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SHORT COMMUNICATION

ENZYME ACTIVITIES IN SOILS: EFFECTS OF LEACHING, IGNITION, AUTOCLAVING AND FUMIGATION

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Enzymes in soil are biologically significant as they participate in the cycling of elements and can influence the availability of nutrients to plants. Microorganisms, active roots and dead cells are the principal sources of soil enzymes. A major proportion of the extracellular enzymes found in soil is adsorbed on to clay and humic colloids. The enzyme fraction that remains in the soil solution is labile and exposed to degradative processes and therefore, only a very small fraction of the total soil enzyme content is found in solution (Burns, 1982). Dehydrogenases, urease and phosphatases are recognized as very important soils enzymes. Measurement of dehydrogenase activity provides an index of the catabolic activity of a soil. Urease and phosphatase are involved in transformations of N and P. As microorganisms are an important source of soil enzymes, the activity of these enzymes correlates with microbial activity, and therefore, measurements of the activities of these enzymes have often been used as an index of microbial activity.

It is difficult to isolate any component of soil and study its effect on the activity of soil enzymes, as removal of one component concomitantly affects other components, and thus the result obtained does not pertain to the effect of removal of the desired component, but also includes its indirect effects on the other components. Probably because of this no attempt has been made to study the relative importance of various soil components such as soil organic matter, soil microorganisms and soil solutes on enzyme activities, although the literature clearly indicates that these components largely determine the rate of activity of soil enzymes. We have attempted to exclude certain components from the soil through various treatments and have measured the activities of enzymes. Our objective was to understand the relative importance of soil organisms, organic matter and soil solutes on enzyme activity.

Cheesecloth pouches containing 200 g freshly-collected soil (moisture 19.0–26.7%) were leached in running tap-water for 24 h. The soils were sterilized by autoclaving (120°C, 60 min) or by $CHCl_3$ fumigation (Jenkinson and Powlson, 1976). Soils were ignited in a muffle furnace at 450°C for 2 h. In another treatment, leached soils were also ignited. Thus, the leached soil was devoid of soil solutes including enzymes that had been present in the soil solution. We assumed that any loss of microbes and any changes in their metabolism and growth during leaching would be negligible. The ignited soils were devoid of organic matter, microbes and enzymes. When leached soils were ignited, in addition to the above, these soils were also deprived of soluble minerals. The autoclaved soil did not contain microbes and enzymes, although the character of the organic matter has been changed. The fumigated soils were devoid of microbes, but abiotic enzymes (Skujins, 1976) and organic matter were not affected.

To determine whether such treatment effects vary with soil type, a broad spectrum of soil types was selected, namely an orchard soil, a forest soil, a garden soil and a grassland soil. The vegetational and physicochemical characteristics of the soils are given in Table 1. Surface soils (0–20 cm) were collected from 15 random sites for each soil type and made into three bulk samples each composed of five subsamples. These soil samples were analyzed separately. Soil pH was determined on a 1:5 soil–water suspension using an electric digital pH meter. The moisture content of soil was estimated on a w/w basis by oven drying soil at 105°C for 24 h.

Dehydrogenase activity was assayed by the 2-3-5 triphenyl tetrazolium chloride (TTC) reduction technique (Casida, 1977) and urease activity by the method of McGarity and Myers (1967). Ammonia released as a result of urease activity was determined by the Indophenol blue

Table 1. Dominant plant species and physicochemical characteristics of various soils

Soil type	Dominant plant species	Soil texture (%) Sand	Silt	Clay	Soil moisture (%)	Soil pH	Organic C (%)	Available P (%)	Available K (%)	Total N (%)
Orchard soil	*Pyrus communis* 30-yr old plantation	27.7	51.5	20.9	24.4	6.4	1.9	0.010	0.0145	0.60
Forest soil	*Pinus kesiya* 50-yr old plantation	50.9	27.6	21.4	19.0	6.0	2.7	0.009	0.0120	0.76
Garden soil	Botanical garden flower bed	40.9	24.6	34.4	25.3	6.0	1.7	0.009	0.009	0.50
Grassland soil	*Paspalum dilatatum* and *Trifolium repens*	35.1	46.3	18.5	26.7	6.2	1.8	0.015	0.0150	0.80

method and assayed colorimetrically at 630 nm. Phosphatase was assayed (Tabatabai and Bremner, 1969) using *p*-nitrophenyl phosphate as the substrate, and nitrophenol produced as a result of phosphatase activity was assayed colorimetrically. For chemical analysis, soils were air-dried and sieved (> 2 mm). Organic C was determined by Walkley and Black's (1934) titration method. A colorimetric molybdenum blue method using Bray's extraction solution was followed for P estimations; K was determined by a flame photometer method using ammonium acetate as the extraction solution (Jackson, 1973). Each value reported is the mean of nine replicate analyses.

The activities of all of the enzymes investigated were lower in the treated soils than in the controls. The net effect of treatments differed with soil type but all of the trends were similar in each of the soils (Figs 1–3).

The lowering of enzyme activities in the leached soil could be attributed to the removal of soluble inorganic and organic substances and free enzymes. Only a very small portion of the total enzyme content of the soil is found in the soil solution and the major part is either present intracellularly or in the immobilized state (Burns, 1982). As expected, the ignition of soil reduced enzyme activities to very low levels as a result of the combustion of all the organic substances including enzymes. Similarly, autoclaving also resulted in a loss of enzyme activities because all microorganisms were killed and enzymes were denatured by heat. The activities recorded in fumigated soils could be assigned to those of abiontic enzymes because most of these are to be found in the immobilized state (Skujins, 1976; Burns, 1982). This was most pronounced in the case of phosphatase, indicating that probably they were present predominantly in the abiotic state. Ramirez-Martinez and McLaren (1966) also considered that soils are enriched in phosphatase enzyme proteins as most soils exhibit greater activity than can be explained by numbers of microorganisms. The appreciable decrease of urease activity in fumigated soils suggests that urease activity is derived from living cells and little urease accumulates (Fig. 2). However, Burns *et al.* (1972a, b) and Pettit *et al.* (1976) proposed that urease accumulates in organo mineral complexes. Thus, living and organo mineral complexes are considered to be the loci of urease activity, and the relative contributions of the two loci may vary depending on the clay content, humus content and cation exchange capacity of soil.

Urea hydrolysis was recorded in the ignited soils even though urease activity was not expected (Fig. 2). Repeated

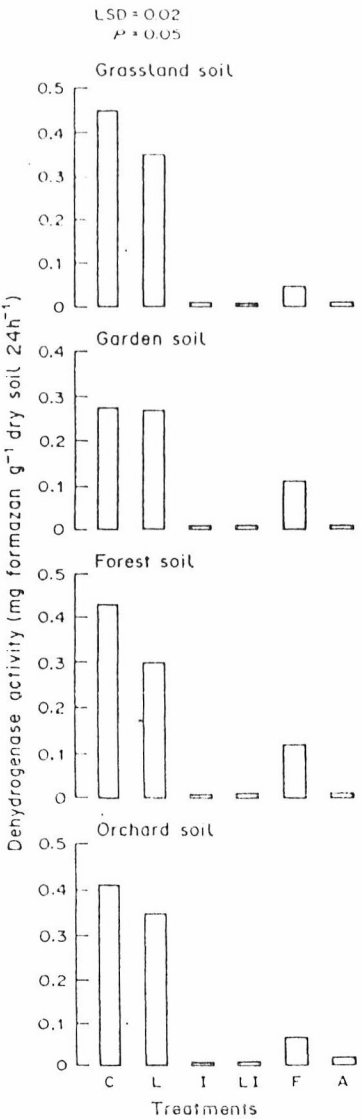

Fig. 1. Dehydrogenase activity in soils: control = C, leached = L, ignited = I, leached + ignited = LI, fumigated = F, autoclaved = A.

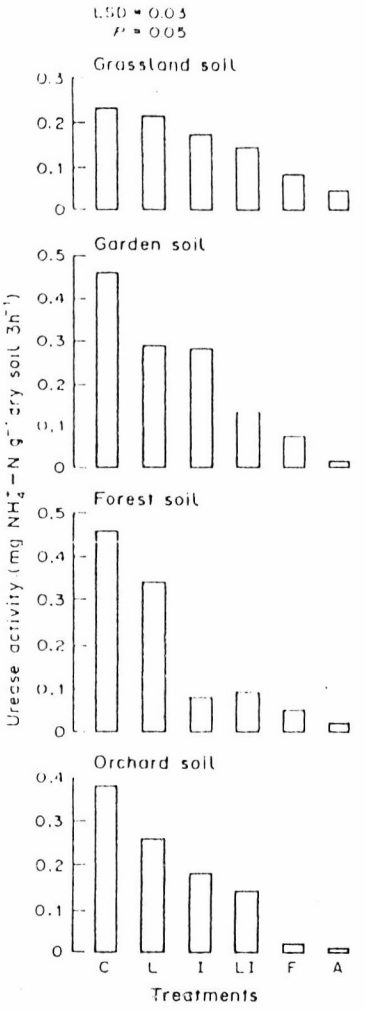

Fig. 2. Urease activity in soils. Symbols as in Fig. 1.

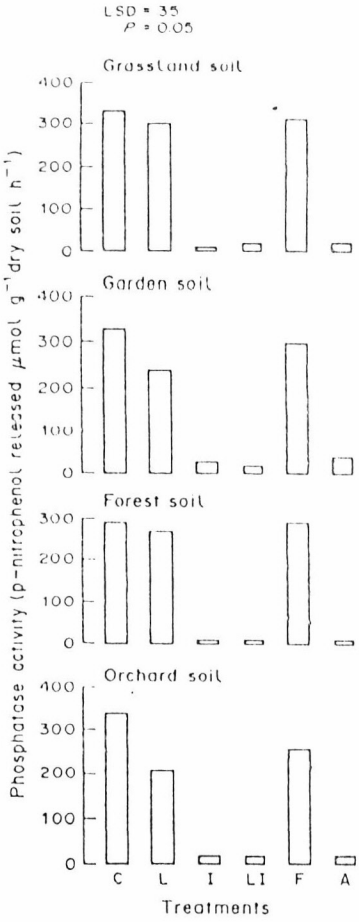

Fig. 3. Phosphatase activity in soils. Symbols as in Fig. 1.

observations with similar results with a variety of soils types suggests the possibility that inorganic catalytic agents are formed in the ignited soils and it is these that are responsible for the hydrolysis of urea. The degree of hydrolysis of urea varied in the different soils and this could be attributed to differences in the quality or quantity of the catalytic agents present.

The results demonstrate that there are at least three different loci of enzyme activity in soil, inside viable cells, on the surfaces of clay–humic colloids and in the soil solution. Of these three, viable cells account for most of the dehydrogenase and urease activities while extracellular enzymes adsorbed on clay–humic colloids are responsible for a major part of the phosphatase activity. The enzymes present in the soil solution account for only a very small fraction of total enzyme activity.

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