# POTENCY OF N-MINERALIZATION IN 2 KINDS OF FOREST SOILS*)

by

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## INTISARI

Penelitian ini dilaksanakan untuk mengukur potensi mineralisasi nitrogen secara *in vitro* pada tanah hutan di bawah tegakan hutan Matebashii (*Pasania edulis*) dan Sudajii (*Castanopsis cuspidata var. Sieboldi*) yang terletak di Propinsi Chiba, Jepang

Potensi mineralisasi tanah hutan dihitung dengan menggunakan model kinetik sederhana secara *in vitro* pada inkubator yang mempunyai suhu 20°C, 25°C dan 30°C. Kadar $NO_3^-$ dan $NH_4^+$ dianalisis secara periodik selama dalam inkubasi.

Mineralisasi pada suhu 30°C ternyata lebih besar bila dibanding dengan pada 20°C dan 25°C untuk Matebashii, tetapi tidak terlalu berbeda untuk Sudajii. Mineralisasi tahunan untuk tanah hutan pada kedalaman 5 cm di bawah tegakan Matebashii hanya sebesar 78,92 $kg.ha^{-1}.th^{-1}$, atau hanya sekitar 50% dari mineralisasi N di bawah tegakan Sudajii (156,6 $kg.ha^{-1}.yr^{-1}$). Karena potensi mineralisasi N pada tegakan Matebashii($N_o$= 40,07 $mg.100g^{-1}$ tanah) adalah lebih rendah bila dibandingkan pada Sudajii ($N_o$= 43,11 $mg.100g^{-1}$ tanah), maka energi aktivasinya (Ea=15.990 $kal.mol^{-1}$) lebih tinggi bila dibanding dengan Sudajii (Ea= 6.072 $kal.mol^{-1}$). Konstanta laju mineralisasi pada tegakan Matebashii (k= 0.006 per hari) mempunyai nilai yang lebih rendah daripada di Sudajii (k= 0.011 per hari). Pengaruh pemanasan global menyebabkan *Day at Temperature Standard* (DTS) pada tegakan Matebashii akan meningkat 42% dan DTS pada tegakan Sudajii akan meningkat 12%. Dengan demikian, mineralisasi N pada tegakan Matebashii di daerah paling utara akan meningkat 17%. Meskipun mineralisasi N pada tegakan Sudajii 2X lebih banyak dibandingkan pada tegakan Matebashii, tetapi pengaruh pemanasan global hanya akan meningkatkan 2% saja.

**Kata kunci:** mineralisasi nitrogen, tanah hutan, *in vitro*, suhu, pemanasan global

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# INTRODUCTION

## Background

Organic N-containing compounds, the products of microbial decomposition of plants and animal remains, account for over 90% of the total N within temperate forests in most soils (Zak *et al.*, 1993). Residue of decomposition and subsequent mineralization-immobili-zation processes is affected by substrate, location, climate, soil texture, pH and temperature (Agus, 1995). In order to determine an accurate prediction of plant-available N and explain N-cycles in forest ecosystems, it is necessary to estimate the amounts of N mineralized from soil organic matter. Any attempt to the prediction by modelling needs some models for mineralization.

Nitrogen mineralization and availability have been studied using numerous biological and chemical techniques, but none of which have been universally adopted (Fyles & McGill, 1987). Haibara (1993) resumed some methods to study the relationship between rate of N mineralization and temperature, i. e. (i) $Q_{10}$ method; (ii) the effective accumulated temperature method, (iii) the law of Arrhenius method and (iv) application of equation in the first order reaction for enzymatic. Longterm aerobic incubation based on the method of Stanford and Smith (1972), however, has the following advantages: (i) it is directly dependent on natural biological mechanism rather than chemical extractants; (ii) the long incubation time reduces the influences of initial N mineralization, which may be affected by sample preparation; and (iii) measurement of total N mineralization over time. The third advantage allows the calculation of theoretical mineralization parameters, namely, potentially mineralizable N ($N_o$) and mineralization rate constant (k).

## Objectives

This study was conducted to determine *in vitro* nitrogen mineralization potential of forest soil under Matesbashii (*Pasania edulis*) and Sudajii (*Castanopsis cuspidata var. Sieboldi*) forests by simple kinetics models. This study employed also nitrification of ammonium ions without leaching. Effects of incubation temperature and the standard temperature were figured as the amounts of N-mineralization. Their correlation to annual soil temperature was also described to determine the annual potential N-mineralization in each study site. Parameters of N-mineralization such as $N_o$, k, and Ea were calculated to explain the characteristic of each material. We also tried to refer effects of global warming on the mineralization of nitrogen in forest soil under evergreen broad-leaved forest in northern limit region.

# REVIEW OF LITERATURE

The law of Arrhenius could determine k as a biological activity or rate constant of mineralization ($day^{-1}$) by an equation:

$$k = A \exp(-Ea/RT) \qquad (1)$$

Where A is a constant value, Ea is apparent activation energy (cal. $mol^{-1}$), R is gaseous constant (1.987 $cal.deg^{-1}.mol^{-1}$), and T is absolute temperature (deg). As a rule, Ea is obtained by Arrhenius plot, but many temperature settings are necessary to obtain Ea. As a reference, Haibara (1993) reviewed that Ea of cultivated soil was at level of 15,000 - 23,000 cal. $mol^{-1}$ and Konno & Sugihara (1986) recommended for Ea of cultivated soil to 15,000 cal. $mol^{-1}$.

Application of equation of the first order reaction for enzymatic reaction to describe amount of mineralized nitrogen ($mg.100g^{-1}$ dried soil) for t time process (day) of N mineralization in soil introduced by Stanford & Smith (1972) was:

$$N = N_o\{1 - \exp(-kt)\} + C \qquad (2)$$

in which N is amount of mineralized nitrogen (mg/100 g dried soil, $N_o$ is N-mineralization potential ($mg.100g^{-1}$ dried soil), k is mineralization rate constant ($day^{-1}$), t is temperature (°C) and C is initial inorganic-N (mg/100 g dried soil). Time required to produce the same amount of inorganic-N is different with temperature, but the mineralization during incubation period at (d) day and mineralization rate (k) is constant. At that time,

$$k_1t_1 = K_2t_2 = k_3t_3 = ¼ = k_nt_n = \text{constant} \qquad (3)$$

Number of days under the condition of standard temperature ($d_s = d_{25}$) and standard contant ($k_s$) corresponding to the amounts of reaction for the days ($d_a$) is shown as follow:

$$\begin{aligned} d_s &= d_a\, k_a/k_s \\ &= d_a \exp\{Ea(T_a - T_s)/RT_aT_s \end{aligned} \qquad (4)$$

$$m(T_a) = \exp\{Ea(T_a - T_s)/RT_aT_s \qquad (5)$$

Because $k_n \cdot d_n$ was constant, so N-mineralization at absolute temperature T after (d) days is equal with {d m(T)} days for T standard by conversion of time axis. Therefore, model equation of the mineralization curve at standard temperature (25°C) was:

$$N = N_o\{1 - \exp(-k_s \times t_{25})\} \qquad (6)$$

and superimposing the mineralization curve at $T_a$ C to that at standard temperature was:

$$N = N_o\{1 - \exp(-k_s \times mT_a)\} \qquad (7)$$

There is a good correlation between soil temperature and transformation day at the standard temperature ($t_{25}$). Konno & Sugihara (1986) calculated number of days at standard temperature (DTS) by equation:

$$DTS = \exp\{EA(T-298)/596T\} \qquad (8)$$

where T is absolute soil temperature and Ea is apparent activation energy (cal.mol$^{-1}$)

Model equation of rate of N-mineralization for one year of each region as follows:

$$Y = N_oA\{1-\exp(-k_st_y)\} \qquad (9)$$

where $t_y$ is cumulative of transformation days at the standard temperature (25°C) for one year.

## MATERIAL AND METHOD

Two parallel transects of 10 m x 10 m were established in 40-yr-old Matebashii and Sudajii forest at Chiba Perfecture, Japan. Two soil samples were collected randomly from each study site from the upper 0-5 cm depth of soil layer on 31st April 1993. Maximum water content was determined separately by 100 cc sampling tube after being saturated. The soil samples were screened under field moist condition to pass through a 4 mm sieve. In 60% of maximum water content condition, 20.0 g of soil were put in sampling bottle (more than 100 ml) to insure optimum microbial activity. The bottle was covered tightly with double layer of parafilm (NOVIX) with two pin holes (1 mm) for ventilation and aeration. By decreasing 0.5 g of soil weight, distilled water was added to set up the water content at 60% of maximum water content.

The samples were incubated at temperature of 20°C, 25°C and 30°C. At each temperature sites, the samples were extracted in 2 replications at 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 19, 22 weeks incubation periods to get amounts of N-mineralization. The extraction of inorganic nitrogen was conducted by mixing 100 ml of 2N-KCl to 20.0 g of moist soil and shaking it for 1 hour. The amount of $NH_4$-N was determined by the indophenol blue method and $NO_3$-N was determined by the phenolate bisulfonic acid method with Flow injection unit (Jasco FIU-300N). Potential net N-mineralization were calculated by the Ammonium-N plus Nitrate-N concentration ($NH_4$-N + $NO_3$-N mg.100g$^{-1}$ dried soil). The N-mineralization process of soil was formulated using a simple kinetics model. Soil temperature at 0 and 10 cm of soil depth were measured hourly by the Automathical KADEC-U2 Multi-channel Soil Temperature (Kona System Co. Ltd.) for at least 1 year. The annual amounts of soil nitrogen mineralization were estimated computerally in relation to daily soil temperatures.

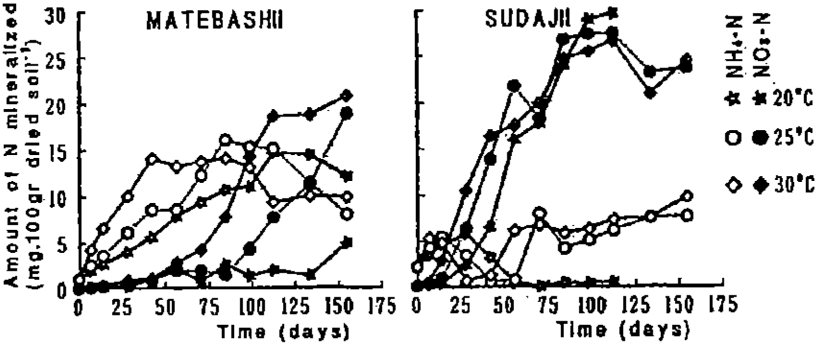
## RESULTS AND DISCUSSION

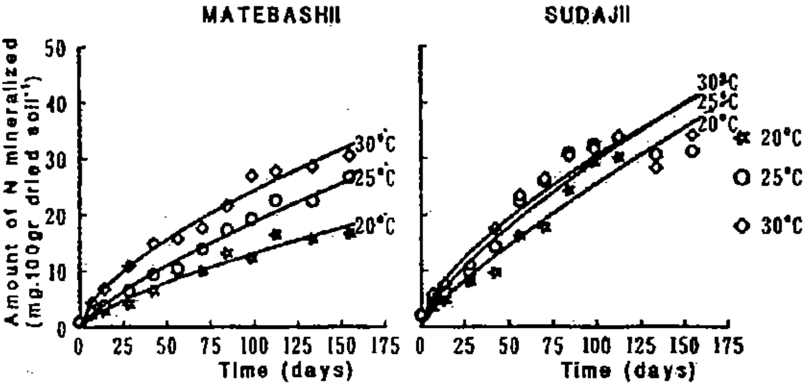
The microorganisms on Matebashii forest tended to convert to nitrate later than those of Sudajii forest. Ammonium production still dominated in Matebashii forest until 13 weeks incubation at 30°C, and longer periods were needed for lower incubation temperature. At 2-3 weeks incubations, nitrate production in Sudajii forest was more than ammonium production (Figure 1). Nitrification bacteria (e.g. *Nitrosomonas* or Nitrobacter) was supposed to be dominant in Matebashii forest, while ammonification bacteria (e.g. *Azotobacter*) was supposed to be dominant in Sudajii forest. Higher ammonium concentration in Matebashii soil might cause a feedback poison to microorganisms. As a result, *Nitrosomonas* or *Nitrobacter* could not convert to nitrate. Other studies concluded that autotrophic nitrifiers were weak competitors for $NH_4^+$ relative to microbial heterotrophs (Vitousek *et al. cit.* Hart *et al.*, 1994). Holmes & Zak (1994) concluded that neither microbial biomass (C or N) nor the change of microbial biomass between sampling dates were significantly inversely correlated with mean daily rates of net N mineralization. He also suggested that N availability was primarily controlled by changes in the turnover rate of microbial biomass such that a relatively constant pool is maintained through time. The accumulation of $NH_4^+$ production in forest soil under Matebashii forest, caused soil pH to be relatively higher, and accumulation of $NO_3^-$ in Sudajii forest caused soil pH to be lower (Table 1).

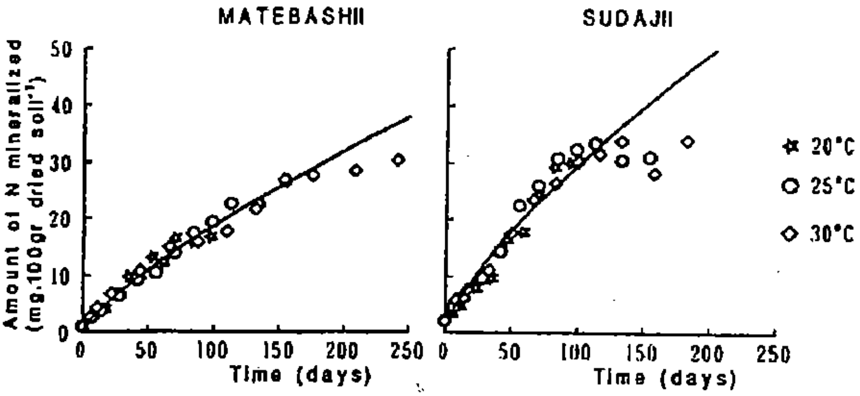
Some early studies demonstrated that in sieved agricultural soils, microbial immobilization of $NO_3^-$ did not occur unless substrates with high carbon to nitrogen ratio were added (Winsor & Pollard *cit.* Hart *et al.*, 1994). The dominant population in certain sieved-soil preferred $NH_4^+$ to $NO_3^-$ as a N source. In addition, soil with low $NO_3^-$ content, $NO_3^-$ generally fails to accumulate in these soils during short-term incubation in laboratory. However, longer incubation of soil from these ecosystems generally resulted in a dramatic increase in $NO_3^-$ pools (Vitousek *et al. cit.* Hart *et al.*, 1994). Such large and rapid increases in $NO_3^-$ pool following a long lag period of small and relatively stable $NO_3^-$ pools have been interpreted as the result of an increase in nitrifier population size that was initially small or the inactivation of allelopathic compounds. Other study indicated that microbial immobilization of $NO_3^-$ can be substantial in forest soils; therefore, lack of net increases in soil $NO_-$ pool sizes during incubation is not unequicoval indication that the nitrification process is insignificant or absent (Hart *et al.*, 1994).

Hart *et al.* (1994) suggested that under field conditions where plant roots and mycorrizae may be competing for $NH_4^+$ and supplying C to microbial heterotrophs during much of the growing season, gross nitrifications rates, and ratio of gross nitrification to gross mineralization are probably better indicator for laboratory incubation.

A cumulated ($NO_3^-$ + $NH_4^+$) production during incubation periods increased to become saturated at each temperature level. Nitrogen mineralization at temperature 30°C was considerably greater than at 25°C and 20°C in Matebashii forest, but


MATEBASHII
SUDAJII
Amount of N mineralized (mg.100gr dried soil⁻¹)
0
5
10
15
20
25
30
0
25
50
75
100
125
150
175
Time (days)
NH₄-N
NO₃-N
20°C
25°C
30°C


MATEBASHII
SUDAJII
Amount of N mineralized (mg.100gr dried soil⁻¹)
0
10
20
30
40
50
30°C
25°C
20°C
0
25
50
75
100
125
150
175
Time (days)
20°C
25°C
30°C


MATEBASHII
SUDAJII
Amount of N mineralized (mg.100gr dried soil⁻¹)
0
10
20
30
40
50
0
50
100
150
200
250
Time (days)
20°C
25°C
30°C

not so different for Sudajii forest (Figure 2). This phenomenon was related to the Arrhenius coefficients that the reaction rate and microbial activity would increase twice with 10°C increase. It is quite interesting to know whether mineralization or nitrification was more sensitive to temperature. Maximum soil temperature in summer at 5 cm depth of forest soil under Matebashii and Sudajii forests were 28.0°C and 26.4°C, respectively. On the other hand, minimum soil temperature in winter ranged between 6-8°C and 4-6°C, respectively. By the low soil temperature in winter, mineralization may occur. Addiscott (1983) reported that nitrification occurred at temperature below 2.8°C, but other study found it to be negligible at this temperature and noticeably active only above 5.5°C.

Superimposing of nitrogen mineralization rate until 22 weeks incubation at three temperatures to standard temperature showed that all of the data could be used to predict the rate of N-mineralization by simplex kinetics models method (Figure 3). Accumulated nitrogen mineralization tended to increase sharply at the first fifty days before reaching maximum. During the first seven days of the incubation, N was rapidly immobilized by a growing microbial biomass, and microbial respiration rate was high during this period, which suggested as an increase in microbial activity (Hart *et al.*, 1994). At the long term incubation period, N-mineralization production tend to be constant, because N-source became empty and microbial activity weaker. Other studies showed similar dynamics of $NH_4^-$ and $NO_3^-$ pool sizes over the course of a forest soil incubation, which have concluded from their results that the microbial demand for N declined, presumably as C availability also declined, more $NH_4^+$ became available to autotrophic nitrifier leading to most of the $NH_4^+$ pool being converted to $NO_3^-$ (Hart *et al.*, 1994).

Annual N-mineralization of forest soil under the Matebashii stand was only about 50% of N-mineralization of forest soil under the Sudajii forest (Table 1). Annual N-mineralization of soil until 5 cm depth was 78.9 $kg.ha^{-1}.yr^{-1}$ for the Matebashii forest, and about 156.6 $kg.ha^{-1}.yr^{-1}$ for the Sudajii forest. Because Matebashii forest had a lower N-mineralization potential ($N_o$) than the Sudajii forest (at level of 40.1 and 43.1 mg.100 $g^{-1}$ dried soil), and the need for activation energy to mineralize organic nitrogen in Matebashi forest was 263% (15,990 $cal.mol^{-1}$), more than in Sudajii forest (6,072 $cal.mol^{-1}$); the mineralization rate constant in Matebashii was 50% than in Sudajii forest (0.006 compared to 0.011) (Table 1). The k value from this study was categorized small compared to other studies (Stanford & Smith, (1972); Stanford *et al.*, (1974), Frankenberger & Abdelmagid (1985), Beauchamp *et al.*, (1986); Sugihara *et al.*, (1986), Robertson *et al.* (1988), Zak *et al.* (1993) and Toda & Haibara (1994). Nevertheless, higher values were reported by Fyles & McGill (1987), Bonde & Lindberg, (1988); Cabrera & Kissel (1988) and Simamrd & N'dayegamiye (1993). Nitrogen mineralization potential ($n_o$) of soil under Matebashii and Sudajii forests was relatively small compared to $N_o$ studied by Simard & N'dayegamiye (1993) and Toda & Haibara (1994), but bigger than reported by Frankenberger & Abdelmagid (1985), Beauchamp *et al.*, 1986; Hornby *et al.* (1986), Bonde & Lindberg (1988). Amounts of N-mineralization tended to reach high value in forest soil which had high $N_o$ value, furthermore Stanford & Smith (1972) reported a low k value for soils with $N_o$ values less than 100 $mg.kg^{-1}$.

**Table 1:** Characteristic parameter and estimated amount of annual nitrogen mineralization in forest soil.

| Soil characteristics | Matebashii forest | Sudajii forest |
|---|---|---|
| - Texture | Clay loam | Sandy loam |
| - pH | 6.4 | 4.8 |
| - Total C (%) | 5.08 | 4.80 |
| - Total N (%) | 0.32 | 0.29 |
| - C/N ratio | 15.88 | 16.55 |
| - $N_o$ ($mg.100g^{-1}$ soil). | 10.07±3.55 | 43.11±3.17 |
| - Constant $k_s$ ($day^{-1}$) | 0.006±0.0009 | 0.011±0.002 |
| - Energy Ea ($cal.mol^{-1}$) | 15,990±1,081 | 6,072±1,526 |
| - C ($mg.100\ g\ soil^{-1}$) | 1.1±0.4 | 0.6±0.9 |
| - AIC | 144.91 | 179.11 |
| - S | 44.81 | 153.32 |
| - half time $t_{0.5}$ (days) | 115 | 58 |
| - N-mineralization ($mg.100g^{-1}$ soil) | 23.87 | 40.45 |
| - Days at temperature standard/DTS (days) | 153 | 253 |
| - Annual N-mineralization ($kg\ ha^{-1}.yr^{-1}$) | 78.92 | 156.62 |

Although total-C content, total-N content and C/N ratio were almost the same (Table 1), clay content on forest soil under Matebashii forest was higher than Sudajii forest, chelating of N organic-clay would cause a stable complexes which can not be released easily by microorganisms. For this reason, the need for apparent activation energy (Ea) was high and N-mineralization of soil in Matebashii forest would be low. This result was consistent with observation by Hammermeister *et al.* (1994) who reported that high rates of N-mineralization were associated with the low clay content. The low maximum water content of soil under Sudajii forest affected N-organic on Sudajii forest to be more converted to $NO_3^-$. Stanford & Epstein (1974) suggested that with adequate aeration, over a broad range of temperatures and soil water contents, soil derived from $NH_4$-N was oxidized to $NO_3$-N rapidly enough so that $NH_4$-N did not accumulate. Thus, the rate of $NO_3$-N accumulation generally reflected accurately the rate of soil N mineralization. Stanford & Epstein (1974) concluded that highest N mineralization rate occurred between matric suctions of 1/3 to 0.1 bar, which ranged 80 to 90% of the total pore space filled with water, and with increasing of drought, N mineralization will decline.

Annual total-nitrogen coming from litterfall in Matebashii and Sudajii forests was 67.0 and 74.8 $kg.ha^{-1}.yr^{-1}$, respectively (Agus, 1995), while total-nitrogen amounts in forest floor ($A_o$ horizon) were 247.7 and 314.3 $kg.ha^{-1}$, respectively (Agus, 1995). It was shown that annual mineralization in Matebashii forest was in balance with annual nitrogen amount which come from litterfall, but in Sudajii forest, mineralization was twice N-litterfall.

Toda & Haibara (1994) found that nitrogen mineralization of forest soil under Established Sugi forest was about twice young forest, and decreasing with soil depth. So it is suggested that mineralization in Intermediate Matebashii was about half on N-mineralization in Established Matebashii.

## CONCLUSIONS

Microorganism in Matebashii forest tended to convert to nitrate later than in Sudajii forest with the assumption that nitrification bacteria (e.g. N*itrosomonas* or *Nitrobacter*) dominated in Matebashii forest and amonification bacteria (e.g. *Azotobacter)* predominated microorganisms in Sudajii forest. Higher ammonium concentration in Matebashii soil might cause a feedback poison to microorganisms. As a result, *Nitrosomonas* or *Nitrobacter* could not convert to nitrate.

Nitrogen mineralization at a temperature of 30°C was considerably greater than at 20°C and 25°C in Matebashii forest, but not so different from the Sudajii forest. This phenomenon was related to the Arrhenius coefficients that the reaction rate and microbial activity would increase twice with 10°C temperature increasing. Annual N-mineralization of 5 cm depth of forest soil under Matebashii forest was only 78.92 kg $ha^{-1}$ $yr^{-1}$, about 50% of N-mineralization of forest soil under Sudajii forest. Chelating of N organic-clay in Matebashii forest would cause stable complexes, which could not be released easily by microorganisms. Since N-mineralization potential ($N_o$) in Matebashii forest was lower, apparent activation energy (Ea) was higher than Sudajii forest, the mineralization rate constant (k) in Matebashii forest had lower value than in Sudajii forest. The lower maximum water content of forest soil under Sudajii forest caused N-organic to be more converted to $NO_3^-$.

Global warming will cause 3±1.5°C increase in temperature in the next century. It is suggested that the effect of global warming cause Day at Temperature Standard (DTS) in Matebashii forest to increase 42% and DTS in Sudajii forest to increase 12%. So the N-mineralization of forest soil under Matebashii forest in northern limit regions would increase 17%. Although N-mineralization of forest soil under Sudajii forest was twice that of under Matebashii forest, the increase was only 2% by global warming.

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