Effect of Cultivation on the Activity of Some Soil Enzymes

V.V.S.R.Gupta and J.J.Germida
Dept. of Soil Science
Univ. of Saskatchewan, Saskatoon.

Introduction :

Microbial activity affects nutrient transformations and availability in soils. Clearing and cultivation of native soils, for eg., prairie grass lands or grey wooded luvisols, causes a decrease in the soil organic matter content (Anderson et al., 1981 and Tiessen et al ., 1982). In addition to a decrease in the organic matter content there is a qualitative change in the nature of organic matter (Voroney et al. 1981). Such changes could affect the microbial biomass and its activity (i.e., enzyme activity) in soil. Information on the effect of clearing and cultivation on soil enzymes is not available in the literature. The objectives of this study were i) to investigate the effect of cultivation on activity of certain soil enzymes, i.e., dehydrogenase, urease, arylsulfatase, acid and alkaline phosphatases, and ii) to determine the kinetic parameters of arylsulfatase.

Materials and Methods :

Soils from adjacent fields of different management systems were sampled (0-10 cm). Site history of the soil samples is given in Table 1. Surface samples of all soils were sieved to pass through a 2mm sieve and stored at 4^0C in polyethylene bags till they were analysed. Sub samples of all soils were air-dried, sieved (<2mm) and stored at 24^0C.

Physical and chemical characteristics were determined on air-dried and sieved soil samples. Organic carbon was determined by the method of Nelson and Sommers (1982). Total carbon, nitrogen and sulfur were determined as described by Tiessen et al. (1981), Bremner (1960) and Tabatabai and Bremner (1970a) respectively. Texture, pH, NO_3 and SO_4 analyses were performed by Saskatchewan soil testing laboratory.

Field moist soil samples were used for all the microbial and biochemical determinations. Serial dilutions of soil samples were prepared and total counts determined by spread plate technique. Bacterial, fungal and actinomycete populations were enumerated on trypticase soy agar (0.3%), czapek-dox agar (pH 3.5) and actinomycete isolation agar respectively. Microbial biomass was determined by the chloroform fumigation- incubation technique (Voroney and Paul, 1984). A kC value of 0.41 was used for conversion of CO_2-C to biomass carbon. Respiration activity (per day) was calculated from the CO_2-C evolved when non-fumigated samples were incubated for a 10 day period at 24^0C.

The activity of dehydrogenase in soil was determined as per the method described by Casida et al (1964). Arylsulfatase activity was measured using p-Nitrophenol sulphate as substrate (Tabatabai and Bremner, 1970b). Urease activity was determined from the rate of urea hydrolysis over a 5 day incubation period at 37^0C (Douglas and Bremner, 1977). Acid and alkaline phosphatases were determined as per the method described by Eivazi and Tabatabai (1977).

Stability of the enzymes in soil was determined at three temperatures, i.e., 4^0C, 25^0C and 37^0C. Moist soil samples were incubated at specified temperatures and enzyme activities were measured after 24hrs. Data reported in this study are results of duplicate analyses on triplicate samples.

Kinetic parameters of arylsulfatase were determined as per the method of Tabatabai and Bremner (1971). Change in the enzyme velocity was measured when the enzyme concentration was held constant but the substrate concentration was allowed to vary from 0.001M to 0.020M. Hanes-Wolf transformation was applied to the results by plotting S/V against S to determine the intercept and the slope of the linear transformtion of the Michaelis-Menten equation (S/V = Km/Vmax + 1/Vmax)(1/S)). From this graph the substrate affinity constant (1/Km) and Vmax values were calculated.

Results :

Clearing and cultivation of native soils decreased the organic carbon content (Table 2). Narrower C:N:S ratios were observed for the cultivated soils as compared to that of native soils. A change in the inorganic nitrate and sulfate concentrations in soil was observed due to cultivation.

Clearing and cultivation of native soil decreased total microbial counts (Table 3) in the Dark Brown Chernozemic soil. However, only minor changes were observed in the Grey Luvisolic soil. Cultivation of native soil decreased the microbial biomass carbon content in soil by 37% and 63% in Dark Brown Chernozem and Grey Lusolic soils respectively (Table 4). The respiratory activity was lower in cultivated soils as compared to that of native soils.

In both soil zones, considerably higher enzyme activities were observed in native soils (Table 5 and 6). Acid phosphatase activity was higher than alkaline phosphatase in all the soils. Clearing alone decreased the acid phosphatase activity by 55% in the Grey Luvisol, but the reduction in alkaline phosphatase was much smaller.

Results from enzyme stability experiment are shown in Fig.1 and 2. A 24hr. incubation of soil at 37^0C decreased activities of urease, arylsulfatase and acid phosphatases in both native and cultivated soils. However, no significant change in the dehydrogenase activity was noted.

Clearing and cultivation of native soil affected the kinetic parameters of arylsulfatase (Table 7). Cultivation increased the substrate affinity constant (1/Km), but decreased the maximum enzyme velocity (Vmax) in both soil zones.

Discussion :

This study investigated the effect of clearing and cultivation on the activity of some soil enzymes. Clearing and cultivation decreased the organic matter content and narrowed C:N:S ratios in both soil zones. These results are in agreement with the findings of Anderson et al.(1981) and Tiessen (1982). Measurements of microbial biomass carbon and its respiratory activity gave a better indication of the negative effect of cultivation on the microbial component in soil. Ayanaba et al. (1976) reported similar results in tropical soils.The inherent errors present in the serial dilution plate count technique could have masked the differences among treatments (Jenkinson and Ladd, 1981).

Soil enzymes originate from animal, plant and microbial sources. Native soils exhibited significantly higher activities of all the enzymes studied. It is likely that the presence of larger amounts of enzyme sources, i.e., plant roots (Skujins, 1967) and microorganisms (Speir and Ross, 1978), could account for the higher enzyme activities in native soils. Temperature-dependant denaturation (37^0C) and reduced activity of enzymes, i.e., urease,

arylsulfatase and acid phosphatase, was noted in both native and cultivated soils. Similar results on the temperature-dependant denaturation of soil enzymes was reported by Zantua and Bremner (1977) and Speir et al.(1980).

The Michaelis constant is one of the fundamental kinetic parameters in enzyme chemistry. The observed differences in the substrate affinity constant (1/Kmax) and maximum enzyme velocity between native and cultivated soils could be the results of changes in state of the enzyme and different factors affecting the substrate availability and product utilization. Tabatabai and Bremner (1971) also reported that Michaelis constant for arylsulfatase and phosphatases were different in different soils.

In conclusion, microbial biomass carbon measurements gave a better indication of changes in microbial populations and activity due to cultivation than that of total counts. The reduction in the enzyme activity in cultivated soils may be due to smaller amounts of enzyme sources such as plant roots and microorganisms. It is also interesting to note that the fundamental enzyme constant (1/Km) of arylsulfatase was affected by the clearing and cultivation of native soils. Thus, the results observed in this study indicate altered potential for biological and biochemical mineralization in cultivated soils.

References :

Anderson, D.W., Sagger, S., Bettany, J.B. and Stewart, J.W.B. 1981. Particle size fractions and their use in studies of soil organic matter: 1. The nature and distribution of forms of carbon, nitrogen and sulfur. Soil Sci. Soc. Am. J. 45: 767-772.

Ayanaba, A., Tuckwell, S.S. and Jenkinson, D.S. 1976. The effects of clearing and cropping on the organic reserves and biomass of tropical forest soils. Soil Biol. Biochem. 8: 519-525.

Bremner, J.M. 1960. Determination of nitrogen in soil by the Kjeldahl method. J. Agric. Sci. 55: 11-33.

Casida, L.E., Jr., Klein, D.A. and Santoro, T. 1964. Soil dehydrogenase activity. Soil Sci. 98: 371-376.

Douglas, L.A. and Bremner, J.M. 1971. A rapid method of evaluating different compounds as inhibitors of urease activity. Soil Biol. Biochem. 3: 309-315.

Eivazi, F. and Tabatabai, M.A. 1977. Phosphatases in soils. Soil Biol. Biochem. 9: 167-172.

Jenkinson, D.S. and Ladd, J.N. 1981. Microbial biomass in soil, measurement and turn over. *In* Soil Biochemistry. vol.5. E.A.Paul and J.N.Ladd (eds.) Marcel Decker Inc., NY. pp. 415-472.

Nelson, D.W. and Sommers, L.E. 1982. Total carbon, organic carbon and organic matter. *In* Methods of Soil Analysis. Part 2. Chemical and Microbiological properties. A.L.Page (ed.) ASM Monograph 9. Madison. pp. 539-579

Skujins, J.J. 1967. Enzymes in soil. *In* Soil Biochemistry. A.D.McLaren and G.H.Peterson (eds.) Marcel Decker Inc., NY. pp. 371-414.

Speir, T.W., Lee, R., Pansier, E.A. and Cairns, A. 1980. A comparision of sulfatase, urease and protease activities in planted and in fallow soils. Soil Biol. Biochem. 12: 281-291.

Speir, T.W. and Ross, D.J. 1978. Soil phosphatase and sulphatase. *In* Soil Enzymes. R.G. Burns (ed.) Academic press, London. pp. 197-250.

Tabatabai, M.A. and Bremner, J.M. 1970a. An alkaline oxidation method for determination of total sulfur in soils. Soil Sci. Soc. Am. Proc. 34: 62-65.

Tabatabai, M.A. and Bremner, J.M. 1970b. Arylsulfatase activity of soils. Proc. Soil Sci. Soc. Am. 34: 225-229.

Tabatabai, M.A. and Bremner, J.M. 1971. Michaelis constants of soil enzymes. Soil Biol. Biochem. 3: 317-323.

Tiessen, H. 1982. Changes in soil organic matter and phosphorus during cultivation of grassland soils. Ph.D. thesis submitted to Dept. of Soil Sci., Univ. of Saskatchewan, Saskatoon.

Tiessen, H., Bettany, J.R. and Stewart, J.W.B. 1981. An improved method for the determination of carbon in soils and soil extracts by dry combustion. Comm. Soil Sci. Plant Anal. 12: 211-218.

Tiessen, H., Stewart, J.W.B. and Bettany, J.R. 1982. Cultivation effect on the amounts and concentrations of carbon, nitrogen and phosphorus in grassland soils. Agron. J. 74: 831-835.

Voroney, R.P., Paul, E.A. 1984. Determination of kC and kN *In situ* for calibration of the chloroform fumigation-incubation method. Soil Biol. Biochem. 16: 9-14.

Voroney, R.P., Van Veen, J.A. and Paul, E.A. 1981. Organic carbon dynamics in grassland soils. 2. Model validation and simulation of long term effects of cultivation and rainfall erosion. Can. J. Soil Sci. 61: 211-214.

Zantua, M.I. and Bremner, J.M. 1977. Stability of urease in soils. Soil Biol. Biochem. 9: 135-140.

Table 1. Site history of the soils used in this study

Soil type	History
Dark Brown Chernozem (Bradwell-loam)	
Native	- virgin site, never broken or stocked
Cultivated (69 y)	- broken in 1915. Wheat-fallow rotation
Grey Luvisol (Loon lake-sandy loam)	
Native	- virgin site, aspen trees & ground cover
Cleared-Unseeded	- trees removed in 1980, Twice disced
Cultivated (5 y)	- Cleared in 1980, Oats and Barley
Cultivated (40 y)	-Wheat, Oats, Barley and Alfalfa

Table 2. General characteristics of the soils.

Soil type	pH	Organic carbon(%)	NO_3 --(ug g^{-1})--	SO_4	C:N:S ratio
Dark Brown Chernozem					
Native	6.5	3.5	2.5	2.0	84: 9:1
Cultivated (69 y)	6.2	1.1	4.6	2.6	72: 7:1
Grey Luvisol					
Native	6.0	9.0	0.5	3.0	150:11:1
Cleared-Unseeded	7.0	4.5	18.0	6.0	135:10:1
Cultivated (5 y)	6.6	1.3	10.5	3.5	127:10:1
Cultivated (40 y)	6.4	1.2	9.0	3.0	120: 9:1

Table 3. Effect of cultivation on microbial populations in soil

Soil type	Bacteria (10^7)	Fungi (10^4)	Actinomycetes (10^6)
		CFU g^{-1} soil	
Dark Brown Chernozem			
Native	5.9	9.5	7.2
Cultivated (69 y)	4.7	2.4	2.6
Grey Luvisol			
Native	10.8	5.5	23.5
Cleared-Unseeded	11.0	5.4	23.0
Cultivated (5 y)	12.1	5.3	11.4
Cultivated (40 y)	10.1	5.2	11.5

Table 4. Effect of cultivation on microbial biomass and respiratory activity in soil

Soil type	Microbial biomass[1]	Respiratory activity[2]
Dark Brown Chernozem		
Native	902	84
Cultivated (69 y)	570	36
Grey Luvisol		
Native	1400	91
Cleared-Unseeded	1259	102
Cultivated (5 y)	750	39
Cultivated (40 y)	517	35

[1] Microbial biomass ug C g^{-1} soil

[2] ug CO_2-C g^{-1} soil day^{-1}.

Table 5. Effect of cultivation on dehydrogenase and urease activities in soil

Soil type	Dehydrogenase[1]	Urease[2]
Dark Brown Chernozem		
Native	127.3	201.8
Cultivated (69 y)	42.0	57.9
Grey Luvisol		
Native	483.0	301.4
Cleared-Unseeded	361.5	168.6
Cultivated (5 y)	216.1	69.0
Cultivated (40 y)	63.7	41.3

[1] ug triphenylformazan g^{-1} soil
[2] ug urea hydrolyzed g^{-1} soil hr^{-1}.

Table 6. Effect of cultivation on arylsulfatase and phosphatase activity

Soil type	Arylsulfatase[1]	Phosphatases[1]	
		Acid[2]	Alkaline[3]
Dark Brown Chernozem			
Native	260.2	2100.0	650.4
Cultivated (69 y)	89.3	1059.1	190.8
Grey Luvisol			
Native	829.3	2256.9	699.9
Cleared-Unseeded	584.4	959.6	621.9
Cultivated (5 y)	95.7	701.7	364.0
Cultivated (40 y)	85.7	329.7	200.8

[1] ug p-Nitrophenol released g^{-1} soil hr^{-1}.
[2] pH - 6.5 [3] pH - 11.0

Table 7. Effect of cultivation on kinetic parameters of arylsulfatase.

Soil type	Michaelis constant Km[1]	Enzyme velocity Vmax[2]
Dark Brown Chernozem		
Native	4.92	863.56
Cultivated (69 y)	2.67	224.37
Grey Luvisol		
Native	7.58	1597.34
Cleared-Unseeded	9.55	774.92
Cultivated (5 y)	2.64	237.64
Cultivated (40 y)	1.72	110.90

[1] 10^{-3}M

[2] ug p-Nitrophenol released g^{-1} soil hr^{-1}.

Fig. 1. Effect of temperature on arylsulfatase and urease activities in soil (Dark Brown Chernozem)

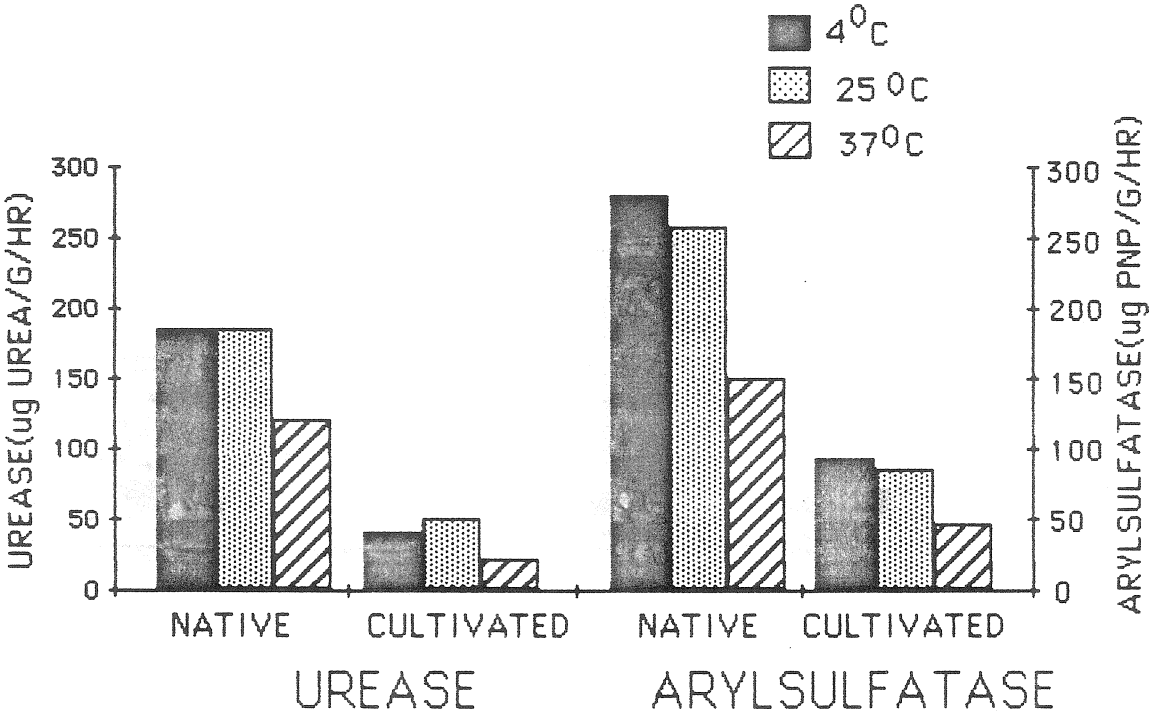

Fig. 2. Effect of temperature on dehydrogenase and acid phosphatase activities in soil (Dark Brown Chernozem)