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FULL PAPER

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Interactions among ammonia fungi on MY agar medium with varying pH

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Abstract Five early-phase ammonia fungi (EP fungi) – *Amblyosporium botrytis*, *Ascobolus denudatus*, *Peziza moravecii*, *Pseudombrophila petrakii*, *Coprinopsis phlyctidospora*, and *Tephrocybe tesquorum*, and one late-phase ammonia fungus (LP fungus), *Hebeloma vinosophyllum* – were co-cultured on malt extract-yeast extract agar media at pH 5.5, 7.0, 8.0, and 9.0. The co-cultures among the early-stage EP fungi *Amblyosporium botrytis*, *Ascobolus denudatus*, *Peziza moravecii*, and *Pseudombrophila petrakii*, generally did not inhibit or accelerate the reproductive structure formation of the opposed fungi. Among the EP fungi, *Am. botrytis*, *As. denudatus*, and *Pe. moravecii* intermingled with each other. The late-stage EP fungus *T. tesquorum* inhibited the growth of other EP fungi. Another late-stage EP fungus, *C. phlyctidospora*, showed ability to invade other EP fungi, but it did not deeply invade into the territories of early-stage EP fungi. The LP fungus *H. vinosophyllum* tended to accelerate basidioma formation of *C. phlyctidospora* at pH 5.5 and 9.0. *H. vinosophyllum* formed the highest numbers of basidomata at pH 5.5. These results show that successive occurrence of ammonia fungi is caused by the interspecific interactions among ammonia fungi as well as by the physiological characteristic of each fungus associated with conditions of its inhabiting soils, such as pH and nitrogen concentration.

Key words Co-culture · Mycelial interactions · pH · Reproductive structure formation · Territory

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Introduction

The interactions among mycelia occupying or attempting to establish themselves on the same resource are critical to the structure of fungal communities (Dix and Webster 1995). It is widely recognized that all organisms in their natural environment come into contact or proximity with a diversity of others, so that common interactions must be a significant feature of their pattern of life. Interactions among fungi colonizing woody debris in forest ecosystems occur when domains occupied by different individuals make contact during vegetative mycelial growth. Mycelial organization is directed by interactions among genotypes in a particular fungal species, and the prevailing external abiotic and biotic environments, and is located at the boundaries of colonies as a whole or individual hyphal cells (Rayner and Boddy 1988).

Inter- and intraspecific interactions among mycelia occur as a consequence of their territory maintenance or expansion when mycelia are competing or searching for limited resources in a shared habitat. Outcomes of interspecific interactions of cord-forming basidiomycetes vary according to microclimatic environment, the location of the interactions (e.g., in wood or in soil), the size of the resources occupied, and the combination of existing fungi (Dowson et al. 1988; Holmer and Stenlid 1996; Boddy and Abdalla 1998; Boddy 2000).

Ammonia fungi occur sequentially on soil after treatment with urea, aqueous ammonia, or nitrogen compounds that release ammonia during decomposition and cause an alkaline condition in the soil (Sagara 1975, 1992). In natural habitats, these fungi occur after the decompositions of urine, feces, or dead bodies of animals (Sagara 1995). The successive occurrence of ammonia fungi generally follows the scheme anamorphic fungi–ascomycota (ascomycetous cup fungi)–smaller basidiomycota (basidiomycetous smaller mushrooms)–larger basidiomycota (basidiomycetous larger mushrooms). This succession is divided into two phases, the early phase (anamorphic fungi, ascomycota, and basidiomycota smaller mushrooms) and late phase (basidiomycota larger mushrooms) (Sagara 1975, 1992, 1995). In the early

Table 1. Fungal isolates used in this experiment

Species	Abbreviation	Isolate number[a]	Successional phase	Nutritional mode[b]
Ascobolus denudatus	Asd	M001	Early-stage EP	Saprotrophy
Amblyosporium botrytis	Amb	M006	Early-stage EP	Saprotrophy
Peziza moravecii	Pem	M004	Early-stage EP	Saprotrophy
Pseudombrophila petrakii	Psp	M015	Early-stage EP	Saprotrophy
Coprinopsis phlyctidospora	Cp	NBRC 30478 (IF0 30478)	Late-stage EP	Saprotrophy
Tephrocybe tesquorum	Tt	M005	Late-stage EP	Saprotrophy
Hebeloma vinosphyllum	Hv	NBRC 31231 (IF0 31231)	LP	Biotrophy (EM)

EM, ectomycorrhizal; EP, early phase; LP, late phase
[a] Isolates indicated by M numbers are stock cultures of Faculty of Education, Chiba University, Japan
[b] Terms after Cooke and Rayner (1984)

Table 2. Buffer components[a] for each pH adjustment

Component	Volume ratio of the component (ml) to adjust the following pH			
	5.5	7.0	8.0	9.0
1 M NH_4OH	0	2.8	10	20
1 M NH_4Cl	0	20	5.5	0.2

[a] 1 ml of either buffer component was added to 99 ml malt extract-yeast extract agar (MYA)

phase, about 1 month after treatment with N compounds, anamorphic fungi, cup fungi, and smaller agarics sporulate or fruit for short periods. In the late phase, larger agarics are observed for a few years.

Investigations on forest soil properties after the addition of a large amount of urea show that the litter layer turns black, ammonia concentration and water content increase, and the pH rises to 8–10. During this period, the early-phase ammonia fungi (EP fungi) sporulate or fruit on the soil under neutral to alkaline conditions caused by an increase in ammonium concentration. The late-phase ammonia fungi (LP fungi) fruit on acidic soil resulting from the decrease of the ammonium concentration and a temporary increase of nitrate (Sagara 1975, 1992, 1995; Yamanaka 1995a–c; Fukiharu et al. 1997; Sato and Suzuki 1997; Suzuki 2000; Suzuki et al. 2002; He and Suzuki 2004).

To elucidate the successive occurrence mechanism of ammonia fungi, physiological experiments are required that are based not only on monoculture of each ammonia fungus but also on dual culture to investigate interactions between fungi. In the former, physiological characteristics of spore germination (Suzuki 1978, 1989, 2006; Suzuki et al. 1982), vegetative growth, and reproductive structure formation (Morimoto et al. 1981, 1982; Enokibara et al. 1993; Soponsathien 1998a,b; Yamanaka 1999, 2001, 2003; He and Suzuki 2003; Suzuki 2006; Licyayo and Suzuki 2006) have been examined. In the latter, only Suzuki (2006) made preliminary studies of interspecific interactions among a few ammonia fungi and reported that the early-stage EP fungi *Amblyosporium botrytis* and *Ascobolus denudatus* showed mutual intermingling under acidic to alkaline conditions and that the LP fungus *Hebeloma vinosophyllum* increased fruiting and invaded into the colony of *Amblyosporium botrytis* under acidic conditions.

The objective of this article is to examine and characterize the in vitro interactions among members of ammonia fungi and to provide insight into how these interactions influence their vegetative and reproductive growths in the natural habitat resulting in their successive occurrence.

Materials and methods

The fungi used in this study are shown in Table 1.

Co-culture of ammonia fungi

Malt extract-yeast extract agar medium [MY agar; malt extract (Difco) 10 g, yeast extract (Difco) 2 g, agar (Nacalai Tesque) 15 g, 1000 ml distilled water] was sterilized for 10 min at 120°C and was kept at 60°C in a water bath for pH adjustment with NH_4OH-NH_4Cl buffer solution (Table 2) in aseptic condition. After the pH adjustment, approximately 30 ml agar media was poured aseptically into a 11.2-cm Petri dish.

Agar discs 5 mm in diameter were bored from the subperipheral region of actively growing colonies of each fungal isolate on MY agar plates at 25.0° ± 0.5°C in darkness. Two discs from different fungal species were inoculated 55 mm apart on a MY agar plate adjusted to different pHs (5.5, 7.0, 8.0, and 9.0). pH 5.5 was used as a control treatment to represent the pH value of most Japanese forest soils (Sagara 1992; Yamanaka 1995 a–c; Sato and Suzuki 1997, Fukiharu et al. 1997, Suzuki 2000; Suzuki et al 2002; He and Suzuki 2004).

Fungal pairings of the same species were used as controls. As slow-growing species were often completely suppressed by fast-growing species when they are inoculated at the same time (Shaw et al. 1995), slow-growing species were inoculated earlier than the fast-growing species (Table 3). All fungal combinations had five replicates. Cultures were incubated at 25.0° ± 0.5°C in darkness, except for 1-h light irradiation at 3-day intervals for the observations.

Observation of mycelial interactions and reproductive structure formation

Outcomes of the interactions in plate culture were observed by a loupe for the culture of *Amblyosporium botrytis* and for other cultures by the naked eye.

Table 3. Inoculation schedule of each fungus

Inoculum A	Days of inoculation of the following fungi (inoculum B) after the inoculation of inoculum A						
	Amb	Asd	Pem	Psp	Cp	Tt	Hv
Amb	0						
Asd	1	0					
Pem	2	0	0				
Psp	2	1	3	0			
Cp	3	3	3	3	0		
Tt	11	11	11	11	6	0	
Hv	x[a]	x	x	x	4	3	0

[a] No co-culture between inoculum A and inoculum B

Table 4. Types of mycelial interactions observed in co-cultured ammonia fungi

Paired inocula A/B	Mycelial interaction under the following pH conditions			
	5.5	7.0	8.0	9.0
Amb/Amb	N	N	N	N
/Asd	N	N	N	N
/Pem	N	N	N	N
/Psp	N	N	N	N
/Cp	Dc	Dc	Dc	Dc
/Tt	Dc	Dc	Dc	Dc
Asd/Asd	N	N	N	N
/Pem	N	N	N	N
/Psp	Da	Da	Da	Da
/Cp	Dc	Dc	Dc	Dc
/Tt	N	Di	N	N
Pem/Pem	N	N	N	N
/Psp	Dc	Dc	Dc	Dc
/Cp	Dc	Dc	Dc	Dc
/Tt	N	Dc	Da	Dc
Psp/Psp	N	N	N	N
/Cp	Di	Di	Di	Dc
/Tt	Da	Di	Da	Da
Cp/Cp	N	N	N	N
/Tt	Di	Di	Di	Di
/Hv	Di	Di	Di	Di
Tt/Tt	Di	Di	N	N
/Hv	Di	Di	Di	Di
Hv/Hv	N	N	N	N

Da, deadlock antagonism before mycelial contact; Dc, deadlock competition sometimes resulting in sparse mycelial growth; Di, deadlock inhibition at contact zone; N, neutral intermingling of mycelia

Definition of interaction pattern among ammonia fungi

When mycelial colonies of paired fungi intermingled completely, we defined such phenomena as neutralistic intermingling; when one fungus covered the other fungus colony without showing any damage to either mycelium, such phenomena were also called neutralistic intermingling.

Deadlock interactions (Rayner and Boddy 1988) were redefined according to the phenomena observed during the interactions of ammonia fungi. When either inoculated fungi invaded the other fungal colony past the contact zone but the invasion stopped within a few centimeters, we defined it as competing deadlock (Dc), whereas when a contact zone was formed between the two fungal colonies and a faint mycelial density was observed behind the contact line, it was called antagonistic deadlock (Da). If a significant growth inhibition was observed before mycelial contact, it was defined as inhibition deadlock (Di), and when standoff inhibition was observed we also defined it as inhibition deadlock. These types of interactions were determined based on the mycelial interaction and occurrence of reproductive structures during the co-culture.

Results

Interactions among ammonia fungi

Intraspecific interactions. The early-stage EP fungi *Amblyosporium botrytis*, *Ascobolus denudatus*, *Peziza moravecii*, and *Pseudombrophila petrakii* and the late-stage EP fungus, *Coprinopsis phlyctidospora*, showed neutralistic intermingling irrespective of pH conditions (Table 4). Another late-stage EP fungus, *Tephrocybe tesquorum*, showed different interactions depending on pH condition, i.e., inhibition deadlock at pH 5.5 and 7.0 and neutral intermingling at a higher pH conditions. The LP fungus *Hebeloma vinosophyllum* also showed neutralistic intermingling at any pH condition.

Interspecific interactions. Amblyosporium (Am.) botrytis showed neutral intermingling with *Ascolobus* (*As.*) *denudatus*, *Pezizal (Pe.) moravecii*, and *Pseudombrophila (Ps.) petrakii* at all pH conditions (Table 4).

The co-culture between *As. denudatus* and *Pe. moravecii* showed neutral intermingling at any pH condition whereas the co-culture between *As. denudatus* and *Ps. petrakii* showed antagonistic deadlock at any pH condition. *Peziza moravecii* showed competing deadlock interaction when cocultured with *Ps. petrakii* at all pH conditions.

In the neutralistic intermingling, *Am. botrytis* was the most combative among the early-stage EP fungi to colonize other fungus colonies, as shown by its fast mycelia expansion and conidiophore formation on the colony of the other fungus with no negative effect on opposed fungus. In the neutralistic intermingling between *As. denudatus* and *Pe. moravecii, As. denudatus* invaded into the colony of *Pe. moravecii*. Among early-stage EP fungi, *Ps. petrakii* showed a different characteristic, i.e., deadlock, when co-cultured with *As. denudatus* or *Pe. moravecii*.

Coprinopsis phlyctidospora showed deadlock interaction when co-cultured with any early-stage EP fungi, irrespective of pH conditions (Fig. 1A–I). Another late-stage EP fungus, *T. tesquorum*, resulted in deadlock interactions when co-cultured with *Am. botrytis* (Fig. 1M–O) and *Ps. petrakii* at any pH conditions. The co-culture of *T. tesquorum* with *As. denudatus* (Fig. 1P–R) or *Pe. moravecii* showed deadlock interactions depending on pH. The co-culture between *C. phlyctidospora* and *T. tesquorum* showed inhibition deadlock interaction where a standoff inhibition of both fungi was observed, irrespective of pH conditions (Fig. 1S–U). The late-stage EP fungus *T. tesquorum* was able to intermingle in some extent with early-stage EP fun-

Table 5. Days required for reproductive structure formation during interspecific co-cultures

Fungus observed/paired	Mean days for the morphogenesis (f, s) after the fungal inoculation at the following pH conditions							
	5.5		7.0		8.0		9.0	
	f[a]	s[b]	f	s	f	s	f	s
Amb/Amb		5.6 ± 0.3		3.0 ± T		3.0 ± T		3.0 ± T
/Asd		4.0 ± T		3.0 ± T		3.0 ± T		3.0 ± T
/Pem		6.0 ± T		3.4 ± 0.3		3.0 ± T		3.4 ± 0.3
/Psp		4.0 ± T		3.0 ± T		3.0 ± T		3.0 ± T
/Cp		4.2 ± 0.2		3.0 ± T		3.0 ± T		3.0 ± T
/Tt		4.0 ± T		3.0 ± T		3.0 ± T		3.0 ± T
Asd/Amb	17.6 ± 3.0		19.0 ± 1.2		9.4 ± 0.6		8.8 ± 0.2	
/Asd	15.4 ± 2.3		16.2 ± 3.0		9.2 ± 0.2		8.4 ± 0.4	
/Pem	11.6 ± 1.0		10.4 ± 0.9		8.2 ± 0.2		7.8 ± 0.2	
/Psp	13.0 ± T		15.7 ± 2.3		11.0 ± 2.0		10.7 ± 0.9	
/Cp	15.9 ± 2.2		16.6 ± 2.7		14.2 ± 2.6		10.6 ± 0.8	
/Tt	11.3 ± 0.7		10.0 ± 2.0		11.3 ± 0.7		10.7 ± 2.4	
Psp/Amb	5.3 ± 0.3		5.3 ± 0.3		5.0 ± T		5.0 ± T	
/Asd	5.7 ± 0.3		5.3 ± 0.3		5.0 ± T		5.0 ± T	
/Pem	6.0 ± T		5.3 ± 0.3		5.3 ± 0.3		5.0 ± T	
/Psp	5.3 ± 0.3		5.7 ± 0.3		5.0 ± T		5.0 ± T	
/Cp	–		–		–		–	
/Tt	5.0 ± T		17.7 ± 1.4		12.7 ± 0.7		5.0 ± T	
Tt/Amb	21.0 ± 1.0		20.0 ± T		21.7 ± 1.7		25.7 ± 0.7	
/Asd	21.7 ± 1.7		21.0 ± 1.0		24.3 ± 0.7		25.0 ± T	
/Pem	23.0 ± T		23.0 ± T		24.0 ± 1.0		23.0 ± T	
/Psp	22.7 ± 1.5		22.0 ± 1.0		23.0 ± T		24.3 ± 0.7	
/Cp	–		–		–		–	
/Tt	22.6 ± 0.6		20.0 ± T		21.0 ± 0.6		23.7 ± 0.4	
/Hv	–		–		–		–	
Cp/Amb	11.8 ± 0.7		12.4 ± 0.4		12.0 ± T		12.0 ± T	
/Asd	12.6 ± 0.4		11.2 ± 0.6		11.6 ± 0.5		10.8 ± 0.6	
/Pem	10.0 ± T		9.0 ± T		9.2 ± 0.3		10.4 ± 0.4	
/Psp	11.0 ± T		13.3 ± 0.7		13.7 ± 1.5		13.2 ± 1.0	
/Cp	12.4 ± 0.3		11.4 ± 0.5		10.6 ± 0.3		11.9 ± 0.1	
/Tt	10.0 ± T		10.5 ± 0.5		9.3 ± 0.3		10.0 ± T	
/Hv	11.0 ± T		8.3 ± 0.3		8.0 ± T		10.7 ± 0.3	
Hv/Cp	15.0 ± 0.6		20.7 ± 0.3		20.7 ± 0.3		24.0 ± 1.0	
/Tt	17.0 ± 1.0		19.7 ± 1.8		23.7 ± 0.7		25.0 ± 1.2	
/Hv	14.2 ± 0.2		16.2 ± 0.2		17.0 ± 0.3		19.8 ± 0.7	

Values indicate means ± SE ($n = 5$)
Peziza moravecii formed no reproductive structure in any fungal combination
–, No reproductive structures formed during the co-culture; T, less than 0.04
[a] Fruiting
[b] Sporulation

gi, whereas *C. phlyctidospora* did not completely invade into the colony of any other EP fungi.

Hebeloma vinosophyllum showed inhibition deadlock when co-cultured with either *C. phlyctidospora* (Fig. 1J–L) or *T. tesquorum* (Fig. 1V–X), irrespective of pH conditions.

Effect of pH on the period required for reproductive structure formation

Conidiophore formation of *Am. botrytis* was not affected by the co-culture with other ammonia fungi at pH 7.0, 8.0, and 9.0 (Table 5). At pH 5.5, conidiophore formation of *Am. botrytis* was faster when co-cultured with *As. denudatus*, *Ps. petrakii*, *T. tesquorum*, or *C. phlyctidospora*, but not with *Pe. moravecii*.

Ascoma formation of *As. denudatus* was accelerated by co-culture with *Pe. moravecii* at pH 8.0 and 9.0 (see Table 5), as well as with a late-stage EP fungus, *T. tesquorum*, at pH 7.0 and 9.0, whereas it was delayed by co-culture with *Ps. petrakii* at pH 7.0, *C. phlyctidospora* at pH 5.5, 7.0, and 8.0, and *T. tesquorum* at pH 5.5 and 8.0. *As. denudatus* fruiting was not affected by the co-culture with *Am. botrytis* at pH 8.0 and 9.0; however, ascoma formation was delayed at pH 5.5 and 7.0. *Ps. petrakii* did not affect the fruiting by co-culture with other EP fungi, irrespective of pH conditions, except for delayed ascomata formation by co-culture with *Pe. moravecii* at pH 5.5, *T. tesquorum* at pH 7.0 and 8.0, and the absolute inhibition by *C. phlyctidospora* at any pH conditions. Basidioma formation of *T. tesquorum* by co-culture with *Am. botrytis* was accelerated at pH 7.0 and with *As. denudatus* at pH 5.5 and 7.0, but was delayed when co-cultured with *Am. botrytis* at pH 9.0, *As. denudatus* and

Table 6. Number of reproductive structures formed during intraspecific co-cultures

Species	Incubation period (day)	Mean number of reproductive structures under the following pH conditions			
		5.5	7.0	8.0	9.0
Am. botrytis	3	0.0 ± T[a]	11.1 ± 0.3[a]	14.6 ± 0.7[a]	19.3 ± 0.5[a]
	5	9.0 ± 0.8[a]	–	–	–
	6	16.7 ± 1.2[a]	–	–	–
As. denudatus	8	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.3	0.7 ± 0.6
	9	0.1 ± 0.1	0.1 ± 0.1	0.8 ± 0.3	26.8 ± 8.3
	12	0.1 ± 0.1	0.3 ± 0.2	8.7 ± 2.9	28.8 ± 8.1
	15	2.5 ± 1.0	1.1 ± 0.7	9.0 ± 2.9	29.9 ± 8.0
	18	46.3 ± 13.9	1.5 ± 0.9	9.7 ± 2.8	31.1 ± 8.2
	21	52.3 ± 14.8	1.7 ± 1.2	9.8 ± 2.8	31.7 ± 8.3
Ps. petrakii	5	2.5 ± 0.9	0.7 ± 0.7	4.2 ± 0.5	14.7 ± 3.1
	6	9.5 ± 2.3	10.3 ± 0.9	18.2 ± 4.2	60.5 ± 5.1
	8	21.7 ± 2.4	21.8 ± 1.5	32.7 ± 4.7	94.5 ± 5.9
	10	45.2 ± 4.2	22.7 ± 1.8	39.5 ± 4.4	104.5 ± 7.7
	12	52.7 ± 5.5	24.8 ± 2.2	39.5 ± 4.4	104.5 ± 7.7
C. phlyctidospora	14	0.2 ± 0.1	1.3 ± 0.3	4.2 ± 1.4	0.0 ± T
	16	5.4 ± 1.4	1.6 ± 0.4	4.7 ± 1.6	7.1 ± 1.5
	18	5.7 ± 1.4	2.3 ± 0.6	4.9 ± 1.6	7.7 ± 1.6
	21	6.1 ± 1.4	2.6 ± 0.7	5.0 ± 1.6	8.5 ± 1.7
H. vinosophyllum	18	3.7 ± 1.0	0.0 ± T	0.0 ± T	0.0 ± T
	22	3.7 ± 1.0	0.0 ± T	0.0 ± T	0.0 ± T
	26	3.7 ± 1.0	1.5 ± 0.7	1.0 ± 0.8	0.2 ± 0.2
	30	3.7 ± 1.0	2.8 ± 0.8	2.0 ± 0.6	0.3 ± 0.2
	35	3.7 ± 1.0	3.5 ± 0.7	2.0 ± 0.6	0.5 ± 0.2

Values indicate means ± SE ($n = 5$)
T, less than 0.04; –, agar surface was already covered by mycelia
[a] Sporulation area (in cm^2)

Ps. petrakii at pH 8.0 and 9.0, and *Pe. moravecii* at all pH conditions. Basidioma formation of *T. tesquorum* was absolutely inhibited when co-cultured with *C. phlyctidospora* and *H. vinosophyllum* at all pH conditions.

Basidioma formation of *C. phlyctidospora* was accelerated by the co-culture with other early-stage EP fungus, *Am. botrytis* at pH 5.5, *As. denudatus* at pH 9.0, *Ps. petrakii* at pH 5.5, and *Pe. moravecii* under all pH conditions. Moreover, basidioma formation of *C. phlyctidospora* was accelerated by co-cultivation with *T. tesquorum* and *H. vinosophyllum* at all pHs.

Basidioma formation of *H. vinosophyllum* was delayed by co-culture with *T. tesquorum* and *C. phlyctidospora*, irrespective of pH conditions.

Effect of pH on number of reproductive structures by intraspecific co-culture

In the culture for 3 days, *Am. botrytis* had the highest number of conidiophores at pH 9.0, then gradually decreased those numbers with decreasing pH value (Table 6). After 5 and 6 days of inoculation, mycelia of *Am. botrytis* covered the whole medium surface at pH 7.0, 8.0, and 9.0. The highest number of ascomata of *As. denudatus* observed at 9 days of co-culture was at pH 9.0, but it was also observed at pH 5.5 after 21 days. *Ps. petrakii* reached the highest number of ascomata at pH 9.0 after 8 days of co-culture. *C. phlyctidospora* reached the highest count at pH 8.0 after 14 days of co-culture and at pH 9.0 after 16 days (Table 6).

Hebeloma vinosophyllum reached the highest number of basidiomata at pH 5.5 after 18 days of co-culture. After 35 days cultivation at pH 5.5 and 7.0, *H. vinosophyllum* similarly had the highest counts (Table 6).

Effect of pH on the site and number of basidioma formation of *C. phlyctidospora*

In co-culture with *Am. botrytis*, *C. phlyctidospora* formed basidiomata both in its territory and in the territory of the opposite fungal species, irrespective of pH conditions, but could not form them at the opposite side of the contact zone with *Am. botrytis* at pH 5.5 (Fig. 1A–C). The number of basidiomata of *C. phlyctidospora* decreased at pH 8.0 and 9.0. In co-culture with *As. denudatus*, *C. phlyctidospora* formed basidiomata both in its territory and in the territory of *As. denudatus* under neutral to alkaline conditions, but at pH 5.5, *C. phylctidospora* did not form basidiomata in the territory of *As. denudatus* (Fig. 1D–F). In co-culture with *Ps. petrakii*, *C. phlyctidospora* formed basidioma on the contact zone at pH 9.0. Basidioma formation of *C. phlyctidospora* decreased at pH 8.0 and 9.0. In contrast, ascoma formation of *Ps. petrakii* was strongly stimulated at the contact zone in this co-culture (Fig. 1G–I). In co-culture with *Pe. moravecii*, the number of basidiomata of *C. phlyctidospora*, which were formed at the contact zone, decreased at pH 5.5, 7.0, and 9.0.

In the co-culture of *C. phlyctidospora* with *T. tesquorum*, *C. phlyctidospora* formed basidiomata within its territory. The number of basidiomata of *C. phlyctidospora* increased

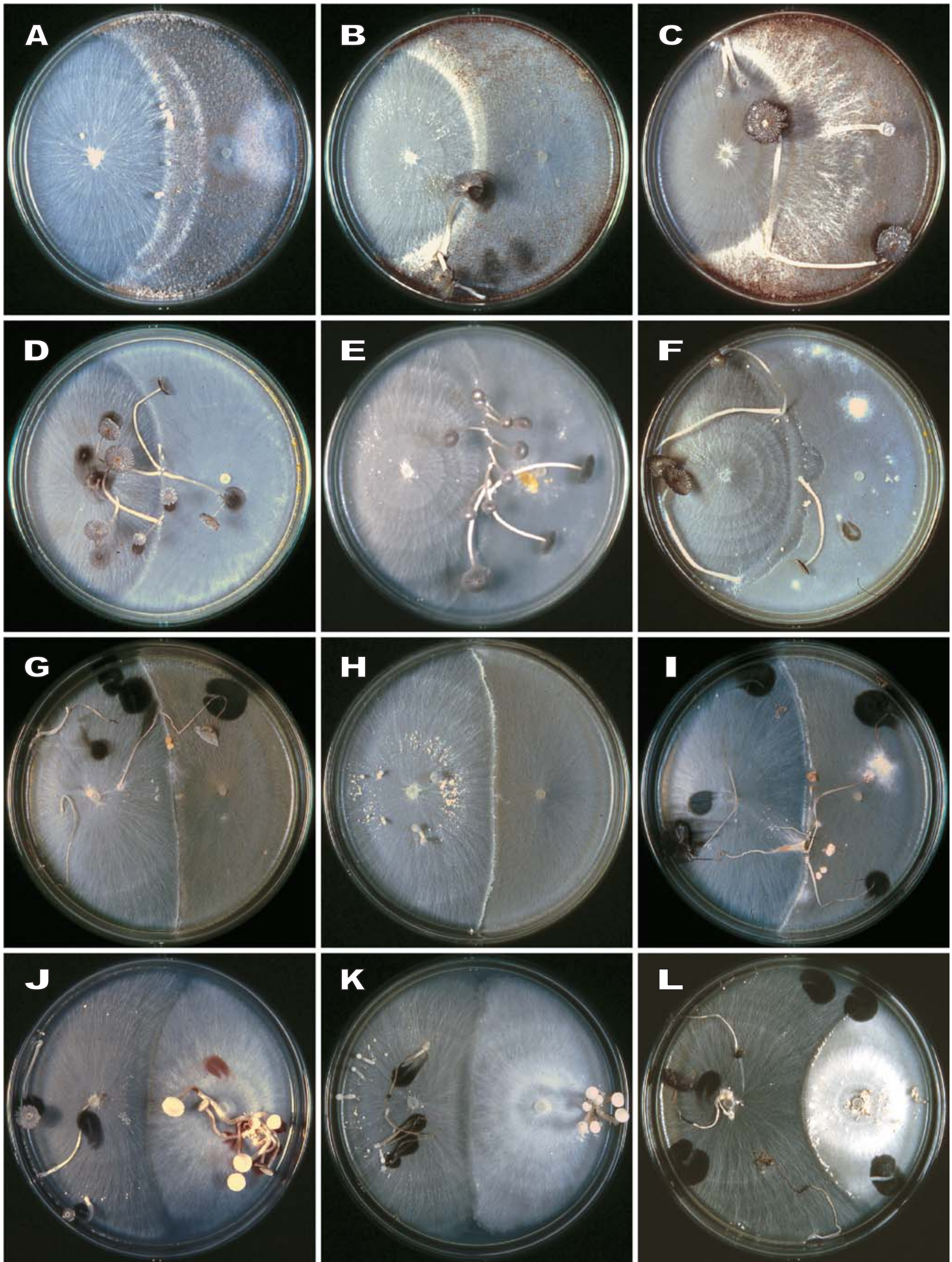
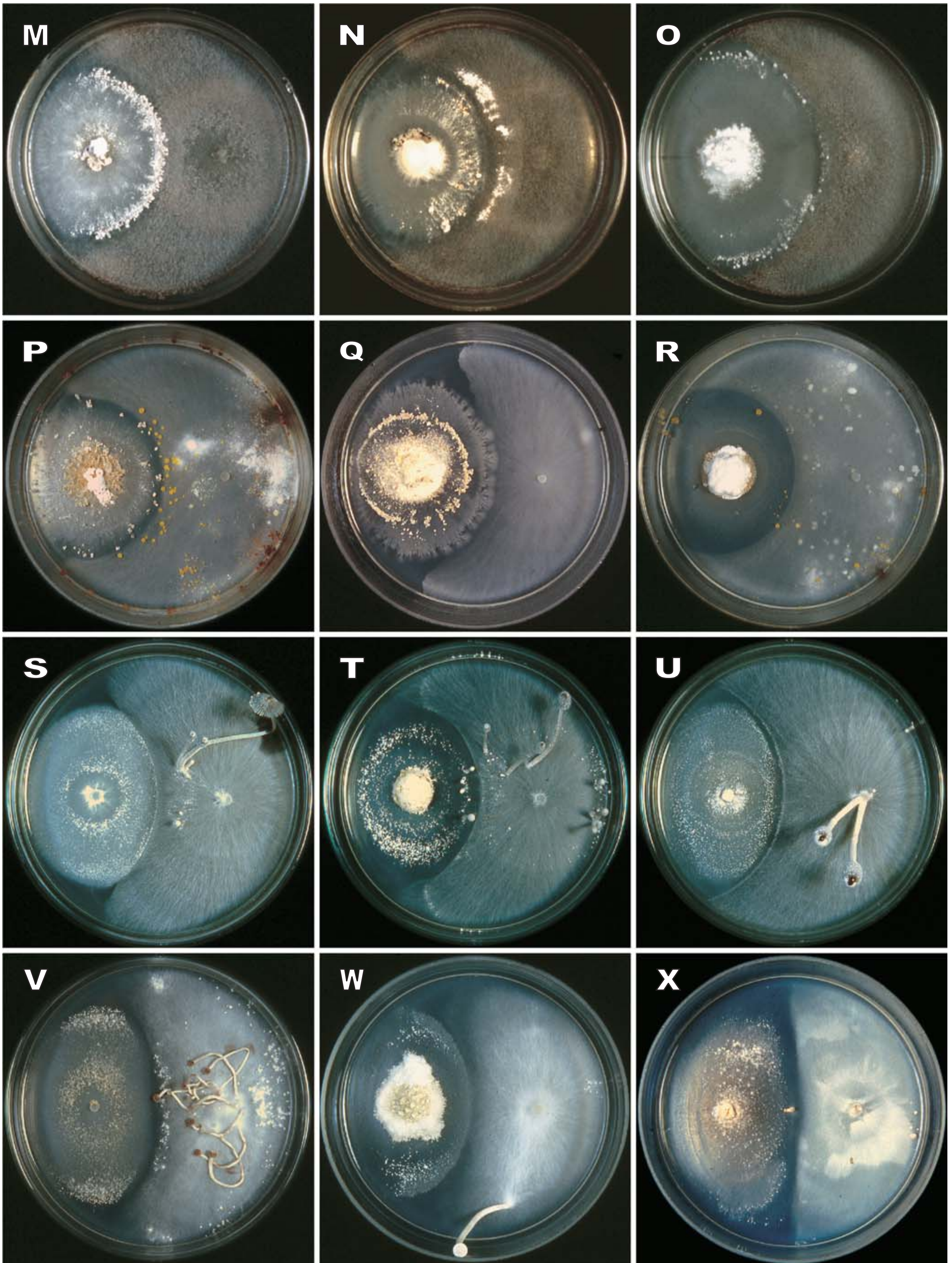


Fig. 1. Interactions between ammonia fungi. **A–C** *Coprinopsis phlyctidospora (left)* and *Amblyosporium botrytis (right)*: pH 5.5, 18th day (**A**), pH 7, 18th day (**B**), pH 9, 18th day (**C**). **D–F** *C. phlyctidospora (left)* and *Ascobolus denudatus (right)*: pH 5.5, 18th day (**D**), pH 7, 18th day (**E**), pH 9, 18th day (**F**). **G–I** *C. phlyctidospora (left)* and *Pseudombrophila petrakii (right)*: pH 5.5, 20th day (**G**), pH 7, 20th day (**H**), pH 9, 20th day (**I**). **J–L** *C. phlyctidospora (left)* and *Hebeloma vinosophyllum (right)*: pH 5.5, 27th day (**J**), pH 7, 30th day (**K**), pH 9, 27th day (**L**).



M–O *Tephrocybe tesquorum (left)* and *Am. botrytis (right)*: pH 5.5, 30th day (**M**), pH 7, 36th day (**N**), pH 9, 30th day (**O**). **P–R** *T. tesquorum (left)* and *As. denudatus (right)*: pH 5.5, 42nd day (**P**), pH 7, 30th day (**Q**), pH 9, 30th day (**R**). **S–U** *T. tesquorum (left)* and *C. phlyctidospora (right)*: pH 5.5, 24th day (**S**), pH 7, 24th day (**T**), pH 9, 24th day (**U**). **V–X** *T. tesquorum (left)* and *H. vinosophyllum (right)*: pH 5.5, 51st day (**V**), pH 7, 36th day (**W**), pH 9, 42nd day (**X**). The days mentioned in this legend refers to the day the photograph was taken. It also refers to days following later inoculation in the co-culture (cf. Table 3)

at pH 7.0 and 8.0. *T. tesquorum* fromed basidiomata within its territory, irrespective of pH conditions (Fig. 1S–U). Interestingly, no matured reproductive structures of *T. tesquorum* were observed in this co-culture.

In the co-culture with *C. phlyctidospora* and *H. vinosophyllum*, basidiomata of *C. phlyctidospora* were not formed in the opposite fungal territory, irrespective of pH conditions (Fig. 1J–L). Moreover, at pH 5.5, 7.0, and 8.0, both fungi formed basidiomata in their territory but not at the contact zone. However, at pH 9.0, basidiomata of *H. vinosophyllum* were observed within the contact zone (Fig. 1J–L).

Discussion

The early-stage EP ammonia fungi, except for *Ps. petrakii*, can invade into the opposed fungal species territory; that is, they have no difficulty in colonization into any ammonia fungal territory under neutral and alkaline conditions (see Table 4). Late-stage EP fungi can invade into a substrate that has been completely occupied by the early-stage EP fungi because they need to have strong invasion abilities against early-stage EP fungi for establishing their territories. In contrast, LP ammonia fungi did not invade into the territories of late-stage EP fungi even if the pH condition was acidic (see Table 4, Fig. 1J–L, V–X). It is likely that their invasion abilities are too weak, and that it is not easy for LP fungi to obtain enough nutrients from the territory of EP fungi if the LP fungi did not produce symbiotic mycorrhizae with the host plant to obtain sufficient carbon and to sustain their colonies.

The present study also shows that pH and concentration of ammonium-nitrogen are principal environmental factors affecting the vegetative growth until reproductive structure formation of ammonia fungi. Interaction between ammonia fungi also functions as a similar factor. Formation of reproductive structures, even in the territory of the opposed ammonia fungus, also agrees with the idea of neutralism. This type of interaction on the same resources allows coexistence of ammonia fungi. Stahl and Christensen (1992) indicated various commensalisms in *Penicillium restrictum–Microdochium bolleyi* co-culture, where both fungi intermingled with each other, accompanied by growth stimulation in *M. bolleyi*. Such phenomenon may be the same mechanism of interaction among *Am. botrytis*, *As. denudatus*, and *Pe. moravecii*.

The reason for the acceleration of reproductive structure formation observed in some co-cultures is not known. It has been shown that in the interactions between saprotrophic cord-forming fungi, even individuals of the same species may release nutrients which the opposing fungus can sequester (Boddy 1993). Rayner and Boddy (1988) described that benefits from fungal interaction can be derived from a variety of causes: (1) waste products or exudates from one organism may provide a resource for the other; (2) products from one organism may stimulate vegetative or reproductive growth of another; or (3) complementary enzyme action may be achieved. We suppose that the acceleration of reproductive structure formation of ammonia fungi in this study may be caused by the benefits they obtain from the other fungus in the co-culture condition.

The interactions among ammonia fungi under different pH regimens either stimulate or inhibit the reproductive structure formation at the interaction area or contact zone during co-culture. Thus, mycelial interactions may be associated with pH.

The duration of vegetative growth until reproductive structure formation of each ammonia fungus was affected by interactions with other ammonia fungi. This study suggests that the period of reproductive structure formation of each ammonia fungus depends not only on the pH of the media, but also the mycelial interaction among ammonia fungi.

Yamanaka (1999, 2003) pointed out that the successive appearance of ammonia fungi from EP to LP species may be controlled by the changes in both pH and the form of inorganic nitrogen. Suzuki (1989, 2006) also proposed that the sequential propagation of each ammonia fungi may be explained by their preference of or tolerance to high concentrations of ammonium nitrogen under alkaline to neutral conditions as well as by their adaptation to different pH conditions.

Successive occurrence of ammonia fungi is caused not only by the physiochemical characteristics of soil, such as pH and nitrogen concentration, but also by the interspecific interactions among ammonia fungi associated with pH and nitrogen concentration.

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References

Boddy L (1993) Saprotrophic cord-forming fungi: warfare strategies and other ecological aspects. Mycol Res 97:641–655

Boddy L (2000) Interspecific combative interactions between wood-decaying basidiomycetes. FEMS Microbiol Ecol 31:185–194

Boddy L, Abdalla SHM (1998) Development of *Phanerochaete velutina* mycelial cord systems: effect of encounter of multiple colonized wood resources. FEMS Microbiol Ecol 25:257–269

Cooke RC, Rayner ADM (1984) Ecology of saprotrophic fungi. Longman, London

Dix NJ, Webster J (1995) Fungal ecology. Chapman and Hall, London

Dowson CG, Rayner ADM, Boddy L (1988) The form and outcome of mycelial interactions involving cord-forming decomposer basidiomycetes in homogeneous and heterogeneous environment. New Phytol 109:423–432

Enokibara S, Suzuki A, Fujita C, Kashiwagi M, Mori N, Kitamoto Y (1993) Diversity of pH spectra of cellulolytic enzymes in Basidiomycetes (in Japanese). Trans Mycol Soc Jpn 34:221–228

Fukiharu T, Sato Y, Suzuki A (1997) The occurrence of ammonia fungi, and changes in soil conditions and decay rate of bamboo in response to application of a large amount of urea in a bamboo grove in Chiba Prefecture, central Japan. Bull Fac Educ Chiba Univ (III Nat Sci) 45:61–67

He X, Suzuki A (2003) Effect of nitrogen resources and pH on growth and fruit body formation of *Coprinopsis phlyctidospora*. Fungal Divers 12:35–44

He X, Suzuki A (2004) Effects of urea treatment on litter decomposition in *Pasania edulis* forest soil. J Wood Sci 50:266–270

Holmer L, Stenlid J (1996) Diffuse competition for heterogenous substrate in soil among six species of wood-decomposing basidiomycetes. Oecologia (Berl) 106:531–538

Licyayo DML, Suzuki A (2006) Growth responses of ammonia fungi to different concentrations of ammonium-nitrogen. Mushroom Sci Biotechnol 14:21–31

Morimoto N, Suda S, Sagara N (1981) Effect of ammonia on fruit-body induction of *Coprinus cinereus* in darkness. Plant Cell Physiol 22:247–254

Morimoto N, Suda S, Sagara N (1982) The effects of urea on the vegetative and reproductive growth of *Coprinus stercorarius* in pure culture. Trans Mycol Soc Jpn 23:79–83

Rayner ADM, Boddy L (1988) Fungal decomposition of wood: its biology and ecology. Wiley, Chichester

Sagara N (1975) Ammonia fungi – a chemoecological grouping of terrestrial fungi. Contrib Biol Lab Kyoto Univ 24:205–276

Sagara N (1992) Experimental disturbances and epigeous fungi. In: Carroll GC, Wicklow DT (eds) The fungal community: its organization and role in ecosystem, 2nd edn. Dekker, New York, pp 427–454

Sagara N (1995) Association of ectomycorrhizal fungi with decomposed animal wastes in forest habitats: a cleaning symbiosis? Can J Bot 73 (suppl 1):S1423–S1433

Sato Y, Suzuki A (1997) The occurrence of ammonia fungi, and changes in soil conditions and decay rate of bamboo in response to application of a large amount of urea in a *Quercus serrata* dominated mixed forest in Meguro, Tokyo. Bull Fac Educ Chiba Univ (III Nat Sci) 45:53–59

Shaw TM, Dighton J Sanders FE (1995) Interactions between ectomycorrhizal and saprotrophic fungi on agar in association with seedling of lodgepole pine (*Pinus contorta*) Mycol Res 29:159–165

Soponsathien S (1998a) Some characteristics of ammonia fungi 1. In relation to their ligninolytic enzyme activities. J Gen Appl Microbiol 44:337–345

Soponsathien S (1998b) Study in the production of acetyl esterase and side-group cleaving glycosidases of ammonia fungi. J Gen Appl Microbiol 44:389–397

Stahl P, Christensen M (1992) In vitro mycelial interactions among member of a soil microfungal community. Soil Biol Biochem 24:309–316

Suzuki A (1978) Basidiospore germination by aqua ammonia in *Hebeloma vinosophyllum*. Trans Mycol Soc Jpn 19:362

Suzuki A (1989) Analyses of factors affecting the occurrence and succession of the ammonia fungi. In: Hattori T, Ishida Y, Maruyama Y, Morita RY, Uchida A (eds) Recent advances in microbial ecology (ISME 5). Japan Scientific Society Press, Tokyo, pp 275–279

Suzuki A (2000) A survey of species assemblage of ammonia fungi (in Japanese). Nat Hist Plant (Planta) 68:27–35

Suzuki A (2006) Experimental and physiological ecology of ammonia fungi: studies using natural substrates and artificial media. Mycoscience 47:3–17

Suzuki A, Motoyoshi N, Sagara N (1982) Effects of ammonia, ammonium salts, urea, and potassium salts on basidiospore germination in *Coprinus cinereus* and *Coprinus phlyctidosporus*. Trans Mycol Soc Jpn 23:217–224

Suzuki A, Uchida M, Kita Y (2002) Experimental analyses of successive occurrence of ammonia fungi in the field. Fungal Divers 10:141–165

Yamanaka T (1995a) Changes in organic matter composition of forest soil treated with a large amount of urea to promote ammonia fungi and the abilities of these fungi to decompose organic matter. Mycoscience 36:17–23

Yamanaka T (1995b) Nitrification in a Japanese red pine forest soil treated with a large amount of urea. J Jpn For Soc 77:232–238

Yamanaka T (1995c) Changes in soil conditions following treatment with a large amount of urea to enhance fungal fruiting-body production in a Japanese red pine forest. Bull Jpn Soc Microb Ecol 10:67–72

Yamanaka T (1999) Utilization of inorganic and organic nitrogen in pure cultures by saprotrophic and ectomycorrhizal fungi producing sporophores on urea-treated forest floor. Mycol Res 103:811–816

Yamanaka T (2001) Fruit-body production and mycelial growth of *Tephrocybe tesquorum* in urea-treated forest soil. Mycoscience 42:333–338

Yamanaka T (2003) The effect of pH on the growth of saprotrophic and ectomycorrhizal ammonia fungi in vitro. Mycologia 95:584–589