KOR. JOUR. MICROBIOL. June, 1985 p. 147-156 Vol. 23, No. 2

Distribution of Abiontic Carboxymethylcellulase in Relation to Microbial Growth and Activity in Forest Soils

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山林土壤內 Carboxymethylcellulase의 分布와 微生物의 生長 및 活性과의 相關에 対하여

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Seasonal and vertical variations of abiontic soil carboxymethylcellulase (CMCase) activities were assessed every other month for a year in two contrasting forest soils and evaluated the relationships between soil CMCase activity and environmental parameters. In climax deciduous soil, variations in CMCase activities caused by differences in sampling time were greater than those caused by differences in soil depth. On the other hand, counter phenomenon was observed in coniferous soil at the stage of development. Correlation analyses showed that soil CMCase activities were significantly ($p>0.01$) correlated with microbial respiration rates (O_2 uptake) and all of the microbial population sizes. From these results, it is suggested that determination of abiontic soil CMCase activity is an useful additional index for evaluating the overall microbial growth and activity in soils.

With the increasing interest in the role of microorganisms in soil, various parameters usable as indices of microbial growth and activity in soil have been proposed. No one parameter, however, has been found to be acceptable because of the complex dynamics of growth and activity of microorganisms in soil. Those parameters currently available for the estimation of microbial growth are microbial counts and microbial biomass (Anderson and Domsch, 1978; Ross, 1983). But such measurements give no indication of the activity of the microorganisms present in soil. Estimates of the total microbial activity have been made mainly by microcalorimetry (Sparling *et al.*, 1981), by respiration rates (Lund and Goksoyr, 1980; Stroo and Jencks, 1982; Valerie and Cook, 1983) and by measurements of the ATP content (Greaves *et al.*, 1973)

Recently, abiontic soil enzymes have been widely considered as a novel parameter for assessing the microbial growth and activity because of their fundamental roles in soil and the simplicities of their estimation (Skujins, 1978; Burns, 1982). Up to date, however, the reliability of soil enzymes as ideal indices is still in doubt and only a few soil enzyme assays have been evaluated as indicators of microbial growth and activity (Kiss *et al.*, 1978; Spalding, 1980; Frankenberger and

Dick, 1983). Therefore, further investigations are needed for a better understanding of the practical use of soil enzyme assays in estimating the relative growth and activity of the microbial population in soil.

Among the various soil enzymes, cellulase, in particular, has attracted considerable attentions, because the decomposition of cellulose which is the major plant polymer on soil and subsequent mineralization of the products have a special significance in the biological cycling of carbon. In addition, the conversion of native cellulose to soluble carbon by the action of cellulase is the initial, and possibly rate-limiting steps in the plant litter decomposition process (Spalding, 1980), and may make an important contribution to the total biological activity and biomass in soil (Knapp *et al.*, 1983; Sato *et al.*, 1984). It is, therefore, of importance to elucidate the relationships of this enzyme activity to microbial growth and activity.

In the present report, ecological characteristics of soil carboxymethylcellulase (CMCase; EC3,2,1,4) were examined bimonthly for a year with soils sampled from two contrasting plant communities, deciduous and coniferous forest communities. Emphasis was placed on the use of this enzyme assay as an available index for assessing the overall microbial growth and activity in soil.

MATERIALS AND METHODS

Soils

Laboratory experiments were carried out with soils sampled from two contrasting plant communities, deciduous (*Quercus mongolia*) and coniferous (*Hamaecyparis obtusa*) forest communities. All of the soil samples from each of the two stands were collected bimonthly, from June 1982 through June 1983, at five randomly selected points within each stand. In order to estimate the CMCase activities with respect to soil depth, optional layers were established as D1 (0-10 cm depth), D2 (10-20 cm), D3 (20-30 cm), D4 (30-40 cm) and D5 (40-50 cm). Details of both soils have been previously described by Rhee and Hong (1984) and some of their characteristics are shown in Table 1.

Assay of soil CMCase

Five grams of field-moist soil sample was placed in a 100 ml flask containing 0.6 ml of toluene. After mixing thoroughly for 15 minutes, 10 ml of 0.1 M acetate buffer (pH 6.0) and 10 ml of 1% sodium carboxymethylcellulose were added. The reaction mixture was then incubated in a water bath shaker (60 strokes/min) for 20 hours at 40°C. At the end of this reaction period approximately 50 ml of D.W. was added and the suspension was filtered. Reducing sugar content was then determined by the Somogyi-Nelson (1944) method. Controls of soil without added CMC and of the CMC substrate

Table 1. *Some physical and chemical properties of studied soils.* (*Mean*± *S. E.*)

	Soil depth	pH	Temperature (℃)	Moisture content (%)	Total organic C (ppm)	Nitrogen				Available P (ppm)
						Nitrite (ppm)	Nitrate (ppm)	Ammonia (ppm)	Total N (%)	
Deciduous	D1	4.80±0.19	10.67±4.30	40.60±2.63	12.91±0.86	0.28±0.04	3.69±0.38	17.33±1.21	0.39±0.02	16.02±0.89
	D2	4.47±0.20	11.00±3.74	38.45±1.85	9.75±1.97	0.27±0.04	3.73±0.32	13.40±0.73	0.29±0.02	12.57±1.20
	D3	4.72±0.24	11.75±3.30	40.40±2.29	7.93±1.67	0.29±0.02	3.66±0.66	11.90±1.35	0.25±0.01	11.33±0.83
	D4	4.77±0.16	11.83±3.01	39.22±3.14	5.77±1.25	0.26±0.01	3.29±0.72	9.45±0.79	0.20±0.03	9.00±1.23
	D5	4.65±0.15	12.25±3.11	38.85±2.97	5.67±1.39	0.32±0.11	2.91±0.71	9.02±0.48	0.20±0.02	6.00±0.58
Coniferous	D1	5.02±0.07	10.83±4.81	27.88±3.97	7.61±1.25	0.20±0.05	1.86±0.18	11.65±0.58	0.24±0.01	14.38±1.10
	D2	5.08±0.07	11.50±4.41	22.27±3.05	4.94±0.83	0.36±0.15	1.59±0.11	12.40±0.42	0.23±0.03	10.80±1.78
	D3	5.08±0.08	11.58±4.14	18.23±0.85	2.71±0.34	0.28±0.06	1.41±0.12	11.53±1.42	0.20±0.02	8.60±1.94
	D4	5.05±0.12	12.17±3.62	19.45±1.55	2.38±0.42	0.24±0.06	1.25±0.25	10.12±0.98	0.16±0.02	7.32±0.82
	D5	5.02±0.08	12.67±3.39	19.53±0.96	1.70±0.21	0.16±0.02	1.19±0.18	8.95±0.38	0.15±0.01	7.07±1.06

without added soil were always included. One unit of CMCase activity was defined as the amount of enzyme releasing 1.0 μg glucose (or glucose equivalent) in 1 min. under standard assay condition.

Chemical Analysis

After screening through a sieve (mesh size 2 mm), soil samples were air-dried at room temperature for the chemical analyses. All analytical results were calculated on the basis of oven-dry (100 °C) weight of soil and at a minimum, analyses were made in duplicate. The moisture content was determined from loss in weight after drying at 100 °C to a constant weight. The soil pH was estimated in a slurry with water (1 part soil to 2.5 parts water) using electrometric pH meter. Soil organic carbon content was measured by Walkley-Black method (Jackson, 1964). The concentration of available phosphorus was determined by Stannous chloride method and measured colorimetrically at 650 nm (APHA, 19 1). Total nitrogen was analyzed by the boric acid . icro-Kjeldahl method. The concentration of NH_4-N was determined colorimetrically at 410 nm after color development by Nesslerization method. Nitrate-nitrogen (NO_3-N) was extracted from soil with a solution of 2 NKC1 and measured colorimetrically after treatment of 1 ml of brucine-sulfanilic acid to 10 ml of sample. The amount of NO_2-N was also determined colorimetrically after color development with sulfanilamide and N-(1-naphtyl) ethylenediamine hydrochloride.

Viable plate counts of microbial populations

Microbial numbers were estimated by the serial dilution plate method. Each of the dilutions was plated out in triplicate using 0.1 ml samples. The medium used for the isolation of bacteria was consisted of 10 g of glucose, 1 g of $NaNO_3$, 1 g of Na_2HPO , 0.5 g of KCl, 0.25 g of albumin, 0.01 g of $FeSO_4 \cdot 7H_2O$, 20 g of agar and 1l of distilled water, while microfungal propagules, modified Czapeck-Dox agar medium (Rhee and Hong, 1984) was used. For the estimation of cellulolytic bacterial or microfungal populations, Na-CMC was substituted for glucose. The plates were incubated under aerobic conditions at 26±1°C. The cultivation times of bacteria and microfungi were 3 and 7 days, respectively.

Estimation of soil respiration rates

Soil respiration rate was estimated by measuring the oxygen uptake in the conventional Warberg apparatus. The mixture of 0.5 g of soil sample, 2 ml of phosphate buffer (pH 5.6) and 0.4 ml of 10 % NaOH were aseptically loaded in stoppered Warberg flasks. The flasks were allowed to equilibrate for 10-15 min at 25°C. Oxygen uptakes were then determined after 3 hours incubation at 25 °C and expressed in terms of μl/hr/g soil.

Statistical analysis

Simple statistics and coefficient of variation (CV%) were computed to valid the significant differences between two values obtained throughout the present study. The relationship between soil CMCase activities and the other parameters estimated in each of two forest soils was established by calculating regression equations and simple linear correlation coefficients.

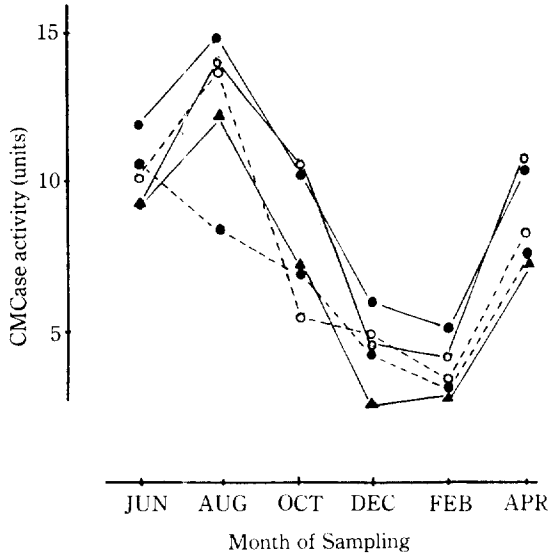

Fig. 1. *Variations of CMCase activities in deciduous forest soil with respect to sampling time and soil depth.*

● - ● ; D1 (0-10cm depth)
○ - ○ ; D2 (10-20cm)
● ··· ● ; D3 (20-30cm)
○ ··· ○ ; D4 (30-40cm)
▲ - ▲ ; D5 (40-50cm)

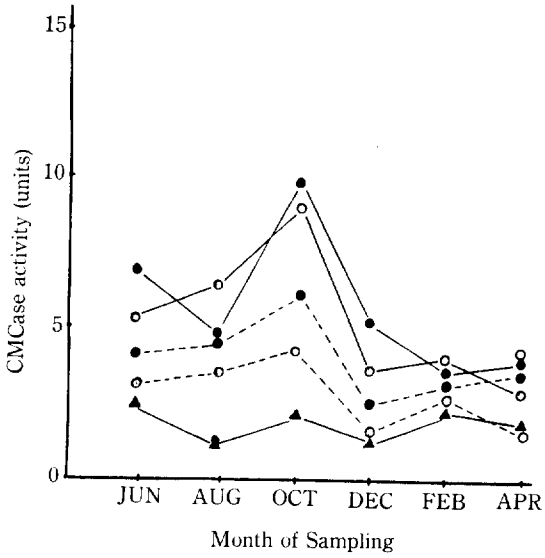

Fig. 2. *Variations of CMCase activities in coniferous forest soil with respect to sampling time and soil depth.*
●—● ; D1, ○—○ ; D2, ●···● ;D3, ○···○ ; D4, ▲—▲ ; D5

RESULTS

Distribution of soil CMCase activities

To compare the significance of sampling time and soil depth on the distribution of abiontic soil CMCase activities, seasonal variations of CMCase activities in two forest soils with respect to soil depth were examined. As shown in Fig. 1 and 2, remarkable variations in CMCase activities with sampling time and soil depth were observed in both forest soils. Most of sample layers (D1-D5) of climax (deciduous) and developmental (coniferous) soil stands showed their highest CMCase activities in August (mean value of 5 layers, 11.8 units) and October (mean value of 5 layers, 6.3 units), respectively, while the lowest CMCase activities in February (mean value of 5 layers, 4.0 units) and December (mean value of 5 layers, 2.9 units), respectively.

The yearly mean values of CMCase activities at the D1 layers of deciduous and coniferous forest soils were 9.8 and 5.9 units, respectively, and the general trends in the decrease of CMCase activity were observed with increasing soil depth. Relatively more conspicuous decrease of CMCase activities occurred in the coniferous forest soil and the activity in D1 were approximately three times greater than D5. For a better understanding of the effects of the sampling time and soil depth, tabulation of the mean coefficient of variation (CV%) was performed on the examined data. The mean CV values for the variation between different soil depths in deciduous and coniferous soils were 20.58 % ANI) 43,66 7, respectivly, while the mean CV values for the activity variation between different sampling times were 41,82 % and 37. 76%, respectively (Table 2). These results indicate the greater effects of sampling time on the CMCase activities in deciduous soil and greater effects of soil depth on the CMCase activities in coniferous soil.

Indices of microbial growth and activity

The sizes of microbial population and respiration values were used in this study as indicators of microbial growth and activity, and were also estimated bimonthly throughout a year. The results are summarized in Table 3. The number of microorganisms estimated by the viable plate count method on various selected media were found to be greater in the deciduous soil and the populations of microfungi were approximately two magnitude lower in numbers when compared to the corresponding populations of bacteria. The celluloytic bacteria and microfungi showed their population sizes of about 28-54 % and 29-63 % of the number of total bacteria and total microfungi, respectively. In general, gradual reductions in population densities with increasing soil depth were commonly examined in two forest soil stands and similar tendencies were also found in soil respiration values measured by O_2 uptake.

Table 2. *Coefficient variations (CV%) of soil CMCase activities caused by different sampling times and soil depths.*

soil	seasonal variation CV%		vertical variation CV%	
	Mean	Range	Mean	Range
Deciduous	41. 82	33. 42-52. 26	20. 58	10. 57-28. 43
Coniferous	35. 76	30. 30-43. 56	43. 66	18. 93-53. 82

Table 3. *Viable plate counts and soil respiration values in two forest stands. (Mean ± S.E.)*

Soil	layer	Viable Plate Counts (colonies/g soil)				Respiration
		Total Bacteria ($\times 10^5$)	CMC-utilizing Bacteria ($\times 10^5$)	Total Fungi ($\times 10^3$)	CMC-utilizing Fungi ($\times 10^3$)	(μl O_2/hr/g soil)
Deciduous	D1	299 ± 35.3	161 ± 20.1	137 ± 15.3	87 ± 13.5	49 ± 3.2
	D2	226 ± 40.4	109 ± 23.1	112 ± 14.1	52 ± 5.9	38 ± 2.7
	D3	206 ± 40.2	97 ± 19.4	78 ± 7.1	31 ± 4.2	32 ± 2.4
	D4	126 ± 19.3	55 ± 14.3	41 ± 5.5	12 ± 1.6	25 ± 3.9
	D5	91 ± 16.4	33 ± 7.4	30 ± 4.3	9 ± 2.3	17 ± 3.4
Coniferous	D1	191 ± 32.9	99 ± 14.6	102 ± 8.2	52 ± 8.6	26 ± 3.1
	D2	184 ± 24.5	91 ± 14.6	73 ± 12.1	29 ± 4.5	19 ± 2.9
	D3	122 ± 25.1	48 ± 14.4	45 ± 7.7	18 ± 3.4	16 ± 2.9
	D4	113 ± 22.2	32 ± 8.2	30 ± 7.3	10 ± 2.9	10 ± 2.7
	D5	83 ± 19.6	24 ± 6.5	16 ± 1.7	6 ± 0.8	10 ± 2.5

Table 4. *Correlation matrix (r values) between soil CMCase, physical and chemical soil parameters and microbiological parameters for deciduous soil.* $n = 30$

Parameters	1	2	3	4	4	6	7	8	9	10	11	12	13	14
1. Moisure														
2. pH	0.423*													
3. Temperature	-0.150	-0.794***												
4. Nitrite	0.408	0.162	-0.064											
5. Nitrate	-0.171	0.423*	-0.648***	-0.003										
6. Ammonia	0.125	0.025	0.099	0.057	0.148									
7. Total N	0.131	0.123	0.175	0.159	0.061	0.568**								
8. Total C	0.255	0.462*	-0.647**	0.098	0.410*	0.345	0.698***							
9. Available P	0.049	0.073	-0.079	-0.048	0.171	0.560**	0.777***	0.546*						
10. Respiration	-0.090	-0.317	0.369*	-0.104	0.164	0.662***	0.606**	0.299	0.737***					
11. Total bacteria	0.004	-0.492**	0.526**	-0.086	-0.223	0.614**	0.522**	0.107	0.604**	0.858***				
12. Cellulolytic bact	0.056	-0.412*	0.496**	-0.001	-0.164	0.676***	0.566**	0.125	0.682***	0.852***	0.950***			
13. Total fungi	-0.012	-0.294	0.289	-0.014	0.039	0.779***	0.621**	0.368*	0.715***	0.865***	0.824***	0.846***		
14. Cellulolytic fun.	0.061	-0.260	0.245	-0.011	0.014	0.774***	0.708***	0.406*	0.724***	0.808***	0.836***	0.869***	0.925***	
15. CMCase activity	0.083	-0.602**	0.771***	-0.112	-0.486*	0.296	0.056	-0.319*	0.227	0.591**	0.658***	0.669***	0.524**	0.538**

*P>0.05, **P>0.01, ***P>0.001

amined in two forest soil stands and similar tendencies were also found in soil respiration values measured by O_2 uptake.

Interrelationships between soil CMCase activity and environmental parameters

Interrelationships between CMCase activities, physical and chemical properties, and microbial populations and activities of two forest soils were assessed by calculating simple linear correlation coefficients. The results obtained from deciduous and coniferous soils were summarized by the correlation matrix in Table 4 and 5, respectively. Among the physical and chemical parameters, soil pH ($r = -0.602^{**}$), soil temperature ($r = 0.771^{***}$), and nitrate ($r = 0.486^{*}$) showed significant ($P > 0.05$) correlations with CMCase activity in

Table 5. *Correlation matrix (r-values) between soil CMCase activity, physical and chemical soil parameters and microbiological parameters for coniferous soil.* $n = 30$

Parameters	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Moisture														
2. pH	-0.121													
3. Temperature	0.237	-0.545**												
4. Nitrite	-0.257	0.534**	-0.398*											
5. Nitrate	0.498*	-0.402*	0.645**	-0.129										
6. Ammonia	0.250	-0.027	0.094	0.037	0.402*									
7. Total N	0.286	0.188	0.149	0.412*	0.417*	0.297								
8. Total C	0.412*	0.025	-0.001	0.221	0.435*	0.508**	0.620**							
9. Available P	0.136	0.087	-0.345*	0.034	0.085	0.266	0.233	0.555**						
10. Respiration	0.488*	-0.464*	0.573**	-0.220	0.832***	0.485*	0.570**	0.606**	0.264					
11. Total bacteria	0.411*	-0.350	0.660***	-0.173	0.838***	0.376*	0.471*	0.444*	0.041	0.832***				
12. Cellulolytic bact.	0.462*	-0.219	0.499**	-0.073	0.758***	0.438*	0.567**	0.582**	0.205	0.823***	0.920***			
13. Total fungi	0.549**	-0.139	0.192	-0.082	0.646***	0.480*	0.519**	0.741***	0.505**	0.764***	0.686***	0.718***		
14. Cellulolytic fun.	0.434*	-0.162	0.269	0.064	0.679***	0.465*	0.573**	0.739***	0.509**	0.781***	0.689***	0.729***	0.924***	
15. CMCase activity	0.387*	0.176	0.347	-0.026	0.630**	0.411*	0.583**	0.683***	0.329	0.743***	0.710***	0.685***	0.790***	0.779***

*P > 0.95, **P > 0.01, ***P > 0.001

deciduous soil. Total organic C and available phosphorus which are known to be important nutritional factors in soil ecosystem were not correlated with CMCase activity in deciduous soil. On the other hand, CMCase activity in deciduous soil showed high (P>0.01)or strongest(P>0.001) correlations with indices of microbial growth and activity such as aoil respiration (r = 0.591**), total bacteria (r = 0.658 ***), cellulolytic bacteria (r = 0.669***), total microfungi(r = 0.524**), and cellulolytic microfungi (r = 0.538**). The regression equations for the relationships between CMCase activity and those indices are shown in Fig. 3.

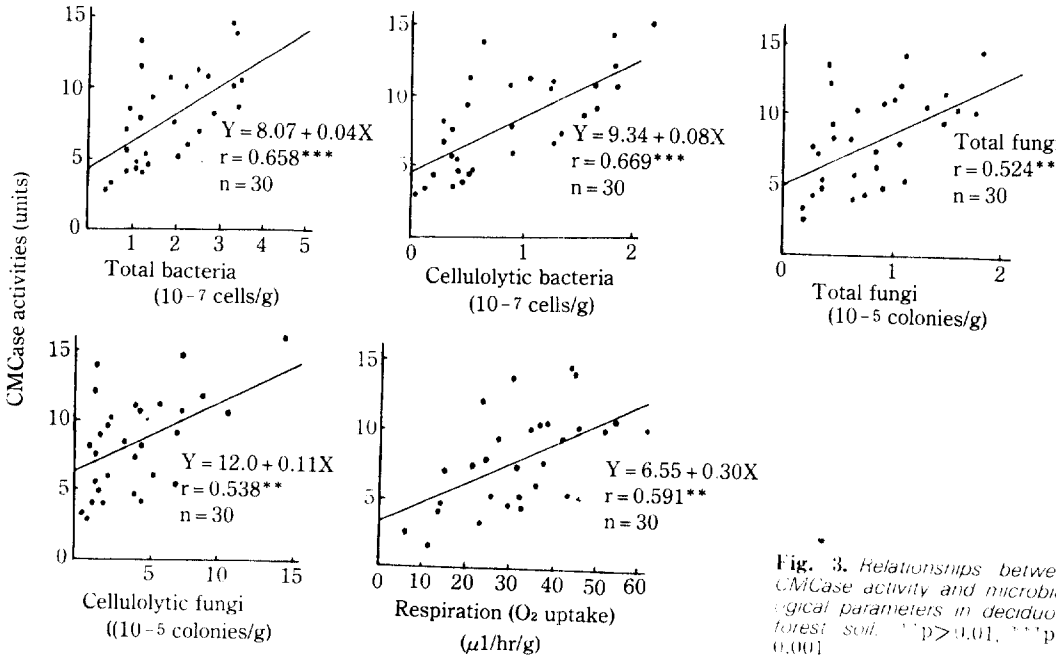

Fig. 3. *Relationships between CMCase activity and microbiological parameters in deciduous forest soil.* **p>0.01, ***p>0.001

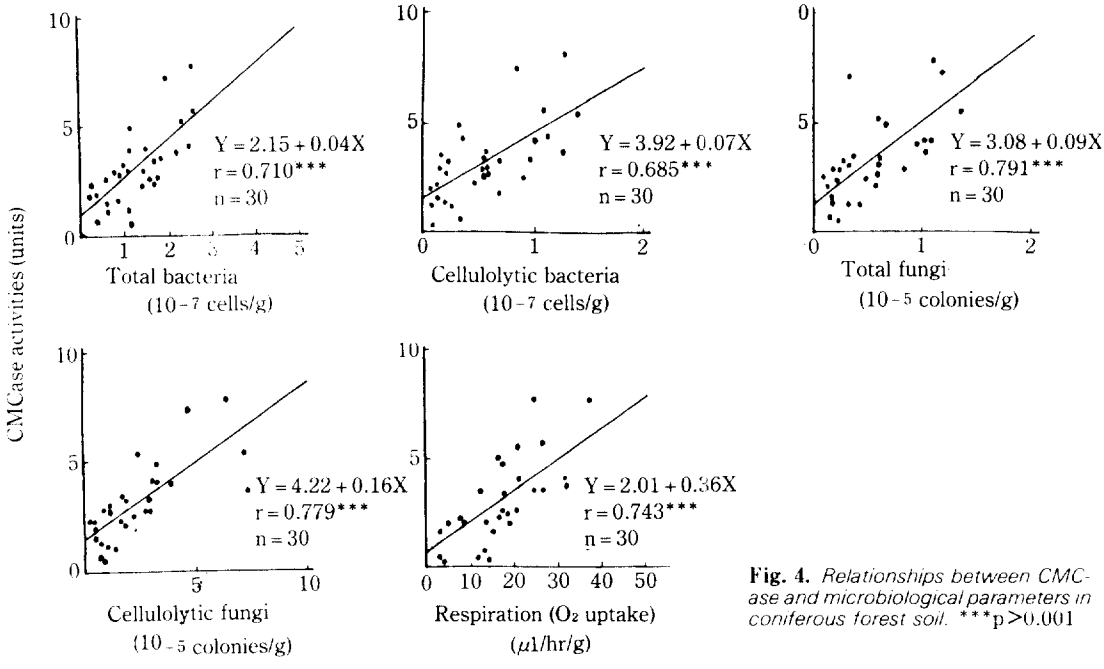

Fig. 4. *Relationships between CMCase and microbiological parameters in coniferous forest soil.* ***p>0.001

Among the physical and chemical parameters, meanwhile, CMCase activity in coniferous soil was correlated with soil moisture (r = 0.387*), nitrate (r = 0.630***), ammonia (r = 0.411*), total nitrogen (r = 0.583**), and total organic C (r = 0.683***). In a same manner with that in deciduous soil, strongest significances were found in the interrelationships between CMCase activity and all of the indices of microbial growth and activity. In Fig. 4, the regression equations for the relationships between CMCase activity in coniferous soil and those microbiological parmeters are presented.

DISCUSSION

Although the occurrence of cellulase activity in soil profiles have been reported by many investigators (review of Ladd, 1978), few broad generalizations can yet be made about the ecology of soil cellulase as indicated by Kiss *et al.* (1975, 1978), who expressed their skeptical view that cellulase accumulation in soil as an extracellular enzyme is not a general phenomenon and needs further studies for a better understanding of cellulase accumulation in soil. Part of the reason for a lack of understanding of the soil cellulase can be attributed to defects in analytical methods and the insufficient considerations for the different properties of cellulase components, and thus reliable informations for every cellulase components are essential to understand the functions of whole cellulase system in soil ecosystem. In the present study, variations in CMCase activities with respect to sampling time and soil depth were observed (Fig. 1 and 2). CMCase activities increased untill summer or autumn and then decreased till winter, and they decreased generally with increasing soil depth. No comparable data for a seasonal profile are available for comparison, but several studies have documented a decrease in soil enzyme activities with soil depth (Duxbery and Tate, 1981; Frankenberger and Tabatabai, 1981). The findings on the seasonal variation in soil CMCase activity reflect the importance of seasonal profile sampling to accurate description of distribution of this enzyme.

Pancholy and Rice (1973a, 1973b) proposed a hyphothesis that cellulolytic microorganisms and cellulase activity are more active and higher in early stages of succession because woody vegetation in the climax forests has a much lower ratio of

polysaccharides to lignin than do the vegetation in succession. It is clear that decomposition of litter of different plants does not occurred at the same rate even under similar environmental conditions. This is undoubtedly due to differences in structure and chemical composition of their leaves and other parts. Leaves of coniferous trees are generally decomposed more slowly than those of deciduous trees (Williams and Gray, 1974). In this study, higher CMCase activities were found in climax (deciduous) forest soil. These informations indicate that CMCase activity in soil is closely related to the type of plant material added.

The marked decrease in soil enzyme activities with increasing soil depth is considered to be the result of either the lack of their substrates or decreased microbial growth as the soil less well aerated (Kiss *et al.*, 1978; Duxbury and Tate, 1981). As can be seen in Table 1, the content of total organic C in coniferous soil was of small quantity and amounted to approximately a half of that of deciduous soil. It seems, therefore, that the lack of fresh substrate is likely one of the factors regulating the CMCase activity in coniferous soil.

Microbiologists studying the growth and activity of microorganisms in nature would very much like to be able to use one or two easily determined indicators of population size or activity. Even though various parameters have been proposed, no one parameter has been found to be an ideal index which would be correlated not only with microbial activity but also with microbial growth. In recent, several attempts to relate the soil enzyme assays to microbial growth and activity in soil have been made with varying results (Skujins, 1978; Nannipieri *et al.*, 1978; Sparling *et al.*, 1981). Burns (1982) indicated the considerable difficulties in relating enzyme activities determined *in vitro* usually involving buffers, constant temperatures and excess substrates, to those occurring *in situ*. Nevertheless, Stroo and Jencks (1982) reported that respiration rate and amylase and phosphatase activities are all significantly correlated with each other. Invertase and sulfatase were suggested to be the best indicators of soil fertility by Ross (1983). Similarly, alkaline phosphatase, amidase and catalase were also proposed as ideal indices in determining the relative activity and mass of microbial population in soils (Frankenberger and Dick, 1983).

Abiontic soil CMCase activities showed significant correlations with microbial indices such as soil respiration and all of the microbial population sizes (Table 4 and 5). There were also strong positive correlation between the soil respiration and various microbial population sizes. Conversion of native cellulosic materials to soluble carbon by the action of cellulase system is the most important process in the biological cycle of carbon and may make significant contribution to the microflora which are severely limited by nutrient availability (Lynch, 1983). Therefore, it is perhaps not surprising that there were good correlations between soil CMCase activities and microbial parameters. These results suggest that determination of abiontic soil CMCase activity is an useful additional index for assessing the overall microbial growth and activity in soil.

적 요

극상상태인 활엽수림토양과 천이가 진행중인 침엽수림토양에서의 abiontic carboxymethylcellulase (CMCase) 의 분포특성과 이 효소활성도와 토양생태계내의 주요 환경요인과의 상관관계를 조사하였다.

활엽수림토양에서는 계절에 따른 환경요인의 변화가 토심에 따른 환경요인의 변화보다 CMCase활성도의 변동에 크게 작용한 반면에, 침엽수림토양에서는 토심에 따른 환경요인의 변화가 더욱 크게 작용하는 것으로 나타났다. 두 토양내의 CMCase활성도는 여러가지 환경요인중 특히 미생물의 호흡율 및 여러가지 주요한 토양미생물의 개체군 크기와 매우 유의한 상관관계를 나타냄으로써 토양내의 CMCase활성도가 토양생태계내에서의 미생물의 생성 및 활성을 나타내줄 수 있는 새로운 지표로서 활용가능함을 보여주었다.

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(Received April 28, 1985)