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Annual Fluctuations and Vertical Distributions of Cellulase, Xylanase Activities and Soil Microorganisms in Humus Horizon of a *Pinus rigida* Stand

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리기다소나무림 부식토내의 Cellulase, Xylanase의 활성과 토양미생물의 연간변동과 수직분포

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ABSTRACT

The annual decomposition of cellulose and hemicellulose by microorganism and distribution of soil microbial flora were investigated in the humus horizon of a *Pinus rigida* stand in Mt. Kwanak.

The cellulase activity was the lowest, 142μg glucose/g/hr from Dec. 1985 to Mar. 1986 and the highest, 760~1,072μg glucose /g/hr in Jul. and Aug. 1985. The xylanase activity was 47% higher than the cellulase activity and was the lowest, 211~275μg xylose /g/hr from Feb. to Mar. 1986 and the highest as 799~1,322μg xylose /g/hr from Jun. to Aug. 1986. The vertical distribution of the enzyme activity was decreased with the order of F, H, L and A_1 in both enzymes and the activities were exponentially decreased below L horizon, which suggests that most decomposition be done in F and H horizons with lots of organic matters. The SEM study showed that the main decomposers of litters were fungi and initial attack into litters was also made by them. The enzyme activities of soil had strong correlations with the temperature and the precipitation. The correlation coefficients were 0.813 and 0.866 in the cellulase, and 0.673 and 0.626 in the xylanase for the temperature and the precipitation, respectively.

INTRODUCTION

The litter decomposition is an important factor in the nutritional cycle, that is a precondition for the functioning of the ecosystem. Leaf detritus is largely composed of lignin, cellulose and hemicellulose (Suberkropp *et al.*, 1976), which are considered as hardly decomposable materials. Kim and Chang (1967) reported that the soil organic matter was mainly decomposed by fungi and the size of the micorobial population was well correlated with the contents of the organic matter, moisture and other various factors. Although the litter decomposition is influenced by the biological factors as well as the physical and chemical ones, more emphasis was layed on the physical and chemical aspects (Chang *et al.*, 1986; Aber *et al.*, 1982). Thus, this study mainly focused on the biodegradational aspects of the decay. Smith and Steyn (1982) suggested that the plate count enumeration technique of the microorganism serves only as a preliminary inspection of the relative abundance of the microorganisms. Therefore we further investigated the hydrolysis enzyme of soil such as cellulase and xylanase reported as good decay indices (Rhee *et al.*, 1985). Cellulase and xylanase, extracellular enzymes, released by the soil microbial flora are responsible for the decay of cellulose and xylan. Cellulose and xylan are the complex of glucose or xylose linked by β-1, 4 bond which cannot be broken by most higher organisms. Their conversion to soluble carbon is a rate limiting step in the litter decay (Sparding, 1980). Therefore, the release of the microbial exoenzyme is essential in recycling of nutrients, and these enzymes are also important to the supplementary parameters for assessing the microbial growth (Burns, 1982).

This study are to examine the annual fluctuation of carboxymethylcellulase (CMCase), xylanase activity and microbial population, and soil enzyme activity according to the soil horizons. It is also of great interest to investigate how closely the cellulase and the hemicellulase hydrolysis are proportional to the biophysical factors as an indicator of the decomposition. In addition, the surface of a pine leaf being decomposed was visualized by a scanning electron microscope study.

MATERIALS AND METHODS

Study site The forest of *Pinus rigida* in Mt. Kwanak, which is at steady state of litter production was selected for study site. Samplings were made at the campus of Seoul National University located in Mt. Kwanak (629) and its annual precipitation and the annual mean temperature is 1, 169 mm and 10. 1°C, respectively.

Samples were collected in the horizon of humus at approximately two weeks intervals from Sep. 1985 to Sep. 1986. In order to determine the enzyme activities of soil microorganisms according to its depth (Wilde and Voit, 1955), each horizon was sampled during the period of the most vigorous soil enzyme activity.

Assay of cellulase and xylanase Five grams of fresh samples were added to 15 ml of 0. 2N Na-acetate buffer, pH 5. 4, containing 0. 5% sodium carboxymethylcellulose (CMC) or xylan. Before incubation, 700 μl of toluene was added to prevent contamination by other microorganisms (Alexander, 1977). Then, the reaction mixture was incubated at 30°C for 10 hours, the solution was filtered through Whatman No. 1 filter paper. And the reducing contents of supernatants was assayed out by Somogyi-Nelson method (1951). Its procedure was as follows; the mixture mixed 1 ml of supernatant with an equal volume of copper reagent, was heated in boiling water bath for 10 min. After cooling, 1 ml of Nelson's reagent was added. The observance of sample, which was made to final volume 5 ml, was measured at 540 nm (Bauch & Lomb Spectronic 70). Blank control which was incubated without substrate, CMC or xylan, was always coprecessed and all samples were run in duplicate.

Cultivation and counting of microorganisms The estimation of microbial population was done by pour plating method after serial dilution. The medium for bacteria was nutrient agar containing 5 g of peptone, 3 g of beef extract and 1,000 ml of distilled water, and the final pH was adjusted to 7.0 (Cappuccino and Sherman, 1983). The medium (pH 5.6) for fungi was sabouraud agar containing 40 g of dextrose, 10 g of peptone and 1,000 ml of distilled water and was added with 10 μg of aureomycin per 1 ml and 0.02% triton X-100 (Cappuccino and Sherman, 1983). The glycerol yeast extract media for actinomycetes contained 5 g of glycerol, 2 g of yeast extract and 1 g of K_2HPO_4 per 1 litter. The medium was added with 10 μl of aureomycin per 1 ml and the pH was adjusted to 7.0 (Cappuccino and Sherman, 1983). The medium for cellulolytic (or xylanolytic) microorganisms was made as follows; carboxymethylcellulose (or xylan), 10 g; NH_4Cl, 1 g ; $NaNO_3$, 0.5 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.3 g; $Fe(II)SO_4 \cdot 7H_2O$, 0.01 g; distilled water, 1,000 ml, pH 5.6 (Schinner, 1982). The plates were incubated at 25°C for 3∼7 days and counted in duplicate by Quebec colony counter.

Photography of scanning electron microscope (SEM) The segments of pine leaves sampled from different depth were cut and immediately fixed in 3% glutaraldehyde in 0.1 N Na-phosphate buffer for 1 hour (Federle *et al.*, 1982). The fixed samples were washed three times with 0.1N Na-phosphate buffer and kept overnight at 4°C. They were postfixed by 0.2% OsO_4 solution for 1 hour and washed three times with buffer and distilled water. After fixation, they were dehydrated in a stepwise succession of 30, 50, 70, 90, 95 and 100 percent ethanol for 10 min, respectively and washed two times with toluene. These samples were coated with gold and examined with SEM (JEOL JSM-35). At first leaf segments were examined in their entireties at low power (× 80) and repersentitive portions were photographed at the higher power.

RESULTS

Annual fluctuation of cellulase activities, xylanase activities and soil microorganisms Annual temperature and precipitation are shown in Fig. 1. The annual means of them were 8.6°C and 1,250 mm, respectively. From Dec. 1985, temperature was below zero and at maximum degree in Jul. and Aug. Precipitation fluctuated with similar to the pattern of temperature.

Annual fluctuations of CMCase activities and cellulolytic microorganisms showed high correlations. As shown in Fig. 2, the lowest CMCase activities occurred between Dec. 1985 and Mar. 1986 (mean value, 142 μg glucose/g/hr). On the other hand, the enzyme activities of cellulase was the highest as the mean value, 916 μg glucose/g/hr between Jul.

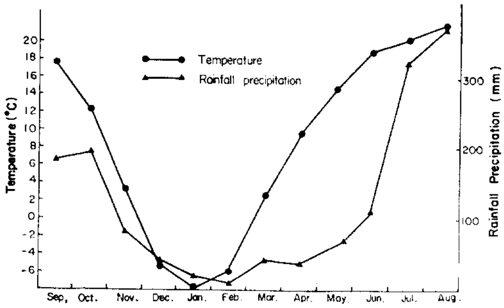

Fig. 1. Annual temperature and precipitation in Mt. Kwanak.

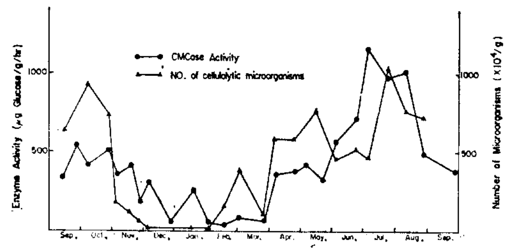

Fig. 2. Annual fluctuation of CMCase activities and cellulolytic microorganisms on humus horizon of a *Pinus rigida* stand in Mt. Kwanak.

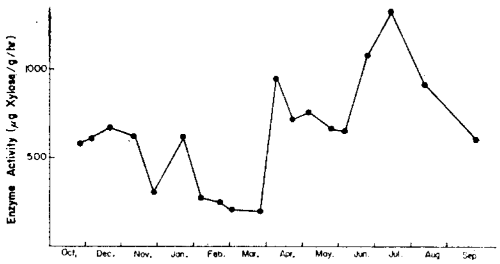

Fig. 3. Annual fluctuation of xylanase activities on humus horizon of a *Pinus rigida* stand in Mt. Kwanak.

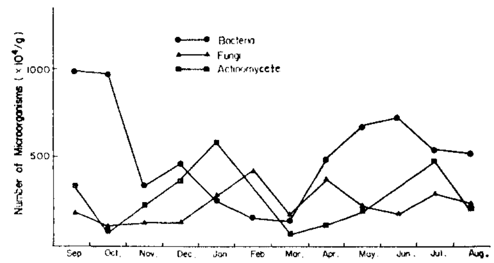

Fig. 4. Annual fluctuation of microorganisms on humus horizon of a *Pinus rigida* stand in Mt. Kwanak.

and Aug. The cellulase activity during that period reached 34% of total annual decay of cellulose. Annual mean value of the cellulase activity was 417 μg glucose/ g/hr. From the results of the enzyme activity in winter, it was also confirmed that the considerable amount of decay was progressed in the cold season.

As with the cellulase activity, xylanase which decomposes xylan as one of hemicellulose exhibited similar pattern of enzyme activity during a year (Fig. 3). In general, xylanase had 47% higher activity than cellulase one, which revealed that hemicellulose is more decomposable than cellulose (Alexander, 1977).

Annual fluctuations of soil microorganisms such as bacteria, fungi and actinomycetes are shown in Fig. 4. The bacterial flora highly fluctuated to compare with fungi and actinomycetes communities. While bacteria flora showed similar fluctuation with environmental factors, fungi and actinomycetes communities were annually little changed. It suggests that fungi and actinomycetes be least affected by environmental stress to compare with bactera, and be main decomposers in the cold season.

Vertical distribution of cellulase and xylanase activities The annual fluctuations of both xylanase and cellulase activities according to its depth were changed showing similar mode and xylanase was more active than cellulase (Fig. 5). The decreasing of enzyme activities was F, H, L and A_1 in order, which coincided wich Kim and Chang's report (1967). The activity bellow L horizon exponentially decreased and this trend was agree with one of content of organic matters (Chang *et al.*, 1986). It could be concluded that most decomposition litters took place in F and H horizons in which a degradation capacity

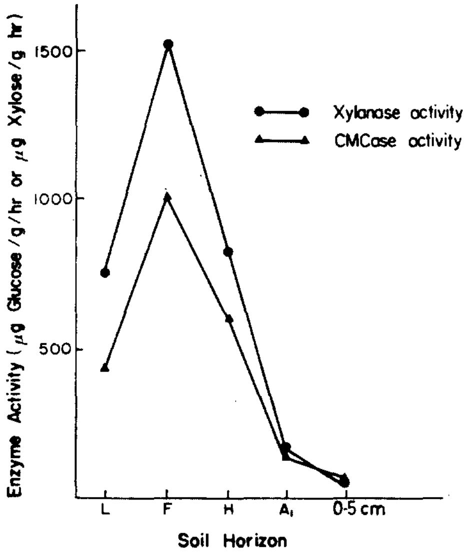

Fig. 5. CMCase and xylanase activities according to soil horizon on a *Pinus rigida* stand in Mt. Kwanak.

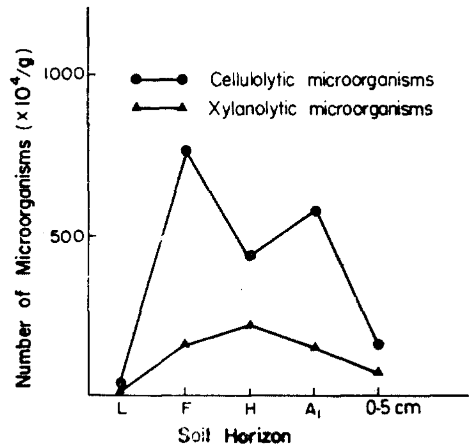

Fig. 6. Microbial population utilizing cellulose and xylan according to soil horizons on a *Pinus rigida* stand in Mt. Kwanak.

was the highest.

Fig. 6 showed that most soil microbial populations decomposing cellulose and hemicellulose were highly distributed in F and H horizons. Consequently, the decay of organic matter might be the most active in these layers.

Interrelationships between soil enzyme and environmental factors as well as microbial populations were assessed by calculating simple linear correlation coefficient. The results

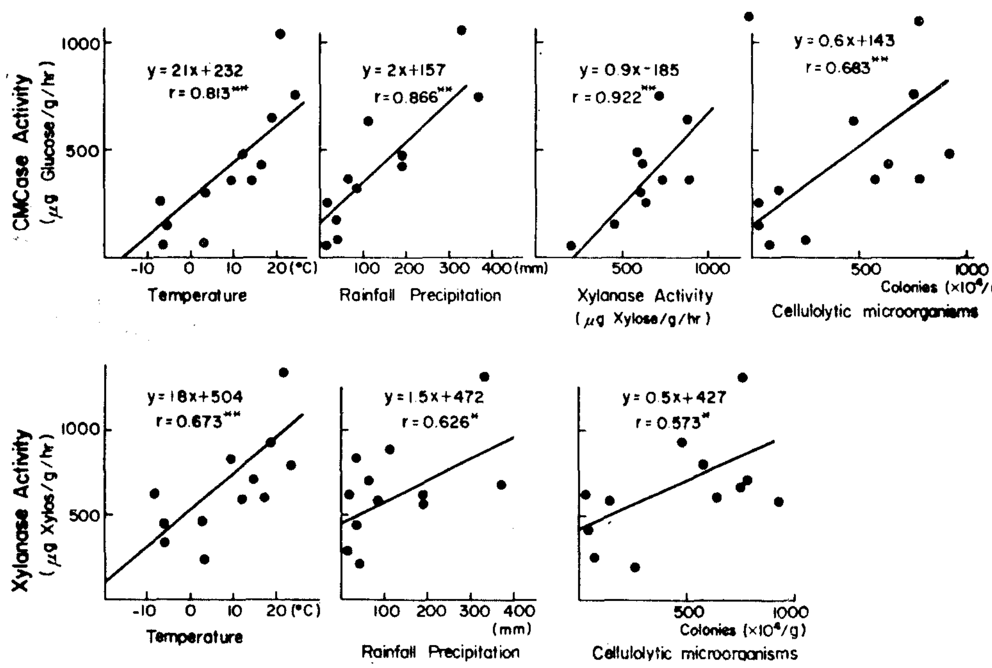

Fig. 7. Relationships between soil enzymes and other parameters.

Table 1. Correlation matrix (r-values) between soil enzymes and other biophysical parameters

n=12

	1	2	3	4	5	6	7	8
1. Temperature								
2. Rainfall Precipitation	0.789**							
3. CMCase Activity	0.813**	0.866**						
4. Xylanase Activity	0.673**	0.626**	0.922**					
5. Bacteria	0.652*	0.438	0.451	0.384				
6. Fungi	−0.182	−0.134	−0.036	0.072	−0.454			
7. Actinomycetes	−0.178	0.104	0.308	0.436	−0.230	0.528		
8. Cellulolytic Microorganisms	0.880**	0.659**	0.683**	0.573*	0.735**	−0.173	−0.293	

* Significant level at 0.05<P<0.01

** Significant level at P<0.01

obtained from the calculations were summarized as the correlation matrix in Table 1. Among many factors, temperature and precipitation showed significant correlation with CMCase activity as r=0.813, r=0.866, respectively. Also, xylanase was strongly correlated with them (r=0.673, 0.626). Consequently, the correlation between CMCase activity and xylanase activity was very significant (r=0.922), too.

Photography of Scanning Electron Microscope (SEM) By the photograph of a pine leaf surface by SEM were observed that the mycelia of fungi were being penetrated into stomata, compositions of which could be easily decomposed. The main decomposer in the pinus stand appears to be the fungi population, which could be confirmed by pictures through SEM. The pine leaf was examined that its morphology was being disappeared in relation to the depths downward.

DISCUSSIONS

The prominent carbanaceous constituents of higher plants and most organic compounds in nature are cellulose and hemicellulose. As a result the decay of these materials is critical step in the cycle of nutrients of the ecosystem.

Meanwhile many ecological approaches concerning the microbial degradation have been done, but most of them are concerned in the soil layer not the litter one (Shinner and Gstaunthaler, 1981 ; Rhee *et al.*, 1985 ; Kauri, 1982).

In this study at first the aspect of the seasonal fluctuation of the soil microbial activities shall be discussed. The fluctuations of enzyme activities were well coincided with the temperature and the precipitation. The most interesting thing was that the significant amount of the decay was progressed at near or below 0°C in spite of the suppression of the low temperature and the precipitation (Boyer and Mahaney, 1985). This result agrees with the study of Sinsababaugh *et al.* (1981) and Tom *et al.* (1983). Tom *et al.* (1983) suggested that the decay of carbohydrate and protein at 0°C be occurred as much as 40 percent of amounts at 20°C. The capacity of decay in the cold season was responsible for the seasonal selection of soil microorganisms (Suberkropp and Klug, 1976; Trentham and

James, 1981). Although bacterial populations among soil microorganisms made a shift during the seasons their degradational actions were not significant. In other words, their seasonal shift was not adequate to permit growth during the coldest winter, but it provided the potential for significant growth during the seasonal transition months of fall and spring.

In the stressed environment, main decomposers were fungi which could endure low temperature and drought periodically changing through year (Joshi and Chauhan, 1982; Alexander, 1977). It was also concluded that, among soil fungi, the selection of species was more effective mechanism for the adaptation to changed environmental conditions than metabolic adaptation (Schinner and Gstaunthaler, 1981). As shown in Fig. 4, while bacterial populations highly fluctuated, fungi and actinomycetes ones were less changed, which nicely supported the high activity of fungi during the cold season.

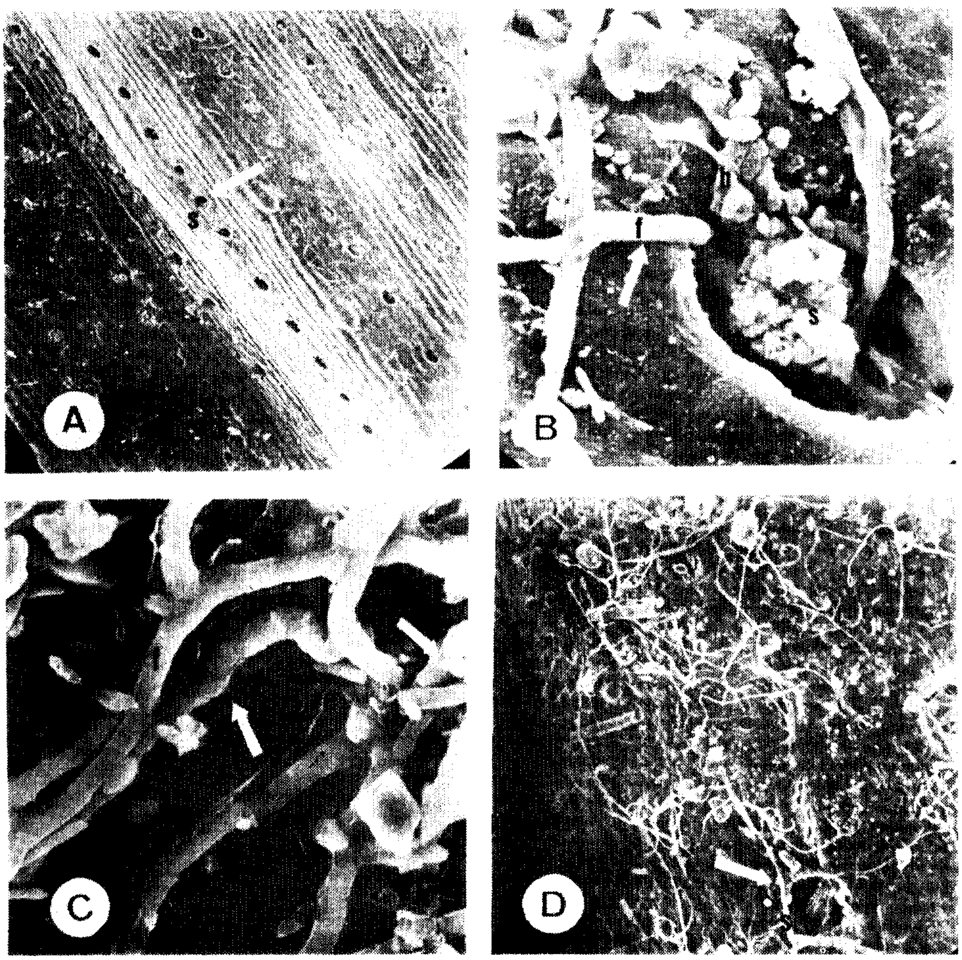

Fig. 8. Scanning electron micrographs of the surface of pine leaves, according to the depths of soils. Each surface was photographed around the stomata of pine leaves.
Various mycelia of fungi and bacteria on the surfaces of pine leaves were observed.
A : L horizon, ×80, B : L horizon, ×2000, C : L horizon, ×2000, D : F horizon, ×360
The arrow description; s: stomata, b: bacteria, f: fungi

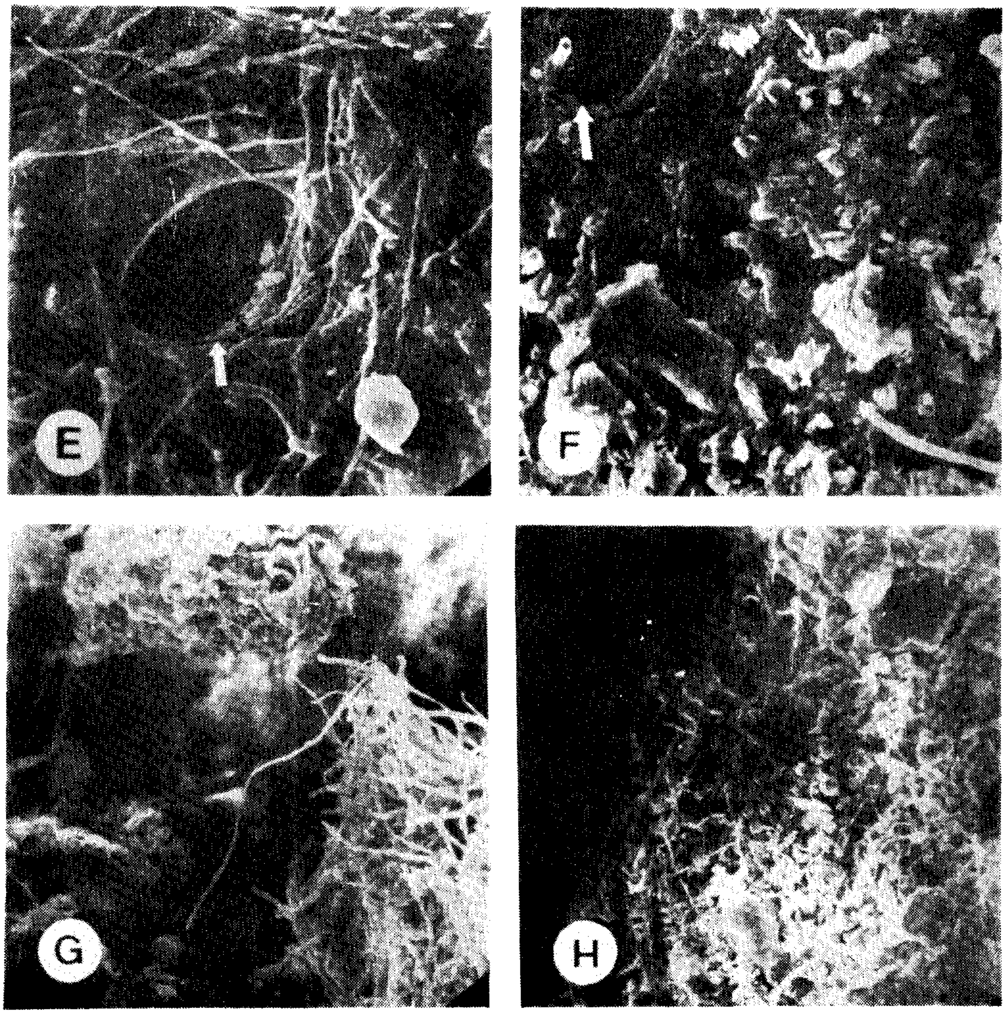

Fig. 8. (Continuous).
E : F horizon, ×900, F : H horizon, ×1000, G : A horizon, ×600, H : 0∼5 cm, ×600.

Most decomposition of litters in the study area was done during a rainy spell in summer. Consequently, 34 percent of decay of cellulose among the total amounts of a year was occurred in that period. According to Federle *et al.*(1982), the increase of 10°C in temperature resulted in doubling of microbial colonization and 50 percent increase in decay. The reason for the highest enzyme activity during that season was that soil moisture caused by high precipitation and temperature were adequate. A rainfall will immediately result in the higher soil moisture content, acting as a trigger for fungal and bacterial activation due to biologically more favorable moisture condition or to nutrient redistribution between microhabitats (Lundgren and Soderstron, 1983). The soil moisture, therefore, appears to be an important factor in determining the seasonal pattern of soil microbial biomass in organic soil (Lundgren and Soderstron, 1983 ; Day, 1982).)

Comparing with cellulase activity, xylanase activity was 47 percent higher, which showed that cellulose was more difficult to decompose and the decay of cellulose was a determining step in decomposition of litters.

The researches of decay in respect to soil horizons also have been done in the layers accumulated most organic matters. The cellulase and xylanase activities according to depths were nearly coincided with the vertical amount of the organic matter except L horizon. Besides the organic matters, as the lots of amounts of N, P and K were accumulated in those layers, those nutrients could accelerate the decay of litters (Srivastava and Rai, 1982). The high increase of the activity in F and H layers appears to be driven by the available nutrients of fallen leaves (Federle and Vestal, 1980).

The inhibition by direct light (Federle *et al.*, 1982), the shortage of moisture and large amount of lignin, hardly decomposable material, explained the reason that there was less decomposition in L horizon. As a rule the high enzyme activity in F horizon ascribed to a favorable environment such as oxygen supply, moisture and the available nutrients.

Through the scanning electron micrograph of pine leaves the phenomenon of penetration of microorganism to crude litters could be visualized. It was significant that the initial attack to the pine leaves was made by fungi. They seem to easily penetrate into stomata which is vulnerable to the attack of soil microorganisms. It was observed that the features of pine needles were being disappeared by the action of soil microorganisms. And it could be confirmed that the fungal populations were the most powerful decomposers with the previous result of annual fluctuation.

Those facts could be supported by the fact that the soils of the pine stand was acidic, pH 5.6, which is favorable for fungi, and the action of the fungal cellulase or xylanase were more prominent than one of bacterial populations (Suberkropp *et al.*, 1976).

From the results of the correlation matrix between the soil enzyme activities and other bio-physical factors, the most important environmental factors in determining the soil enzyme activities were the moisture and the temperature.

The xylanase activities were well correlated with the cellulase activities seasonally as well as the vertical depths. Thus, the xylanase activity was proposed to be a good indicator of the soil microbial growth and activity.

摘 要

관악산 ***Pinus rigida***림의 부식토에서 1년간, 낙엽의 대부분을 차지하는 cellulose와 hemicellulose의 분해양상 및 이에 따르는 미생물의 소장 관계를 조사하였다. Cellulase 및 xylanase의 활성은 기온과 강우량의 변화와 높은 상관이 있으며 cellulase의 경우에는 12−3월의 동절에 가장 적은 값인 142 μg glucose/g/hr을 나타내었고 7−8월에 760∼1,072 μg glucose/g/hr로 가장 큰 활성을 나타내었다. Xylanase의 경우에는 cellulase보다 47%정도 높은 분해율을 나타내었으며 2−3월에 211∼275 μg xylose/g/hr, 6−8월에 799∼1,322 μg xylose/g/hr로 최고, 최저의 분해율을 나타내었다. 깊이에 따르는 분해양상은 두 분해요소 모두 F,H,L,A_1의 순으로 높은 활성도를 나타내었고 F층 이하의 활성도는 지수함수적으로 감소하여 대부분의

분해가 유기물의 함량이 많은 F, H층에서 일어남을 알 수 있었다. SEM을 통하여 낙엽의 주요 분해자는 fungi임을 알 수 있었으며 그들에 의하여 초기침투가 일어나 분해가 진행됨을 알 수 있었다. 토양분해 효소는 기온 및 강우량과 가장 높은 상관을 나타내었다. 즉 cellulase의 경우에는 각각 0.813, 0.866의 상관은 나타내었으며 xylanase의 경우에는 0.673, 0.626의 상관을 나타내었다.

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