Experimental analyses of successive occurrence of ammonia fungi in the field

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Suzuki, A., Uchida, M. and Kita, Y. (2002). Experimental analyses of successive occurrence of ammonia fungi in the field. In: *Fungal Succession* (eds. K.D. Hyde and E.B.G. Jones). Fungal Diversity 10: 141-165.

Sequential appearance of the ammonia fungi after urea treatment (800 g/m^2) was observed in the field and following 20 C incubation of soils from L-A_1 horizons collected at different days after the treatment. The results indicate that successive occurrence of saprobic ammonia fungi in the field results from the combination of sequential propagation (colonisation) of ammonia fungi and the time needed for each fungus to produce reproductive structures. The sequential propagation and the fruiting time of each ammonia fungus may be explained by their degree of tolerance to high concentrations of NH_4-N under alkaline to neutral conditions. The duration of occurrence of saprobic ammonia fungi in the field was shortened by interactions between organism(s) and changes in soil conditions, especially pH and NH_4-N concentration, resulting from activities of soil organisms including ammonia fungi. Two hundred soil core samples (5 × 5 × 5 cm) collected from a plot (50 × 100 cm) were separately placed in sterilized flasks. Twenty-two mg urea/g dry soil was added to each flask and the water content was adjusted to 60%. Following incubation at 20 C, migrule (spores and mycelia, etc.) frequencies plotted for *Amblyosporium botrytis*, *Ascobolus denudatus*, *Tephrocybe tesquorum*, and *Coprinopsis phlyctidospora* were estimated as 4/200, 38/200, 52/200 and 9/200, respectively. These four ammonia fungi showed no specific co-existence between each other.

Key words: ammonia fungi, pH, spatial distribution, successive occurrence, urea.

Introduction

Ammonia fungi are defined as a chemoecological group of fungi which sequentially develop reproductive structures exclusively or relatively luxuriantly on the soil after a sudden addition of ammonia or some other nitrogenous materials which react as bases by themselves or on decomposition, or alkalis (Sagara, 1975). The sequential occurrence of ammonia fungi generally proceeds as follows: anamorphic fungi (deuteromycetes) →

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ascomycetes → smaller basidiomycetes → larger basidiomycetes (Sagara, 1975). The mechanism of this sequential occurrence has been indirectly inferred by the results obtained from the physiological characteristics of spore germination (Suzuki, 1978, 1989; Suzuki *et al.*, 1982), vegetative growth (Suzuki, 1989; Soponsathien, 1998; Yamanaka, 1999, 2001), and fruiting (Morimoto *et al.*, 1981, 1982; Suzuki, 1989; Yamanaka, 2001) of ammonia fungi. The data indicate that ammonia fungi, especially saprobic ammonia fungi, are well adapted to high concentrations of NH_4-N under weak alkaline to neutral conditions, but do not fully explain the mechanism of successive occurrence of ammonia fungi in the field. The present study was, therefore, conducted to elucidate the mechanism of successive occurrence of ammonia fungi by the examination of the propagation (colonisation) periods of ammonia fungi in the field.

Materials and methods

Field experiments

The field experiments were carried out in a mixed forest in Mt. Kiyosumi, Chiba, Japan. The location and vegetation of experimental sites is shown in Table 1. This region has a warm temperate monsoon climate. In sites I and II, two parallel quadrats (15 × 1 m at 10 m intervals) were placed along a contour line, with one area for urea treatment and the other for the control. Each quadrat was divided into 15 sub-quadrats (1 × 1 m). Fertilizer urea (granular form, N 45%) was spread over on the upper surface of the quadrat of each site by hand scattering at the rate of 800 g per sub-quadrat on 3 April 1984 in site I, and on 8 April 1989 in site II, respectively. After the urea treatment, fungal occurrence was observed with the unaided eye on the sampling dates described below for 12 months at site I and for 9 months at site II. Soils for physical and chemical analyses were collected separately from L-A_1 and H-A_1 horizons, and soils for incubation was collected as follows. Soil sampling was carried out from one quarter of the area of the urea-treated and the control sub-quadrats at the same time as fungus observation. The soil analyses were carried out over 9 months at both sites.

Laboratory experiment (soil incubation)

At site I, soil for laboratory incubation was collected from L-A_1 horizon and then placed into aseptic plastic bags and mixed well under aseptic conditions. A sterilized test tube (30 mm diam. × 200 mm long) was 3/4 filled with soil from each sub-quadrat (*ca.* 25 g in fresh weight) and stoppered with a sterilized cotton plug. The soil sampling at site I was carried out over 12

Table 1. Location, soil characters, and vegetation characters of the quadrats in Mt. Kiyosumi, Chiba, Japan.

Quadrat	Site I (Arakashizawa)	Site II (Hinokio)	Site III (Arakashizawa)
Location	35º10'N 140º8'E	35º11'N 140º7'E	35º9'N 140º9'E
Altitude (m)	*ca.* 280	*ca.* 230	*ca.* 320
Position on slope	Middle	Upper	Middle
Shape of slope	Concave	Flat	Flat
Slope aspect	N50W	S44E	N68E
Inclination (º)	34	26	12
Depth of horizons	L-F: 4-5 cm; H: 5-9cm	L-F: 3-5 cm; H: 2-4cm	L-F: 1-2 cm; H: 1-3 cm
Soil type	B_D	B_D	B_D
Substrate	Sandstone and granule conglomerate	Mudstone with interbeds of tuff and sandstone	Sandstone and granule conglomerate
Canopy trees	*Abies firma* *Quercus acuta* *Quercus salicina* *Quercus serrata*	*Abies firma* *Quercus acuta* *Castanopsis cuspidata* *Machilus thunbergii* *Cinnamomum japonicum* *Mallotus japonicus*	*Castanopsis cuspidata* *Quercus acuta* *Quercus salicina*
Vegetation cover (%)	80	80	95

months (3 April 1984 – 17 April 1985; In Fig. 1, data obtained between on 3 April 1984 and on 16 December are shown) at designated intervals. At site II, soils collected from L-F and H-A_1 horizons were placed separately into the sterilized test tubes in the field. The tube was 3/4 filled with the soil and stoppered with a sterilized cotton plug. The sampling for soils at site II was carried out for 5 months (8 April 1989 – 12 August 1989) at designated intervals. The soils collected from sites I and II were incubated at 20.0 ± 0.5 C for 120 or more days under a light regime of 16 h light / 8 h dark. Appearance of reproductive structures of fungi in incubated soils was observed using a dissecting microscope at 3 days intervals for 153 or more days at site I and for 120 or more days at site II. Seven replicates were prepared for each kind of soil incubation. The first appearance date of each fungus, are expressed as the earliest fruiting date of each fungus from the seven replicates. The final disappearance date of each fungus is expressed as the latest date of disappearance of reproductive structures of each fungus from the seven replicates.

At site III, a quadrat (100 × 50 cm) was prepared on the forest floor and divided into 200 sub-quadrats (5 × 5 cm). The soils of L-A_1 horizon of the sub-

quadats were collected separately using 200 sterilized cubic soil-samplers (5 × 5 × 5 cm). Soil samples were placed separately in 200 aseptic plastic bags and returned to the laboratory. We selected an area for soil sampling that contained no visible fungal fruit-bodies. Each soil was placed into a 200 ml sterilized conical flask. An adequate concentration of membrane filtered (cellulose nitrate; pore size, 0.2 µm, Avantec) sterilized urea solution was introduced to the soil sample in order to adjust the sample to 22.1 mg urea/g dry soil with a water content to 60%. A sterilized foamed silicon rubber plug (Silico-plug, Shin-etsu Chemical Co.) was added to the conical flask. All procedures were carried out under aseptic conditions. The soils were incubated for 65 days under the same conditions as described above.

Analyses of physical and chemical properties of soils

pH

Two g of the fresh soil was mixed with 8 mL distilled water and stirred for a few min. The pH value was measured using a glass electrode (Horiba 6028).

Water content

One hundred g of the fresh soil was dried at 105 C for 24 h and left to cool to room temperature in a desiccator. Water content was expressed as a percentage of fresh weight.

Inorganic nitrogen

Ten g of fresh soil was mixed with 50 mL of distilled water and shaken reciprocally (160 times / min) for one h. The concentration of NH_4-N, NO_3-N, and NO_2-N in each water extract was analyzed with a Technicon Autoanalyser II by the indophenol method, hydrazine reduction-sulfanilamide method and sulfanilamide method, respectively (Hiroki *et al.*, 1983).

Results and discussion

Successive occurrence of ammonia fungi

Site I

Occurrence of ammonia fungi in the field

Seven fungi appeared sequentially after urea treatment. The order of occurrence was: *Amblyosporium botrytis* (18 April 1984) → *Ascobolus denudatus*, *Peziza moravecii* (30 April) → *Pseudombrophila petrakii* [Syn: *Pseudombrophila deerata*] (3 May) → *Tephrocybe tesquorum* [Syn:

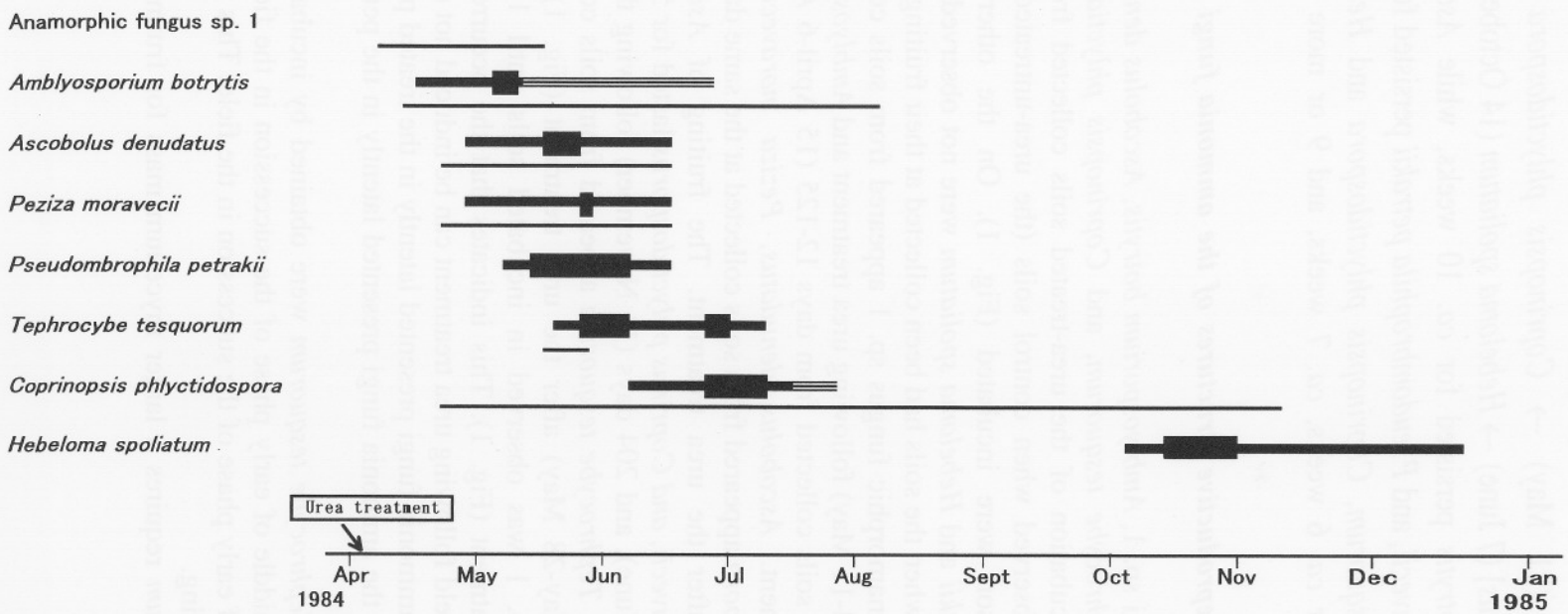


Fig. 1. Successive occurrence and estimated duration of propagation of ammonia fungi in soils of L-A_1 horizon.
In site I, 800 g/m^2 of urea was applied on 3 April 1984.

: Duration of the occurrence of each ammonia fungus.

: Duration of the occurrence peak of each ammonia fungus.

: Duration of the occurrence fall 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 : It is expressed as the duration between sampling date of soils, which showed the first appearance of reproductive structures of each fungus in soil incubation, and that of soils, which showed final disappearance of reproductive structures of each fungus in soil incubation.

Lyophyllum tylicolor] (21 May) → *Coprinopsis phlyctidospora* [Syn.: *Coprinus phlyctidosporus*] (7 June) → *Hebeloma spoliatum* (14 October) (Fig. 1). *Amblyosporium botrytis* persisted for *ca.* 10 weeks, while *Ascobolus denudatus*, *Peziza moravecii*, and *Pseudombrophila petrakii* persisted for *ca.* 7 weeks. *Tephrocybe tesquorum*, *Coprinopsis phlyctidospora* and *Hebeloma spoliatum* persisted for *ca.* 6 weeks, *ca.* 7 weeks, and 9 or more weeks, respectively (Fig. 1).

Appearance of reproductive structures of the ammonia fungi by soil incubation

Anamorphic fungi sp. 1, *Amblyosporium botrytis*, *Ascobolus denudatus*, *Peziza moravecii*, *Tephrocybe tesquorum*, and *Coprinopsis phlyctidospora* appeared following incubation of the urea-treated soils collected from the forest floor but not observed when control soils (the urea-untreated soils) collected in any season were incubated (Fig. 1). On the other hand, *Pseudombrophila petrakii* and *Hebeloma spoliatum* were not observed on the urea-treated soils even when the soils had been collected at their fruiting period in the field (Fig. 1). Anamorphic fungus sp. 1 appeared from soils collected from days 3-38 (6 April-11 May) following urea treatment and *Amblyosporium botrytis* appeared from soils collected from days 12-125 (15 April-6 August) following urea treatment. *Ascobolus denudatus, Peziza moravecii,* and *Coprinopsis phlyctidospora* appeared from soils collected at the same date, i.e., 18 days (21 April) after the urea treatment. The fruiting of *Ascobolus denudatus, Peziza moravecii, and Coprinus phlyctidosporus* lasted for 57 days (17 June), 47 days (7 June), and 204 days (11 November) following the urea treatment, respectively. *Tephrocybe tesquorum* appeared from soils collected on days 43-55 (16 May-28 May) after the urea treatment (Fig. 1). Only anamorphic fungus sp. 1 was observed in incubated soils until 12 days following the urea treatment (Fig. 1). This indicates that the occurrence of ammonia fungi in the field following urea treatment can be induced not only by the propagation of the ammonia fungi presented latently in the treated plot, but also by the invasion of the ammonia fungi presented latently in the peripheral area of the plot.

Fruit-bodies of *Tephrocybe tesquorum* were obtained by incubation of soils collected at the middle of early phase of the succession in the field, but not during other time of early phase of the succession in the field. This may be explained by the following.

1. *Tephrocybe tesquorum* requires a larger mycelium mass for fruting body formation.

2. *Tephrocybe tesquorum* grows and fruits at narrow physical and chemical properties ranges of soils.
3. *Tephrocybe tesquorum* requires interactions between other organisms including other ammonia fungi, to fruit.
4. *Tephrocybe tesquorum* is not resistant to drought, as water was not added during soil incubation.

Six anamorphic fungi (e.g. *Penicillium* sp., *Doratomyces* sp.) appeared in the urea-treated soils. Except for Anamorphic fungus sp. 1, these fungi are not shown in Fig. 1 because they did not appear before the occurrence of *Amblyosporium botrytis* and they were not easily observed from the outside of glass surface of the test tube. Fruit bodies of *Mycena* sp. 1, 2, *Marasmius* sp. 1, two kinds of basidiomycete mycelial strands (nos. 1, 2), *Bisporella* sp. 1, 2, Anamorphic fungus sp. 2 were observed on the incubated-soils collected from the control plot at different seasons, but not observed on any incubated soils collected from the urea plot. In particular, fruit-bodies of *Mycena* sp. 3 and *Marasmius* sp. 2, and another kind of mycelial strand (no. 3) were observed following incubation of soils collected 125 or more days after the urea treatment, as well as in the soils collected from the control plot as described above. This result suggests that litter-decomposing fungi (non-ammonia fungi) start to propagate earlier than 125 days following the urea treatment.

Site II

Occurrence on ammonia fungi in the field

Nine fungi appeared sequentially after urea treatment. The order of occurrence was: Anamorphic fungus sp. 4 (19 April 1989) → *Cladorrhinum foecundissimum*, *Amblyosporium botrytis*, *Ascobolus denudatus*, *Peziza moravecii*, *Pseudombrophila petrakii* (1 May) → *Humaria velenovskyi* and *Coprinopsis phlyctidospora* (20 May) → *Hebeloma spoliatum* (8 October) (Fig. 2). *Cladorrhinum foecundissimum* and *Amblyosporium botrytis* occurred for *ca.* 1 week. The other ammonia fungi persisted for the following periods, Anamorphic fungus sp. 4, *Ascobolus denudatus*, *Peziza moravecii*, *Pseudombrophila petrakii*, *Humaria velenovskyi*, *Coprinopsis phlyctidospora* and *Hebeloma spoliatum*, for *ca.* 2 weeks, for *ca.* 5 weeks, *ca.* 6 weeks, *ca.* 2 weeks, *ca.* 6 weeks, *ca.* 10 (-20) weeks, and *ca.* 9 weeks, respectively (Fig. 2).

Appearance of reproductive structures of the ammonia fungi by soil incubation

Among the ammonia fungi that occurred in the field, *Amblyosporium botrytis*, *Ascobolus denudatus*, and *Coprinopsis phlyctidospora* also appeared following incubation of the urea-treated soils collected separately from L-F and

H-A_1 horizons (Fig. 2). This indicates that at least these three ammonia fungi inhabit both L-F and H-A_1 horizons. The results obtained from soil incubations at sites I and II suggest that occurrence of ammonia fungi occur irrespective of the presence of the living ammonia fungi in the urea-treated-plot. In other words, the propagation of ammonia fungi can result from invasion of ammonia fungi from the outside of the urea-treated-plot and / or growth of ammonia fungi already inhabiting the urea-treated-plot.

Anamorphic fungus sp. 4 and *Pseudombrophila petrakii, Humaria velenovskyi,* and *Hebeloma spoliatum* were not observed following incubation of urea-treated-soils in either sites even when the soil had been collected at the periods of pre-fruiting and fruiting of each fungus (Figs. 1, 2). Sagara (1975) divided ammonia fungi into two groups: one comprising species, which occurred following laboratory experiments, and the other, which did not. The ammonia fungi, which belong to the former group are saprobic, while most ammonia fungi belonging to the latter are symbiotic (Sagara, 1975, 1992, 1995; Fukiharu and Horigome, 1996). *Cladorrhinum foecundissimum* and *Pseudombrophila petrakii* belong to the former group, while *Humaria velenovskyi* and *Hebeloma spoliatum* belong to the latter group. *Hebeloma spoliatum* is categorized as endomycorrhizal (Sagara, 1992, 1995; Fukiharu and Horigome, 1996). *Hebeloma spoliatum* did not form any fruit-bodies following incubation of the urea-treated-soils that had been collected from sites I and II (Figs. 1, 2) because no living host tree(s) were present. Other ammonia fungi that formed reproductive structures following soil incubation (Figs. 1, 2) are saprobic (Sagara, 1975; Fukiharu and Horigome, 1996), but *Humaria velenovskyi* and *Pseudombrophila petrakii* did not appear in incubation of the urea-treated-soils even when the soils had been collected at the periods of pre-fruiting and fruiting in each fungus in the field (Figs. 1, 2). The lack of fruiting of these saprobic ammonia fungi following soil incubation may be explained by the following:

1. The temperature used in soil incubation was not optimum for vegetative growth and / or fruiting.

Sagara (1975) observed the occurrence of *Humaria velenovskyi* in the field following urea treatment both in winter and summer. However, the temperature factor cannot completed by dismissed in the case of *Humaria velenovskyi* which did not fruit in our laboratory experiments because there is no sufficient data concerning the temperature range for the growth and the fruiting not only in *Pseudombrophila petrakii* but also in *Humaria velenovskyi.*

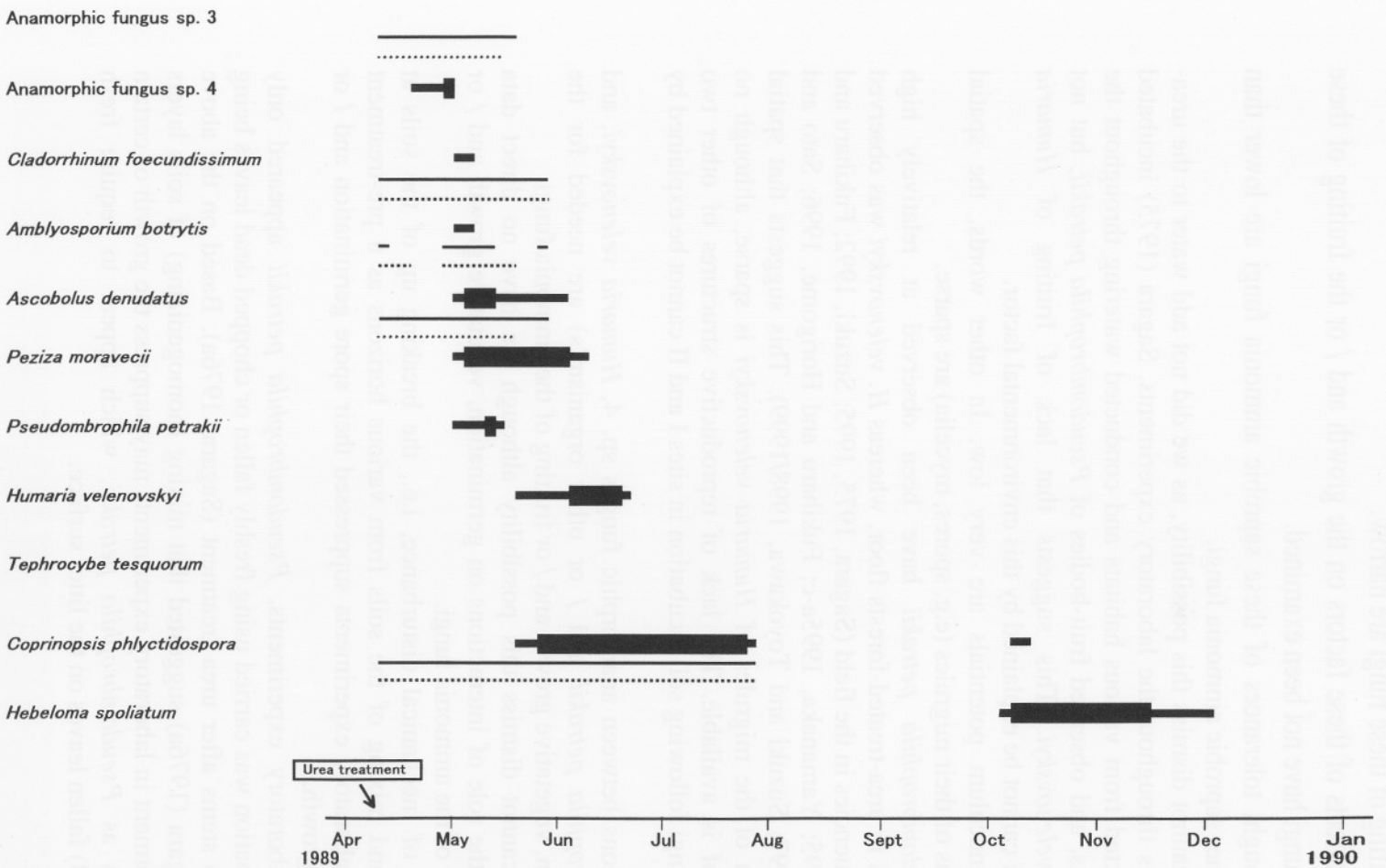


Fig. 2. Successive occurrence and estimated duration of propagation of ammonia fungi in soils of L-F and H-A_1 horizons.
In site II, 800 g/m^2 of urea was applied on 8 April 1989.
: Duration of the occurrence of each ammonia fungus. : Duration of occurrence peak of each ammonia fungus.
: Duration of propagation of each ammonia fungus, estimated by incubation of soils that were collected from L-F horizon (P).
: Duration of propagation of each ammonia fungus, estimated by incubation of soils that were collected from H-A_1 horizon (P).
P : See legend in Fig. 1.

2. The pH and NH_4-N ranges that are suitable for the vegetative growth and / or their fruiting of these fungi are narrow.

The effects of these factors on the growth and / or the fruiting of these ammonia fungi have not been examined.

3. The drought tolerances of these saprobic ammonia fungi are lower than those of other saprobic ammonia fungi.

We cannot dismiss this possibility, as we did not add water to the urea-treated-soils throughout the laboratory experiments. Sagara (1975) incubated soils collected from various habitats and conducted watering throughout the experiments, and observed fruit-bodies of *Pseudombrophila petrakii,* but not *Humaria velenovskyi.* This suggests that lack of fruiting of *Humaria velenovskyi* cannot be explained by this environmental factor.

4. Their inoculum potentials are very low. In other words, the spatial distributions of their migrules (e.g. spores, mycelia) are sparse.

Pseudombrophila petrakii have been observed at relatively high frequencies in urea-treated-forests floor, whereas *H. velenovskyi* was observed at low frequencies in the field (Sagara, 1975, 1995; Suzuki, 1992; Fukiharu and Hongo, 1995; Yamanaka, 1995a-c; Fukiharu and Horigome, 1996; Sato and Suzuki, 1997; Suzuki and Toyokawa, 1998/1999). This suggests that spatial distribution of the migrules of *Humaria velenovskyi* is sparse, although no direct proof is available. The lack of reproductive structures of other two saprobic fungi following soil incubation in sites I and II cannot be explained by this factor.

5. Interactions between anamorphic fungus sp. 4, *Humaria velenovskyi*, and *Pseudombrophila petrakii* and / or other organism(s) are needed for the germination, vegetative growth and / or fruiting of the ammonia fungi.

We cannot dismiss this possibility although we have no direct data indicating the role of interactions on germination, vegetative growth and / or the fruiting of the ammonia fungi.

6. A kind of mechanical disturbance, i.e., the breaking up of the soils at sampling and mixing of the soils from various horizons as a pre-treatment made the laboratory experiments suppressed their spore germination and / or vegetative growth.

In laboratory experiments, *Pseudombrophila petrakii* appeared only when incubation was carried using freshly fallen or chopped dead leaves being attached to stems after urea treatment (Sagara, 1976a). Based on the above results, Sagara (1976a) suggested that mixing (homogenizing) of soils layers for pre-treatment in laboratory experiments may suppress the growth of certain fungi such as *Pseudombrophila petrakii,* which appear to require fresh (undecayed) fallen leaves on the litter surface.

7. The sampling date might be undesirable for the litter harvesting to obtain the fruiting of *Pseudombrophila petrakii* in the laboratory.

We collected the fallen leaves with other litter components in L-A_1 horizon in the early April and mixed them well. This means that the sample soils used for our incubation are unsuitable for the fruiting of this fungus if we follow Sagara's assumption (Sagara, 1976a) described above.

Changes in the soil properties

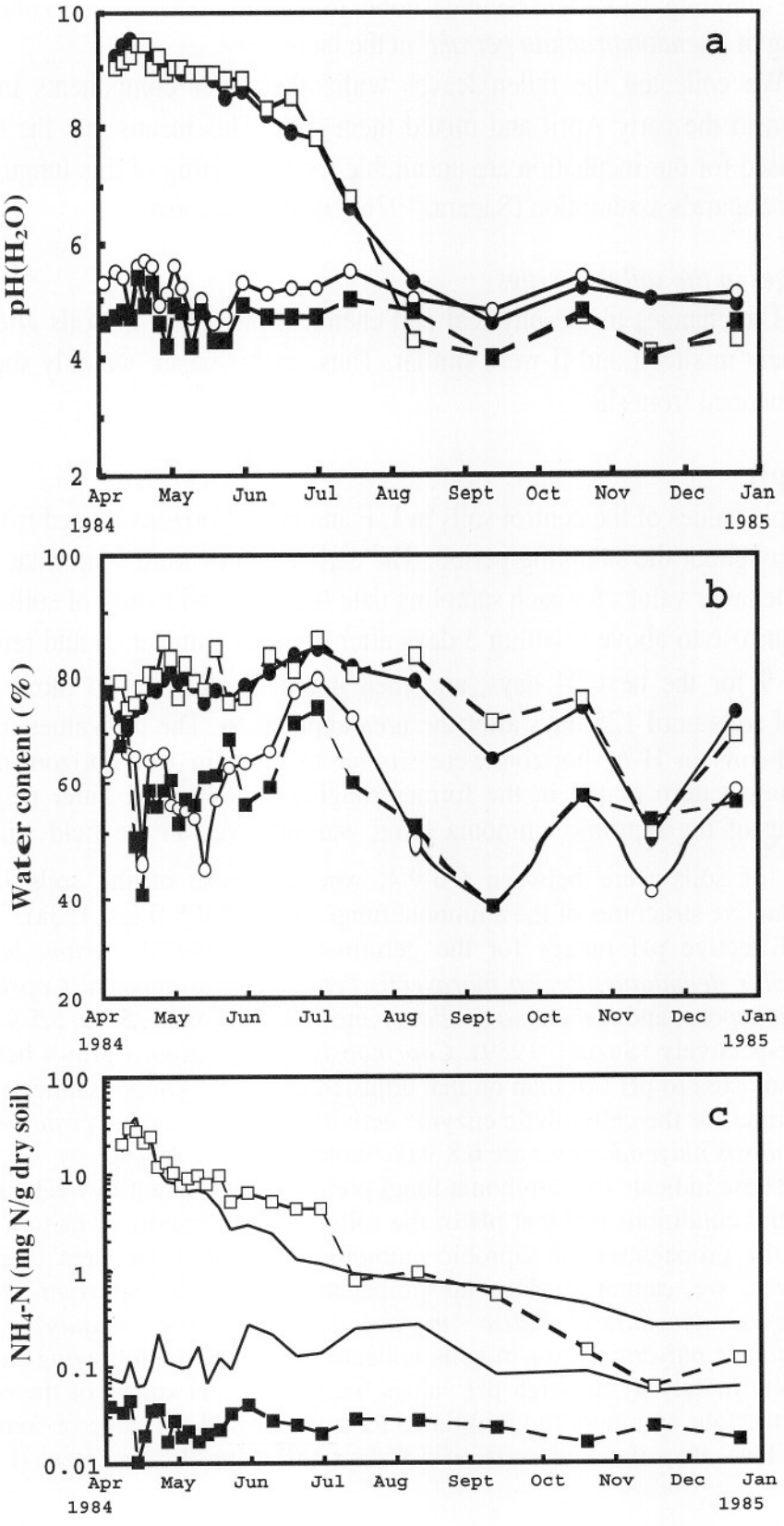
The changes in the physical and chemical properties of soils after urea treatment in sites I and II were similar. Thus, in this paper, we only show the data obtained from site I.

pH

pH values of the control soils in L-F and H-A_1 horizons ranged from 4.0-5.7 throughout the sampling period. The earlier values were somewhat higher than the latter values for each sampling date (Fig. 3a). pH values of soils in L-F horizon rose to above 9 within 3 days after the urea application and remained above 9 for the next 24 days, and then gradually declined to those of the control soils until 125 days after the urea application. The pH values in urea-treated-soils in H-A_1 horizon were similar to those in L-F horizon and the changing pattern of pH in the former roughly followed the latter (Fig. 3a). Fruiting of the saprobic ammonia fungi was observed in the field when pH values of soils were between 6.6-9.4, whereas those of the soils lacking reproductive structures of the ammonia fungi were 9.0-9.5 (Figs. 1, 3a).

Effective pH ranges for the germination of *Amblyosporium botrytis*, *Ascobolus denudatus*, *Peziza moravecii*, *Tephrocybe tesquorum*, *Coprinopsis phlyctidospora*, and *Hebeloma spoliatum* are 7-9, 5-11, 6-11, 5-12, 5.5-9.5, and 4-9, respectively (Suzuki, 1989). *Coprinopsis phlyctidospora* grows better on PDA adjusted to pH 6-8 than on that adjusted to pH 3-5 (Soponsathien, 1998). pH optima for the cellulolytic enzyme activities of *Tephrocybe tesquorum* and *Coprinopsis phlyctidospora* are 6.8-9.0 (Enokibara *et al.*, 1993).

These indicate that ammonia fungi prefer or are tolerant to weak alkaline to neutral conditions and that pH of the soils is one of important factors which cause the propagation of saprobic ammonia fungi after the urea treatment. However, we cannot explain no propagation of *Amblyosporium botrytis*, *Ascobolus denudatus*, *Peziza moravecii*, *Tephrocybe tesquorum*, and *Coprinopsis phlyctidospora* in soils collected on 3-9 days following the urea treatment merely by the high pH values because the pH values of the soils in which the four ammonia fungi initiated to grow showed the same or somewhat higher level than those of soils on 3-9 days following the treatment (Figs. 1, 3a).


a
pH(H2O)
10
8
6
4
2
Apr
May
Jun
Jul
Aug
Sept
Oct
Nov
Dec
Jan
1984
1985
b
Water content (%)
100
80
60
40
20
c
NH4-N (mg N/g dry soil)
100
10
1
0.1
0.01

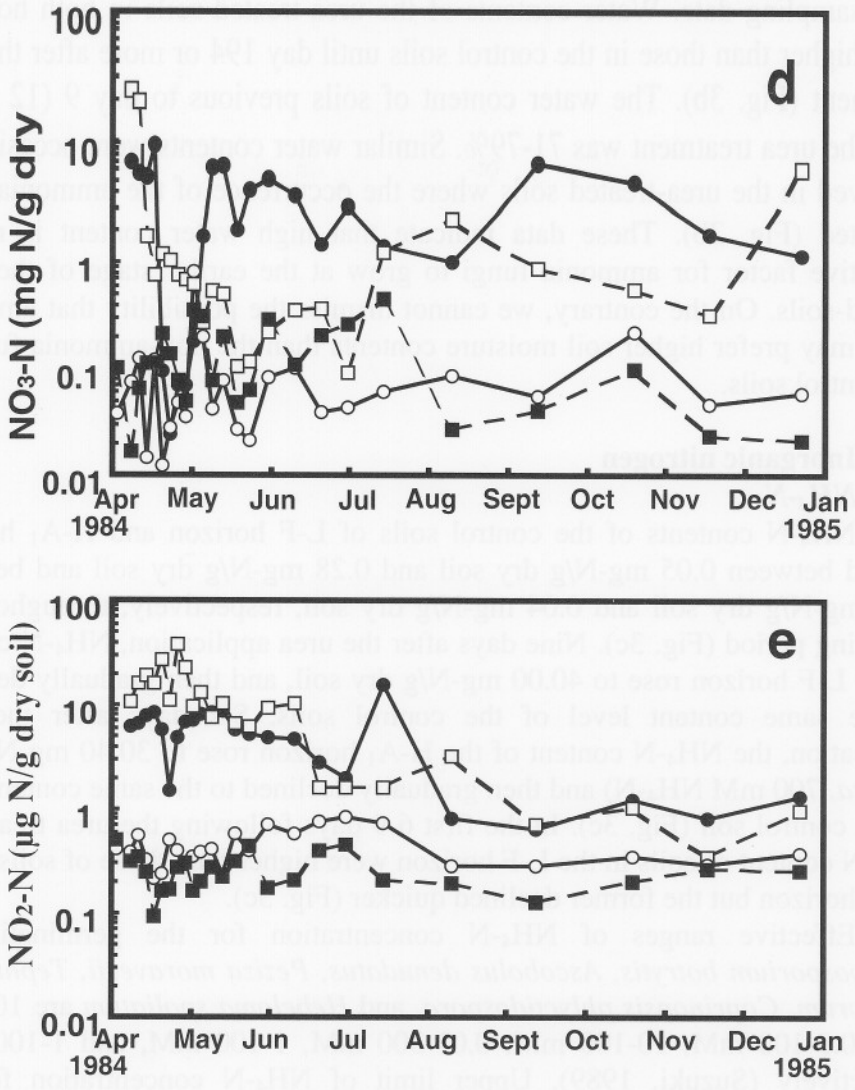


Fig. 3. Changes in physical and chemical properties of soils following urea treatment in site I. In site I, 800 g/m^2 of urea was applied on 3 April 1984. **a.** pH; **b.** Water content; **c.** NH_4-N content; **d.** NO_3-N content; NO_2-N content.

: Urea treated, L-F horizon; : Control, L-F horizon

: Urea treated, H-A_1 horizon; : Control, H-A_1 horizon

Water content

Water content of the control soils in L-F and H-A_1 horizons ranged from 38 to 79% throughout the sampling period (Fig. 3b). In the urea-treated-plot, water contents of soils in L-F and H-A_1 horizons also showed similar values at

each sampling date. Water contents of the urea-treated-soils in both horizons were higher than those in the control soils until day 194 or more after the urea treatment (Fig. 3b). The water content of soils previous to day 9 (12 April) after the urea treatment was 71-79%. Similar water contents were occasionally observed in the urea-treated soils where the occurrence of the ammonia fungi persisted (Fig. 3b). These data indicate that high water content is not an inhibitive factor for ammonia fungi to grow at the earlier stage of the urea-treated-soils. On the contrary, we cannot dismiss the possibility that ammonia fungi may prefer higher soil moisture contents than the non-ammonia fungi in the control soils.

Inorganic nitrogen

NH_4-N

NH_4-N contents of the control soils of L-F horizon and H-A_1 horizon ranged between 0.05 mg-N/g dry soil and 0.28 mg-N/g dry soil and between 0.02 mg-N/g dry soil and 0.04 mg-N/g dry soil, respectively, throughout the sampling period (Fig. 3c). Nine days after the urea application, NH_4-N content of the L-F horizon rose to 40.00 mg-N/g dry soil, and then gradually declined to the same content level of the control soils. Six days after the urea application, the NH_4-N content of the H-A_1 horizon rose to 30.40 mg-N/g dry soil (*ca.* 700 mM NH_4-N) and then gradually declined to the same content level of the control soil (Fig. 3c). In the first 6-9 days following the urea treatment, NH_4-N content of soils in the L-F horizon were higher than those of soils in the H-A_1 horizon but the former declined quicker (Fig. 3c).

Effective ranges of NH_4-N concentration for the germination of *Amblyosporium botrytis*, *Ascobolus denudatus*, *Peziza moravecii*, *Tephrocybe tesquorum*, *Coprinopsis phlyctidospora*, and *Hebeloma spoliatum* are 10-1000 mM, 0.3-100 mM, 10-100 mM, 0.01-300 mM, 1-100 mM, and 1-100 mM, respectively (Suzuki, 1989). Upper limit of NH_4-N concentration for the vegetative growth of each fungus is 455 mM for *Amblyosporium botrytis* and *Coprinopsis phlyctidospora* and 68 mM for *Ascobolus denudatus*, *Tephrocybe tesquorum*, and *Hebeloma spoliatum* (Suzuki, 1989).

These data indicate that the NH_4-N concentration is one of important factors which regulates the germination and vegetative growth of ammonia fungi, namely which cause the propagation of ammonia fungi to the urea-treated-plot after the treatment.

NO_3-N

NO_3-N contents of the control soils of the L-F and H-A_1 horizons ranged from 0.01 mg-N/g dry soil to 0.81 mg-N/g dry soil throughout the sampling

period (Fig. 3d). NO_3-N content of urea-treated soils in the L-F and H-A_1 horizons rose quickly to maximum values of 9.62 mg-N/g dry soil and 24.00 mg-N/g dry soil, respectively, on day12 and day 3 after the urea treatment. Thereafter, NO_3-N contents of the urea-treated-soils in the L-F horizon rose and then rapidly declined to the lower level (0.02 mg-N/g dry soil) at day 18 (21 April) after the urea application. Thereafter it rose again to 6.27 mg-N/g dry soil on day 34 (7 May) and remained around this level throughout the experiment. NO_3-N contents of the urea-treated-soils in the H-A_1 horizon gradually declined to the level of the control experiment on day 43 (16 May) and remained at this level for subsequent 37 days (11 July), and then rapidly rose to the same level to the urea-treated-soils in the L-F horizon throughout the sampling period (Fig. 3d). Total NO_3-N contents of the urea-treated soils in L-F and H-A_1 horizons showed lower values at the period of the early phase of fruiting of *Amblyosporium botrytis, Ascobolus denudatus, Peziza moravecii,* and *Pseudombrophila petrakii* (Figs. 1, 3d).

Yamanaka (1999) reported that *Amblyosporium botrytis*, *Pseudombrophila petrakii*, *Tephrocybe tesquorum*, and *Coprinopsis phlyctidospora* cultured at pH 7 as well as *Hebeloma* spp. cultured at pH 5 grew better on nitrate than on ammonium. These results give us the assumption that the decline of NO_3-N content during the fruiting periods of *Amblyosporium botrytis, Ascobolus denudatus, Peziza moravecii, Pseudombrophila petrakii*, and *Coprinopsis phlyctidospora* is due to utilization of nitrate by these saprobic ammonia fungi and / or an ectomycorrhizal fungus, *Hebeloma spoliatum*. However, we cannot ascertain the role of *Hebeloma spoliatum* in the utilization of nitrate during this period because the propagation period of ectomycorrhizal fungi, such as *Hebeloma spoliatum*, could not be estimated by soil incubation as described above.

NO_2-N

NO_2-N content of the control soils of the L-F and H-A_1 horizons ranged from 0.13-0.83 µg-N/g dry soil throughout the sampling period (Fig. 3e). On 3 days (6 April) following the urea treatment, NO_2-N contents of the urea-treated-soils in the L-F and H-A_1 horizons rapidly rose to 6.27 µg-N/dry soil and to 10.80 µg-N/dry soil, respectively, and then remained at a higher level than those of the control plot for the next 96 days (7 November) or longer. Maximum values (27.40 µg-N/dry soil) of NO_2-N content was obtained from the urea-treated-soil in the H-A_1 horizons collected on 15 days (21 April) following the urea treatment (Fig. 3e). However, ammonia fungi occurred even when the contents of NO_2-N showed higher values (Figs. 1, 3e). Although nitrite is utilized as a nitrogen source by some fungi, it is toxic to many fungus

species (Garraway and Evans, 1984). For example, the growth of *Neurospora crassa* is inhibited above 5 mM NO_2^- (Nicholas, 1965).

The nitrite utilization of ammonia fungi have not been examined but the maximum nitrite concentration obtained here (less than 0.5 mM) may be too low level to evaluate the inhibitive effect of nitrite on the vegetative growth of ammonia fungi.

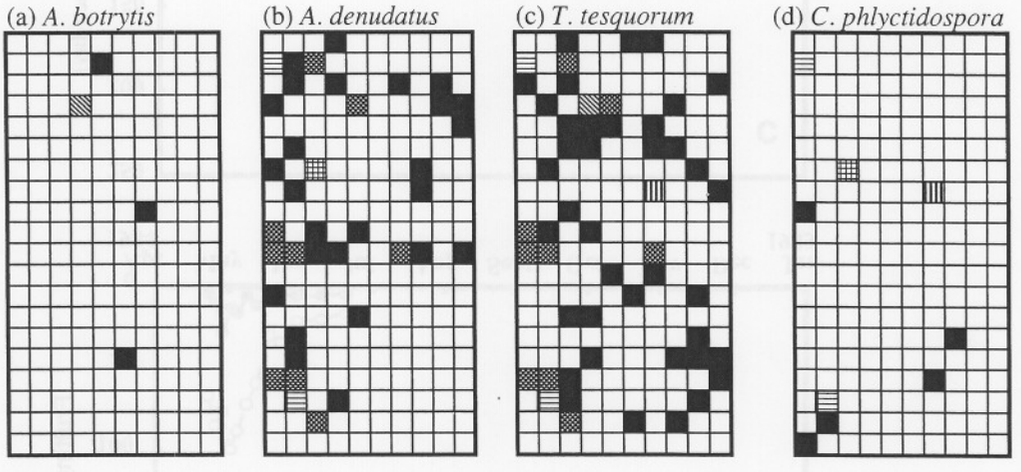
Spatial distribution of ammonia fungi

Site III

Amblyosporium botrytis, *Ascobolus denudatus*, *Tephrocybe tesquorum*, and *Coprinopsis phlyctidospora* appeared from the soils collected from sub-quadrats at the frequency of 4/200, 38/200, 52/200 and 9/200, respectively. The four ammonia fungi showed no specific co-existence (Fig. 4). *Amblyosporium botrytis* was isolated from soils collected from the separate sub-quadrats (Fig. 4a). *Ascobolus denudatus* was isolated from three groups of adjacent sub-quadrats and diagonally adjacent sub-quadrat(s), three groups of adjacent sub-quadrats, one group of diagonally adjacent sub-quadrats, and four separate sub-quadrats (Fig. 4b). *Tephrocybe tesquorum* was isolated from six groups of adjacent sub-quadrats and diagonally adjacent sub-quadrats, two group of adjacent sub-quadrats, and four separate sub-quadrats (Fig. 4c). *Coprinopsis phlyctidospora* was also isolated from one group of two adjacent sub-quadrats and one diagonally adjacent sub-quadrat, and six separate sub-quadrats (Fig. 4d). The minimum size of migrules of each fungus may be less than 5 cm cubic although we cannot know the latent forms, i.e., spores and / or mycelia, of each ammonia fungus by this kind of experiment.

The occurrence frequencies of *Amblyosporium botrytis, Ascobolus denudatus*, *Tephrocybe tesquorum*, and *Coprinopsis phlyctidospora* have been shown to be high in various geographical areas of Japan, when large amounts of urea are applied (Sagara, 1975; 1976b; Suzuki, 1992; Yamanaka, 1995a-c; Fukiharu and Hongo, 1995; Fukiharu and Horigome, 1996; Fukiharu *et al.*, 1997; Sato and Suzuki, 1997; Suzuki and Toyokawa, 1998/1999). These indicate that the frequencies of occurrence of ammonia fungi in the field are not proportional to the frequencies of spatial distribution of ammonia fungi in a small area.

These observations support the assumption that the occurrence of ammonia fungi in the low and medium level of urea-treated plot can be due to propagation of the ammonia fungi latently inhabiting treated plots. The propagation commences just after both the pH value and NH_4-N concentration become lower than the upper limit values for the germination and the vegetative growth of ammonia fungi.

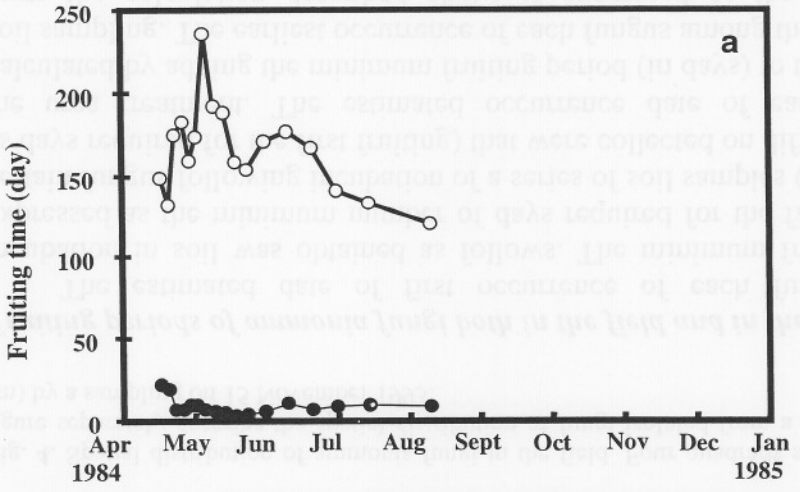


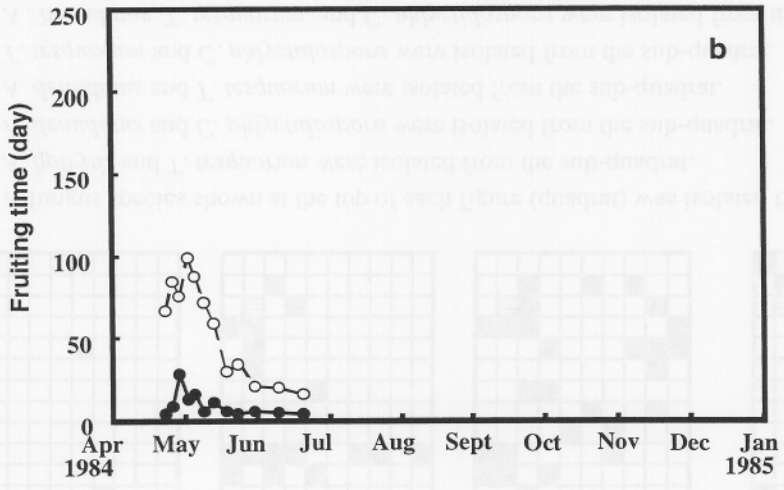
A fungus species shown at the top of each figure (quadrat) was isolated from the sub-quadrat.
A. botrytis and *T. tesquorum* were isolated from the sub-quadrat.
A. denudatus and *C. phlyctidospora* were isolated from the sub-quadrat.
A. denudatus and *T. tesquorum* were isolated from the sub-quadrat.
T. tesquorum and *C. phlyctidospora* were isolated from the sub-quadrat.
A . denudatus, *T. tesquorum*, and *C. phlyctidospora* were isolated from the sub-quadrat.

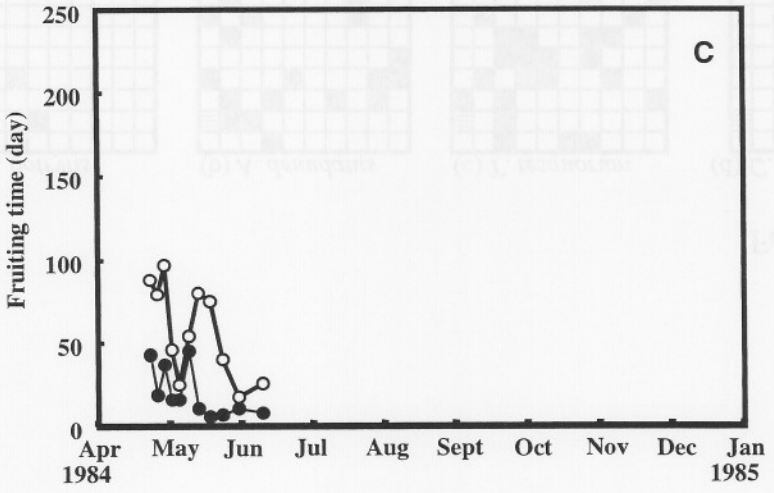
Fig. 4. Spatial distribution of ammonia fungi in the field. Four quadrads shown in the above figure separately describe the spatial distribution of fungi isolated from a quadrad (100 × 50 cm) by a sampling on 15 November 1993.

Fruiting periods of ammonia fungi both in the field and in the laboratory

The estimated date of first occurrence of each fungus following incubation in soil was obtained as follows. The minimum fruiting period is expressed as the minimum number of days required for the first fruiting of a certain fungus following incubation of a series of soil samples (In Fig. 5 shown as days required for the first fruiting) that were collected on different days after the urea treatment. The estimated occurrence date of each fungus was calculated by adding the minimum fruiting period (in days) to the date for each soil sampling. The earliest occurrence of each fungus among the dates obtained from the calculation described above is expressed as the estimated first occurrence date of the fungus following soil incubation. At site I, the estimated first occurrence dates of *Amblyosporium botrytis, Ascobolus denudatus, Peziza moravecii, Tephrocybe tesquorum* and *Coprinopsis phlyctidospora* were 27 April (on 24 days after the urea treatment), 25 April (on 22 days after the urea treatment), 13 May (on 40 days after the urea treatment), 28 May (on 55 days after the urea treatment), and 6 June (on 64 days after the urea treatment), respectively.


a
Fruiting time (day)
250
200
150
100
50
0
Apr
1984
May
Jun
Jul
Aug
Sept
Oct
Nov
Dec
Jan
1985


b
Fruiting time (day)
250
200
150
100
50
0
Apr
1984
May
Jun
Jul
Aug
Sept
Oct
Nov
Dec
Jan
1985


C
Fruiting time (day)
250
200
150
100
50
0
Apr
1984
May
Jun
Jul
Aug
Sept
Oct
Nov
Dec
Jan
1985

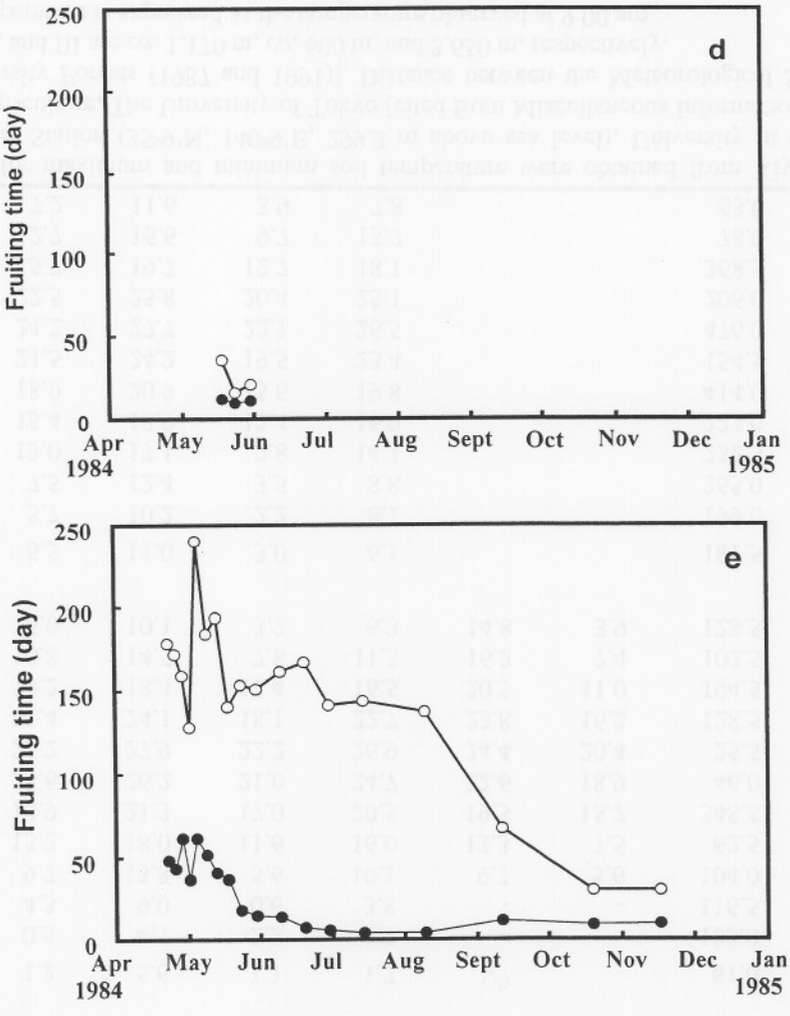


Fig. 5. The days required for the appearance and disappearance of reproductive structures of ammonia fungi in incubation of soils (L-A_1 horizon) that were collected on different days following the urea treatment in site I. In site I, 800 g/m^2 of urea was applied on 3 April 1884. **a.** *Amblyosporium botrytis*; **b.** *Ascobolus denudatus*; **c.** *Peziza moravecii*; **d.** *Tephrocybe tesquorum*; **e.** *Coprinopsis phlyctidospora*.

—●— : The days required for the first fruiting of each ammonia fungus by incubation of soils that were collected on different days following the urea treatment.

—○— : The days required for the final disappearance of each ammonia fungus by incubation of soils that were collected on different days following the urea treatment.

Table 2. Meteorological data[a] in Kiyosumi, Chiba, Japan.

Date	Air temperature[b] (C)			Soil temperature[c] (C)			Precipitation (mm)
	Mean	Max.	Min.	Mean	Max.[d]	Min.[d]	
1984							
January	1.2	5.6	-1.1	1.7	-[d]	-	81.0
February	0.5	4.7	-2.2	1.7	-	-	103.0
March	4.3	9.0	0.6	3.8	-	-	116.5
April	9.7	13.8	5.6	10.1	9.7	5.6	104.0
May	15.2	18.0	11.6	16.0	13.3	7.5	62.5
June	18.9	21.3	17.0	20.5	19.3	15.7	348.5
July	23.6	26.2	21.0	24.7	22.6	18.9	46.0
August	25.2	27.9	22.2	26.9	24.4	20.4	25.5
September	21.4	24.1	18.1	22.7	23.8	16.2	128.5
October	15.2	18.4	12.4	16.5	20.5	11.0	194.5
November	10.8	14.7	7.8	11.3	16.3	7.4	102.5
December	6.0	10.1	3.2	6.3	14.8	3.9	128.5
1989							
January	6.5	11.0	3.0	6.1			181.5
February	5.7	10.2	2.2	6.1			199.0
March	7.5	12.4	3.5	8.8			265.0
April	13.0	17.1	9.8	14.1			232.5
May	15.4	18.9	12.4	16.9			223.0
June	18.0	20.9	15.6	19.8			414.0
July	21.5	24.2	19.5	23.4			154.5
August	24.2	27.7	22.1	26.5			476.0
September	22.5	25.8	20.4	25.1			206.0
October	15.7	19.7	12.7	18.1			368.5
November	12.7	16.6	9.7	13.7			78.0
December	7.2	11.6	3.9	7.8			63.0

[a] Data except for maximum and minimum soil temparature were obtained from Kiyosumi Meteorological Station (35º9'N, 140º9'E, 299.8 m above sea level), University in Chiba, Faculty of Agriculture, The University of Tokyo [cited from Miscellaneous Information, The Tokyo University Forests (1987 and 1991)]. Distance between the Meteorological Station and sites I, II, and III are *ca.* 1,170 m, *ca.* 600 m, and 3,650 m, respectively.

[b] Mean air temperature is expressed as the temperature observed at 9:00 am.

[c] Maximum and minimum soil temperature was observed at site I. The temperature was measured by a thermometer placed in a tin box. The box was placed 8 cm under the ground surface and the lid of the box was covered by 1 cm of litter. Mean soil temperature was observed at 10 cm under the ground surface of the Meteorological Station.

[d] Not observed.

The estimated first occurrence dates of these fungi should be similar to or earlier than the actual dates for the first occurrence of those fungi in the field. This is because during the experimental period the incubation temperature for

these ammonia fungi was higher than the average soil temperature at 8 cm under the ground surface (Table 2). The estimated first occurrence dates of *Ascobolus denudatus* and *Coprinopsis phlyctidospora* were somewhat earlier than the actual dates of the first occurrence of those fungi in the field (Fig. 1). The estimated first occurrence dates of *Amblyosporium botrytis, Peziza moravecii,* and *Tephrocybe tesquorum* however, were day 9, 13, and 7 after actual dates for the first occurrence of these fungi in the field, respectively (Fig. 1). This may have resulted due to the inhibitive effects of drought and / or mixing of soil samples for incubation, and / or the absence of the interactions between those fungi and the organism(s) that had not collected by the soil samplings.

The soil sampling dates which gave the above estimated first occurrence dates for saprobic ammonia fungi following soil incubation do not fully coincide with the sequences of occurrence of those fungi in the field; these were, 15 April (on day 12 after the urea treatment) for *Amblyosporium botrytis,* on 21 April (on day 18 after the urea treatment) for *Ascobolus denudatus*, on 24 April (on day 21 after the urea treatment) for *Peziza moravecii,* and both on 21 April and on 30 April (on day 18 and 27 after urea treatment) for *Coprinopsis phlyctidospora*, and on 16 May (on day 43 after the urea treatment) for *Tephrocybe tesquorum,* respectively (see Figs. 1, 5).

These results indicate that the successive occurrence of ammonia fungi in the field mainly results from the combination of sequential propagation of ammonia fungi and the time needed for each fungus to produce reproductive structures.

The maximum latent fruiting period of each fungus by soil incubation is expressed as the number of days between the estimated first occurrence date (X) of a certain fungus for each soil incubation and the final date (Y) marking the latest disappearance of the fungus of each soil incubation. Therefore, the maximum latent fruiting period of each fungus is calculated as (Y) – (X).

The final date marking the disappearance of the fungus following particular soil incubation was determined as follows. The maximum number of days required for the disappearance of the fungus in incubated soil samples collected on different days following urea treatment is added to the date of each soil sampling (In Fig. 5 this is shown as the days required for the final disappearance of reproductive structures of each fungus) The latest disappearance date of a fungus among the dates obtained from the calculation described above is expressed as the final disappearance date of the fungus following soil incubation. The maximum latent fruiting periods of *Amblyosporium botrytis, Ascobolus denudatus, Peziza moravecii*, and *Coprinopsis phlyctidospora* estimated using the above calculation are days

232, 104, 81, and 202, respectively. The maximum latent fruiting period of *Tephrocybe tesquorum* cannot be estimated in the present experiment because not enough soil samples resulted in fruiting. In the above ammonia fungi, the actual fruiting duration of each fungus in the field, i.e., 42 days for *Amblyosporium botrytis*, 38 days for *Ascobolus denudatus* and *Peziza moravecii,* and 51 days for *Coprinopsis phlyctidospora* are shorter than the maximum latent fruiting period described above (Fig. 1). These results indicate that the fruiting period of fruiting of each ammonia fungus is shorter and this may be due to interactions among soil animals and soil microbes. It is likely that these animals and microbes had been collected less frequently or were absent in soils used for incubation in the laboratory.

At both sites I and II, conidiophores of *Amblyosporium botrytis* were observed following incubation of soils collected even after the disappearance of this fungus in the field (Figs. 1, 2). In site I, the fruiting bodies of *Coprinopsis phlyctidospora* were also obtained after a long duration following incubation of the soils collected even after the disappearance of this fungus in the field (Fig. 1). The difference in the length of the latent fruiting periods of *Coprinopsis phlyctidospora* at both sites and the intermittent pattern of the fruiting period of *Amblyosporium botrytis* obtained from soil incubation in site II (Figs. 1, 2) may be explained by the sparse spatial distribution of the migrules of both fungi (Fig. 4).

In conclusion, the main reason for the successive occurrence of saprobic ammonia fungi in urea-treated soils may be derived from the combination of sequential propagation of ammonia fungi and the time needed for each fungus to produce reproductive structures, and not merely sequential invasion and / or propagation. The duration of occurrence of each saprobic fungus in the field was shorter because of interactions between organisms and changes in soil conditions resulting from activities of soil organisms including the ammonia fungi themselves. The propagation of ammonia fungi results from spore germination and mycelial growth, which is base primarily on their preference or tolerance to high concentration of NH_4-N under neutral to alkaline conditions. Unfortunately we could not obtain any information about the propagation period of *Hebeloma spoliatum* using the present experiments because ectomycorrhizal fungus, *Hebeloma spoliatum* (Sagara, 1992, 1995; Fukiharu and Horigome, 1996) does not fruit without presence of living host tree(s). The hypothesis here is partially similar to that proposed for fungal succession in coprophilous fungi as shown by Harper and Webster (1964) and reviewed by Richardson (2002) in this volume. Namely, they proposed that sequential appearance of fruit-bodies of coprophilous fungi on the dung could

be explained by the minimum time taken by the various species to produce them, and not primarily the result of competition for substrata.

Acknowledgements

This work was partially supported by a Grant-in-Aid for Scientific Research (No. 63560141) from the Ministry of Education, Science, Sports and Culture, Japan (Now known as the Ministry of Education, Culture, Sports, Science and Technology, Japan). We are thankful to University Forest in Chiba, Faculty of Agriculture, The University of Tokyo (Now known as University Forest in Chiba, Graduate School of Agricultural and Life Sciences, The University of Tokyo), for their support and making the experimental sites available. We would like to acknowledge the technical assistance of K. Takahashi, M. Maruta and J. Sakai, Faculty of Education, Chiba University. We are very grateful to T. Kuboi, Faculty of Agriculture, Shizuoka University, for his help in soil analysis. We are grateful to K.D. Hyde for helping to improve the English.

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(Received 18 March 2002, accepted 7 April 2002)