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

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**ABSTRACT**

This research investigated the occurrence of ammonia fungi, and changes in soil conditions and the decay rates of wood pieces in response to application of urea (800 g/m²) on a forest floor of *Quercus serrata* dominated mixed forest. Research was conducted in Tokyo from 7 May 1993 to 6 January 1994. Seven species of ammonia fungi were collected on the urea-treated plot (urea plot): *Amblyosporium botrytis*, *Ascocholorus denudatus*, *Peziza moravecii*, *Pseudomorphilicia deereata*, *Coprinus phylctidosporus*, *Hebeloma spoliatum*, and *Lepista* sp. pH values of soils in the urea plot increased to about 9 by 12 days after the urea application and then decreased to that of the control level, 5.0-6.2 by 109 days after the application. Water contents of the soils in the urea plot showed somewhat higher values than those of the soils in the control plot throughout sampling period. Decay rates of balsa board pieces embedded in the urea plot were somewhat higher than those of the pieces embedded in the control plot during the occurrence of the early stage of ammonia fungi.

Key Words — ammonia fungi; decay rate; pH; *Quercus serrata*; urea treatment; water content.

**INTRODUCTION**

Ammonia fungi (Sagara, 1975), a chemoeocological group of fungi, develop sporophores sequentially on forest soil that has been treated with such nitrogenous materials as urea and ammonia. Naturally, these fungi appear after the decomposition of both the bodies and/or faeces of animals, or on other substrata (Sagara, 1992). Sagar (1975) divided the ammonia fungi into two groups: one comprises species which occur in both laboratory and field experiments at the early stage of succession, and the other comprises those which appear only in the field at the later stage of succession. Ammonia fungi were observed in various habitats such as broad-leaved forests and needle-leaf forests etc. from Hokkaido to Taiwan (Sagara, 1975; Suzuki, 1992; Fukiharu and Hongo, 1995; Yamanaka, 1995a, b; Fukiharu et al., 1996). The ammonia fungi in the Kanto plain, where soil profiles are characterized by the surface covering loam layer, were examined only at Nishinasuno in Tochigi Prefecture, and at Kemigawa and Kiyosumi in Chiba Prefecture (Suzuki, 1992).

After the urea-treatment, blackening of the litter layer and increases in pH, NH₄-N concentration were observed on the soils of the L-A layer, and ammonia fungi appeared even in high NH₄-N concentrations with weak alkaline conditions (Sagara, 1975; Suzuki, 1989; Yamanaka, 1995b). Enokihara et al. (1993) reported the ammonia fungi having higher cellulolytic enzyme activities under neutral to alkaline conditions. These results suggest that ammonia fungi are mainly responsible for the decomposition of organic matters in the urea plot. Suzuki and Ogawa (1981) reported that percentage losses in dry weight of square balsa pieces (4 × 5 × 200
mm) reached to 50% within 7 weeks after embedding in litter without urea treatment and that decay rates of balsa pieces were higher than those pieces from other tree species.

For these reasons, we selected a *Quercus serrata* Murray dominated mixed forest in Tokyo to gather and add information about the biogeographical distribution of ammonia fungi in Japan and placed balsa pieces on the forest floor to examine the wood decay rates after the urea treatment in the field.

**MATERIALS AND METHODS**

**The study site**

The investigation was carried out in a *Q. serrata* dominated mixed forest at the Institute for Nature Study, National Science Museum, which is situated at a latitude of 35°38’N and a longitude 139°43’E and has an altitude of about 20 m above sea level, in Minato Ward, which is located in the west part of the Tokyo metropolitan area. This region has a warm-temperate monsoon climate. The average annual mean air temperatures and annual precipitation during the last 10 years at Tokyo District Meteorological Observatory (ca. 7 km northeast of the studying site) were 15.9°C and 1,544 mm, respectively. The study site is located on a gentle slope (N60°W, 3.0-11.5°) and is covered with ca. 18 m high *Q. serrata*, ca. 10 m high *Cornus controversa* Hemsley, *Neolitsea sericea* (Bl.) Koidzumi, and *Prunus grayana* Maxim. (Fig. 1). A pond is situated at ca. 17 m in a north-west-by-westerly direction from the site. The canopy enclosing the site was closed and the ground was bare for the most part except for sparse herbaceous plants, some shrubs such as *Aucuba japonica* Thunb., *Ligustrum japonicum* Thunb. and *Trachycarpus fortunei* (Hook.) H. Wendl. etc., and a few ferns (Fig. 1).

**Profile of the organic horizons**

At the site, both the L and F layers (ca. 1.5-5.0 cm) of the organic horizon were recognized throughout the year. The H layer and A1 layer were fused together, and the boundary between them was indistinguishable. The upper part of the mineral soil horizon is called black horizon (Hirayama et al., 1978).

**Urea treatment**

Six quadrats (2 m × 2 m) were placed along the contour line at 2 m intervals. Each quadrat was divided into four subquadrats of 2 m × 0.5 m (Fig. 1). Three quadrats were treated with urea by means of hand

![Fig. 1. Arrangement of urea-treated quadrats (urea quadrats) and non-urea-treated quadrats (control quadrats) in a *Quercus serrata* dominated mixed forest in the botanical garden of the Institute for Nature Study, National Science Museum, Japan.](image-url)

- : urea quadrat,  : control quadrat,  : site for initial soil sampling.

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scattering at the rate of 800 g/m² on 7 May 1993; others were not treated by urea to be the controls. The urea quadrat and the control quadrat were placed alternately along the contour line (Fig. 1).

Preparation of the balsa wood piece and their placement on the forest floor

Balsa (*Ochroma lagopus* Sw.) wood boards of 1 cm in thickness were cut into wood pieces of 11 cm long and 2 cm wide. They were dried at 80°C for 24 hours and then weighed. Bunches of wood pieces were spread on the surface of the H-A₁ layer where prepared by stripping off the L-F layer by hand, and then uniformly covered with the litter from the L-F layer on 7 May 1993, just before the urea application.

Observation of fungal occurrence

The fungi that form reproductive structures, which can be observed by naked eye, on the soil surface were observed on the sampling dates described below.

Sampling and observation dates

Five hundreds grams of soils from the L-F layer and H-A₁ layer in the urea and control quadrats were collected separately from the top part of the slope from a subquadrat on 19 May, 2 June, 2 July, 24 August, 7 October and 22 October in 1993, and 6 January in 1994. On 7 May, soil samples of the control were collected from 10 different sites which are located between each of the two quadrats (Fig. 1). Both urea treated and control soils were collected from each one of the subquadrats from 19 May 1993 to 6 January 1994. Seven to fourteen wood pieces were also collected in turn from each one of the urea and control subquadrats at the northeast side of the study site. Soil samples and wood pieces were stored in a refrigerator when they could not immediately be subjected to the analysis just after the sampling. On 25 June and 29 October, only the observation of fungal occurrence was done not any sampling.

Analysis of soil samples

Twenty grams of fresh soil from the L-F layer and 10 g of fresh soil from the H-A₁ layer were stirred for a few minutes in 80 ml and 40 ml of distilled water, respectively. Soil pH was then measured by a glass electrode after placing the stirred soil suspension for 30 minutes. One hundred grams of the fresh soil of the L-F layer and 10 g of the fresh soil of the H-A₁ layer were dried using a drying-oven at 105°C for 24 hours and then cooled down to room temperature in a desiccator. Water content was expressed as the percentage of fresh weight basis. Wood pieces were washed and gently rubbed to remove soils and hyphae from their surfaces. They were then dried at 80°C for 24 hours and then cooled down to room temperature in a desiccator. The decay rate was expressed as the percentage of the dry weight loss (the percentage of the dry weight loss of each wood piece at each sampling date/initial dry weight of the piece).

RESULTS AND DISCUSSION

The following seven species of fungi occurred successively in the urea plot, i.e., *Amblyosporium botrytis* Fres., *Ascobolus denudatus* Fr., *Peziza moravcii* (Syrek) Donadini, *Pseudomicrophila beerta* (Kurst.) Seaver, *Coprinus phyllyctidosporus* Romagni, *Hoheloma spoliatum* (Fr.) Karst., and *Lepista* sp. (Fig. 2). In the control plot, *Cryptotrama asperta* (Berk.) Redhead et Ginns and *Russula* sp. were observed on 25 June 1993 and *Marasmius* sp. on 24 August 1993.

Blackening of litter was observed during the first 12 days and then returned to their original color after a short while. The scent of ammonia was detected on 19 May and 2 June. The difference of pH values in soils
of the L-F and H-A1 layers in each control plot were few (Figs. 3, 4). pH levels in the L-F and H-A1 layers of the urea plot showed similar values throughout the sampling period, and those in both layers increased up to about 9 by 12 days after the urea application and remained at 7-9 for the next 44 days. After that, it then declined to almost the same value as that of the control (pH 5.0-6.1) by 109 days after the urea application (Figs. 3, 4). The pH levels in both layers in the 10 control sub-quadrats showed similar values on 7 May 1993. Only the soil in the L-F layer on 19 May 1993 showed a pH level of 7.1, but the pH levels of soils in other cases were below 6.0. This may be from the influence of crow faeces on the subquadrat.

The water content of soils in the urea plot showed somewhat higher values than those of soils in the control plot in both layers throughout sampling period (Figs. 5, 6).

Decay rates of the wood pieces in the urea plot showed significantly higher values than those in the control plot on 2 July (56 days after urea application) and 7 October 1993 (153 days after urea application) (Fig. 7; Table 2; Newman-Keuls test, P<0.05). After 7 October the number of wood pieces which could not be collected, gradually increased in both plots (Table 2). Therefore, decay rate obtained after 7 October may be underestimated, especially in those of the urea plot because quicker decomposed groups of wood pieces could not be used to calculate them. On 22 October 1993, all wood pieces in the urea plot were difficult to be collected. On 29 October 1993 and 6 January 1994, all wood pieces even in the control plot became also impossible to be collected because they were broken during their handling. These mean that wood pieces embedded in the urea plot are decomposed more quickly than those used in the control.

The pH optima of the activities of cellulolytic enzymes, i.e., avicelase, CMCase and β-glucosidase, of ammonia fungi during the early stage of succession are neutral to alkaline regions, while those of cellulolytic enzymes of wood rotting fungi are acidic regions (Enokibara et al. 1993). The vegetative growth of ammonia fungi during the early stage of succession is accelerated from neutral to alkaline conditions (Suzuki, 1989). A. botrytis and C. phyllyctidosporus grow vigorously at
higher concentration of NH$_3$-N (154 mM) (Suzuki, 1989). These results suggest that ammonia fungi in the early stage of succession may adapt well to high concentrations of NH$_3$-N with neutral to alkaline conditions.

From this information, the changes in decay rates of wood pieces in both plots may be explained as follows; 1) Decomposing activities of ammonia fungi in the early stage of succession can be maintained at a higher level until summer because NH$_3$-N concentration and pH values for litter soils are suitable for their vegetative growth and reactions in their cellulolytic enzymes (Figs. 2-4). 2) Then cellulolytic activities of those ammonia fungi become weaker by the lowering of NH$_3$-N concentration and pH values of litter (Figs. 2-4). Under acidic conditions, ammonia fungi, which play a main role in the decomposition of plant materials, may gradually be substituted to the fungi which grow in the control plot. Moreover, the shortness of precipitation during the summer season (Table 1) suggests the possibility that the decomposing activities of the ammonia fungi in the early stage of the succession are weakened more remarkably than those of fungi in the control. This is supposed from the observation that ammonia fungi usually have opportunities to grow under higher water contents based on the urea effect than the fungi in the control plot (Figs. 2, 5, 6), (Sagara, 1975; Suzuki, 1989; Yamanaka, 1995b) and not adapt to drought conditions. To confirm the above hypothesis and to know the decomposition activities of ammonia fungi in the field, it is necessary to measure the quantities of growing vegetative hyphae on embedded wood pieces.

ACKNOWLEDGEMENTS

The authors are grateful to the Institute for Nature Study, National Science Museum, Japan for allowing us to conduct research on their Botanical Garden. We sincerely thank Dr. Nobuhiko Ohga, Faculty of Science, Chiba University for his kindness in suggesting us the suitable area for urea application in the Botanical Garden. We also wish to thank Tokyo-
Table 1. Monthly mean temperature and precipitation during the sampling period (April 1993—January 1994) at Tokyo District Meteorological Observatory.

<table>
<thead>
<tr>
<th>Month</th>
<th>Temperature (°C)</th>
<th>Precipitation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Maximum</td>
</tr>
<tr>
<td>1993</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apr.</td>
<td>13.4</td>
<td>26.9</td>
</tr>
<tr>
<td>May.</td>
<td>18.1</td>
<td>30.7</td>
</tr>
<tr>
<td>Jun.</td>
<td>21.7</td>
<td>31.9</td>
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<tr>
<td>Jul.</td>
<td>22.5</td>
<td>32.3</td>
</tr>
<tr>
<td>Aug.</td>
<td>24.8</td>
<td>32.9</td>
</tr>
<tr>
<td>Sep.</td>
<td>22.9</td>
<td>32.1</td>
</tr>
<tr>
<td>Oct.</td>
<td>17.5</td>
<td>25.3</td>
</tr>
<tr>
<td>Nov.</td>
<td>14.1</td>
<td>24.0</td>
</tr>
<tr>
<td>Dec.</td>
<td>8.5</td>
<td>21.1</td>
</tr>
<tr>
<td>1994</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan.</td>
<td>5.5</td>
<td>16.8</td>
</tr>
</tbody>
</table>

Table 2. Changes in decay rates of wood pieces of balsa embedded between the L-P and H-A1 layers in the study site of Quercus serrata dominated mixed forest following application of urea (800 g/m²) on 7 May 1993.

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Urea plot</th>
<th>Control plot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Decay rate (%)</td>
<td>Number of samples</td>
</tr>
<tr>
<td>1993</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May. 19</td>
<td>0.7±0.4**</td>
<td>11</td>
</tr>
<tr>
<td>Jun. 2</td>
<td>4.5±0.9**</td>
<td>13</td>
</tr>
<tr>
<td>Jul. 2</td>
<td>18.8±1.3**</td>
<td>10</td>
</tr>
<tr>
<td>Aug. 24</td>
<td>44.0±2.1**</td>
<td>12</td>
</tr>
<tr>
<td>Oct. 7</td>
<td>49.2±5.2**</td>
<td>8</td>
</tr>
<tr>
<td>Oct. 22</td>
<td>46.1±3.2**</td>
<td>7</td>
</tr>
</tbody>
</table>

Decay rate for each sampling is shown as an average with standard error.
The same English letter appended to values indicates no significant difference at the 5% level among sampling dates.
The same Greek letter appended to values indicates no significant difference at the 5% level between urea and control plots on same sampling date.

District Meteorological Observatory allowing us to cite the meteorological data.

REFERENCES


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