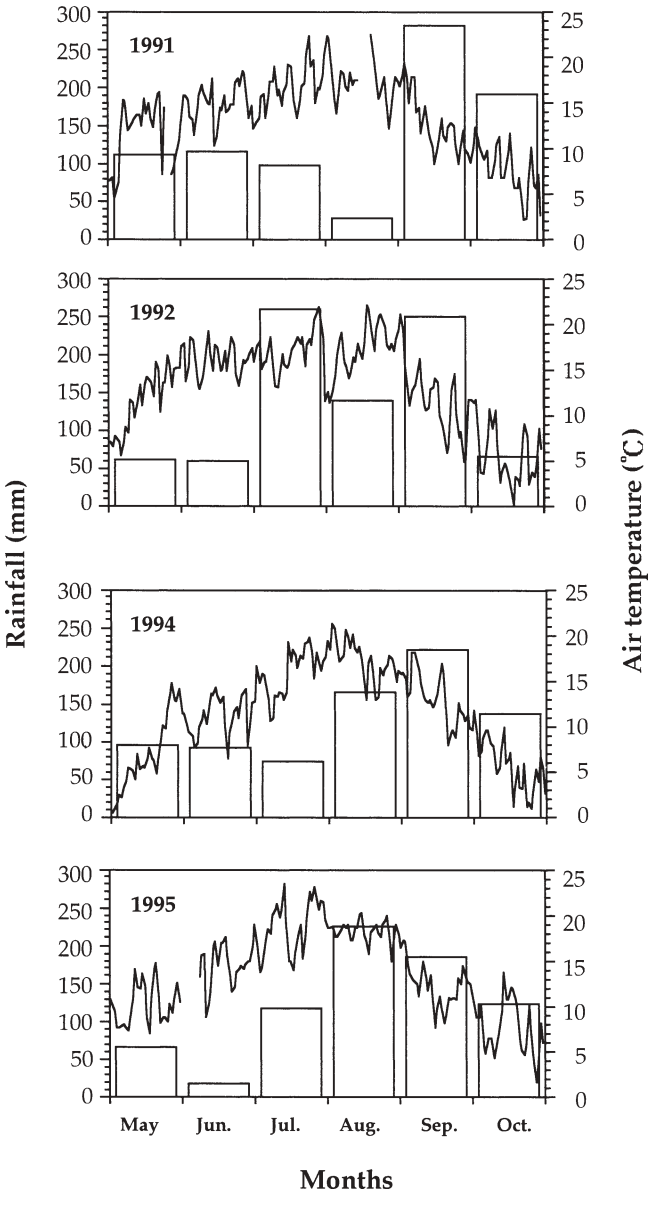

Fig. 1. Monthly rainfall (*open bars*) and daily mean air temperature (*solid lines*) at the weather station of Uryu Experimental Forest of Hokkaido University during the study

Counting and mapping the sporophores

Sporophores of *C. pinastris* were counted and mapped in each of the subplots throughout the snowfree period (May/July to Oct./Nov.) during 1991–1992 and 1994–1995. The plot was visited at about 10-day intervals, and observations were carried out at shorter intervals after rainfalls. The sporophores were cut off at the stem with scissors to avoid duplication of records after counting and mapping.

Although the sporophores of *C. pinastris* are not cespitose, they are small and occur close to each other, and therefore are difficult to count. We mapped the outline of a group of neighboring sporophores within 5 cm of each other as a single patch on graph paper, and counted the number of sporophores inside the outline in the field. The area of patches on the map were measured, and then a regression line was calculated between the area of patches and the number of sporophores. In some patches the number of sporophores were estimated by the regression line ($F = 110.88$, *d.f.* = 1, 39, $P < 0.0001$). However, all sporophores were counted in the whole plot in 1992 and 1995.

Measurement of the litter layer thickness in the forest floor

In September 1995, the spatial distribution of the thickness of the litter layer was investigated. The thickness of the litter layer (L and F layers, but the humus layer was excluded) at each plot was measured at four spots at regular intervals in each subplot within the 4 m × 10 m permanent plot. The thickness of the litter layer at each subplot was represented by the average of four spots.

Statistical analysis

A Friedman test was performed to determine whether there were statistically significant differences ($P < 0.05$) in the total number of sporophores among sampling years. Cochran's Q test was performed to determine whether there were significant differences ($P < 0.05$) in the total number of the subplots in which sporophores occurred among each of the sampling years. The goodness of fit of the spatial distribution of subplots with and without sporophores to the expected, which were based on the null hypothesis of independent, in successive or non-successive years was tested by G test (with Williams' correction) of independence (Sokal and Rohlf 1995). A sequential Bonferroni procedure (Rice 1989) was employed to control for the multiple, nonindependent, simultaneous tests performed. The significance of differences in the mean thickness of the litter layer between the subplots with and without sporophores was determined by a t test. The data were $\log_{10}$ transformed for normality and constant variance.

Results

Relationship between the number of sporophores and air temperature or rainfall

There was significant correlation ($r = -0.987$, $n = 4$, $P < 0.05$) between the number of sporophores and the accumulated rainfall during May to October of each year. There was no significant correlation ($r = 0.342$, $n = 4$, $P = 0.658$) between the number of sporophores and the average daily air temperature during May to October of each year.

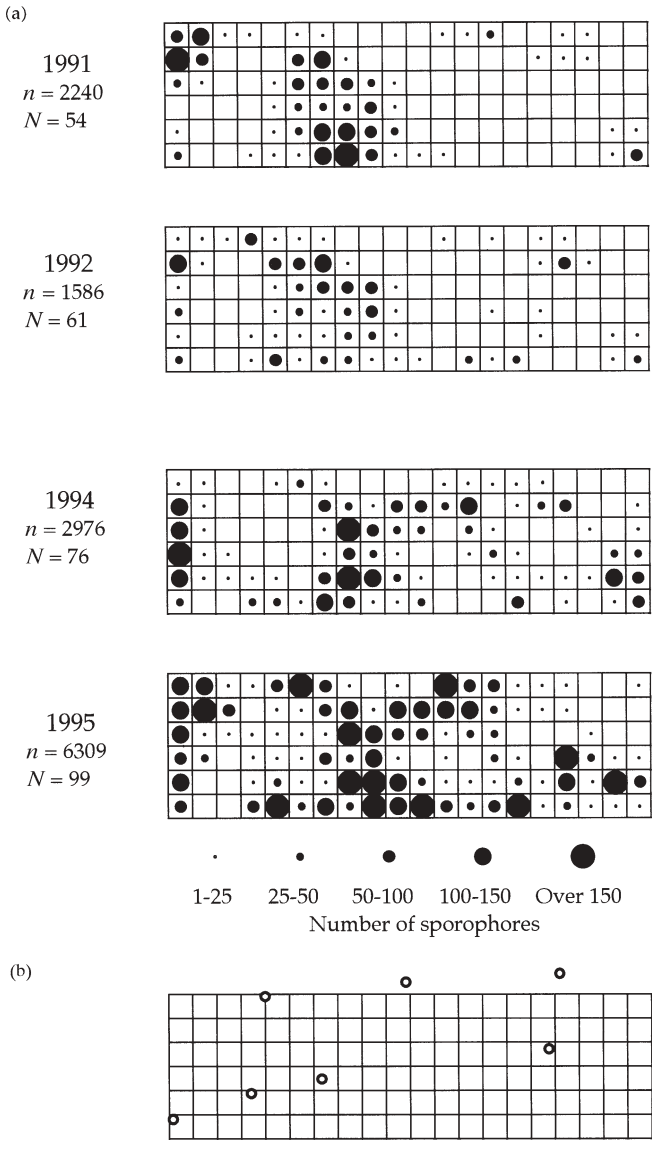

Fig. 2. Spatial distribution of (**a**) sporophores of *Collybia pinastris* and (**b**) the spruce tree trunks in (and close to) the study plot in a *Picea abies* plantation, 1991–1995. Grid shows the permanent plot (3 m × 10 m) consisting of 0.5 m × 0.5 m subplots. The size of the *dot* in a subplot represents the number of sporophores collected at the subplot during the season of fruiting in each sampling year; n is the total number of sporophores collected in each year; N is the total number of subplots where sporophores were collected in each year

Spatial distribution of sporophores

The patches of sporophores had round or irregular outlines and did not have empty centers. The size sometimes reached more than 1 m in diameter. A large aggregation of subplots with high density of sporophores was constantly located near the center of the permanent plot during the years 1991–1995, and some subplots with high-density sporophores newly appeared during this investigation period (Fig. 2a). The distribution of sporophores was not influenced by the position of spruce trunks (Fig. 2b). The total number of sporophores observed during this investigation was highest in 1995 ($n = 6309$) and lowest in 1992

Table 1. Spatial dynamics of sporophore occurrence in each subplot with *Collybia pinastris* in successive and nonsuccessive years

Interval (years)	Number of subplots[a]				*G* value	*P*
	A	B	C	D		
1 (1991–1992)	4[b] (27)[c]	11 (34)	50 (28)	55 (33)	77.35	<0.001*
1 (1994–1995)	6 (13)	29 (36)	70 (63)	15 (8)	12.51	<0.01*
2 (1992–1994)	14 (22)	29 (37)	47 (39)	30 (22)	10.05	<0.02*
3 (1991–1994)	12 (20)	34 (42)	42 (34)	32 (24)	8.95	<0.05*
3 (1992–1995)	2 (11)	40 (49)	59 (50)	19 (10)	19.07	<0.001*
4 (1991–1995)	2 (9)	47 (54)	53 (46)	18 (11)	13.89	<0.01*

[a] A, sporophores occurred in first sampling year alone; B, sporophores occurred in later sampling year alone; C, sporophores occurred in both sampling years; D, sporophores occurred in neither sampling year
[b] Observed number of subplots
[c] Expected number (rounded off) of subplots, based on the null hypothesis of independent, given in parentheses
* Significant difference between the composition of observed number of subplots and expected number using sequential Bonferroni test procedure

($n = 1586$). The total number of sporophores was significantly different among the sampling years (Friedman test, $P < 0.05$). The total number of the subplots in which sporophores occurred increased constantly, and there was a significant difference in the subplot number among sampling years (Cochran's Q test; $P < 0.05$).

Table 1 shows the dynamics of sporophores occurrence in each subplot between successive and nonsuccessive years. The G values are significant ($P < 0.05$), indicating that the spatial distribution of the subplots with sporophores showed agreement with the distributions in subsequent years. When sporophores were observed in a subplot, they were usually observed again in the same subplot in the subsequent sampling years. However, there were a few cases in which sporophores were not observed in the following year.

Relationship between the number of sporophores and the thickness of the litter layer

The thickness of the litter layer in the subplots ranged from 6.25 to 40.0 mm, and the spatial distribution of the litter layer was not even. The numbers of sporophores in the subplots ranged from 0 to 335 in 1995. The mean thickness of the litter layer in the subplots with sporophores (15.0 mm, $n = 118$) was significantly smaller than that in the subplots with no sporophores (17.6 mm, $n = 42$) (t test, $P < 0.01$). There was no significant correlation ($r = 0.11$, $P = 0.165$) between the number of sporophores and the thickness of the litter layer in the subplots.

Discussion

The locations at which the sporophores of *C. pinastris* occurred did not change much over the 5-year period of the study, and the total area increased gradually. These results possibly indicate that the perennial or renewable mycelia of *C. pinastris* occupied the same or close locations year to year for at least 5 years, although it is not known which mycelia were the same genets or regenerated from spores in this study. An in vitro study confirmed that *C. pinastris* decomposes the needle litter of *P. abies*, including the lignin content of the litter (Miyamoto et al. 2000). Thus, the existence of the mycelia at the same location of this species and the litter that it decomposes will have an effect on nutrient dynamics and the heterogeneity of nutrients due to the release of extracellular enzymes from the mycelia in the soil. Based on the large area that this species occupies in the *P. abies* forest in this study and its frequent occurrence in the *Picea glehnii* (Fr. Schmidt) Mast. forests, *C. pinastris* may be one of the most important members of the litter-decomposing mycoflora in the spruce forests in Hokkaido.

Another litter-decomposing basidiomycete is *Mycena galopus* (Pers. ex Fr.) Kummer. This species, similar to *C. pinastris*, has perennial mycelia and shows a slight annual movement (Swift 1982; Frankland 1984). Swift (1982) studied an 8 m × 16 m plot consisting of 0.5 m × 0.5 m subplots over a 2-year period. He reported that the subplots with sporophores in the second year significantly overlapped the subplots with sporophores in the first year, which he took as evidence for the perennial existence of mycelia. However, only 23.8% of the subplots with sporophores that fruited in the first year were observed to fruit in the second year. In contrast, in the present study of *C. pinastris*, a greater percentage of the subplots showed repeated fruiting in successive years: 82.0% in 1991–1992 and 70.7% in 1994–1995.

The absence of a sporophore at a location does not always indicate the absence of mycelia in the soil, because it could be caused by some restriction of fruiting while the mycelia are present. In any case, some unsuitable conditions seem to restrict the growth of mycelia or the fruiting when a sporophore is absent. The most likely factors that could restrict the growth of mycelia or fruiting include (a) insufficient resource, (b) less than optimal moisture content, temperature, or light conditions, and (c) the presence of a competitor or mycophagous arthropod. Some evidence of and some hypotheses concerning biotic restrictions on the

distribution of basidiomycetes have been presented (Newell 1984; Murakami 1987; Tyler 1994). To our knowledge, there have been no quantitative or analytical studies of the distribution of litter-decomposing basidiomycetes in relation to the abiotic micro-environments in the field. However, a few studies have investigated the relations in vitro (Wilson and Griffin 1979; Dix 1984).

In the present study, *C. pinastris* was distributed aggregately and unevenly. Our observations seem to indicate that the amount of substrate was sufficient for *C. pinastris* to live or fruit throughout the study plot, because the thickness of the litter layer was >6.25 mm in this plot, and the sporophores were observed to originate from at least 1 mm in depth. However, the litter layers were significantly thinner in the subplots with sporophores than in the subplots with no sporophores. It seems that in the places with a thick litter layer (L and F layers) the moisture content was in excess and this fungus was suppressed in growing or fruiting. The negative correlation between the production of sporophores and the accumulated rainfall during the season might also indicate that the excess in moisture content of litter layer inhibits the growth of this fungus. To determine the factors that limit the distribution of the fungi, more detailed investigations are needed. However, the substrate for this fungus was abundantly distributed over the plot, and an insufficiency of the substrate did not restrict the distribution of this fungus.

C. pinastris forms rhizomorph-like thickened mycelial threads, and its hyphal cell wall, which is very thick (Miyamoto et al. 1998), seems to be resistant to unsuitable environments. This resistance may be one of the characteristics of this species that enables it to occupy a large area in this plot and not be strongly affected by the heterogeneity of some microenvironmental conditions. In view of the findings that the area of this species was increasing in this plot, the highly aggregated patches, which were present throughout the 5-year study period, may have resulted from the initial conditions that were suitable for migration and establishment of the mycelia.

Agaricus, *Clitocybe*, *Collybia*, and *Marasmius* have been reported to be fairy-ring type fungi (Shantz and Piemeisel 1917; Smith 1957; Weaver 1975; Ogawa 1985; Dowson et al. 1989). The fairy-ring type mycelial system has an empty center due to radial growth and to decay of the trailing edge. The decay of the trailing edge is thought to be caused by autoinhibition, resource depletion, or polarity. Mycelial systems of this type tend to move rapidly, foraging for new resources. On the other hand, resources in the form of litterfall in the forest are supplied annually. If the resources are sufficient, mycelia can continue to live at a location waiting for new resources from the canopy and do not need to move. *C. pinastris* has perennial or renewable mycelia that seem to remain in the originally occupied area when the amount of annual litterfall is sufficient. Indeed, they are able to consume the litter over most of this plot every year.

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