Changes in Soil Conditions Following Treatment with a Large Amount of Urea to Enhance Fungal Fruiting-body Production in a Japanese Red Pine Forest

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There have been many reports of changes in fungal fruit-body flora following treatment of forest soil with chemicals or fertilizers^{2,6,9-11,15,16,24}. These reports are from an orchard, coniferous forests, and deciduous forests. According to these investigations, the number and kind of fungus species occurring after chemical treatment changed depending on vegetation, the intensity of treatment, and the type of substances added.

Communities of other soil-organisms, which are important as biotic factors in fruit-body production, as reported in *Agaricus bisporus* (Lange) Sing.³¹) and some coprophilous fungi²¹), would change after these chemical treatments. However, such changes in soil treated to enhance production of fleshy fungi have received little attention. Changes in populations of soil-organisms has been well documented after use of fertilizers^{1,4,12,20}). Among these reports, Franz and Loub⁴) noted changes in fruit-body occurrence without referring to the period of the occurrence.

The "ammonia fungi"²²), a chemoecological group of fungi, develop sporophores sequentially on forest soil after treatment with ammonia water

or some ammonia-releasing substances, or after decomposition of dead bodies or faeces of animal. Until now approximately 35 species of ammonia fungi have been reported in Japan. In the U.K. and the U.S.A., this group of fungi have been observed after urea application^{11,23}). According to Sagara²²) and Suzuki²⁹), after urea treatment, litter became black and $\text{NH}_4^+\text{-N}$ concentration in soil increased, resulting in an increase in soil pH to 8-10, and the water content increased; afterwards, the pH value and $\text{NH}_4^+\text{-N}$ concentration decreased to nearly normal, and water content often decreased below that of the control.

Treatment with nitrogenous materials as described above should also stimulate build up of other soil-organisms, and this in turn should affect changes in the previously-described soil parameters, and have an important effect on fungus occurrence. Furuya⁵) isolated fungi from the soil treated with 1,000 g/m² of urea by plating, and most of the fungi obtained differed from those occurring on the soil surface and from those isolated from an untreated soil. In the present study I investigated changes in the numbers of bacteria and nematodes in soil treated with urea in relation to the occurrence of ammonia fungi, and changes in soil pH, water content and $\text{NH}_4^+\text{-N}$ concentra-

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tion.

The study was carried out in a 45-year-old Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) forest at the Kamigamo Experimental Station of Kyoto University Forest (35°04'N, 135°43'E; 120–140 m above sea level), about 12 km north of Kyoto City. Air temperature at the station in 1989 ranged from 4.2°C in December to 24.3°C in August, and annual rain fall was 1,430 mm. The parent rock consists of Paleozoic sandstone and slate. The soil surface is covered with a litter layer (A_0), with three zones, i.e., L, F and H layers. The H layer and mineral soil layer (A_1 layer) were fused together and the boundary between them was indistinguishable. The B layer was below an 8–13 cm depth.

Eleven plots, each 50 cm wide and 200 cm long, were marked out along a slope in the forest. Six

such plots were each treated with 700 g of urea by hand-scattering on 10 April 1989; the remaining plots served as controls. After treatment, the occurrence of fungal fruit-bodies was observed. Soil sampling began on 10 April, 1989, in the control plots and on 17 April, 1989, in the urea-treated plots, and repeated 12 times in the first year, and then once or twice a year in the subsequent years until 21 October, 1991. Samples were collected from the organic soil (F+H) layer in five of the six urea-treated plots and in the five untreated plots.

Soil samples were thoroughly mixed and supplied for the following analyses. Soil pH was measured by a glass electrode after stirring 5 g fresh soil in 20 ml distilled water for 3 min. Water content was determined by oven-drying 5 g of fresh soil at 105°C for 5 hrs and expressed as the

Fig. 1a-c. Fungi occurring in urea-treated (700 g/m²) forest soil. a. *Amblyosporium botrytis*. Scale=1 cm. Photo on 3 May 1989. b. *Lyophyllum tylicolor*. Scale=5 cm. Photo on 29 May 1989. c. *Laccaria bicolor*. Fruiting was observed within the urea-treated plot (50 cm×200 cm) shown with white lines. Photo on 15 Oct. 1990.

percentage of the weight of fresh soil. $\text{NH}_4^+\text{-N}$ was extracted by suspending 10 g of soil in 100 ml of 2 N KCl for 60 min, and determined using the indophenol blue method of Keeney and Nelson⁷⁾. Bacteria were counted using 10 g of fresh soil by the dilution plate technique. Nematodes were extracted from 10 g of fresh soil using a modified Baermann funnel technique³⁾, and counted using a dissecting microscope.

The following species of fungi occurred successively in the urea-treated soil, i.e., *Amblyosporium botrytis* Fres., *Ascobolus denudatus* Fr., *Peziza moravecii* (Svrček) Donadini, *Peziza* sp., *Pseudombrophila deerata* (Karst.) Seaver, *Lyophyllum tylicolor* (Fr.: Fr.) Lange and Sivertsen, *Coprinus echinosporus* Buller, *Hebeloma vinosophyllum* Hongo, *Laccaria bicolor* (Maire) Orton, *Hebeloma* sp.²⁵⁾, *Mycena pura* (Pers.: Fr.) Kummer and *Collybia cookei* (Bres.) J.D. Arnold (Figs 1 and 2). All were absent in the control plots, and all except *M. pura* have been reported to be ammonia fungi²³⁾. The occurrence of most ammonia fungi started from the periphery of the plot. The following changes in surface soil were concurrent with the occurrence of fungi. The litter turned blackish and smelled of ammonia soon after the urea was added. Within 6 months after the treat-

ment, fine roots were newly growing there, and ectomycorrhizas had formed in some of the roots.

While the pH value of untreated (control) soils remained at 3.6–4.4 throughout the sampling period, pH in the urea-treated plots increased to 7.8 seven days after treatment (Fig. 3a) and remained at pH 7–8 for the next 98 days, then declined to 4.5 at 191 days after treatment.

The water content of control plot soil ranged between 59 and 73% throughout the sampling period (Fig. 3b). The water content in the urea-treated plots was higher than that in the control plots for 434 days following urea treatment.

$\text{NH}_4^+\text{-N}$ concentration in the control plots ranged from 0.004 to 0.074 mg N/g dry soil throughout the sampling period (Fig. 3c). Sixteen days after the addition of urea, the $\text{NH}_4^+\text{-N}$ concentration rose to 15 mg/g dry soil and remained there until 142 days after treatment, thereafter it gradually declined to 0.02 mg/g dry soil by the end of the study period.

In the control plots, the number of bacteria ranged from 2.1×10^8 and 9.9×10^8 /g dry soil throughout the sampling period (Fig. 3d). Eighty-six days after adding urea the number of bacteria rose to 2.4×10^{11} /g dry soil. The population then declined slowly to 9.7×10^8 /g dry soil by

Fig. 2. Occurrence of fungi (+) in plots, where the forest soil was treated with 700 g/m² urea on April 10, 1989.

the end of the study period.

In the control plot, nematode numbers were between 170–312/g of dry soil throughout the sampling period (Fig. 3e). In the urea-treated plots the number of nematodes decreased dramatically soon after the addition of urea. In some extreme cases, the number temporarily dropped to zero. It then rose to 2,700/g dry soil 42 days after urea treatment, and then declined again to 94/g dry soil 191 days after the treatment. Most of the nematodes which increased after the urea addition were bacterium-feeding nematodes judged from the shape of their mouths.

At the end of the observation period, there were no significant (*t*-test, $p=0.05$) differences, except for the number of bacteria, in levels of any of the parameters in the treated and control plots ($n=5$).

Urea is extensively employed for forest-fertilization, and its effects on soil-organisms as well as soil pH and N content have been well studied^{18–20,26}. In these studies urea addition resulted in an increase of soil pH and ammonium nitrogen and of soil microbial activity estimated by microbial respiration. In the present study, urea was applied at a higher rate than that for fertilization, and damaged the nematode population temporarily, but thereafter, nematodes and bacteria increased considerably (Figs 3d and 3e).

Ammonia was formed by urease soon after addition to soil. Although some of the increased ammonia might be lost by volatilization or by leaching after nitrification, that remaining should have been immobilized by soil-organisms, and then released via excretion or from their decomposed bodies. Thus, ammonia levels in the soil would remain high for a specific time after treatment (Fig. 3c). In laboratory experiments, ammonia in alka-

Fig. 3a-e. Changes in soil parameters following treatment with 700 g/m² of urea on 10 April 1989. a. soil pH. b. soil water content. c. NH₄⁺-N concentration. d. the number of bacteria. e. the number of nematodes. ○—○, treated; ●—●, control. Values are the means of observations ± the standard errors of the means.

line conditions promotes spore germination, vegetative growth and fruit-body initiation in some ammonia fungi^{13,14,27,30}. In the present study, the direct effects of other microorganisms on the development of ammonia fungi were not examined, e.g., as reported for *Agaricus bisporus* where fruit-body initiation and hyphal growth were stimulated by some bacteria^{17,31}. Such microorganisms supposedly support the growth of ammonia fungi indirectly by maintaining high ammonia levels in the soil.

The increase in soil water content following urea treatment (Fig. 3b) may be due to the increase in the numbers of soil-organisms. Soil organisms could increase the water-holding capacity of soil by decomposing insoluble organic matters into particles. The increase in the fresh weight of the microbial population may also increase water content²².

It is not known how ammonia fungi survive in untreated field soil. Although their occurrence under natural conditions is rare²², they can be readily isolated from the tissue of fruit-bodies, or by germinating spores. In pure culture, without an excess supply of nitrogen, these fungi grow well and some of them fruit^{8,28}. These observations show that these fungi could exist as both mycelium and spores.

The ammonia fungi usually develop as follows²². In the early stage, soon after urea treatment, imperfect fungi, cup fungi and smaller agarics appear for a short period; later, larger agarics appear. This pattern of fungus succession also occurred in the present study (Fig. 2). The early stage fungi appeared in response to high soil $\text{NH}_4^+\text{-N}$ and pH (Figs 3a and 3c), which has been documented by an in vitro study²⁹. The late stage species might be considered as "pioneers in the recolonization of disturbed soils"²³, because they were observed when the soil returned to an acidic condition (Fig. 3a) and fine roots which were killed by the treatment, had recovered. Many late stage fungi, i.e., *Laccaria bicolor*, *Hebeloma* spp., are mycorrhizal species, and likely form mycorrhizas with new roots. The sequence and timing of

occurrence of each fungus is likely determined by other, yet unidentified endogenous and exogenous factors.

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