Mycoscience (2006) 47:3–17
DOI 10.1007/s10267-005-0270-8

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PRESIDENTIAL ADDRESS

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Experimental and physiological ecology of ammonia fungi: studies using natural substrates and artificial media

Received: October 19, 2005 / Accepted: November 21, 2005

Abstract Experiments using natural substrates and artificial media were categorized into three groups. Using experiments in these three categories, we investigated dispersion, invasion sequence, and decomposing ability of ammonia fungi in the field on natural substrates. Spore germination and vegetative and reproductive growth of ammonia fungi derived from monocultures and five-species cultures were assessed on natural substrates and on artificial media. These features assist understanding of the colonization and successive occurrence of ammonia fungi in the field based on estimation of interactions among the fungi and the physiological characteristics of each ammonia fungus. The sequential colonization and fruiting of ammonia fungi in the field can be explained primarily by the preference or tolerance of a species to high concentrations of ammonium-N under alkaline to neutral conditions. Succession of ammonia fungi results from their sequential colonization associated with the time needed for each fungus to produce reproductive structures. Colonization likely initiates from mycelia and/or spores preinhabiting the soil and from newly invading airborne propagules. Duration of occurrence of ammonia fungi in the field is shortened by the interactions among them. Saprobic ammonia fungi are the principal agents of litter decomposition under alkaline to neutral conditions.

Key words Ammonia fungi · Colonization · Interspecific interactions · Multiple species culture · Spore germination

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Parts of this review were presented as the presidential address of the Mycological Society of Japan (MSJ) at the Mycological Society of America (MSA) and MSJ Joint Meeting 2005 held in Hawaii in August 2005

Introduction

Research on ammonia fungi, first initiated by Dr. Naohiko Sagara (Professor Emeritus of Kyoto University, Japan) (Sagara 1975; cf. Sagara and Hamada 1965; Sagara 1973), is one of the major achievements in the 50-year history of the Mycological Society of Japan (MSJ) and has maintained leadership by Japanese mycologists. With this background and following my personal research interests, I have chosen ammonia fungi as my topic for the presidential address of MSJ. In this article, I review knowledge of the experimental and physiological ecology of ammonia fungi, attained because for these 35 years I have been studying the physiology of basidiomycota, especially the effect of environmental factors on fungal morphogenesis, with many colleagues (Kitamoto et al. 1972, 1974, 1986; Suzuki et al. 1976; Fujimoto et al. 1982; Kinugawa et al. 1986, 1994; Watanabe and Suzuki 1995; Watanabe et al. 1994a,b, 1996; Koyama et al. 1997, 2002; Ban-nai et al. 1997, 2005; Murata et al. 2001; Arai et al. 2004). I then began research on ammonia fungi based on this background in physiology research.

Ammonia fungi are defined as a chemoecological group of fungi that sequentially develop reproductive structures exclusively or relatively luxuriantly on the soil after the sudden addition of ammonia, or of some other nitrogenous material that reacts as a base, or of alkalis (Sagara 1975). The sequential appearance of reproductive structures (=succession) of these fungi in the field generally proceeds as follows: anamorphic fungi → Ascomycota → smaller Basidiomycota → larger Basidiomycota (Sagara 1975). Ammonia fungi can be divided into two groups based on their succession in the field. One group comprises species that appear in the early phase in the succession (EP fungi; anamorphic fungi → Ascomycota → smaller Basidiomycota) while those of the second group appear in the late phase of the succession (LP fungi; larger basidiomycota) (Sagara 1995; Yamanaka 1999, 2003; Imamura and Yumoto 2004). All the EP fungi are saprobic (=saprotrophic) (Yamanaka 1999, 2003), mostly litter-decomposing fungi as speculated based on their observed litter-decomposing abilities

(Enokibara et al. 1993; Yamanaka 1995a; Soponsathien 1998a,b; cf. He and Suzuki 2004). In contrast, most of the LP fungi are biotrophic and are characterized as ectomycorrhizal symbionts (Sagara 1995). Ammonia fungi can be also divided into two groups based on the appearance of the reproductive structures (=occurrence) in the laboratory and in the field. One group comprises species that occur under laboratory conditions and another group comprises species that occur only in the field when nitrogenous materials such as urea are applied (Sagara 1975).

In ecosystems, ammonia fungi have a role in the immobilization of nitrogenous degradation products from animal wastes such as urine, feces, and dead bodies (cf. Sagara 1976a,b, 1981, 1984, 1992, 1995; Morimoto et al. 1981; Sagara et al. 1985, 2000; Miller and Hilton 1987; Yamanaka 1995a, 1999; Wang and Sagara 1997; Fukiharu et al. 2000a,b; Harmaja 2002; He and Suzuki 2003; Tibbett and Carter 2003; Suzuki 2004) as well as the decomposition of plant materials (Fukiharu et al. 1997; Sato and Suzuki 1997; He and Suzuki 2004).

Ammonia fungi have been recorded in Asia, Oceania (Australia, New Zealand), Hawaii, Europe, North Africa, North America, and South America (Fukiharu and Horigome 1996; Suzuki et al. 2003). In other words, ammonia fungi are distributed from the sub-Arctic to south temperate region, and possibly also in sub-Antarctic and tropical lowland regions. Many ammonia fungi have been recognized as belonging also to other ecological categories, such as fungicolous fungi, coprophilous fungi (dung fungi), pyrophilous fungi (fireplace fungi), etc. (Sagara 1975, 1976a, 1992; Suzuki 2004).

Field experiments versus laboratory experiments

Not only field experiments but also laboratory experiments are indispensable to analyze the ecology of fungi. For both field and laboratory experiments, natural substrates as well as artificial media have been adopted as useful experimental methods to examine fungus ecology. Experiments using natural substrates and artificial media have advantages and disadvantages.

In the field, fungi grow three dimensionally and many of them decompose and utilize organic polymers such as cellulose, hemicellulose, lignin, and chitin (Dickinson and Pugh 1974). Therefore, their cultivation using natural substrates provides the most appropriate conditions for examination of fungal growth, especially for saprobic fungi. Experiments using natural substrates are relevant to understanding the growth of each fungus in the field and for examination of interactions among different species of microorganisms; natural substrates provide similar conditions to those in the field by maintaining heterogeneity and a three-dimensional structure. For mixed cultures (multiple species cultures), natural substrates also have the same advantages as already described. On the other hand, experiments using natural substrates have a disadvantage in quantitative examination of vegetative growth of each fungus, because it is difficult to observe mycelia and to distinguish fungus cells (hyphae) from the natural substrates. Cultivation of fungi on artificial media is more suitable for quantitative research on the physiological characteristics of each fungus, especially for examination for potential growth rates of each fungus. A challenge with artificial media, however, is controlling moisture conditions, one of the most important environmental factors for fungal growth.

In the following, I show several examples of experiments using natural substrates and artificial media to examine mechanisms of colonization (=propagation) and occurrence of ammonia fungi. The experiments using natural substrates and artificial media are categorized into three kinds of experiments: field experiments, laboratory experiments associated with field experiments, and laboratory experiments. The categories "laboratory experiments associated with field experiments" and "laboratory experiments" are strictly overlapping when natural substrates are used for both kinds of experiments. Here, I categorize cultivation using natural substrates from the field without any treatments as "laboratory experiments," whereas those using natural substrates after some treatment of the soils are designated "laboratory experiments associated with field experiments."

Field experiments

Chemical treatments in the field

The mycobiota (=fungal species assemblage, fungal flora) of some ecological groups of fungi has been examined by the application of chemicals on the surface of the forest floor and that of a weed community (Sagara 1975, 1976a, 1992). Such treatment is a kind of field-based enrichment culture. This method has the advantage over laboratory-based enrichment culturing in that it allows the efficient survey of mycorrhizal fungi (cf. Sagara 1975, 1976a, 1992; Fukiharu and Horigome 1996; Suzuki 2000a; Suzuki et al. 2003) along with saprobic fungi, especially macromycetes from large domains (cf. Sagara 1975). However, it is likely that there are many fungal species growing vigorously in the substrates and stimulated by the chemical treatments but which do not produce any reproductive structures.

As previously described, the mycobiota of ammonia fungi has been examined by application of substances such as aqueous ammonia, ammonium salts yielding alkalinity in soil, and urea in different vegetation types (Sagara 1975; Suzuki 1992, 2000a; Fukiharu and Hongo 1995; Yamanaka 1995a–c; Fukiharu and Horigome 1996; Fukiharu et al. 1997; Sato and Suzuki 1997; Imamura 2001; Suzuki et al. 2002a; Nagao et al. 2003; He and Suzuki 2004; Imamura and Yumoto 2004) as well as by chemical treatment of substrates in the laboratory level (Sagara 1975, 1976a,c, 1992; Fukiharu and Horigome 1996; Suzuki et al. 2002a; Suzuki 2004).

Fig. 1. Embedding litter bags and wood blocks in the field (experiment 1)

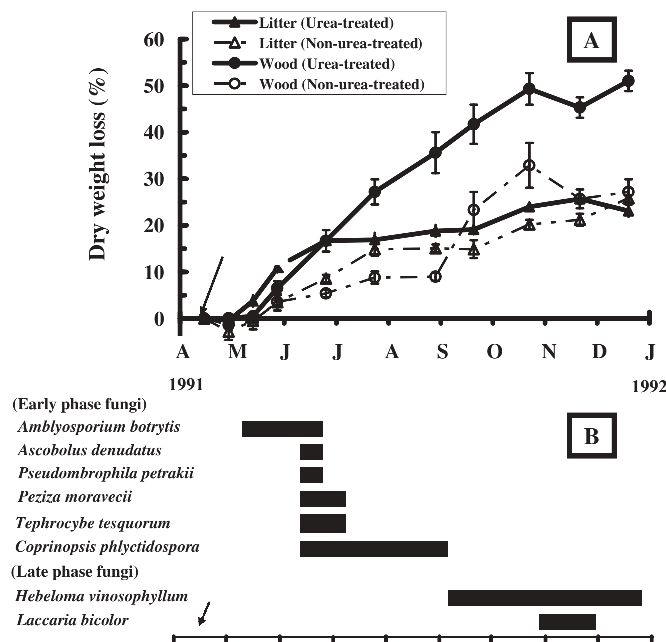

Fig. 2. Decomposition of litter and wood blocks in urea-treated soils (experiment 1). *Arrow*, urea application. **A** Percentage dry weight loss of litter and wood blocks. **B** Occurrence of ammonia fungi in the field, where both urea-treated samples were embedded

Embedding litter bags and wood pieces in the field

The litter bag method is appropriate for field analysis of the litter decomposition capabilities of microorganisms (Robertson and Paul 2000; Wall and Reichman 2000), although inevitably the method involves disturbance when the bags are embedded in soil and restrict the movement of soil animals. The rate of decay of embedded litter is also affected by the seasonal timing of experimentation and selection of the soil horizon for the experiment.

Litter decomposition abilities of ammonia fungi were studied by the examination of changes in the decomposition rates of a mixture of fallen leaves of broadleaf trees (leaf litter) and wood blocks that were embedded in a mixed forest soil after urea application (Fig. 1). The results indicated that urea application stimulates decomposition of leaf litter and wood, especially when EP fungi are present. This tendency was more marked in the wood blocks than in the leaf litter (Fig. 2). In *Pasania edulis* forest, the decomposition of embedded stem sticks of *P. edulis* was accelerated by urea application whereas the decomposition of embedded fallen leaves (leaf litter) was not accelerated (He and Suzuki 2004).

These results indicate that decomposition, at least in part, involves saprobic ammonia fungi. Such a conclusion is also supported by the specific activities of cellulolytic and ligninolytic enzymes in saprobic ammonia fungi, with cellulolytic enzymes having an optimal pH between 7 and 9 (Enokibara et al. 1993) and ligninolytic enzymes having a wide optimal spectra from weakly acidic to weakly alkaline conditions (Soponsathien 1998a,b).

Laboratory experiments associated with field experiments

Experiments using natural substrates

Cultivation of soils treated in the field

Invasion sequence of fungi in the field. The invasion sequence of saprobic ammonia fungi in the field following urea application was examined by the cultivation of packed soils, which had been collected from the forest floor at different days after urea application, in sterilized test tubes with cotton plugs. These results suggest that the first to invade was *Amblyosporium botrytis* 12 days after the application of urea, followed by *Ascobolus denudatus*, *Peziza moravecii*, and *Coprinopsis phlyctidospora*, which invaded 18 days after the urea application. Finally, *Tephrocybe tesquorum* invaded 43 days after urea was applied (Fig. 3). This sequence of invasion by the five ammonia fungi roughly equated with the observed succession in the forest, although the sequence of *C. phlyctidospora* and *T. tesquorum* was reversed (see Fig. 3).

These results suggest that the succession of ammonia fungi in the field partially results from the sequential colonization of each ammonia fungus accompanied by the time needed for formation of reproductive structures by each fungus in urea-treated soils (cf. Suzuki 1989).

Invasion of ammonia fungi by airborne spores/hyphae. The invasion of ammonia fungi by airborne propagules, such as spores and pieces or fragments of hyphae, was examined by sampling sterilized soils collected at different times after urea application (Figs. 4, 5). Reproductive structures of the three saprobic ammonia fungi *Amblyosporium botrytis*, *Ascobolus denudatus*, and *Coprinopsis phlyctidosora* were observed on the sample soils. Of the three fungi, *C. phlyctidospora* was isolated at the highest frequency (Table 1).

This observation suggests that airborne spores and/or hyphae of saprobic ammonia fungi can be the invasive

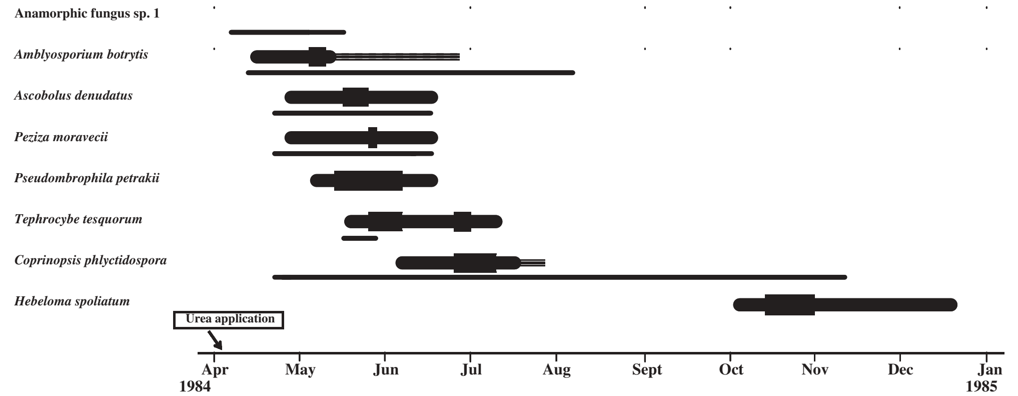

Fig. 3. Invasion sequence of ammonia fungi in the field (experiment 2). In site I, 800 g/m^2 of urea was applied on April 3, 1984 (*arrow*). ⬬, Duration of occurrence of each ammonia fungus; ■, duration of occurrence peak of each ammonia fungus; ═, duration of reduction in occurrence of each ammonia fungus; ▬, duration of propagation of each ammonia fungus, estimated by incubation of soils that were collected from L-A_1 horizon (P). P is expressed as the duration between two soil sampling dates, the first being the date of initial appearance of reproductive structures of each fungus in incubated soil and the second being the date at which final disappearance of the reproductive structures of each fungus was observed. (From Suzuki et al. 2002b, with permission)

Fig. 4. Sampling experiment in the field: sampling apparatus (experiment 3)

propagules, although we cannot deny the possibility that the invasion resulted from spores and/or hyphae carried by animals from the air and/or as contaminants on the sampling equipment. Unfortunately, it is not possible to test for airborne propagules of mycorrhizal ammonia fungi by this kind of sampling experiment.

Spore germination of ammonia fungi on sterilized soils

Monocultures. Spore germination of five ammonia fungi, the saprobic *Amblyosporium botrytis*, *Ascobolus denudatus*, *Tephrocybe tesquorum*, and *Coprinopsis phlyctidospora*, and the ectomycorrhizal *Hebeloma vinosophyllum*, was evaluated on the sterilized soils (L-F horizons) collected at different times after urea application in the field. They were then incubated at 20.0° ± 0.5°C in a light (16 h) and dark (8 h) regime.

All tested ammonia fungi formed reproductive structures on several urea-treated soils (Suzuki et al., unpublished data). These results indicate that at least the spores of selected saprobic and mycorrhizal ammonia fungi germinate on urea-treated soils.

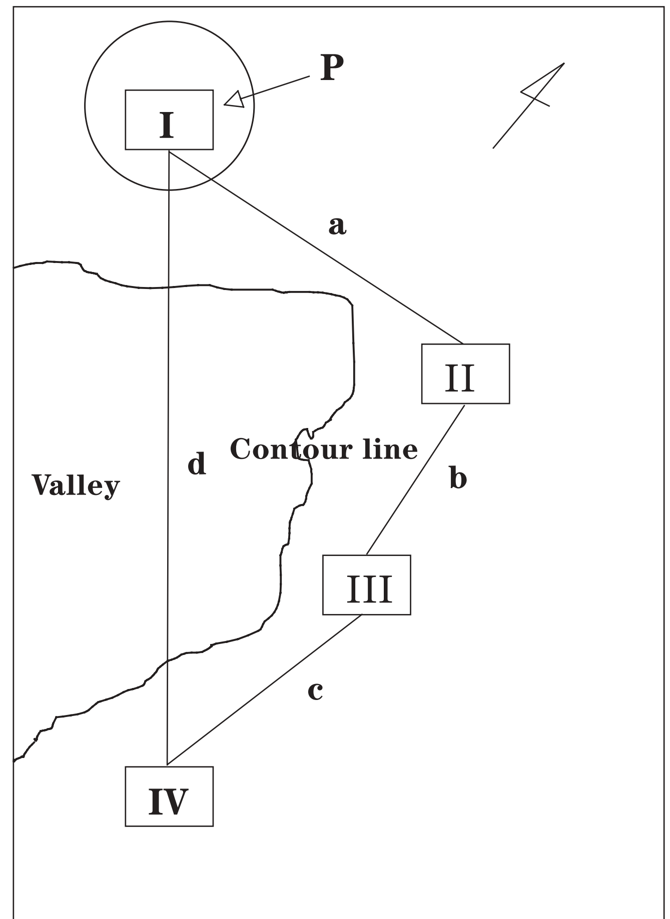

Fig. 5. Sampling experiment in the field: distance between sampling sites (experiment 3). *P*, area where urea treatment (800 g/m^2) was applied 2, 3, 4, and 5 years before the sampling experiment. Each of the urea plots in the area was 2–5 m apart from the spot where a desk was placed. *a*, 31 m; *b*, 18 m; *c*, 21 m; *d*, 56 m

Table 1. Invasion of ammonia fungi by airborne spores/hyphae in the field (experiment 3)

a.

Sample site	Sample soil	Frequency of occurrence of:			
		Unidentified fungi[a]	*Amblyosporium botrytis*	*Ascobolus denudatus*	*Coprinopsis phlyctidospora*
I	A[b]	43/47	0/47	1/47	1/47
	B[c]	16/24	0/24	7/24	8/24
II	A	17/45	0/45	0/45	0/45
	B	7/21	0/21	0/21	1/21
III	A	27/46	0/46	0/46	1/46
	B	6/21	0/21	0/21	1/21
IV	A	6/46	0/46	0/46	1/46
	B	3/23	0/23	0/23	0/23

b.

Sample site	Sample soil	Frequency of occurrence of:			
		Unidentified fungi[a]	*Amblyosporium botrytis*	*Ascobolus denudatus*	*Coprinopsis phlyctidospora*
I	A	8/47	0/47	0/47	2/47
	B	10/24	1/24	3/24	1/24
II	A	1/47	0/47	0/47	0/47
	B	6/23	0/23	2/23	0/23
III	A	0/47	0/47	0/47	0/47
	B	7/23	0/23	0/23	0/23
IV	A	18/47	0/47	0/47	0/47
	B	19/24	0/24	0/24	0/24

c.

Sample site	Sample soil	Frequency of occurrence of:			
		Unidentified fungi[a]	*Amblyosporium botrytis*	*Ascobolus denudatus*	*Coprinopsis phlyctidospora*
I	A	8/38	3/38	0/38	5/38
	B	6/14	0/14	0/14	0/14
II	A	0/37	0/37	0/37	1/37
	B	5/12	0/12	0/12	0/12
III	A	3/36	0/36	0/36	1/36
	B	1/12	0/12	0/12	0/12
IV	A	1/39	0/39	0/39	0/39
	B	0/13	0/13	0/13	1/13

Numerator: number of bottles in which occurrence of each ammonia fungal species was observed
Denominater: number of bottles placed in the field
[a] Anamorphic fungi
[b] Mixture of soils of the L-F and H-A horizons collected 6 days after urea application: moisture content 66.0%, pH 8.8, 1570.7 mg NH_4-N/100 g dry soil, 0.4 mg NO_2-N/100 g dry soil, 648.4 mg NO_3-N/100 g dry soil
[c] Mixture of soils of the L-F and H-A horizons collected 16 days after urea application: moisture content 71.0%, pH 8.3, 1255.2 mg NH_4-N/100 g dry soil, 0.1 mg NO_2-N/100 g dry soil, 803.2 mg NO_3-N/100 g dry soil

Vegetative growth and formation of reproductive structures of ammonia fungi on sterilized soils

Monoculture and five-species culture. The mycelia of four EP fungi, *Amblyosporium botrytis*, *Ascobolus denudatus*, *Tephrocybe tesquorum*, and *Coprinopsis phlyctidospora*, and the mycelium of the LP fungus *Hebeloma vinosophyllum* were inoculated separately (monoculture) or together (five-species culture) on the surface of sterilized soils collected at different times after urea application. As a control, the mycelia of the five ammonia fungi were also inoculated separately or together on the surface of gamma ray-sterilized non-urea-treated soils collected on the same date as the urea-treated soils. In monoculture, both EP and LP fungi grew well on all urea-treated soils except for the soils collected just a few days after urea application (Fig. 6). The growth rate of vegetative mycelia of all tested fungi increased for soils collected at the time of normal occurrence of each species in the field (Suzuki et al., unpublished data; cf. Suzuki 1989).

Reduced formation of reproductive structures was observed in five-species culture of the EP fungi *Ascobolus denudatus*, *Tephrocybe tesquorum*, and *Coprinopsis phlyctidospora*, and also for the LP fungus *H. vinosophyllum*, but not the EP fungus *Amblyosporium botrytis* (Fig. 6). This tendency was more drastic in *T. tesquorum*; that is, its fruiting was absolutedly inhibited in five-species cultures except for the five-species cultures using the urea-treated soils collected at its fruiting period in the field (see Fig. 6).

It appears, therefore, that ammonia fungi grow vigorously on soils with relatively high ammonium nitrogen concentra-

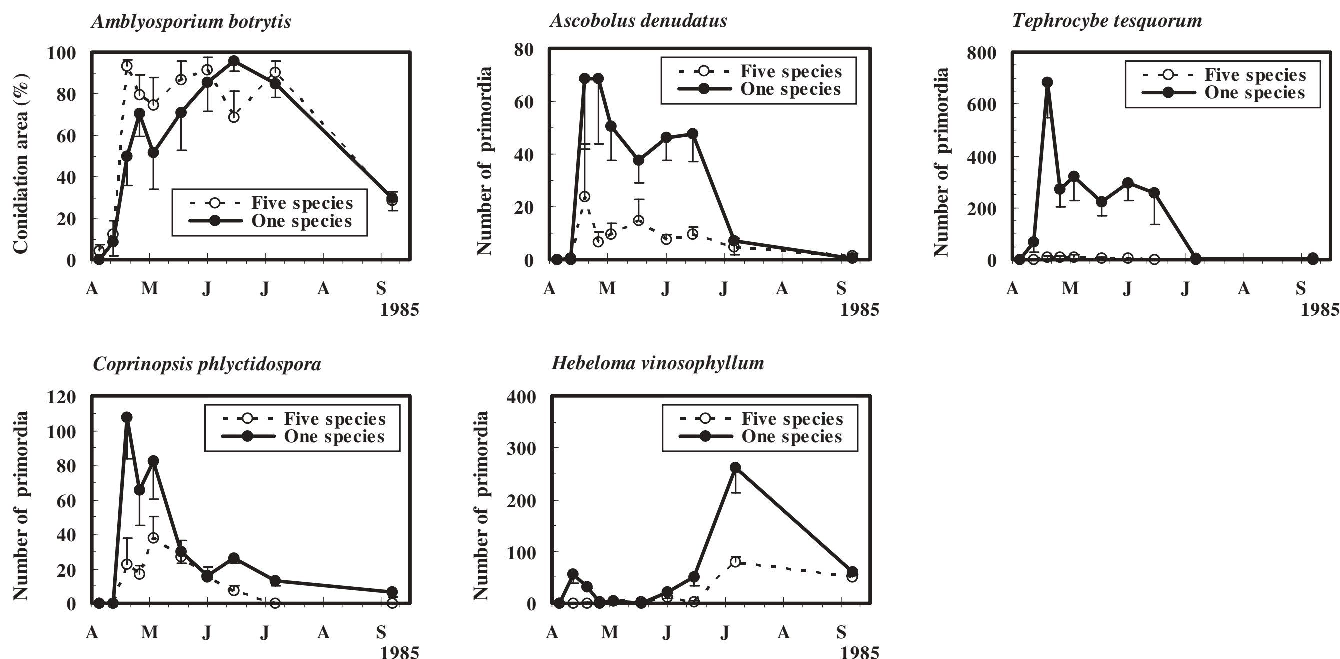

Fig. 6. Vegetative growth and formation of reproductive structures of five ammonia fungi on sterilized soils (experiment 4)

Table 2. Spore germination of ammonia fungi in water extract of soils (experiment 5)

Fungal species	Isolate no.	Urea-treated soil[a]	Control soil[b]	Positive control[c]	Negative control[d]
Amblyosporium botrytis	M 006	62.5 ± 3.6[e]	2.3 ± 0.5	29.0 ± 0.3	0.8 ± 0.4
Ascobolus denudatus	M 007	94.5 ± 0.9	0	77.0 ± 3.1	0
Coprinopsis phlyctidospora	NBRC 30478	82.8 ± 2.5	0	85.6 ± 1.8	0
Hebeloma vinosophyllum	NBRC 31231	67.0 ± 3.3	0	73.6 ± 3.5	0
Hebeloma spoliatum	–	74.1 ± 2.6	0	66.0 ± 1.9	0

[a] pH 9.2, 820 mM NH_4-N, 0.4 mM NO_3-N
[b] Non-urea-treated soil; pH 5.2, 0.46 mM NH_4-N, 0.04 mM NO_3-N
[c] 100 mM $(NH_4)_2HPO_4$ (pH 8.0) for *A. botrytis*, *C. phlyctidospora*, *H. vinosophyllum*, and *H. spoliatum*; 10 mM NH_4Cl (pH 9.0) for *A. denudatus*
[d] H_2O (double-distilled water)
[e] Average of five replicates with standard error

tion under neutral to weakly alkaline conditions. The results obtained from monocultures and five-species cultures also suggest that the timing of occurrence of each ammonia fungus in the field is partly related to interactions with other ammonia fungi. I hypothesize that the acquisition of symbiosis with plants (i.e., the formation of ectomycorrhizae) is a propagation strategy of *Hebeloma vinosophyllum* to overcome potentially competitive interactions with soil microbes, especially with saprobic ammonia fungi.

Experiments using extract of natural substrates

Cultivation by extract of natural substrates treated in the field

Spore germination of ammonia fungi in sterilized extracts of urea-treated soils. Spore germination of ammonia fungi in the field was examined by incubation of spores in sterilized water extracts of urea-treated soils. Results confirm that spores of ammonia fungi, both saprobic and ectomycorrhizal species, are stimulated by urea-treated soils. In other words, no strongly inhibitory factors for spore germination are present in urea-treated soils (Table 2). Further experiments involving a combination of cation-exchange residues and anion-exchange residue suggest that the major stimulatory component in urea-treated soils is ammonium nitrogen (Suzuki et al., unpublished data).

Laboratory experiments

Experiments using natural substrates

Cultivation of soils treated in the laboratory

Survey of mycobiota of saprobic ammonia fungi. As already described, most saprobic ammonia fungi can be surveyed by cultivation of soils collected from the A_0 horizon followed by application of nitrogenous chemicals, such as urea (cf. Sagara 1975, 1976a,c, 1992; Fukiharu and

Horigome 1996; Suzuki 2004). Cultivation can also be conducted in a small vessel such as a flower pot or a planter, and in scientific glassware such as a conical flask, even when soils in various horizons are completely mixed (cf. Sagara 1976c). Sagara (1976a) conducted soil cultivation trials at various constant temperatures or under different temperature regimes following application of different amounts of urea. From these results, he speculated the mechanism of the occurrence of ammonia fungi. Namely, he advocated that, in principle, the occurrence of ammonia fungi is caused by the predominant growth induced at the early stage of cultivation. His experiments also revealed that the effective application rate of urea for occurrence of ammonia fungi is in a remarkably narrow range, with the upper limit in laboratory experiments well below that in the field (cf. Sagara 1975, 1976a,c, 1992). This difference may be caused by the narrower ranges of concentration gradient of ammonium nitrogen, the absence of leakage of urea and its derivative ammonia from treated soil, and by lack of invasion of the same fungal species from outside the treated area; the soil packed in a laboratory vessel represents a kind of closed system at the substrate level.

The effective application rate of urea for occurrence of ammonia fungi was also examined by the soils (L–H horizons) obtained from lodgepole pine (*Pinus contorta* var. *latifolia*) forests using the flower pot cultivation method described previously. In the lodgepole pine forest soils, the effective range for urea application that supports ammonia fungi was 2.5–40 mg/g dry soil (Fig. 7). The saprobic fungi appearing with the smallest amount of urea was *Ascobolus denudatus* (Fig. 7B) and that appearing with the largest amount was *Coprinus* sp. (Fig. 7C).

Spatial distribution of saprobic ammonia fungi in the field. The spatial distribution of each saprobic ammonia fungal species can be studied by incubation of soil cores. *Amblyosporium botrytis*, *Ascobolus denudatus*, *Tephrocybe tesquorum*, *Coprinopsis neolagopus*, and *Coprinopsis phlyctidospora* appeared from soils collected from subquadrats at the frequencies of 77/200, 43/200, 25/200, 11/200, and 144/200, respectively (Fig. 8). These data show the minimum frequency of existence of each ammonia fungus in the soil because there are no data about the recovery rate, i.e., the efficiency of sporulation and fruiting of each fungal species from a living hypha and/or a spore.

Similar results have been reported from the same kind of experiment undertaken in Kiyosumi, Chiba (Suzuki et al. 2002b), although the distribution frequency of each saprobic species differed somewhat between the two sampling sites.

These results indicate that the inoculum sizes of saprobic ammonia fungi are relatively large in the field, although we cannot exactly know the spatial distribution of living propagules of each ammonia fungus in the field by this method, as already described. The effectiveness of colonization by ammonia fungi, i.e., by means of spores and hyphae, etc., has not been completely resolved by this experiment. Even on the assumption that one of the latent presence forms of ammonia fungi in the soil is hyphae, we cannot determine a genet size in these experiments. We cannot know how many adjacent subquadrats are occupied by the same fungus genet, nor whether a certain fungal species obtained from a subquadrat soil sample is derived from one or more hyphae of different genets. Using artificial media in association with molecular techniques will likely give us more precise information about the spatial distribution of both saprobic and mycorrhizal fungi.

Growing ability of ammonia fungi. Yamanaka (2001) treated forest soils of the A_0 horizon with different amounts of urea in the laboratory. Five days after the urea treatment, he sterilized the soils by gamma-ray radiation and inoculated the sterilized soil with vegetative hyphae of *Tephrocybe tesquorum.* He found that vegetative growth and fruit-body development occurred when the ammonia concentration in the soil was high. This result is supported by the results of similar experiments using sterilized urea-treated soils collected from different days after urea application in the field (see earlier).

Experiments using artificial media

Cultivation using agar media

Vegetative growth and fruiting ability of ammonia fungi. Cultivation of vegetative mycelia on nutritional agar media is a suitable method to assess certain physiological characteristics of each fungus, especially the growth rate of each fungus on the basis of hyphal extension. The mycelial growth rate of each fungus provides an indication of the potential territory acquiring ability of each fungus in the field. Cultivation on nutrient agar media generally results in formation of fungal reproductive structures at a higher frequency than that in liquid media. Although agar cultivation helps our understanding of physiological characteristics of fungi and characteristics of reproductive structure formation of each fungus, we should be aware that artificial cultivation methods only demonstrate the physiological characteristics of reproductive structure formation arising from vegetative growth. We should introduce other culture methods using liquid media when we want to examine more closely the physiological characteristics of each fungus at the stage of reproductive structure formation (cf. Kitamoto et al. 1980). In addition, cultivation on nutrient agar enables the coculture (dual culture, or two-species culture) of two fungi to evaluate interactions between them in a two-dimensional system (Boddy 2000), although interactions between fungi in the field always happen in three dimensions.

Monocultures. Hyphal growth rates (linear growth) of five ammonia fungi, *Amblyosporium botrytis*, *Ascobolus denudatus*, *Humaria velenovskyi*, *Coprinopsis echinospora*, and *Hebeloma vinosophyllum*, were measured on nutrient agar media adjusted to pH 8.0, compared with agar at pH 7.0, 6.0, 5.0, and 4.0, respectively. Correlations of growth rate with pH were less profounced for species that occurred in the field later in the sequence of ammonia fungi (Fig. 9, Table 3).

Fig. 7. Occurrence of saprobic ammonia fungi on urea-treated soils packed in a flower pot (experiment 6). **A** Appearance of conidiophore of *Amblyosporium botrytis* (20 mg-N urea/g dry soil; incubation for 50 days at 10°C). **B** Appearance of ascomata of *Ascobolus denudatus* (10 mg-N/g dry soil; incubation for 34 days at 25°C). **C** Appearance of basidiomata of *Coprinopsis* sp. (20 mg-N/g dry soil; incubation for 94 days at 10°C)

a) ***Amblyosporium botrytis*** **b)** ***Ascobolus denudatus*** **c)** ***Tephrocybe tesquorum*** **d)** ***Coprinopsis neolagopus*** **e)** ***Coprinopsis phlyctidospora***

: The fungal species shown at the top of each figure (quadrat) was isolated from the subquadrat
: *A. botrytis* and *A. denudatus* were isolated from the subquadrat
: *A. botrytis* and *C. phlyctidospora* were isolated from the subquadrat
: *A. botrytis* and *C. neolagopus* were isolated form the subquadrat
: *A. botrytis, A. denudatus, and C. phlyctidospora* were isolated from the subquadrat
: *A. botrytis* , *T. tesquorum* , and *C. phlyctidospora* were isolated freom the subquadrat
: *A. botrytis, T. tesquorum,* and *C. neolagopus* were isolated from the subquadrat
: *A. botrytis* , *C. phlyctidospora* , and *C. neolagopus* were isolated from the subquadrat
: *A. botrytis, A. denudatus, C. phlyctidospora* , and *C. neolagpopus* were isolated from the subquadrat
: *A. denudatus* and *C. phlyctidospora* were isolated from the subquadrat
: *A. denudatus* and *T. tesquorum* were isolated from the subquadrat
: *A. denudatus, T. tesquorum,* and *C. phlyctidospora* were isolated from the subquadrat
: *T. tesquorum* and *C. phlyctidospora* were isolated from the subquadrat
: *T. tesquorum* and *C. neolagopus* were isolated from the subquadrat
: *C. phlyctidospora* and *C. neolagopus* were isolated from the subquadrat

Fig. 8. Spatial distribution of saprobic ammonia fungi in the field (experiment 7). Five quadrats shown in the figure separately describe the spatial distribution of fungi isolated from a quadrat (100 cm × 50 cm) by sampling on April 24, 1994

The saprobic ammonia fungus *Coprinopsis stercorea* formed basidiomata on 0.25–1.0 g urea/l under light (Morimoto et al. 1982). The ectomycorrhizal ammonia fungus, *Hebeloma vinosophyllum*, formed tiny basidiomata in synthetic media containing ammonium nitrogen and urea as a single nitrogen source, but did not form them in media containing nitrite or nitrate nitrogen as a single nitrogen source.

These results agree well with the observed ecological characteristics of *Hebeloma vinosophyllum* as a pioneer, facultative ectomycorrhizal fungus.

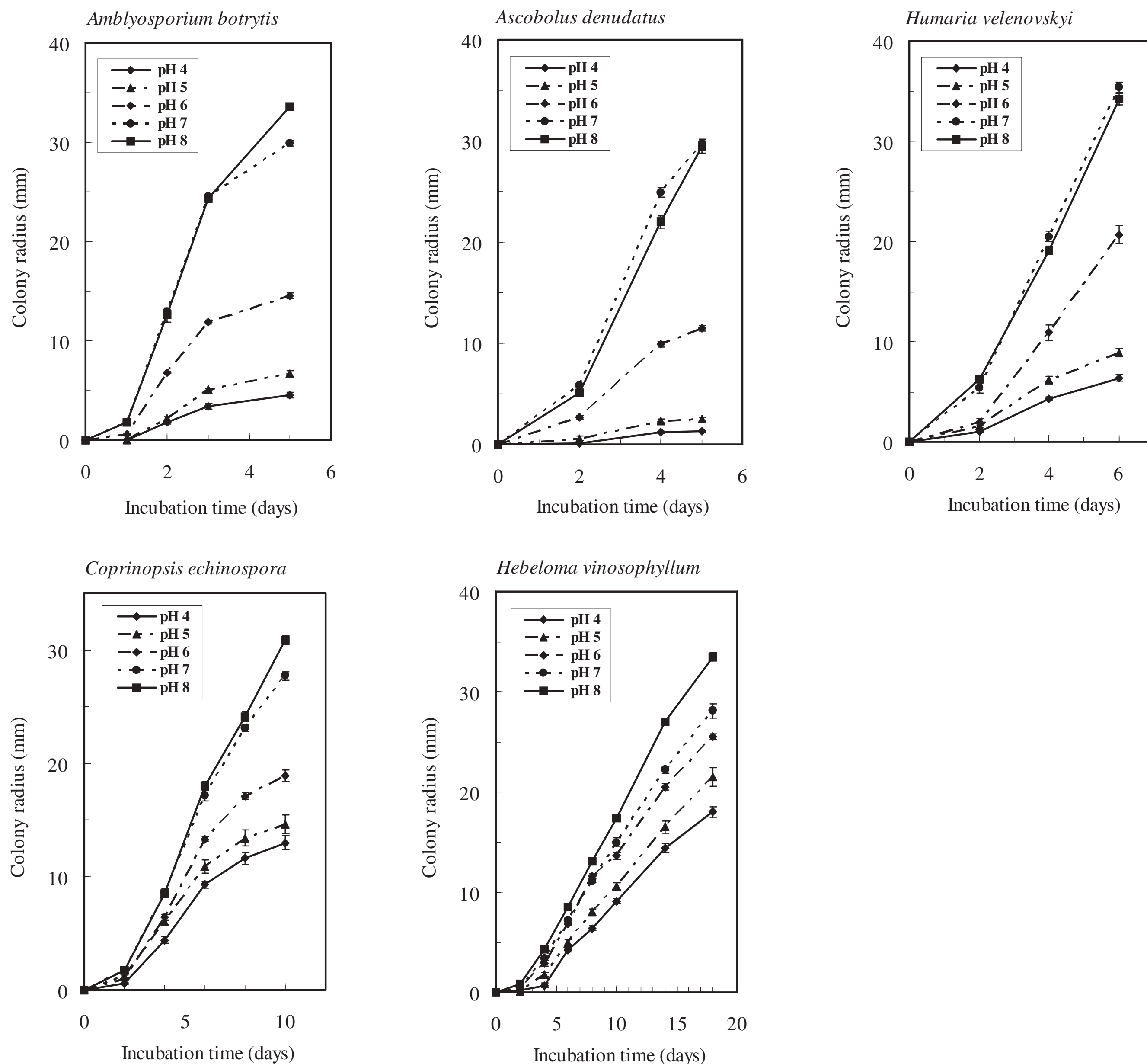

Fig. 9. Effect of pH on vegetative growth of ammonia fungi (experiment 8)

Table 3. Changes in pH in cultivation of different fungal species (experiment 8)

Fungal species	Isolate no.	Incubation period (days)	Final pH[a] of the medium cultured at initial pH of:				
			4	5	6	7	8
Amblyosporium botrytis	NAO 578	4	4.4 ± T[b]	4.5 ± T	4.6 ± T	4.8 ± 0.1	5.5 ± 0.1
Ascobolus denudatus	NAO 575	5	4.4 ± T	4.6 ± T	5.0 ± T	5.7 ± 0.1	6.3 ± 0.1
Humaria velonovskyi	NAO 567	6	4.5 ± T	4.6 ± T	4.9 ± T	5.5 ± 0.1	5.9 ± T
Coprinopsis echinospora	NBRC 30630	10	4.3 ± T	4.3 ± T	4.5 ± T	4.9 ± T	5.6 ± 0.1
Hebeloma vinosophyllum	NBRC 31231	18	4.1 ± T	4.0 ± T	4.0 ± T	4.0 ± 0.1	3.8 ± 0.1

[a] Average of five replicates with standard error
[b] Less than 0.1

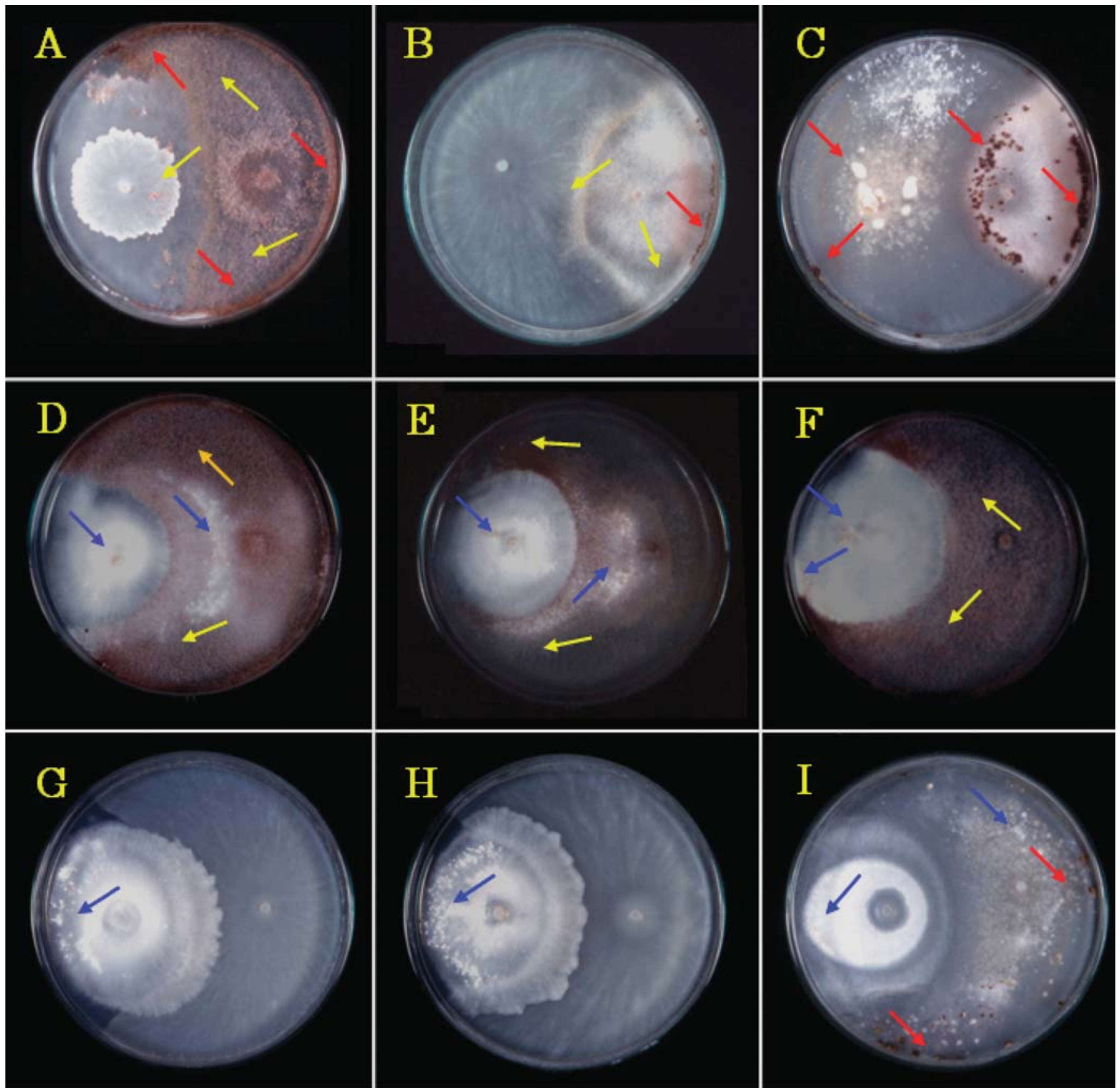

Fig. 10. Interactions between ammonia fungi (experiment 9). **A–C** *Ascobolus denudatus* (*left*) and *Amblyosporium botrytis* (*right*): pH 5 (**A**), pH 6 (**B**), pH 7 (**C**). **D–F** *Hebeloma vinosophyllum* (*left*) and *A. botrytis* (*right*): pH 4 (**D**), pH 5 (**E**), pH 6 (**F**). **G–I** *H. vinosophyllum* (*left*) and *A. botrytis* (*right*): pH 5 (**G**), pH 6 (**H**), pH 7 (**I**). *Red arrows* indicate the ascomata of *A. denudatus*; *yellow arrows* indicate conidiophores of *A. botrytis*; *blue arrows* indicate basidiomata of *H. vinosophyllum*

Multiple species cultures. By the coculture method, we found that the EP fungi *Amblyosporium botrytis* and *Ascobolus denudatus* showed mutual intermingling under acidic to alkaline conditions (Fig. 10A–C; cf. Suzuki 2000b). Both EP fungi produced vigorous vegetative growth and reproductive structures at pH above 7.0 whether cultured together with LP fungus *Hebeloma vinosophyllum* or as a monoculture (Fig. 10F,I; Licyayo et al., unpublished data). In contrast, the LP fungus *H. vinosophyllum* showed strongest invasion ability and fruiting ability at pH 4.0–6.0 (Fig. 10D,E,G,H), but inhibited both vegetative growth and fruiting by opponent EP fungi *A. botrytis* and *A. denudatus* at pH 8.0.

Cultivation using liquid media

The physiological characteristics of each fungus, especially the latent growth rate of the fungus as assessed by biomass, can be examined by using liquid cultures.

Spore germination of ammonia fungi. The principal chemical components that stimulates spore germination of ammonia fungi were determined by incubation of spores in different aqueous solutions of chemicals chosen from results from urea-treated soils as described earlier. Spore germination was stimulated by solutions containing 10–100 mM NH_4-N under alkaline to neutral conditions (Suzuki 1978, 1989, 2004; Suzuki et al. 1982).

Vegetative growth of ammonia fungi. Based on measurement of dry weight, Yamanaka (2003) found that many of the saprobic ammonia fungi grew well at pH 7 and 8, whereas the ectomycorrhizal ammonia fungi showed optimum growth at pH 5 and 6. In his study, pH of the liquid media was adjusted by different kinds of buffers. A pH value of 5–6 or somewhat higher aligns with that of most Japanese forest soils without urea application (cf. Sagara 1992; Yamanaka 1995a–c; Sato and Suzuki 1997; Fukiharu et al. 1997; Suzuki 2000a; Suzuki et al. 2002b; He and Suzuki 2004).

Both inorganic nitrogen, NH_4-N and NO_3-N, and organic nitrogen such as L-asparagine and urea were highly effective for the growth of saprobic ammonia fungi, especially *Coprinopsis cinerea* (Morimoto et al. 1981) and *C. phlyctidospora* (Suzuki 1989; Yamanaka 1999; He and Suzuki 2003), at somewhat higher concentrations than the optimal nitrogen concentration for growth of many wood decay and litter-decomposing fungi. Other saprobic ammonia fungi, *Amblyosporium botrytis*, *Tephrocybe tesquorum*, and *C. echinospora*, grow well on NH_4-N, some amino acids (L-asparagine, glycine, etc.), urea, and bovine serum albumin but not nitrate (Yamanaka 1999). In contrast, the saprobic ammonia fungi *Peziza urinophila* and *Pseudombrophila petrakii* (=*Pseudombrophila deerta*) do not grow on any of these nitrogen sources (Yamanaka 1999).

The effect of nitrogen sources on the growth of an ectomycorrhizal ammonia fungus, *Hebeloma vinosophyllum*, was examined on a basal synthetic medium. *Hebeloma vinosophyllum* grew when associated with the following nitrogen sources: NH_4Cl, KNO_3, KNO_2, and urea. pH optima for vegetative growth of *H. vinosophyllum* using each of these four nitrogen sources were 7–8, 5–6, above 8, and 4–6, respectively (Fig. 11, Table 4). Another ectomycorrhizal ammonia fungus, *Laccaria bicolor*, also grows well on ammonium, nitrate, and urea (Yamanaka 1999). The major initial form of nitrogen in urea-treated soils is ammonium nitrogen, and this gradually changes to nitrate nitrogen (cf. Yamanaka 1995a; Suzuki 2000a; Suzuki et al. 2002b).

These results fit the ecological character of *Hebeloma vinosophyllum* as a pioneer, facultative ectomycorrhizal fungus. These results also suggest that the major nitrogen source for vegetative growths of *H. vinosophyllum* and *Laccaria bicolor* in urea-treated soils is ammonium nitrogen, gradually replaced by nitrate nitrogen along with an accompanying decrease in soil pH.

Reproductive structure formation of ammonia fungi. Urea and NH_4-N stimulated fruit-body formation of the saprobic ammonia fungus *Coprinopsis cinerea* when applied to potato dextrose liquid (Morimoto et al. 1981). Urea was also effective for fruit-body formation of the saprobic ammonia fungus *Coprinopsis phlyctidospora* when it was applied to the synthetic medium (He and Suzuki 2003).

The foregoing results indicate that urea is an effective stimulant for fruiting of ammonia fungi. However, urea cannot be one of the major determinants for the occurrence of ammonia fungi in the field, because urea concentrations in the soils are inadequate, based on the concentration of ammonium nitrogen in the urea-treated soil of several days after application of urea (cf. Sagara 1992; Yamanaka 1995a–c; Sato and Suzuki 1997; Fukiharu et al. 1997; Suzuki 2000a; Suzuki et al. 2002b; He and Suzuki 2004).

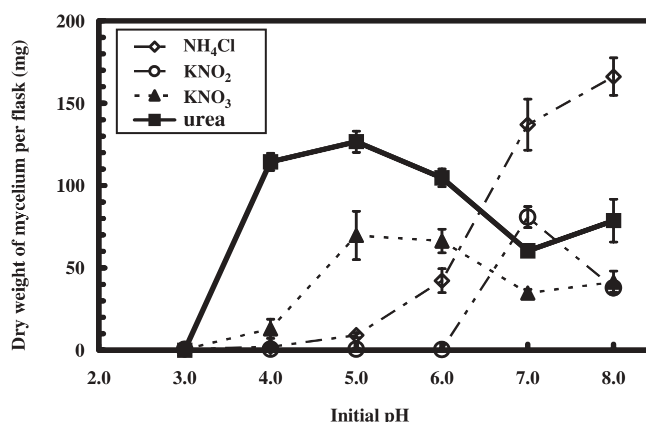

Fig. 11. Effects of nitrogen sources on vegetative growth of *Hebeloma vinosophyllum* (experiment 10)

Table 4. Changes in pH by cultivation of different nitrogen sources (experiment 10)

Nitrogen source	Final pH[a] of the medium cultured at initial pH of:					
	3	4	5	6	7	8
NH_4Cl	3.1 ± T[b]	3.9 ± 0.1	3.9 ± 0.1	3.8 ± 0.2	3.1 ± 0.1	3.7 ± 0.4
KNO_2	2.5 ± T	5.8 ± T	6.0 ± T	6.1 ± T	7.4 ± T	7.2 ± T
KNO_3	3.1 ± T	4.2 ± 0.3	6.5 ± 0.1	6.7 ± 0.1	6.8 ± T	7.2 ± T
Urea	3.2 ± T	4.2 ± 0.4	6.5 ± 0.2	6.7 ± 0.2	6.9 ± T	7.3 ± T

Incubation period: 12 days
[a] Average of five replicates with standard error
[b] Less than 0.1

Conclusions

Based on these results and the discussion, our current understanding of the mechanism of colonization (=propagation) and succession of ammonia fungi and its role in the field is summarized as follows.

1. The invasion and colonization of ammonia fungi is initiated mainly by spores and small mycelial colonies inhabiting in the ammonia-treated area and/or the area adjacent to the ammonia-treated area, and probably also from airborne and/or animalborne spores and fragments of mycelium.
2. The successive occurrence of ammonia fungi in the field results from the sequential colonization of ammonia fungi associated with the time needed for each fungus to produce its reproductive structures.
3. The duration of the occurrence of ammonia fungi in the field is shortened by interactions between ammonia fungi and other soil microbes, especially interactions between different ammonia fungi themselves.
4. The sequential propagation of each ammonia fungus and the time needed for development of reproductive structures may be explained by their preference of and tolerance to, high concentrations of ammonium nitrogen under alkaline to neutral conditions.
5. Some saprobic ammonia fungi are the principal decomposers of plant materials on forest floors that have been disturbed by deposition of a large amount of ammonium nitrogen (compensatory effects on nutrient cycling; cf. Suzuki 2002).

Sagara (1995) proposed cleaning symbiosis for the role of ectomycorrhizal postputrefaction fungi (Sagara 1992), which are composed of ammonia fungi.

Future knowledge of the ecophysiology of ammonia fungi will likely develop from ideas and methods that combine the results obtained from experiments using artificial media and those using natural substrates.

Experiments

Experiment 1. Litter was collected from the area adjacent to the site of embedding in the litter bags 3 days before they were installed. Thereafter, only leaves of broadleaf trees were sorted from the litter. They were mixed and dried at room temperature, and then about 17 g litter was packed in each bag made of nonwoven fabric (size, 15 cm × 9 cm; mesh size, 0.5 mm). The fabric is made of string composed of 70% polyester and 30% nylon). The balsa (*Ochroma lagopus*) wood blocks (2 cm wide, 10 cm long, 1 cm thick) were also dried at room temperature. Both samples were embedded between L–F horizons and H–A horizons in the mixed forest (see Fig. 1) in Ninosawa, Kiyosumi, Japan (35°10′ N, 140°9′ E; 220 m alt.) on April 14, 1992, followed by application of fertilizer urea (800 g/m^2).

Experiment 2. Detailed information about the methods of the experiment is shown in Suzuki et al. (2002b).

Experiment 3. A desk (~90 cm × ~60 cm) covered with a sheet of plastic film (140 cm × 120 cm) hanging about 30 cm (from the lowest part) above the desk surface was placed in a mixed forest (see Fig. 4) in Arakashizawa, Kiyosumi, Japan (detailed information about this plot is shown as site III in Table 1) (Suzuki et al. 2002b). Four desks were fixed 30–70 cm apart over the surface of the forest floor (see Fig. 4). A mixture of soils of the L–F and H–A horizons were collected 6 days (A) and 16 days (B) after urea application (1600 g/m^2) on October 27, 1990. Soils were packed separately to fill 90% of the volume of screw-cap bottles (22 mm in diameter, 65 mm long; 30 ml), and soils were sterilized using gamma-ray irradiation (1.5 × 10^3 C/kg). The sterilized soils were stored at −30°C. The sterilized soils in the bottle were put on desks placed in the field on August 4, 1991 (a), October 15, 1991 (b), and October 22, 1991 (c), respectively. The caps of the bottles were left open. About half the bottles were kept open for 1 week and others for 2 weeks (see Fig. 4). Thereafter, the bottles were sealed with the sterilized screw caps. The soils in the bottles were incubated for 60 days at 20.0° ± 0.5°C in a light (16 h) and dark (8 h) regime. Invasion of each ammonia fungal species was evaluated by appearance of its reproductive structures (see Table 1).

Experiment 4. The EP fungi *Amblyosporium botrytis* M006, *Ascobolus denudatus* M007, *Tephrocybe tesquorum* NBRC 30487, and *Coprinopsis phlyctidospora* NBRC 30478, and the LP fungus *Hebeloma vinosophyllum* NBRC 31231, were separately cultured on MY agar medium [malt extract (Difco) 10 g, yeast extract (Difco) 2 g, agar (Nakalai Tesque) 15 g, distilled water 1000 ml, pH 5.5] at 25.0° ± 0.5°C in darkness. Inoculum was an agar disk of mycelium (6 mm in diameter, 3 mm deep) bored from the subperipheral region of an agar culture as already described. For monoculture, an agar disk of each ammonia fungal species was separately inoculated on the surface of gamma ray-sterilized (1.5 × 10^3 C/kg) soils collected from H–A horizons at different times after urea application (800 g urea/m^2; applied on April 6, 1985) in a mixed forest in Arakashizawa, Kiyosumi, Chiba, Japan) (detailed information about this plot is shown as site III in Table 1) (Suzuki et al. 2002b). For the five-species culture, the agar disks of five ammonia fungal species (one disk for each fungus species) were inoculated together on the surface of gamma ray-sterilized soils prepared by the same procedure as previously described. The monocultures and five-species cultures were incubated at 20.0° ± 0.5°C in light (16 h) and dark (8 h) regime. Occurrence of each ammonia fungus in the field was *A. botrytis*, April 17 to June 12; *A. denudatus*, May 1 to May 15; *T. tesquorum*, May 8 to June 26; *C. phlyctidospora*, May 29 to September 18; and *Hebeloma spoliatum*, July 17 to December 22). The ability of reproductive structure formation for the five ammonia fungal species was evaluated as conidiation area for *A. botrytis*, and for the others as total numbers of primordia of ascomata or basidiomata during

the whole cultivation period. The conidiation area is shown as the percentage of the area of conidiophore formation to a full surface area of medium, including its top and bottom surfaces (100%), at 6 days of cultivation. The number of primordia of *A. denudatus*, *T. tesquorum*, *C. phlyctidospora*, and *H. vinosophyllum* were counted at 22, 39, 120, and 50 days after inoculation, respectively.

Experiment 5. *Amblyosporium botrytis* M 006, *Ascobolus denudatus* M007, *Coprinopsis phlyctidospora* NBRC 30478, and *Hebeloma vinosophyllum* NBRC 31231 were separately cultured on slants made of MY agar at 25.0° ± 0.5°C in darkness. Conidia of *A. botrytis* and ascospores of *A. denudatus* were collected from conidiophores and ascomata, respectively, by scraping, and were then made separately into a water suspension. The mixture of conidia and fragments of conidiophores, and that of ascospores and fragments of ascomata, were suspended in pure water, and spores were separated out by filtration through folded gauze. The spores were washed three times in sterilized pure water. Dispersed spores of *C. phlyctidospora* and *H. vinosophyllum* were collected aseptically from the cultures. Basidiospores of *Hebeloma spoliatum* were collected from basidiomata collected from a urea-treated plot in the field. The spores were washed three times in sterilized pure water. The spore suspensions of *A. botrytis*, *A. denudatus*, *C. phlyctidospora*, *H. vinosophyllum*, and *H. spoliatum* were prepared at densities of 1.5×10^5/ml, 1.2×10^5/ml, 3.2×10^5/ml, 5.0×10^5/ml, and 5.2×10^5/ml, respectively. The urea-treated soil was collected from the H–A horizons 6 days after the application of urea (800 g urea/m^2; March 22, 1983) in a mixed forest, Arakashizawa, Kiyosumi, Chiba, Japan (35°10′ N, 140°8′ E; 290 m alt.). Non-urea-treated soil was collected on March 28, 1983 by the same way as already described. Pure water, twice the weight of the fresh soil sample, was added to the soil, and the soil suspension was shaken (180 times reciprocal shaking/min) for 1 h at room temperature followed by filter paper filtration (no. 6; Advantec). Water extracts of the soils were sterilized using a membrane filter (cellulose nitrate; pore size, 0.2 μm; Advantec). Five milliliters of the sterilized water extract was added to 0.5 ml of spore suspension of each fungal species. The spore suspensions were incubated at 20.0° ± 0.5°C in darkness for 20 days. Spore germination was examined at designated intervals. The results are shown according to the maximum percentage of germination in each fungal species cultivation (see Table 2).

Experiment 6. Litter (A_0 horizon) was collected from lodgepole pine forest near Cold Creek Ranger Station, Alberta, Canada (53°36′ N, 115°35′ W; 825 m alt.). About 100 g fresh soil was packed in a flower pot (850 ml). Then, 0.01, 0.3, 1, 2.5, 5, 10, 20, or 40 mg-N urea/g dry soil was applied separately on the surface of the soil. As control, non-urea-treated soils were prepared. The mouth of the pot was covered with a plastic film having many pinholes. Water was sprayed on the litter surface at suitable intervals. The soils were incubated at 10.0° ± 0.8°C or 25.0° ± 0.8°C for 103 days in a light (16 h) and dark (8 h) regime.

Experiment 7. Fifty soil core samples (5 cm × 5 cm × 5 cm) collected on April 24, 1994, from the floor (0.5 m × 1.0 m) of a *Quercus acuta* forest in Kan-nami, Shizuoka, Japan (139°1′ E, 35°9′ N; 600 m alt.) were separately packed in sterilized 200-ml conical flasks, which were then sealed with sterilized cotton plugs. Sterilized aqueous urea solution was applied to each soil sample to adjust the urea concentration to 21 mg urea/g dry weight of soil and achieve a water content in the final range of 60%–75%. The treated soils were incubated at 20.0° ± 0.5°C for 39 days in a light (16 h) and dark (8 h) regime. This incubation temperature is the most suitable temperature for induction of fruiting of most saprobic ammonia fungi.

Experiment 8. Five ammonia fungi were grown on nutrient agar media (glucose, 20.0 g; NH_4Cl, 0.9 g; KH_2PO_4, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.3 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; $ZnSO_4 \cdot 7H_2O$, 0.30 mg; $FeSO_4 \cdot 7H_2O$, 0.15 mg; $CuSO_4 \cdot 5H_2O$, 0.05 mg; $MnSO_4 \cdot 5H_2O$, 0.05 mg; $(NH_4)_6Mo_7O_{24}$, 0.02 mg; thiamine hydrochloride, 0.50 mg; niacin, 0.10 mg; agar (Difco), 15.0 g; and distilled water, 1 l, pH 5.5) at 25.0° ± 0.5°C for 7 days in darkness (preculture). A mycelial disk (5 mm in diameter) obtained from the subperipheral region of the preculture was inoculated on nutrient agar media adjusted to different pHs. pH adjustment was achieved using 1 M HCl and 1 M NaOH. The cultures were incubated at 25.0° ± 0.5°C in darkness, and linear growth of the mycelia was measured. The pH of each medium was recorded just after harvesting mycelium (see Table 3).

Experiment 9. The mycelial blocks of *Amblyosporium botrytis* M 006, *Ascobolus denudatus* M 001, and *Hebeloma vinosophyllum* NBRC 31231 were prepared as shown in the methods described in experiment 4. Mycelial agar blocks of *A. botrytis* and of *A. denudatus*, those of *A. botrytis* and of *H. vinosophyllum*, and those of *A. botrytis* and of *H. vinosophyllum* were separately inoculated at 2-cm intervals on MY agar adjusted at pH 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0, respectively: pH 5.0 was adjusted with 1 M HCl and 1 M NH_4Cl; pH 6.0 was adjusted with 1 M NH_4Cl; pH 7.0–9.0 was adjusted with 1 M NH_4Cl and 1 M NH_4OH. The cocultures were incubated at 20.0° ± 0.5°C in the dark for 21 days. Observation of cocultures was done every day under light for 1 h.

Experiment 10. The basal synthetic medium consisted of maltose, 40.0 g; KH_2PO_4, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.3 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; $ZnSO_4 \cdot 7H_2O$, 0.30 mg; $FeSO_4 \cdot 7H_2O$, 0.15 mg; $CuSO_4 \cdot 5H_2O$, 0.10 mg; $MnSO_4 \cdot 5H_2O$, 0.10 mg; $Na_2MoO_4 \cdot 2H_2O$, 0.02 mg; thiamine hydrochloride, 0.50 mg; niacin, 0.10 mg; and double-distilled water, 1 l. Then, 0.26 g nitrogen was added as NH_4Cl, KNO_3, KNO_2, or as urea to the basal medium. Initial pH was adjusted from 3 to 8 by 1 M NaOH and 1 M HCl. Media exceeding pH 9 were not prepared because sedimentation was observed above pH 8.5. The medium was sterilized using a membrane filter (cellulose nitrate; pore size, 0.25 μm; Advantec), and 20 ml filtered media was separately poured into a 50-ml sterilized conical flask. The dikaryotic isolate of *Hebeloma*

vinosophyllum NBRC 31231 was cultivated on nutrient agar (see experiment 8) for 11 days at 25.0° ± 0.5°C. An inoculum plug (5mm in diameter, 3mm deep) obtained from the subperipheral region of the mycelium was inoculated on 3-mm-deep water agar. An inoculum plug (5mm in diameter, 3mm deep) of *Hebeloma vinosophyllum* obtained from the water agar plate was inoculated in each liquid medium. The culture was incubated at 25.0° ± 0.5°C in darkness for 12 days. Each mycelial sample was dried at 80°C. The pH of each medium was recorded just after harvesting of mycelium (see Table 4).

Acknowledgments This work was financially supported in part by Grants-in-Aid for Scientific Research (nos. 61560156 and 63560141) from the Ministry of Education, Science, Sports and Culture, Japan (now known as Ministry of Education, Science, Sports and Technology, and Culture, Japan) and by grants (nos. 14560112 and 16570073) from the Japan Society for the Promotion of Science (JSPS). This work was also financially supported in part by the Fujiwara Natural History Foundation, Japan (fiscal year 1994–1995). Gamma-ray sterilization of soils was financially supported by the Inter-University Program for the Joint Use of JAERI Facilities by Research Center for Nuclear Science and Technology, The University of Tokyo (now known as the Graduate School of Engineering and Systems Science, The University of Tokyo) (fiscal year 1985–1995). I am thankful to The University Forest in Chiba, The University of Tokyo, and Cold Creek Ranger Station, Alberta, Canada, for their support and making the experimental sites available. I thank Professor Emeritus Naohiko Sagara (Kyoto University, Japan) for provision of the fungal strains and useful information. I also express appreciation to Dr. Toshimitsu Fukiharu, Natural History Museum and Institute, Chiba, Japan, Dr. Siriphan Soponsathien (Pathumwan Institute of Technology, Bangkok, Thailand), and many students of our laboratory, Chiba University, Japan, and those of Toho University, Japan, for their collaboration with me. I wish to express sincere thanks to Professor Randolph S. Currah (University of Alberta, Canada), to emeritus research scientist Dr. Yasuyuki Hiratsuka (Canadian Forest Service, Northern Forestry Centre, Edmonton, Canada), and to Dr. Hideji Ono (Land and Forests Division, Alberta Sustainable Resource Development, Alberta, Canada) for their support and making the experiment in Canada available. I also thank Dr. Neale L. Bougher (Department of Conservation and Land Managemnet Western Australia, Australia) for their collaboration with us. I am grateful to Dr. Peter K. Buchanan (Landcare Research, Auckland, New Zealand) for improving the English, collaborating with me, and other valuable comments.

References

Arai Y, Suzuki A, Kitamoto Y, Shirasaka N, Yoshikawa K, Sakamoto R, Terashita T (2004) Acceleration of the fruit-body formation of edible mushrooms by the extract from corn fiber. Mushroom Sci Biotechnol 12:171–177

Ban-nai T, Muramatsu Y, Yoshida S, Uchida S, Shibata S, Ambe S, Ambe F, Suzuki A (1997) Multitracer studies on the accumulation of radionuclides in mushrooms. J Radiat Res 38:213–218

Ban-nai T, Yoshida S, Muramatsu Y, Suzuki A (2005) Uptake of radiocesium by hypha of Basidiomycetes: radiotracer experiments. J Nucl Radiochem Sci 6:111–113

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

Dickinson CH, Pugh GJF (eds) (1974) Biology of plant litter decomposition, vol 1. Academic Press, London

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

Fujimoto H, Yamazaki M, Suzuki A (1982) Survey of the toxic *Hebeloma* and isolation of the toxic principle of *Hebeloma vinosophyllum* (in Japanese). Trans Mycol Soc Jpn 23:405–412

Fukiharu T, Hongo T (1995) Ammonia fungi of Iriomote Island in the southern Ryukyus, Japan and a new ammonia fungus, *Hebeloma luchuense* (in Japanese). Mycoscience 36:425–430

Fukiharu T, Horigome R (1996) Ammonia fungi in the Abukuma Mountains and its biogeographical distribution around Japan (in Japanese). Mem Natl Sci Mus Tokyo 29:105–112

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

Fukiharu T, Osaku K, Iguchi K, Asada M (2000a) Occurrence of ammonia fungi on the forest ground after decomposition of a dog carcass. Nat Hist Res 6:9–14

Fukiharu T, Yokoyama G, Oba T (2000b) Occurrence of *Hebeloma vinosophyllum* on the forest ground after decomposition of crow carcass. Mycoscience 41:401–402

Harmaja H (2002) *Alciphila vulgaris*, a new genus and species of deutromycetes. Karstenia 42:33–38

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

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

Imamura A (2001) Report on *Laccaria amethystina*, newly confirmed as an ammonia fungus. Mycoscience 42:623–625

Imamura A, Yumoto T (2004) The time of urea treatment and its effects on the succession of ammonia fungi in two warm temperature forests in Japan. Mycoscience 45:123–130

Kinugawa K, Takamatsu Y, Suzuki A, Tanaka K, Kondo N (1986) Effects of concentrated carbon dioxide on the fruiting of several cultivated basidiomycetes (in Japanese). Trans Mycol Soc Jpn 27: 327–340

Kinugawa K, Suzuki A, Takamatsu Y, Kato M, Tanaka K (1994) Effects of concentrated carbon dioxide on the fruiting of several cultivated basidiomycetes (II). Mycoscience 35:345–352

Kitamoto Y, Suzuki A, Furukawa S (1972) An action spectrum for light-induced primordium formation in a basidiomycete, *Favolus arcularius* (Fr) Ames. Plant Physiol 49:338–340

Kitamoto Y, Horikoshi T, Suzuki A (1974) An action spectrum for photoinduction of pileus formation in a basidiomycete, *Favolus arcularius*. Planta (Berl) 119:81–84

Kitamoto Y, Ohshita K, Noboru H, Ichikawa Y (1980) Nutritional requirement for fruit-body development of *Psilocybe panaeoliformis* in replacement culture (in Japanese). Trans Mycol Soc Jpn 21:369–380

Kitamoto Y, Suzuki A, Matsumoto T, Ichikawa Y (1986) The role of light on fruit-body initiation in *Favolus arcularius*. Trans Mycol Soc Jpn 27:451–461

Koyama K, Imaizumi T, Akiba M, Kinoshita K, Takahashi K, Suzuki A, Yano S, Horie S, Watanabe K, Naoi Y (1997) Antinociceptive components of *Ganoderma lucidum*. Planta Med 63:224–227

Koyama K, Akiba M, Imaizumi T, Kinoshita K, Takahashi K, Suzuki A, Yano S, Horie S, Watanabe K (2002) Antinociceptive constituents of *Auricularia polytricha*. Planta Med 68:284–285

Miller OK Jr, Hilton RN (1987) New and interesting agarics from Western Australia. Sydowia 39:126–137

Morimoto N, Suda S, Sagara N (1981) Effect of ammonia on fruit-body induction of *Coprinus cinereus* in darkness. Trans Mycol Soc Jpn 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

Murata M, Nagai M, Takao M, Suzuki A, Sakai T, Terashita T (2001) Purificaion and characterization of an extracellular acid trehalase from *Lentinula edodes*. Mycoscience 42:479–482

Nagao H, Udagawa S, Bougher NL, Suzuki A, Tommerup IC (2003) The genus *Thecotheus* (Pezizales) in Australia: *T. urinamans* sp. nov. from urea-treated jarrah (*Eucalyptus marginata*) forest. Mycologia 95:688–693

Robertson GP, Paul EA (2000) Decomposition and soil organic matter dynamics. In: Sala OE, Jackson RB, Mooney HA, Howarth, RW (eds) Methods in ecosystem science. Springer, New York, pp 104–116

Sagara N (1973) Proteophilous fungi and fire place fungi (a preliminary report). Trans Mycol Soc Jpn 14:41–46
Sagara N (1975) Ammonia fungi: a chemoecological grouping of terrestrial fungi. Contrib Biol Lab Kyoto Univ 24:205–276
Sagara N (1976a) Growth and reproduction of the ammonia fungi (in Japanese). In: Biseibutsu-seitai Kenkyukai (ed) Ecology of microorganisms, vol 3. University of Tokyo Press, Tokyo, pp 153–178
Sagara N (1976b) Presence of a buried mammalian carcass indicated by fungal fruiting bodies. Nature (Lond) 262:816
Sagara N (1976c) Supplement to the studies of ammonia fungi (I) (in Japanese). Trans Mycol Soc Jpn 17:418–428
Sagara N (1981) Occurrence of *Laccaria proxima* in the grave site of a cat. Trans Mycol Soc Jpn 22:271–275
Sagara N (1984) On "corpse finder." Mcllvainea 6(2):7–9
Sagara N (1992) Experimental disturbances and epigeous fungi. In: Carroll GC, Wicklow DT (eds) The fungal community: its organization and role in the 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
Sagara N, Hamada M (1965) Responses of higher fungi to some chemical treatments of forest ground. Trans Mycol Soc Jpn 6:72–74
Sagara N, Kitamoto Y, Nishio R, Yoshimi, S (1985) Asssociation of two *Hebeloma* species with decomposed nests of vespine wasps. Trans Br Mycol Soc 84:349–352
Sagara N, Hongo T, Murakami Y, Hashimoto T, Nagamatsu H, Fukiharu T, Asakawa Y (2000) *Hebeloma radicosoides* sp. nov., an agaric belonging to the chemoecological group ammonia fungi. Mycol Res 104:1017–1024
Sato Y, Suzuki A (1997) The occurrence of ammonia fungi, and changes in soil conditions and wood decay rate 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
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 on the production of acetyl esterase and side-group cleaving glycosidases of ammonia fungi. J Gen Appl Microbiol 44:389–397
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 Societies Press, Tokyo, pp 275–279
Suzuki A (1992) Physiological characteristics of the ammonia fungi and their role in bioscience. In: The Asian Mycological Symposium, Seoul, ROK, October 1–4, pp 238–247
Suzuki A (2000a) A survey of species assemblage of ammonia fungi (in Japanese). Nat Hist Plant (Planta) 68:27–35
Suzuki A (2000b) Recent topics in fungal ecology (in Japanese). Newslett Natl Sci Mus 375:6–7
Suzuki A (2002) Fungal succession at different scales. Fungal Divers 10:11–20
Suzuki A (2004) Physiology and ecology of ammonia fungi and their biogeographical distribution. In: Kobayashi T, Takahashi H (eds) Natural history of fungi. The Museum of Hokkaido University, Asahitosho Kanko Center, Sapporo, pp 5–12
Suzuki A, Horikoshi T, Kasai Z (1976) Growth and development in isolated stipes of *Favolus arcularius*. Trans Mycol Soc Jpn 17:63–73
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, Tanaka C, Bougher NL, Tommerup IC, Buchanan PK, Fukiharu T, Tsuchida S, Tsuda M, Oda T, Fukada J, Sagara N (2002a) ITS rDNA variation of the *Coprinopsis phlyctidospora* (syn.: *Coprinus phlyctidosporus*) complex in the Northern and the Southern Hemispheres. Mycoscience 43:229–238
Suzuki A, Uchida M, Kita Y (2002b) Experimental analyses of successive occurrence of ammonia fungi in the field. Fungal Divers 10:141–165
Suzuki A, Fukiharu T, Tanaka C, Ohono T, Buchanan PK (2003) Saprobic and ectomycorrhizal ammonia fungi in the Southern Hemisphere. N Z J Bot 41:391–406
Tibbett M, Carter DO (2003) Mushrooms and taphonomy: the fungi that mark woodland graves. Mycologist 17(pt 1):20–24
Wall DH, Reichman OJ (2000) Biotic manipulations involving belowground animals. In: Sala OE, Jackson RB, Mooney HA, Howarth RW (eds) Methods in ecosystem science. Springer, New York, pp 318–329
Wang YZ, Sagara N (1997) *Pezia urinophila*, a new ammonophilic discomycete. Mycotaxon 65:447–452
Watanabe T, Suzuki A (1995) Effects of exposure of concentrated carbon dioxide on chemical components of bunashieji mushroom (*Hypsizigus marmoreus* Bigelow) during growing of fruit body (in Japanese). Nippon Shokuhin Kagaku Kogaku Kaishi 42:656–660
Watanabe T, Takai Y, Tanaka K, Suzuki A (1994a) Effects of ozone exposure during cultivation of nameko mushroom (*Pholiota nameko*) on fatty acid composition of the fruit bodies (in Japanese). Nippon Shokuhin Kogyo Gakkaishi 41:702–704
Watanabe T, Tsuchihashi N, Takai Y, Tanaka J, Suzuki A (1994b) Effects of ozone exposure during cultivation of oyster mushroom (*Pleurotus ostreatus*) on chemical components of the fruit bodies (in Japanese). Nippon Shokuhin Kogyo Gakkaishi 41:705–708
Watanabe T, Tsuchihashi N, Suzuki A (1996) Effects of ozone exposure during the different cultivation processes of winter mushroom (*Flammulina velutipes*) on their chemical components (in Japanese). Nippon Shokuhin Kagaku Kogaku Kaishi 43:110–116
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 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 Microbial 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