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ORIGINAL ARTICLE

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Effects of urea treatment on litter decomposition in *Pasania edulis* forest soil

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Abstract Five kinds of ammonia fungi were observed in a *Pasania edulis* forest after treatment with 1600 g·m^{-2} of urea. The number of fungal fruiting bodies decreased with time, and disappeared completely within 6 weeks. The population of cellulose decomposing bacteria also decreased after urea treatment. Urea treatment brought about marked changes in soil pH, redox potential (Eh), and nitrogen content, but no significant changes were observed in carbon content. In this experiment, urea treatment promoted decomposition of branches, but inhibited decomposition of leaves. In urea-treated plots, the decomposition rate of leaves was lower than that of branches, and the decomposition rate of large branches was greater than that of small branches. However, in the control plots, the decomposition rate of leaves was greater than that of branches, and the decomposition rate of large branches was lower than that of small branches. This experiment indicated that litter (branch and leaf) decomposition was dependent on the texture and size of the litter components, and that ammonia fungi and cellulose decomposing bacteria were not closely related to the litter decomposition.

Key words Ammonia fungi · Cellulose-decomposing bacteria · C/N ratio · Litter decomposition · Urea disturbance

Introduction

Urea treatment of forest soil increases soil pH and nitrogen content. Ammonia fungi have also been observed in forest soil after urea treatment,[1] and urea treatment has been reported to markedly affect the microbial population.[2]

Urea at a level of 1600 g·m^{-2} is sufficient to disturb forest soil.[3,4] Nitrogen concentration and alkaline conditions are reported to be important factors influencing the occurrence of ammonia fungi[5] and litter decomposition.[6]

Among the factors that control the rates of litter decomposition in forests, the influences of climate (temperature, humidity, and moisture) and litter quality (e.g., lignin/N and C/N ratios, and availability of N and P) have been well documented.[7–9] However, further studies are required to determine how urea treatment influences litter decomposition, and the relationship between ammonia fungi and litter decomposition.

The present study was performed to examine the effects of urea treatment on litter decomposition in a pure forest of *Pasania edulis* Makino.

Materials and methods

This study was conducted in a forest of *Pasania edulis* Makino (N 35°07′49″, E 40°11′11″), located in Kominato, Chiba Pref., Japan. The tree density in this forest was about 7 trees per 10 m^2, and the trees were 3–5 m in height. The forest soil was a dry brown type. During the experiment period, the temperature of the forest ranged from 3.8°C to 29.8°C, and the annual precipitation was 1470 mm. The soil surface had a litter layer (0–7 cm in depth). Twenty plots (each 1 × 1 m) on a 36° slope elevated 85 m above sea level were selected. Ten of these plots were treated with urea at 1600 g·m^{-2} on June 13, 1999, and the remaining plots served as untreated controls. Fungi were isolated and identified in the laboratory. Soil samples were collected separately from depths of 0–7 cm and 7–12 cm from three of the urea-treated plots and from three control plots. All samples were transported and kept at 4°C if not utilized immediately. LFC and LFU represent soil samples from depths of 0–7 cm, and HAC and HAU represent samples from depths of 7–12 cm, where C and U

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indicate samples from control and urea-treated plots, respectively.

NH_4-N and NO_3-N were extracted from 30-g samples of fresh soil suspended in 100 ml of 10% KCl solution and 100 ml of 0.1% Ag_2SO_4 solution, respectively. NH_4-N and NO_3-N levels were determined using an ammonia electrode (95-12, Orion, USA) and a nitrate electrode (93-07, Orion, USA), respectively. Total contents of carbon and nitrogen were measured using a C-N corder (MT-500, Yanaco). Soil pH and soil redox potential (Eh) were measured in 5-g samples of fresh soil suspended in 15 g of pure water using a pH electrode (6367-10D, Horiba, Japan) and an Eh electrode (6861-10C, Horiba, Japan), respectively. The water content of soil samples was determined after oven-drying at 80°C for 24 h according to Eq. 1.

$$\text{Water content (\%)} = \left[(\text{fresh weight} - \text{dry weight})/\text{fresh weight}\right] \times 100 \quad (1)$$

Decomposition rate was calculated by the litterbag method. Dry leaves and three kinds of dry branches from *P. edulis* trees were prepared before urea treatment: branch 1 (length 1–5 cm, diameter 2.0–4.8 cm), branch 2 (length 6–13 cm, diameter 0.9–1.9 cm), and branch 3 (length 14–22 cm, diameter 0.3–0.9 cm). One sample of each of branches 1, 2, and 3 were packed together into one plastic litterbag (size, 20 × 25 cm; mesh, 1.5 × 1.5 mm) with 10 g of leaves. On June 13, 1999, litterbags were buried between the LF and HA layers just before urea treatment. Seven replicates of the litterbags were sampled randomly at 3-month intervals. The bagged litter samples were collected, cleaned of soil materials, and oven-dried at 80°C for 24 h. Decomposition rates of the branches and leaves were calculated according to Eq. 2.

$$\text{Decomposition rate (\%)} = \left[(\text{initial dry weight} - \text{final dry weight})/\text{initial dry weight}\right] \times 100 \quad (2)$$

Cellulose decomposing bacteria were counted by the most probable number (MPN) method. Samples of fresh soil (10 g) were suspended in 90 ml of pure water. The soil suspension was shaken for 30 min at a speed of 180 rpm, and then diluted tenfold with pure water. Ten milliliters of Dubos medium[10] was added to test tubes (16.5 mm in diameter, 165 mm in length). Three pieces of filter paper (5 × 100 mm, No. 6, Advantec, Japan) were soaked in the medium with the upper part extending above the surface, and the test tubes were capped with silicon rubber plugs (Shirikosen, Shinetsu Chemical). After autoclaving, 1 ml of each diluted solution was added to the test tubes (5 replicates per dilution). These test tubes were cultivated at 30°C for 30–90 days. Breakage or collapse of the filter paper was taken to indicate the presence of cellulose decomposing bacteria.

An investigation period of 540 days was selected because soil properties (pH, water content, N content, etc.) returned sufficiently to the level of controls within 540 days after urea treatment.

Results

After urea treatment, soil pH increased rapidly from 6.7 to 9.0 (Fig. 1A). Eight weeks later, soil pH decreased to 5.7, which was lower than that in the control samples. NH_4-N and NO_3-N contents increased (Fig. 1B,C) but there were no significant changes in carbon content (*t*-test, $P < 0.05$).

Soil Eh decreased from 152 mV to 56 mV (Fig. 1F), and the water content of urea-treated plots increased (Fig. 1D). The C/N ratio of LFU soil samples decreased from 21:1 to 12:1, while that of HAU soil samples decreased from 15:1 to 4:1 (Fig. 1E).

Five ammonia fungi were observed after the urea treatment. These fungal fruiting bodies decreased with time, and they disappeared within 6 weeks (Table 1). These fungi were not observed in the control plots.

Three months after urea treatment, the decomposition rate of branch 1 was the highest in the urea-treated plots (38.1%), while the decomposition rate of leaves showed the lowest value (30.4%). In the control plots, the decomposition rate of leaves was the highest (31.0%), followed by branch 3 (18.3%), with branch 1 showing the lowest rate (15.4%). Decomposition rate of the litter in the urea-treated plots increased in the order: leaves < branch 3 < branch 2 < branch 1. Decomposition rate of the litter in the control plots decreased in the order: leaves > branch 3 > branch 2 > branch 1 (Fig. 2). The decomposition rate of branches was greater in the urea-treated plots than in the controls, while that of leaves was lower in the urea-treated plots than in the controls. These observations indicated that urea treatment enhanced the decomposition of branches and inhibited that of leaves.

The number of cellulose decomposing bacteria in the soil at a depth of 0–7 cm was much higher than that at a depth of 7–12 cm. After urea treatment, the bacterial number in LFU soil samples decreased from $6.6 \times 10^3\,g^{-1}$ dry soil to $1.5 \times 10^3\,g^{-1}$ dry soil (Table 2).

Table 1. Number of fungal fruiting bodies(mature and immature) after urea application in the forest of *Pasania edulis*

Fungal species	Days after urea treatment			
	8	15	30	45
Amblyosprium botrytis[a]	[b]	[c]	0	0
Ascobolus denudates	97 ± 15	57 ± 7	0	0
Peziza moravecii	88 ± 10	48 ± 5	0	0
Lepista sordida	77 ± 8	57 ± 6	0	0
Coprinopsis phlyctilospora	73 ± 11	49 ± 9	19 ± 5	0

Values are the means per m^2 with the standard errors of five plots
[a] Reproductive structures
[b] Present in large numbers
[c] Present in low numbers

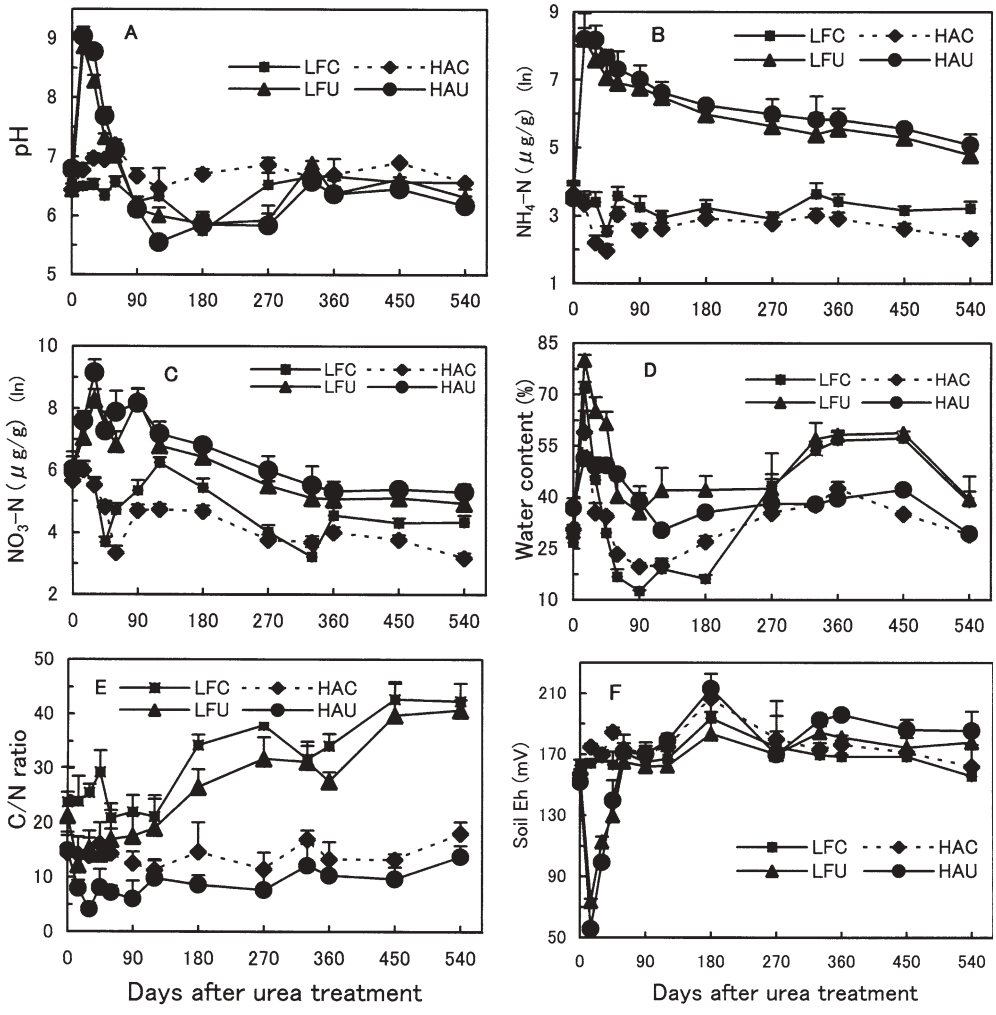

Fig. 1A–F. Changes in soil properties in the forest of *Pasania edulis* treated with urea. **A** Soil pH. **B** NH_4-N. **C** NO_3-N. **D** Water content. **E** C/N ratio. **F** Soil Eh. *LFC*, samples from 0–7 cm from control plots; *LFU*, samples from 0–7 cm from urea-treated plots; *HAC*, samples from 0–7 cm from control plots; *HAU*, samples from 7–12 cm from urea-treated plots. Values shown are means with standard errors

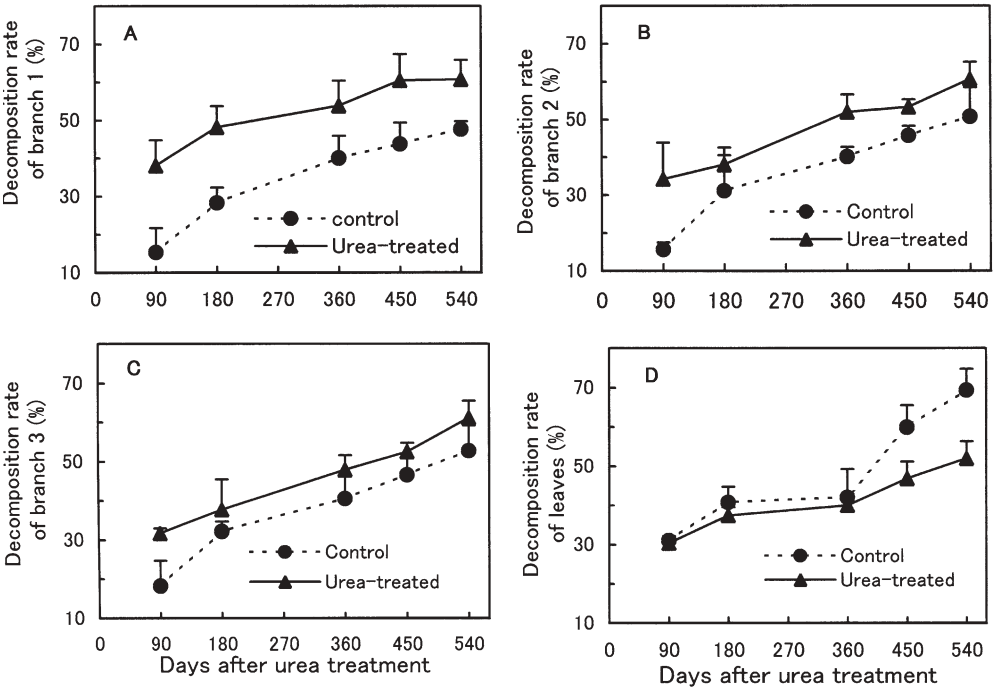

Fig. 2A–D. Changes in decomposition rates of branches and leaves in the forest of *Pasania edulis* treated with urea. **A** Branch 1. **B** Branch 2. **C** Branch 3. **D** Leaves. Values shown are means with standard errors

Table 2. Cellulose decomposing bacteria numbers in the forest of *Pasania edulis* treated with urea

Sample	Days after urea treatment					
	0	15	30	45	80	120
LFC	6070 ± 334	6740 ± 549	6170 ± 663	6270 ± 231	6398 ± 332	2670 ± 112
HAC	622 ± 68	870 ± 31	765 ± 23	870 ± 77	840 ± 91	800 ± 71
LFU	6570 ± 242	1530 ± 48	2000 ± 121	2150 ± 211	2354 ± 412	1020 ± 73
HAU	510 ± 90	400 ± 33	360 ± 65	400 ± 33	440 ± 29	340 ± 17

Values are the means (per gram of dry soil) with the standard errors of three replicates
LFC, soil samples from 0–7 cm from control plot; HAC, soil samples from 7–12 cm from control plot; LFU, soil samples from 0–7 cm from urea-treated plot; HAU, soil samples from 7–12 cm from urea-treated plot

Discussion

Soil properties

After treatment with a large amount of urea, soil properties (pH, water content, nitrogen content, etc.) changed, and ammonia fungi were observed, which was in agreement with the results of previous studies.[1,4,7] Soil pH increases mainly because of hydrolysis of urea by urease.[11] In the present study, soil pH decreased to below that of controls 3 months after urea treatment, and this acidification was a result of soil nitrification. Plants taking up ammonium ions release protons to maintain their charge balance, which also leads to soil acidification.

Decomposition of leaves and branches

In the controls, the decomposition rates of branches were lower than those of leaves (Fig. 2). This is because branches have a higher content of substances that are difficult to decompose, such as lignin, terpenoids, tannic acid, etc. A high concentration of lignin has been shown to significantly affect decomposition rate.[12,13]

In the urea-treated plots, decomposition rates of branches were greater than those of leaves, and decomposition rates of large branches were greater than those of small branches. This was considered to be caused by suppression of microbial activity by treatment with a large amount of urea,[14,15] which was responsible at least partially for changes in soil pH,[16,17] osmotic effects, and other phenomena, such as "ammonium metabolite repression".[18] It is clear that the microbial population and activity in leaves and small branches were influenced by urea treatment to a greater extent than in large branches.

Decomposition rates of branches in urea-treated plots were greater than those in control plots (Fig. 2). Exogenous supply of nitrogen has been reported to enhance decomposition under both laboratory[19] and field conditions.[20,21] Furthermore, urea and ammonium-based fertilizers, such as NH_4Cl, have been reported to solubilize some hemicellulose, pectic materials, and saponified ester linkages of plant cell walls.[22] Soponsathien[23] also reported that application of urea stimulated a marked increase in the enzyme activity of β-glucosidase of *Coprinus phlyctidospora* and *Tephrocybe tesquorum.*

The availability of nitrogen determines the speed of litter decomposition. Litter with an appropriate C/N ratio (e.g., a C/N ratio of 20, as in leguminous plants) would decompose quickly. However, when the supply of nitrogen is poor (e.g., a C/N ratio of >200, as in wood), the rate of decomposition will be slow and nitrogen may be taken up from the surrounding soil.[24] Old branches generally have higher carbon content and low nitrogen content than young branches, and therefore old branches would decompose more slowly.

The relationship between decomposition and C/N ratio implies that the C/N ratio is a good indicator of decomposition rate. The C/N ratio has also been widely used as an index of resource quality for the microbial population.[25] However, the differences in the form of carbon, i.e., lignin carbon or nonlignin carbon, may be of greater importance than the gross C/N ratio[26,27] and the lignin concentration or the lignin/N ratio may be a better predictor of decomposition rate.[28,29]

The number of cellulose decomposing bacteria decreased after urea treatment (Table 2). Henriksen and Breland[30] also reported that high nitrogen concentration inhibited cellulase-producing, colony-forming bacteria. This suggested that cellulose decomposing bacteria had no effect on the decomposition in urea-treated plots.

The reproductive structures of five ammonia fungi were observed after urea treatment, but they disappeared within 6 weeks (Table 1). These fungi were not observed in the control plots and the results suggested that ammonia fungi were not closely related to litter decomposition, especially 6 weeks after the urea treatment. Although the five ammonia fungi observed in this experiment had little effect on litter decomposition, fungi were still considered to play an important role in the decomposition of branches. Eijsackers and Zehder[31] reported that fungi had a major influence on decomposition in plants with high lignin ratios. Swift et al.[32] and Slapokas[33] reported that fungal populations began to grow and biological decomposition increased after urea treatment.

Urea treatment stimulated the decomposition of branches but inhibited that of leaves in the forest. The results of the present study indicated that five ammonia fungi and cellulose decomposing bacteria were not closely

related to litter decomposition, and suggested that other fungi played important roles in the decomposition process. Further studies are required to characterize these fungi and determine their roles in the decomposition process.

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