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Spatiotemporal distribution of fruitbodies of ectomycorrhizal fungi in an *Abies firma* forest

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Abstract Spatial associations between ectomycorrhizal (ECM) fungi and their presumed host trees, and spatiotemporal associations among ECM fungi were surveyed for 3 years in an *Abies firma*-dominated forest in central Japan. A total of 39 species in 13 genera of ECM fungi were recorded, with more species in the Russulaceae than any other family. *Russula ochroleuca*, *Russula* sp.1 and *Strobilomyces confusus* tended to produce their fruitbodies on the forest floor directly under the crown of *A. firma*, whereas those of *Inocybe cincinnata*, *Gomphus floccosus* and *G. fujisanensis* were aggregated in limited areas outside the *A. firma* crown. Interspecific spatial associations were analysed for *Russula* sp.1, which was the most dominant species, and three other frequent species, *I. cincinnata*, *S. confusus* and *R. ochroleuca*. Pairwise, *Russula* sp.1 with *I. cincinnata*, with *S. confusus* or with *R. ochroleuca* showed an association which was exclusive, overlapping or independent, respectively. Fruiting phenologies differed in that *S. confusus* showed a peak density in the summer, whereas the other three species peaked in the autumn. These results suggest that the formation of ECM fruitbodies can be partitioned among the species both spatially and temporally.

Key words *Abies firma* · Ectomycorrhizal fungi · Fruitbodies · ω index · Spatiotemporal distributions

Introduction

Most woody plants have ectomycorrhizal (ECM) roots associated with a large number of fungal species (Smith and Read 1997) and there is an increasing body of knowledge about the degree of specificity in ECM associations, based mainly on field observations (Trappe 1962; Molina et al. 1992). For instance, Trappe (1977) estimated that Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] may have ECM associations with as many as 2000 species of ECM fungi. The biological traits and ecological significance of most potential ECM fungi in forest ecosystems, however, still remain unclear. To better understand the role of ECMs in forest ecosystems, characterization of the fungal species and elucidation of fungal community structure are required by monitoring the spatial and temporal distribution of ECM fungi in various types of forest.

Some studies have reported changes in the species composition of ECM fungi as forest stands age (Dighton et al. 1986; Chu-Chou and Grace 1988; Keizer and Arnolds 1994). Other studies have examined the spatial distribution of ECM fungi based on the appearance of their fruitbodies (Murakami 1987; Dahlberg and Stenlid 1990; Tyler 1994). The concept of two ecological groups of ECM fungi termed "early-stage fungi" and "late-stage fungi" originated from a series of studies on the spatial and temporal patterns of ECM fungi associated with birch trees (*Betula pendula* Roth and *B. pubescens* Ehrh.) in Scotland (Deacon and Fleming 1992). However, Newton (1992) questioned the applicability of the classification to mature trees in a forest soil, because the concept had been established from studies of younger trees (up to 14 years old) growing in agricultural soil. Moreover, only a few tree species have been surveyed with respect to the spatial and temporal distribution of ECM fungi in mature forests.

Momi fir (*Abies firma* Sieb. et Zucc.) is a major tree species occurring only in warm-temperate forests in Japan (Oohata 1994). The fir sporadically forms pure stands at the late vegetational stage, but occurs more frequently mixed with other tree species (Kitamura and Murata 1984). Although some studies of ECMs have been conducted on the *Abies* genus (Masui 1926; Vogt et al. 1982; Berndt et al. 1990; Kernaghan et al. 1997),

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there is no information about the distribution pattern of associated ECM fungi.

The purpose of this study was to elucidate both spatial associations between sporophores of ECM fungi and their presumed host trees, and the spatiotemporal sporophore distributions among ECM fungi by monitoring the species composition and abundance of fruitbodies in an *A. firma* forest.

Materials and methods

Study site

The study site, located in Inabu Town, Aichi Prefecture in central Japan (680 m a.s.l., 35°11'N, 137°33'E), was first largely occupied by a man-made coniferous stand of 30- to 35-year-old Japanese cedar (*Cryptomeria japonica* D. Don) and Japanese cypress (*Chamaecyparis obtusa* Endl.), which form vesicular-arbuscular mycorrhizas (Mizoguchi 1996), and followed by a naturally regenerated forest of unknown age consisting mainly of *A. firma*. A study plot of 10×30 m was set on a marginal area of the regenerated forest facing the man-made coniferous stand (Fig. 1a), because a preliminary survey showed that far fewer fruitbodies were produced inside the regenerated forest than in the marginal area (Matsuda 1994). The plot had a tree density of about 1500 ha^{-1} with mean diameters at breast height of 13.5 cm for *C. japonica* and *C. obtusa* ($n=41$) and 68.9 cm for *A. firma* ($n=3$). In addition, it contained one mature hornbeam tree [*Carpinus laxiflora* (Sieb. et Zucc.) Blume] and one suppressed *A. firma* tree. The forest floor was sparsely dominated by *Lindera triloba* Blume. Seedlings of *Vaccinium hirtum* Thunb., *Rhus trichocarpa* Miquel, *Hydrangea hirta* Sieb. et Zucc. and *Akebia trifoliata* Koidzumi occurred infrequently on the forest floor. The soil was classified as a slightly wetted black soil (B*l* on the criteria of soil type in Japan) or a slightly wetted brown forest soil (B_E). The pH (H_2O) of the soil 10 cm below the litter layer was 4.7.

The data for mean air temperature and monthly rainfall during the study period (1994–1996) were taken at the nearest weather station (3 km away from the study site; Fig. 2). Peak rainfall occurs in July or September in every year. The total rainfall from

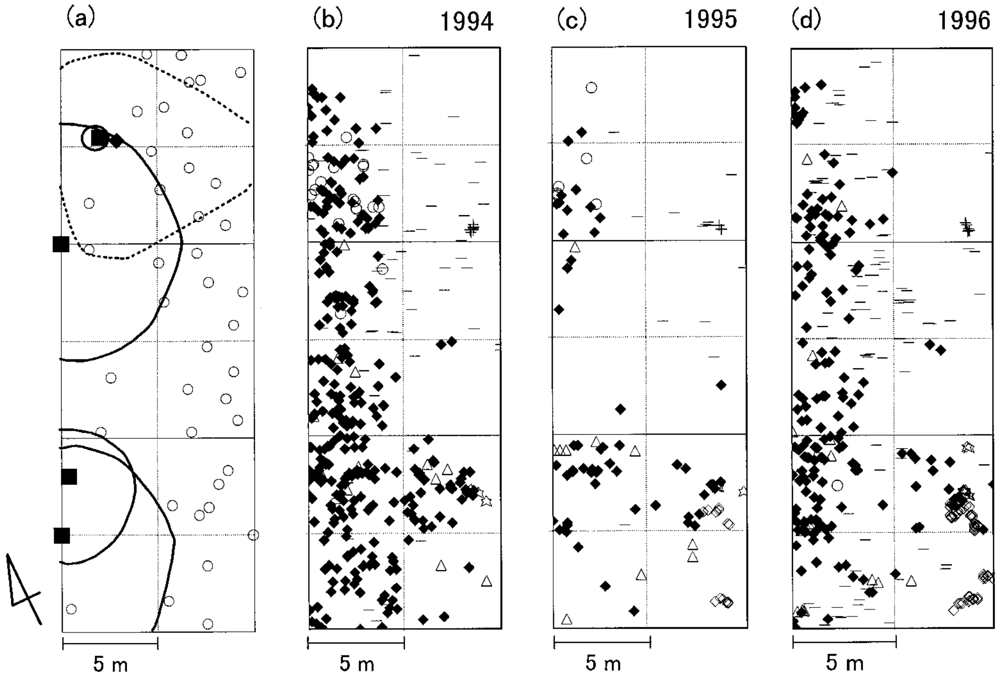

Fig. 1 **a** Distribution of standing trees and the crown projection areas of *Abies firma* and *Carpinus laxiflora* in the study plot (10×30 m; ■ *A. firma*, ◆ *C. laxiflora*, ○ *Cryptomeria japonica* or *Chamaecyparis obtusa*, – crown area of *A. firma*, ··· crown area of *C. laxiflora*. **b–d** Spatial distribution of fruitbodies of ectomycorrhizal fungi in the plot in 1994 (**b**), 1995 (**c**) and 1996 (**d**); ○ *Russula ochroleuca*, ◆ *Russula* sp. 1, △ *Strobilomyces confusus*, ◇ *Inocybe cincinnata*, ☆ *Gomphus floccosus*, + *G. fujisanensis*, – other species

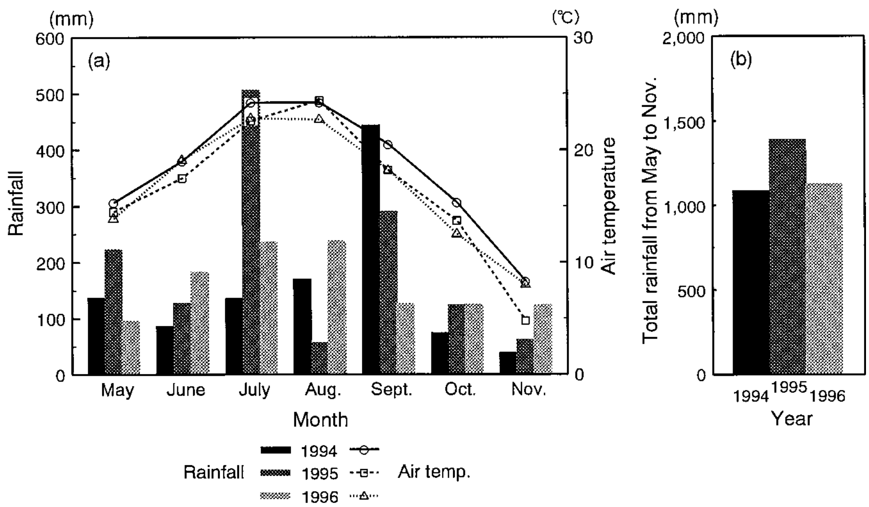

Fig. 2a, b Climatic conditions during the study period (1994–1996). **a** Seasonal changes in mean air temperature (*lines*) and monthly rainfall (*bars*). **b** Total rainfall from May to November in each sampling year

May to November was higher in 1995 than in the other two years. In 1995, however, a severe drought lasting for 38 days under relatively high air temperatures was observed during the summer.

Sampling of fruitbodies

Surveys were performed 67 times at about 10-day intervals from May to November in 1994, 1995 and 1996. In each survey, the species, position and number of individuals were recorded for all fruitbodies considered as ectomycorrhizal (Trappe 1962). Some fruitbodies were collected for further examination of their morphological features and as voucher specimens, but otherwise sporocarps were clipped at ground level and left in the plot. Because of uncertainty about the ecological roles of *Entoloma* spp., fruitbodies of this genus were excluded from the analysis. Determination of the fungal nomenclature was according to Imazeki and Hongo (1987, 1989). After oven-drying at 75 °C for 48 h, selected dried voucher specimens were stored at the Nagoya University Forest.

Analysis of the spatial distribution of ECM fruitbodies

Interspecific associations as reflected by reproductive efforts with respect to the spatial distribution were analysed by the ω index (Iwao 1977) as follows. Mean crowding (m^*) within each species, defined as the mean number of other individuals per quadrat per individual, is denoted by

$$m^*_X = \sum x_{Xi}(x_{Xi}-1)/\sum x_{Xi} \tag{1}$$

and

$$m^*_Y = \sum x_{Yi}(x_{Yi}-1)/\sum x_{Yi} \tag{2}$$

where m^*_X and m^*_Y are mean crowding of species X and Y, respectively, and x_{Xi} and x_{Yi} are the number of individuals of species X and Y in the ith quadrat ($i=1, 2, ..., Q$). When individuals belonging to species X and Y are distributed over the same space, the mean crowding on species X by Y is given as

$$m^*_{XY} = \sum x_{Xi}x_{Yi}/\sum x_{Xi} \tag{3}$$

and that on species Y by X is

$$m^*_{YX} = \sum x_{Xi}x_{Yi}/\sum x_{Yi} \tag{4}$$

If there is no spatial overlapping between species X and Y

$$m^*_{XY} = m^*_{YX} = 0 \tag{5}$$

If the distributions of two species are completely overlapped

$$\begin{aligned} m^*_{XY} &= m^*_Y + 1 \\ m^*_{YX} &= m^*_X + 1 \end{aligned} \tag{6}$$

and if two species are distributed independently of each other

$$\begin{aligned} m^*_{XY} &= m_Y \\ m^*_{YX} &= m_X \end{aligned} \tag{7}$$

From Eq. 6, the ratios, $m^*_{XY}/(m^*_Y+1)$ and $m^*_{YX}/(m^*_X+1)$, indicate the degree of overlapping of Y on X and vice versa, and the geometric mean of these ratios

$$\gamma = \sqrt{\{m^*_{XY}/(m^*_Y+1)\}\{m^*_{YX}/(m^*_X+1)\}} \tag{8}$$

gives an index of overlapping between two species, which has a maximum value of 1 when the distributions of species X and Y are completely overlapped and a minimum value of 0 when they are completely exclusive of each other (Iwao 1977). From $\gamma = \sqrt{\{(m^*_{YX}/m_X)(m^*_{XY}/m_Y)\}/[\{(m^*_X+1)/m_X\}\{(m^*_Y+1)/m_Y\}]}$, transformed from Eq. 7 and Eq. 8, the γ expected for independent distributions of two species is $\gamma_0 = \sqrt{\{m_X/(m^*_X+1)\}\{m_Y/(m^*_Y+1)\}}$.
A measure of the degree of spatial correlation, or the degree of overlapping relative to the independent distributions is given by,

$$\omega = (\gamma - \gamma_0)/(1-\gamma_0) \text{ for } \gamma \geqq \gamma_0$$
$$\omega = (\gamma - \gamma_0)/\gamma_0 \text{ for } \gamma < \gamma_0$$

The value of ω changes from its maximum of 1 for complete overlapping, through 0 for independent distributions, to the minimum of –1 for complete exclusion (Iwao 1977).

The analysis was not based on numerical abundance but on the presence or absence of ECM fruitbodies in a unit area, because the numbers of fruitbodies observed do not always reflect the actual abundance of ECM fungi belowground. Thus, prior to analysis of the distribution map of ECM fruitbodies, the whole plot was modified into a mesh-data map with a 0.25-m mesh: if one or more fruitbodies occurred in one unit (0.25 × 0.25 m) of the mesh map, it was defined as being present, i.e. the unit was assumed to be equivalent to one "individual", and absence was considered to be 0. The ω index was calculated for each combination of *Russula* sp.1 and one of the other most frequent fungal species at five different sizes of quadrat representing different numbers of units in the same plot: 0.25 (0.5 × 0.5) m^2 [the number of units involved in a quadrat (u) = 4, the number of quadrats involved in the plot (Q) = 1200], 1 m^2 (u = 16, Q = 300), 4 m^2 (u = 64, Q = 75), 6.25 m^2 (u = 100, Q = 48) and 25 m^2 (u = 400, Q = 12). Changes in the ω index with increasing quadrat size will reflect a characteristic pattern of distribution with increasing density per quadrat according to the type of distribution for each species, and may thus provide more detailed information on the spatial associations between species than analysis based on a single ω value at a given quadrat size (Iwao 1972).

Results

Species composition and abundance of ECM fungi

The species composition of fruitbodies of ECM fungi and their frequency of occurrence in each year are given in Table 1. A total of 39 species in 13 genera were identified during the study period. *Cortinarius* sp.1, *Strobilomyces confusus*, *Russula ochroleuca*, *Russula* sp.1, *Gomphus floccosus* and *G. fujisanensis* consistently fruited every year (Table 1). The family Russulaceae contained more species than any other family, with 28% of all species in the genus *Russula* and 18% in *Lactarius*. Fruitbodies of an undescribed *Russula* species, *Russula* sp.1 with a very acrid flavor, occurred most frequently, and this species with *Inocybe cincinnata*, *S. confusus* and *R. ochroleuca* accounted for 76% of all the fruitbody occurrences.

The number of species and fruitbodies of ECM fungi confirmed at the site varied between years, with a maximum in 1994 and a minimum in 1995 (Fig. 3). The cumulative number of species increased from 29 to 39 over the three successive years.

Fruiting phenology of ECM fungi

Seasonal changes in numbers of species and fruitbodies both showed a bimodal pattern in each year, with one peak from late June to early August and the other larger peak from late September to early October (Fig. 4). The peak values for species richness and fruitbody production were much lower in 1995 than in 1994 and 1996 (Fig. 4), probably because of a severe drought from late July to the end of August in 1995 (Fig. 2).

Table 1 Species of ectomycorrhizal fungi fruited in an *Abies firma* study plot (10×30 m) from 1994 to 1996 and their frequencies in each year. *Asterisked* species were used for the analysis of spatial distribution

Species	1994	1995	1996	Total
Laccaria sp. 1		1		1
Tricholoma saponaceum (Fr.) Kummer	3			3
T. imbricatum (Fr.: Fr.) Kummer	1			1
Amanita sychnopyramis Corner & Bas f. *subannulata* Hongo	1			1
A. vaginata (Bull.: Fr.) Vitt var. *vaginata*			8	8
A. volvata (Peck) Martin			3	3
Inocybe cincinnata (Fr.: Fr.) Quél.*		14	55	69
I. kobayashi Hongo			7	7
Inocybe sp. 1		10	1	11
Cortinarius ssp.	2			2
Cortinarius sp. 1	3	3	9	15
Strobilomyces confusus Sing.*	13	11	20	44
Austroboletus gracilis (Peck) Wolfe	2		1	3
Pulveroboletus ravenellii (Berk. & Curt.) Murr.	3			3
Boletus ornatipes Peck	1		4	5
Boletus sp. 1	1			1
Tylopilus neofelleus Hongo		1	4	5
T. vinosobrunneus Hongo			2	2
Tylopilus sp. 1	1			1
Russula japonica Hongo	1			1
R. rubescens Beaydslee	3			3
R. compacta Frost & Peck apud Peck	15		12	27
R. laurocerasi Melzer		1	11	12
R. metachroa Hongo	1			1
R. pectinatoides Peck	1		1	2
R. ochroleuca (Pers.) Fr.*	24	7	1	32
R. alboareolata Hongo	1	1		2
R. emetica (Schaeff.: Fr.) S. F. Gray	8		15	23
R. omiensis Hongo	3			3
Russula sp. 1*	309	56	191	556
Lactarius piperatus (Scop: Fr.) S. F. Gray	1			1
L. volemus (Fr.) Fr.	5		2	7
L. gerardii Peck	1			1
L. chrysorrheus Fr.	2			2
Lactarius sp. 1	1		25	26
Lactarius sp. 2			2	2
Lactarius sp. 3	2		7	9
Gomphus floccosus (Schw.) Sing.	3	3	10	16
G. fujisanensis (Imai) Parmasto	9	3	6	18

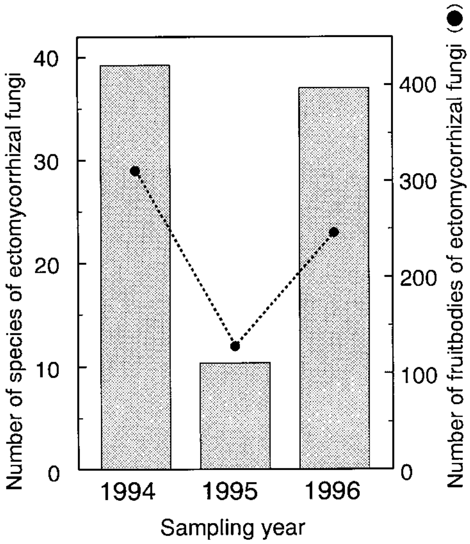

Fig. 3 Number of species (*broken line*) and fruitbodies (*columns*) of ectomycorrhizal fungi confirmed at the plot during the survey period

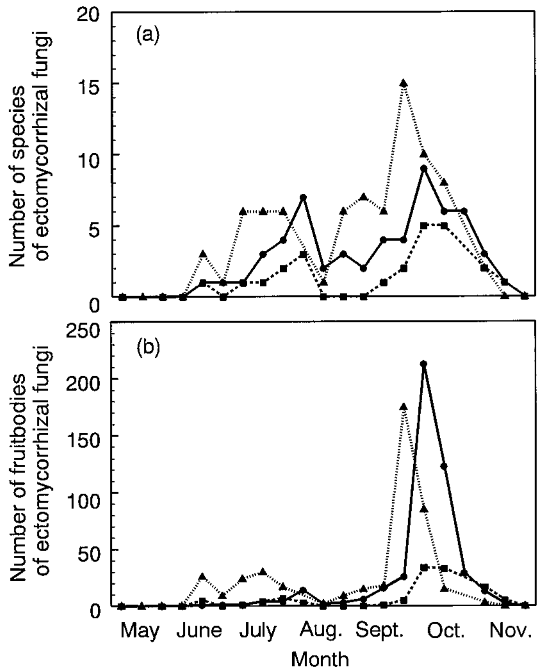

Fig. 4a, b Fruiting phenology of ectomycorrhizal fungi during the survey periods: 1994 (●–●), 1995 (■···■) and 1996 (▲▲). **a** Number of species. **b** Number of fruitbodies

At the species level, the seasonal abundance of fruitbodies tended to show one striking peak for all four major ECM fungi (Fig. 5). *Inocybe cincinnata* fruited most frequently from late September to early October in every year, *R. ochroleuca* and *Russula* sp.1 fruited most abundantly in October, and *S. confusus* most abundantly in July. For other less frequent species, *R. emetica* for instance occurred in July or August, whereas *Lactarius* sp.1, *R. compacta* and *G. fujisanensis* fruited in September and/or October (not shown).

Spatial association between ECM fruitbodies and standing trees

The fruitbody mapping data from 1994 to 1996 revealed a spatial distribution pattern of ECM fungi related to

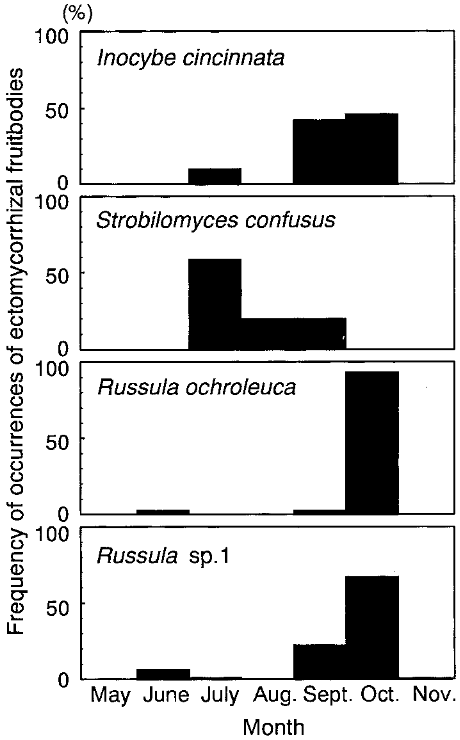

Fig. 5 Frequency of monthly occurrences of the four dominant species of ectomycorrhizal fungi (*Inocybe cincinnata*, *Strobilomyces confusus*, *Russula ochroleuca*, *Russula* sp.1) at the plot during the survey periods from 1994 to 1996. Frequency of monthly occurrences is given as the proportion of the number of fruitbodies in each month relative to the total number of fruitbodies in each species

the position of trees (Fig. 1b–d). Although there was a large difference in fruitbody production from year to year (Fig. 3), their spatial distribution showed a similar pattern in each year. A large number of fruitbodies were observed on the forest floor of the west side of the plot, where *A. firma* and *C. laxiflora* were dominant. On the other hand, fewer fruitbodies occurred on the east side of the plot, occupied exclusively by the non-ECM trees, *C. japonica* and *C. obtusa*.

At the species level, *R. ochroleuca*, *Russula* sp.1 and *S. confusus* tended to produce their fruitbodies on the forest floor directly under the crown of *A. firma* (Fig. 1). Although several ECM morphotypes were observed on *C. laxiflora*, no ECM association has yet been successfully confirmed. However, *Boletus ornatipes*, which has been recorded under various hardwood trees (Imazeki and Hongo 1989), was observed only on the forest floor directly under the crown of *C. laxiflora*. On the other hand, individuals of *I. cincinnata*, *G. floccosus* and *G. fujisanensis* were independently aggregated in limited areas outside the crowns of *A. firma* and *C. laxiflora* (Fig. 1).

Spatial and temporal associations among ECM fruitbodies

The four most frequent species of ECM fungi, *I. cincinnata*, *S. confusus*, *R. ochroleuca* and *Russula* sp.1 (Table 1), were selected for analysis of interspecific spatial associations. *Russula* sp.1 was the most dominant species with respect to fruitbody frequency every year, and thus the analysis was made combining *Russula* sp.1 and one of the other three species.

The pair *Russula* sp.1 and *I. cincinnata* showed an exclusive and an independent distribution pattern relative to each other in 1995 and 1996, respectively (Fig. 6c, d), although no fruitbodies of *I. cincinnata* were observed in 1994. The ω values ranged between 0 and –0.4 for the two years, and tended to increase with quadrat size (Fig. 6a). The peak abundance of *Russula*

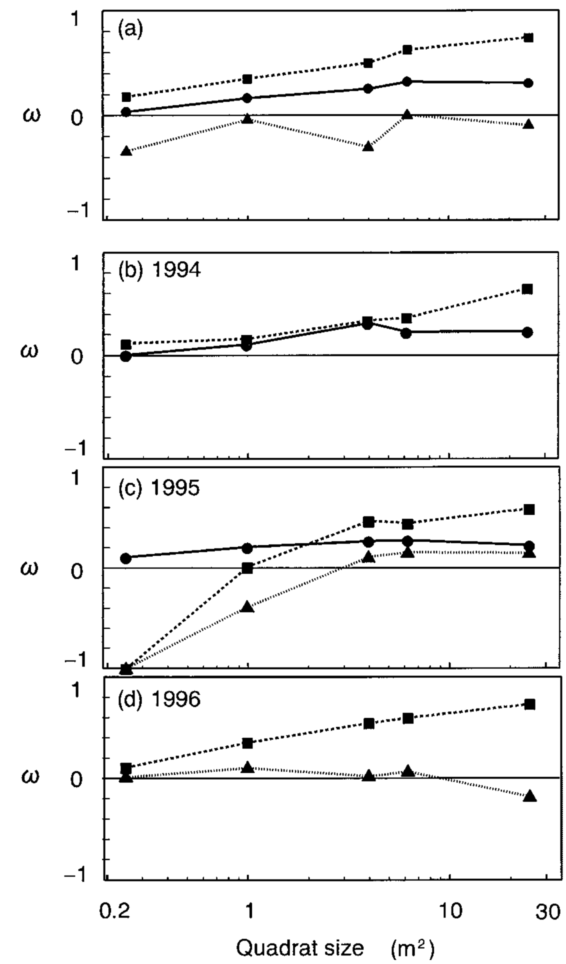

Fig. 6 Changes in the degree of overlap in relation to quadrat size between *Russula* sp.1 and each of the three ECM fungi during the study period (**a**), in 1994 (**b**), in 1995 (**c**) and in 1996 (**d**); ● *R. ochroleuca*, ■ *S. confusus*, ▲ *I. cincinnata*. The ω index was calculated based on the m^*-m method (Iwao 1977). The ω value changes from 1 (complete overlap), through 0 (independent occurrence) to –1 (complete exclusion). No fruitbodies of *I. cincinnata* were observed in 1994. Only one fruitbody of *R. ochroleuca* was observed in 1996 and, thus, the interspecific analysis of the species with *Russula* sp.1 was not made

sp.1 and *I. cincinnata* occurred during a similar period, early October, suggesting the likelihood of spatial partitioning between the two species. A similar distribution pattern was also observed between *Russula* sp.1 and *G. fujisanensis* (Fig. 1b–d), where the ω value for the smallest quadrat size showed complete spatial exclusion ($\omega = -1$) between the two species (not shown).

In the case of *Russula* sp.1 and *R. ochroleuca*, fruitbodies of both species appeared to occur in similar positions under the crown of ECM trees (Fig. 1b–d), but on a local scale, a positive spatial association between these species was less distinctive. The ω value for 1994 and 1995, and combined data for the two years, at the smallest quadrat size (0.25 m^2), clearly showed that the two species were distributed independently during the study period (Fig. 6a–c). That the increase in the ω value at larger quadrat sizes was only slight also indicated that the spatial overlap between the two species was small. In 1996, *R. ochroleuca* produced only one fruitbody, and hence the analysis was not conducted.

The spatial distribution of fruitbodies of *Russula* sp.1 overlapped greatly with that of *S. confusus* every year (Fig. 1b–d). Values of the ω index in 1994 and 1996 increased from 0.1 to 0.7 with the quadrat size (Fig. 6b, d) and although complete spatial exclusion was suggested by the smallest quadrat sizes in 1995, the ω index increased with quadrat size to 0.6 (Fig. 6c). The ω index showed a positive value even at the smallest quadrat size for the overall 3-year data of fruitbody occurrence, and increased with the quadrat size towards a maximum (Fig. 6a). However, the fruiting phenology of the two species showed different patterns of temporal distribution: *Russula* sp.1 had a peak abundance in the autumn and *S. confusus* in the summer (Fig. 5).

Discussion

Among the 39 species of presumed ECM fungi recorded during the study period in the 300 m^2 site (Table 1), *Tricholoma saponaceum*, *S. confusus*, *Austroboletus gracilis*, *R. rubescens*, *G. floccosus* and *G. fujisanensis* are known to fruit on the *A. firma* forest floor (Imazeki and Hongo 1987, 1989). Masui (1926) confirmed a mycorrhizal association by detecting mycelial hyphae connecting *G. floccosus* (renamed from *Cantharellus floccosus* Schw.) and roots of *A. firma*. Thus, it is most likely that *G. floccosus* has mycorrhizal associations with *A. firma* at this study site. Although some of the other species may associate with roots of *C. laxiflora* (personal observation), they have not been listed previously as mycorrhizal fungi associated with *A. firma*.

The rise in the cumulative number of ECM fungal species, from 29 to 39, for the three successive years suggests that the duration of the survey may have been insufficient to reveal all potential ECM species in the study plot. However, Arnolds (1992) estimated that 75% of the total number of species of macrofungal representatives in fungal communities could be observed over 3 years with a survey every 2 weeks at a selected site. Moreover, Dahlberg et al. (1997) surveyed epigeous ECM fungal species on a 100-m^2 plot for 6 years in a mature Norway spruce [*Picea abies* (L.) Karst.] forest and observed up to 86% of the total number of species in the plot in the first 3 years. In the present study, particular species of ECM fungi, such as *Russula* sp.1 and *S. confusus*, remained dominant throughout the study period in spite of large fluctuations in the numbers of species and fruitbodies between years. Moreover, the stand dominated by mature firs was characterized almost all the time by the family Russulaceae. Therefore, although some species of potential ECM fungi remain unknown, major species of ECM flora indicated by fruitbody occurrence, were recorded in association with *A. firma* in this 3-year survey.

In the present study, the frequency of fruitbodies of ECM fungi tended to decrease with the distance from ECM host trees. Nevertheless, there were distinct differences in fruiting positions among different fungal species. Studies conducted on a single site have revealed spatial partitioning among species of ECM fungi related to the distance from the stem of an associated host tree (Ford et al. 1980; Mason et al. 1982; Gibson and Deacon 1988). In the present study, the genus *Russula* and *S. confusus* fruited near the stems of ECM trees, whereas the genera *Inocybe* and *Gomphus* occurred at some distance from them. A similar trend was previously found for *G. floccosus*, which fruited in areas far from the standing position of an *A. firma* tree (Masui 1926). However, according to the succession concept (Deacon and Fleming 1992), the genera *Russula* and *Gomphus* are considered as late-stage fungi and tend to occur at the proximal part of host tree root systems, whereas the genus *Inocybe* is considered as early-stage and tends to occur at the distal part. This suggests a limit to the applicability of the concept to the whole forest succession, as argued by Arnolds (1991), and although some studies on the succession of ECM fungi have indicated a sequence of ECM occurrence with increasing stand age (Keizer and Arnolds 1994; Visser 1995), results suggest that the question of ECM succession is more complicated than previously supposed.

Interspecific associations among ECM fungi have previously been examined mainly for members of the Russulaceae. Murakami (1987) showed exclusive associations between *R. laurocerasi* and *R. densifolia* in an evergreen broad-leaved [*Castanopsis cuspidata* var. *sieboldii* (Makino) Nakai] forest in Japan. Tyler (1994) obtained similar results in a pure stand of hornbeam (*Carpinus betulus* L.) based on fruitbody occurrence, where species of *Russula* and *Lactarius* tended to exclude each other. On the other hand, Brunner et al. (1992) demonstrated spatial coexistence between *R. alnicrispae* and *R. subarctica* in an *Alnus crispa* forest. In this study, the spatial association between *Russula* sp.1 and *R. ochroleuca* was independent, irrespective of quadrat size, whereas that of *S. confusus* and of *I. cin-*

cinnata to *Russula* sp.1 varied depending on the quadrat size, suggesting that adequate quadrat size may be an important factor in studies of associations between congeners.

Using the ω index analyses based on the 0.25-m-mesh map data, it was possible to quantify the degree of spatial associations among the species of ECM fungi in the field plot. Moreover, periodic survey of fruitbody occurrence enabled characterization of the fruiting phenology of the ECM fungi. These combined approaches revealed that *Russula* sp.1 and *S. confusus* fruitbodies did not coincide temporally, but did so spatially. Although this study has focused on ephemeral fruitbodies, their spatiotemporal relationship could provide insight into the characteristics of fungi and may explain their habitat segregation.

The occurrence of fruitbodies of a confirmed ECM fungus indicates the presence of the fungal species underground. However, the relationship between production of fruitbodies aboveground and formation of ectomycorrhizas belowground can vary among fungal species. Fruitbody production by an ECM fungus may not always reflect frequency of ECM associations formed by same fungus and vice versa (Menge and Grand 1978; Danielson 1984; Jansen and Nie 1988). Recent studies based on ECM morphotypes and on molecular approaches have confirmed that the abundance of fruitbodies formed by a particular species of ECM fungus does not reflect the ECM fungal mass belowground (Gardes and Bruns 1996; Kårén and Nylund 1996; Dahlberg et al. 1997). Thus, detecting the patterns of fruitbody occurrence may be less useful for revealing the spatiotemporal patterns of relationships between ECM fungi. Only a small proportion of ECM fungi have so far been investigated in this respect, compared with the number of potential ECM species (5000–6000) (Molina et al. 1992). Further research is needed to clarify the correspondence of aboveground fruitbodies with belowground ECMs using molecular and morphological characterization techniques; these in turn will contribute to a better understanding of the structure of communities of ECM fungi in forest ecosystems.

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References

Arnolds E (1991) Decline of ectomycorrhizal fungi in Europe. Agric Ecosyst Environ 35:209–244

Arnolds E (1992) The analysis and classification of fungal communities with special reference to macrofungi. In: Winterhoff W (ed) Fungi in vegetation science. Kluwer, Dordrecht, pp 7–47

Berndt R, Kottke I, Oberwinkler F (1990) Ascomycete mycorrhizas from pot-grown silver-fir seedlings (*Abies alba* Mill.). New Phytol 115:471–482

Brunner I, Brunner F, Laursen GA (1992) Characterization and comparison of macrofungal communities in an *Alnus tenuifolia* and an *Alnus crispa* forest in Alaska. Can J Bot 70:1247–1258

Chu-Chou M, Grace LJ (1988) Mycorrhizal fungi of radiata pine in different forests of the north and south islands in New Zealand. Soil Biol Biochem 20:883–886

Dahlberg A, Stenlid J (1990) Population structure and dynamics in *Suillus bovinus* as indicated by spatial distribution of fungal clones. New Phytol 115:487–493

Dahlberg A, Jonsson L, Nylund J-E (1997) Species diversity and distribution of biomass above and below ground among ectomycorrhizal fungi in an old-growth Norway spruce forest in south Sweden. Can J Bot 75:1323–1335

Danielson RM (1984) Ectomycorrhizal associations in jack pine in northeastern Alberta. Can J Bot 62:932–939

Deacon JW, Fleming LV (1992) Interactions of ectomycorrhizal fungi. In: Allen MF (ed) Mycorrhizal functioning. Chapman & Hall, London, pp 249–300

Dighton J, Poskitt JM, Howard DM (1986) Changes in occurrence of basidiomycete fruit bodies during forest stand development: with specific reference to mycorrhizal species. Trans Br Mycol Soc 87:163–171

Ford ED, Mason PA, Pelham J (1980) Spatial patterns of sporophore distribution around a young birch tree in three successive years. Trans Br Mycol Soc 75:287–296

Gardes M, Bruns TD (1996) Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and belowground views. Can J Bot 74:1572–1583

Gibson F, Deacon JW (1988) Experimental study of establishment of mycorrhizas in different regions of birch root systems. Trans Br Mycol Soc 91:239–251

Imazeki R, Hongo T (1987) Colored illustrations of mushrooms of Japan, vol I (in Japanese). Hoikusha, Osaka

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

Iwao S (1972) Application of the m^*-m method to the analysis of spatial patterns by changing the quadrat size. Res Popul Ecol 14:97–128

Iwao S (1977) Analysis of spatial association between two species based on the interspecies mean crowding. Res Popul Ecol 18:243–260

Jansen AE, Nie HW (1988) Relations between mycorrhizas and fruitbodies of mycorrhizal fungi in Douglas fir plantations in The Netherlands. Acta Bot Neerl 37:243–249

Kårén O, Nylund J-E (1996) Effects of N-free fertilization on ectomycorrhiza community structure in Norway spruce stands in southern Sweden. Plant Soil 181:295–305

Keizer PJ, Arnolds E (1994) Succession of ectomycorrhizal fungi in roadside verges planted with common oak (*Quercus robur* L.) in Drenthe, The Netherlands. Mycorrhiza 4:147–159

Kernaghan G, Currah RS, Bayer RJ (1997) Russulaceous ectomycorrhizae of *Abies lasiocarpa* and *Picea engelmannii*. Can J Bot 75:1843–1850

Kitamura S, Murata G (1984) Colored illustrations of woody plants of Japan, vol II (in Japanese). Hoikusha, Osaka

Mason PA, Last FT, Pelham J, Ingleby K (1982) Ecology of some fungi associated with an ageing stand of birches (*Betula pendula* and *B. pubescens*). For Ecol Manage 4:19–39

Masui K (1926) A study of the mycorrhiza of *Abies firma*, S. et Z., with special reference to its mycorrhizal fungus, *Cantharellus floccosus*, Schw. Mem Coll Sci Kyoto Imp Univ Ser B 2:15–84

Matsuda Y (1994) Seasonal occurrence and spatial distribution of fruitbodies of ectomycorrhizal fungi on the border of a manmade and a naturally regenerated forest. Bull Nagoya Univ For 13:109–118

Menge JA, Grand LF (1978) Effect of fertilization on production of epigeous basidiocarps by mycorrhizal fungi in lobolly pine plantations. Can J Bot 56:2357–2362

Mizoguchi T (1996) Growth and nutritional responses of some Japanese conifers for inoculation of arbuscular mycorrhizal fungi and different soil phosphorus regimes (abstract). In: First International Conference on Mycorrhizae, Berkeley, p 88

Molina R, Massicotte HB, Trappe JM (1992) Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In: Allen MF (ed) Mycorrhizal functioning. Chapman & Hall, London, pp 357–423

Murakami Y (1987) Spatial distribution of *Russula* species in *Castanopsis cuspidata* forest. Trans Br Mycol Soc 89:187–193

Newton AC (1992) Towards a functional classification of ectomycorrhizal fungi. Mycorrhiza 2:75–79

Oohata S (1994) Speciation and distribution in the genus *Abies* with reference to speciation of the genus *Pinus* (in Japanese with English summary). Bull Kyoto Univ For 66:24–36

Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd ed. Academic Press, London

Trappe JM (1962) Fungus associates of ectotrophic mycorrhizae. Bot Rev 28:538–606

Trappe JM (1977) Selection of fungi for ectomycorrhizal inoculation in nurseries. Annu Rev Phytopathol 15:203–222

Tyler G (1994) Spatial sporophore pattern of ectomycorrhizal fungi in a hornbeam (*Carpinus betulus* L.) forest. For Ecol Manage 65:165–170

Visser S (1995) Ectomycorrhizal fungal succession in jack pine stands following wildfire. New Phytol 129:389–401

Vogt KA, Grier CC, Meier CE, Edmonds RL (1982) Mycorrhizal role in net primary production and nutrient cycling in *Abies amabilis* ecosystems in western Washington. Ecology 63:370–380