Pergamon

0038-0717(95)00045-3

Soil Biol. Biochem. Vol. 27, No. 9, pp. 1161–1166, 1995

0038-0717/95 $9.50 + 0.00

THERMAL STABILITY AND ACTIVITIES OF SOIL ENZYMES AS INFLUENCED BY CROP ROTATIONS

MORTEN MILLER[1] and RICHARD P. DICK[2]*

[1]Microbiology Section, The Royal Veterinary and Agricultural University, Rolighedsvej 21, DK-1958 Frederiksberg C, Copenhagen, Denmark and [2]Department of Crop and Soil Science, Agriculture and Life Sciences Building, Room 3067, Oregon State University, Corvallis, OR 97331-7306, U.S.A.

(Accepted 16 February 1995)

Summary—Soil samples were collected to a depth of 10 cm in 1991 and 1993 from a vegetable crop rotation experiment initiated in 1989. The two cropping treatments, with either 0 or 280 kg N ha^{-1}, represented the traditional vegetable rotation (TVR) and an alternative legume vegetable rotation (LVR) when a vegetable crop alternated with a red clover (*Trifolium pratense* L.) seed-crop that was incorporated as green manure in the following spring. The enzyme activities of L-asparaginase, amidase and β-glucosidase were determined on whole soil and five soil aggregate size fractions: 1.00–2.00, 0.50–1.00, 0.25–0.50, 0.10–0.25 and <0.1 mm. Thermal stability of the enzymes was determined by conditioning soil samples at 85°C for 2 h or by exposing soil samples to five successive freeze–thaw cycles prior to enzyme assays. Enzyme activities for LVR were significantly greater ($P < 0.05$) than TVR for β-glucosidase and amidase at both N-rates in 1991. This difference in activity for these enzymes was sustained only at the high N-rate in 1993. The activity of L-asparaginase was significantly higher ($P < 0.01$) in the LVR in 1993. Sixty to seventy percent of the soil enzyme activity (on a mass basis) was associated with macroaggregates with specific distribution of activity across aggregate fractions varying with enzyme. Average β-glucosidase activity decreased by 50% from 1991 to 1993. In contrast, amidase activity increased 1.43-fold over the same period. β-glucosidase activity was sensitive to temporal trends, showing proportional decreases in activities in each system that were consistent with decreases in organic C. Furthermore, β-glucosidase activity showed significantly higher ($P < 0.05$) resistance to heat-induced thermal stress in the LVR in both sampling years. Amidase and L-asparaginase activities showed no such treatment effects. The results showed that soil enzyme activity is a sensitive biological indicator of the effects of soil management practices.

INTRODUCTION

Soil enzymes play an important role in soil mineralization processes (Tate, 1987) and have been related to other soil biological properties (Frankenberger and Dick, 1983). In soil, enzymes can be associated with viable cells or as abiontic enzymes, a term first used by Skujins (1976), which are associated with dead cells, cell debris and immobilized enzymes in the soil matrix (Burns, 1982). Soil enzymes can be stabilized in two locations: adsorbed to internal or external clay surfaces; and complexed with humic colloids by adsorption and cross-linking, microencapsulation, ion exchange, entrapment or co-polymerization (Weetal, 1975; Boyd and Mortland, 1990). Clay and humic colloids have been shown to play an important role in the stability of abiontic enzymes to thermal stress, such as heating and repeated freezing–thawing cycles (Lähdesmäki and Piispanen, 1992).

Studies from long-term sites have shown that cropping systems that have higher C inputs (e.g. crop ration, additions of green or animal manure) or conserve C inputs (e.g. minimum tillage) promote enzyme activity (Dick, 1994). For example, a comparison of on-farm cropping systems showed that an alternative system that included a legume green manure improved soil structure (Reganold, 1988) and increased soil enzyme activities (Bolton *et al.*, 1985). The increased activity of soil enzymes in these soils may be due to enhanced protection and survival of abiontic enzymes in humic complexes of these soils (Martens *et al.*, 1992). We hypothesized that legume green manure would enhance soil aggregation and that this would be reflected in the activities of soil enzymes and their stability to thermal stress. The objectives of our study were to determine if green manure applications would enhance the stabilization of enzymes and to determine whether a relationship exists between soil aggregate development and soil enzyme activities.

*Author for correspondence.

MATERIALS AND METHODS

Sample collection and preparation

Soil samples were collected from two treatments in a vegetable crop rotation experiment initiated in 1989 at the North Willamette Research and Extension Center in Oregon. The two treatments represent the traditional vegetable rotation (TVR) currently being practiced with a winter fallow and an alternative

Table 1. Traditional and alternative crop rotations

Year	Legume-vegetable rotation	Traditional-vegetable rotation
Fall 89–winter 90	Red clover	Fallow
Spring 90	Red clover	Corn
Fall 90–winter 91	Red clover	Fallow
Spring 91	Broccoli†	Broccoli
Fall 91–winter 92	Red clover‡	Wheat‡
Spring 92	Red clover	Wheat
Fall 92–winter 93	Red clover	Fallow
Spring 93	Broccoli†	Broccoli
Fall 93	Fallow	Fallow

†Red clover was plowed under as green manure prior to establishment of broccoli crop.
‡Planted September 1991.

vegetable rotation that includes a legume crop (LVR). In the LVR, a vegetable crop was alternated with red clover (*Trifolium pratense* L.) harvested for seeds in the summer, allowed to regrow over the winter and then plowed down in spring as a green manure (Table 1). The two cropping treatments were subdivided into two N-rates of 0 (N_0) or 280 kg N ha^{-1} (N_{280}). The experimental design was a randomized complete-block split-plot, with cropping system as the main plot and N-rate as the subplot with four replications. Two of the four replications were included in this study. The soil is a Willamette silt loam (Pachic Ultic Argixerolls). The climate is Mediterranean, characterized by cool winters and hot dry summers. The mean temperature is 11.1°C and the annual precipitation is 1040 mm, with 70% occurring during the winter months (November–April). The average soil temperature at a depth of 5 cm is 7.2°C (winter) and 19.3°C (summer).

Soil was collected in early September 1991 (post broccoli harvest prior to red clover or wheat planting) and 1993 (post broccoli harvest) (Table 1) to a depth of 10 cm and a composite sample was obtained from 30 cores that had been thoroughly homogenized. The composite samples were stored at 4°C and analyzed within 4 days. Field-moist soil samples, with a volumetric water content of ca. 10%, were carefully sieved by hand to obtain five soil aggregate size fractions: 1.00–2.00, 0.5–1.00, 0.25–0.5, 0.1–0.25 and <0.1 mm. Samples were air-dried for chemical and biochemical analyses. Chemical characteristics of these soil samples for both 1991 and 1993 were reported by Miller and Dick (1995). All results are expressed on a g^{-1} oven-dry (105°C, 24 h) wt basis. Standard analysis of variance procedures were conducted with a SAS statistical software package (SAS Institute, Cary, NC). The data presented are the mean values (coefficient of variation <5%) of triplicate analyses of each field replication.

Microbial biomass C was determined by the chloroform fumigation-incubation method described by Jenkinson and Powlson (1976) on a 12 g (fresh wt) soil sample.

Enzyme activity on whole soil and aggregates

Air-dry samples of both aggregates and whole soil were analyzed for enzyme activity. Amidase (acylamide amidohydrolase, EC 3.54), L-asparaginase (L-asparaginase amididohydrolase, EC 3.5.1.1), and β-glucosidase (β-glucosidase, EC 3.2.1.2) were determined as described by Frankenberger and Tabatabai (1980a, 1991a) and Eivazi and Tabatabai (1988), respectively. Because of the short assay times (<2h), no microbial inhibitors were added.

The resistance of soil enzyme activity to heat-induced stress was determined by placing 1.00 g of air-dried soil in a 50-ml Erlenmeyer flask which was then stoppered and kept for 2 h at 85°C. The samples were allowed to cool for 20 min and then assayed for soil enzyme activity as described above. The resistance to freeze–thaw cycles was determined by weighing 1.00 g of soil into a polypropylene centrifuge tube that was sealed with o-ring caps. The samples were exposed to five successive freeze–thaw cycles by placing the centrifuge tube into liquid N_2 for 1 min and then transferred to a 50°C waterbath for 10 min. In the fifth cycle, the frozen samples were transferred to 50-ml Erlenmeyer flasks and allowed to thaw for 1 h. The samples were then assayed for enzyme activity.

RESULTS

There was a significant crop rotation effect on enzyme activity of whole soil in both 1991 and 1993 (Table 2). In 1991, the activity of β-glucosidase was higher in the LVR than the TVR at 0 ($P < 0.05$) and at the 250 kg N ha^{-1} ($P < 0.10$) treatments. Amidase activity in the LVR was significantly higher at both N-rates in 1991 and at the control N-rate in 1993. L-asparaginase was significantly higher in the LVR at the high N-rate in 1993. Average β-glucosidase activity decreased by 50% from 1991 to 1993 in contrast to amidase activity, which increased by 43% in the same period. There was a major shift in the natural fabric

Table 2. Enzyme activities in 1991 and 1993 as affected by N-rate and crop rotation

		1991		1993	
N-rate (kg ha^{-1})	Enzymes	LVR	TVR	LVR	TVR
0	β-glucosidase†	79.4**	65.4	43.0*	38.2
	Amidase‡	134.2***	112.3	196.4***	178.3
	L-asparaginase‡	12.6	13.8	17.7	20.6
280	β-glucosidase	93.5*	77.6	44.1	45.5
	amidase	129.3***	113.6	162.4	164.7
	L-asparaginase	20.1	16.0	17.1***	9.9

*,**,***Significant difference at $P < 0.05$, $P < 0.1$ and $P < 0.01$ between columns for each year.
†μg ρ-nitrophenol g^{-1} soil h^{-1}.
‡μg NH_4^+ g^{-1} soil 2 h^{-1}.

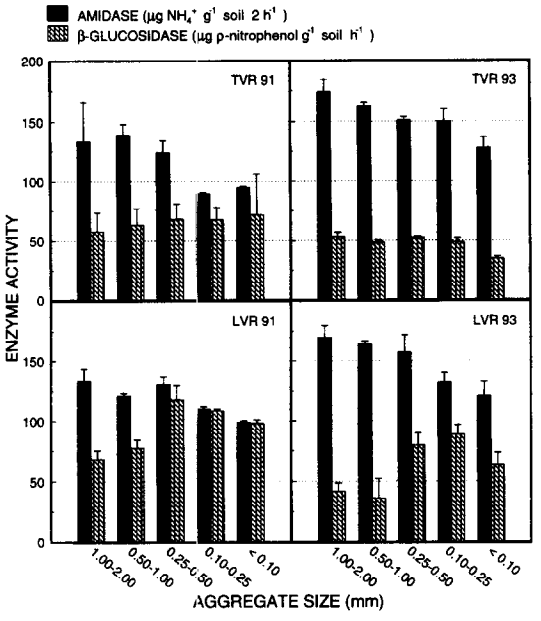

Fig. 1. Amidase and β-glucosidase activity for 1991 and 1993 as affected by crop rotation and aggregate size at high rate of N (calculated on g^{-1} of aggregate fraction).

aggregate size distribution in the LVR, with a 35% decrease in microaggregates (< 0.25 mm) and a similar percentage increase in macroaggregates (> 0.25 mm) (data not shown).

The relative distribution among different aggregate size classes showed that 60–70% of the total enzyme activity could be attributed to the macroaggregates (> 0.25 mm). There were substantial differences between individual enzyme activities in the various aggregate size classes (activity g^{-1} aggregate fraction) (Figs 1 and 2). Amidase activity, regardless of rotation and N-rate, increased with aggregate size. In contrast, L-asparaginase and β-glucosidase activity generally showed enhanced activity in the smaller aggregates (Figs 1 and 2).

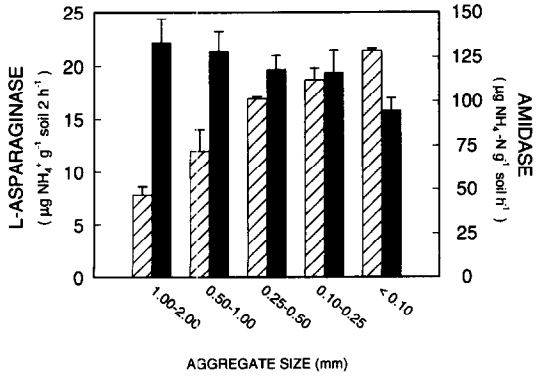

Fig. 2. L-asparaginase activity and amidase activity in 1991 in the TVR low N-rate as affected by aggregate size (calculated on g^{-1} of aggregate fraction).

In 1991 β-glucosidase activity in the LVR showed significantly higher ($P < 0.05$) resistance to heat-induced thermal stress (Table 3), with similar results found in 1993 ($P < 0.05$) (data not shown). These observations are corroborated by data from other green manure (winter cover crops) treated soils, where β-glucosidase activity showed greater resistance to heat-induced thermal stress (1991, data not shown). Amidase activity was sensitive to heat exposure, but did not show any significant crop rotation treatment effects. In contrast, L-asparaginase was unaffected by heat-induced thermal stress (Table 3). Amidase and β-glucosidase activities were both unaffected by repeated freeze–thaw cycles.

DISCUSSION

Enzyme activity on whole soil and aggregates

β-glucosidase activity was significantly higher in LVR soil than TVR in 1991, 2 years after treatments began. Furthermore, β-glucosidase activity was sensitive to temporal trends, decreasing proportionally under each management practice in a manner consistent with the decreases in organic C reported by Miller and Dick (1995) where LVR decreased by 6.5% (not significant at $P < 0.05$) and TVR decreased by 11.9% ($P < 0.05$). The higher enzyme activities in the LVR treated soil are likely related to having a rhizosphere nearly year around and biennial green manure inputs which resulted in greater soil aggregation.

Sixty to seventy percent of the soil enzyme activity (on a mass basis) was associated with macroaggregates which may be because macroaggregates probably maintain and stabilize soil enzyme activity by increasing the amount of potential habitat for the microbial biomass. Evidence for this is provided by Gupta and Germida (1988) who found that macroaggregates have a greater microbial biomass and enzyme activities per mass of aggregate. This may be because macroaggregates increase potential microbial habitat and offer a greater potential to protect abiontic enzymes. Other studies have shown correlations between enzyme activity and soil structural properties; Dick *et al.* (1988) found highly significant negative correlations between bulk density and activities of dehydrogenase, phosphatase and arylsulfatase. In seven out of ten enzymes tested, Martens *et al.* (1992) found significant negative correlations with soil bulk density, and for five enzymes, significant positive correlations with cumulative water infiltration rates.

The inverse trends between β-glucosidase and amidase activities suggest that these two enzymes reflect different soil characteristics. Microbial biomass C (data not shown) showed a highly positive correlation between microbial biomass and amidase activity ($r = 0.90$***). Conversely, β-glucosidase activity showed no correlation with specific microbiological factors. The major source of β-glucosidase

Table 3. Resistance to thermal stress in 1991 as a percentage change in enzyme activity (averaged across N-rates)

	β-glucosidase†		Amidase‡		L-asparaginase‡	
Thermal stress	LVR	TVR	LVR	TVR	LVR	TVR
Original activity	86.4	71.4	131.7	112.9	16.3	14.9
Heat (2 h 85 C)	74.0	46.6	69.5	48.1	16.9	14.4
Percentage change	− 14.3*	− 34.7	− 47.2	− 57.3	+ 3.6	0.0
Freeze/thaw (5 cycles)	79.5	64.0	145.3	113.6	ND	ND
Percentage change	− 8.0	− 10.3	+ 10.3	+ 0.0	ND	ND

*Significant difference between rotations at $P < 0.05$.
†μg ρ-nitrophenol g^{-1} soil h^{-1}.
‡μg NH_4^+ g^{-1} soil 2 h^{-1}.

activity in soil is fungi, in particular, mucoraceous fungi (Hayano and Katami, 1977; Hayano and Tubaki, 1985). Thus, the greater β-glucosidase activity in microaggregates in the LVR may indicate that fungi played a role in the aggregate formation observed in this rotation. Foster (1994) reported that fungal hyphae were readily identified in microaggregate structure and Gupta and Germida (1988) showed the highest fungal biovolume and hyphae length in the 0.5–0.1 mm size aggregate fractions on cultivated soils. If β-glucosidase activity is of fungal origin, our results would be consistent with these reports.

Between 1991 and 1993 there were small decreases in the soil C pools in the LVR and significant decreases in the soil C pools in the TVR (Miller and Dick, 1995). Concurrent with these changes, we observed a definite decrease of β-glucosidase activity on whole soil and among aggregate size classes, particularly in the smaller aggregates. The fact that decreases in β-glucosidase activities among aggregate size classes were much less pronounced in the LVR suggests that the changes in activity over time are a likely reflection of changes in the soil C.

Contrasts of two amidohydrolases

Amidase and L-asparaginase are both amidohydrolases catalyzing the hydrolysis of amides with the production of NH_4^+ and the corresponding carboxylic acids. They have been found in a wide variety of plants and microorganisms (Frankenberger and Tabatabai, 1980a,b). Despite the similarities between the two enzymes, we observed a number of intriguing differences in their interaction with soil. Amidase and L-asparaginase showed contrasting trends in distribution among different aggregate size classes (Fig. 2). Amidase activity was strongly affected by heat-induced thermal stress whereas L-asparaginase was unaffected. We observed more than 10 times greater activity of amidase than L-asparaginase, regardless of N-rate or crop rotation. Frankenberger and Tabatabai (1980b, 1991b) determined the kinetic property of maximum velocity (V_{max}) (an indicator of active enzyme sites) for these enzymes in eight soils. Amidase V_{max} values ranged from 138 to 438 (formamide used as substrate), whereas L-asparaginase ranged from 9 to 114 (Frankenberger and Tabatabai, 1980b, 1991b). These large differences in V_{max} are consistent with the higher amidase activity compared to L-asparaginase we observed in our study.

Toluene decreased amidase activity 40–50% but increased L-asparaginase activity 10–20% (data not shown). Frankenberger and Tabatabai (1991a) observed a similar increase in L-asparaginase activity in toluene-treated soils. They suggested that the increased activity in the presence of toluene may be due to a change in the permeability of the microbial-cell membrane to substrate and enzyme reaction products because it can be used as an unmasking agent in enzyme assays (Herzenberg, 1959; Jackson and DeMoss, 1965; Levinthal *et al.*, 1962). In a study by Beck and Poschenrieder (1963), toluene was shown to reduce the number of microorganisms in the presence or absence of asparagine. The extracellular asparaginase, however, remained active after the toluene treatment. The increase in L-asparaginase activity after exposure to toluene may thus reflect an association with cellular debris in the soil that is consistent with the findings of Mouraret (1965), who found asparaginase activity was bound to cell constituents and did not accumulate outside cells.

The persistence of soil enzymes has traditionally been attributed to stabilization in organo-mineral complexes. Accordingly, amidase has been reported to have a very strong correlation to clay ($r = 0.69$***) (Frankenberger and Tabatabai, 1981), but no such correlation has yet been found for L-asparaginase activity (Frankenberger and Tabatabai, 1991b).

Based on our data and evidence from the literature, we conclude that the interaction of L-asparaginase with soil is fundamentally different from that of a closely related amidohydrolase. The most likely explanation is that L-asparaginase has no persistence outside biological tissue and cells. The L-asparaginase activity in soils may thus originate from enzymes in viable cells, intact dead cells and cell debris. The constant turnover of living tissue in soils ensures that, at any time, a certain amount of enzyme activity is associated with this fraction.

These results suggest that L-asparaginase which, is associated with microbial tissue, is not susceptible to heat stress (85 C) but amidase, which apparently is complexed or stabilized by colloids, is susceptible to heat stress. This would appear to contradict previous work that has shown enzymes to be more stable when complexed with clay or humic colloids (Boyd and

Mortland, 1990; Lähdesmäki and Piispanen, 1992). However, it should be kept in mind that these studies compared complexed enzymes to free enzymes in solution. L-asparaginase likely is found in cellular tissue which may provide protection to the enzyme.

Green manure and enzyme stabilization

The differential thermal effect among enzymes as a function of soil management provides indirect evidence for the location of enzymes. Thermal stress had a greater effect on β-glucosidase enzyme activity in soil with lower green manure inputs, indicating a "significant" amount of its activity is associated with enzymes stabilized with soil colloids. Amidase decreased similarly among the crop rotations, and L-asparaginase was unaffected by thermal stress of C-inputs. The mechanisms of enzyme stabilization in soils are not well understood. Soil-bound or immobilized enzymes are conceptualized as enzymes adsorbed to clay minerals or associated with humic colloids due to adsorption, entrapment or co-polymerization (Burns, 1982).

Hayano and Katami (1977) extracted β-glucosidase enzymes from soil. The extract was brown and the results indicated that β-glucosidase was adsorbed on the surface of soil particles, suggesting that the stabilization of β-glucosidase may involve the interaction of both mineral colloids and humic colloids. In corroboration, Lähdesmäki and Piispanen (1992) found that the resistance to heat-induced thermal stress on proteolytic, cellulolytic, and amylytic activities decreased considerably in soil fractions from which the clay and humus colloids had been removed. They concluded that the stability of the enzymes to seasonal climatic effects is based on a built-in protection mechanism that relies on the existence of clay and humus colloids. The significantly higher content of particulate organic matter, dissolved organic C aggregate formation, and microbial biomass and its activity (Miller and Dick, 1995) in the LVR over the TVR, indicates that the application of green manure increased stabilization of organic matter and biological activity.

Acknowledgements—We thank Dr Del Hemphill for his assistance with the research and management of long-term plots, and the Danish Agricultural and Veterinary Research Council and Knud Hoejgaards Fond for support.This is Oregon State University AES Technical Publication 10,506.

REFERENCES

Beck T. and Poschenrieder H. (1963) Experiments on the effect of toluene on the soil microflora. *Plant and Soil* **18,** 346–357.

Bolton H., Eliott L. F., Papendick R. I. and Bezdicek D. F. (1985) Soil microbial biomass and selected soil enzyme activities: effect of fertilization and cropping practices. *Soil Biology & Biochemistry* **17,** 297–302.

Boyd S. A. and Mortland M. M. (1990) Enzymes interactions with clays and clay-organic matter complexes. In *Soil Biochemistry* (J. M. Bollag and G. Stozsky, Eds), Vol. 6, pp. 1–28. Marcel Dekker, New York.

Burns R. G. (1982) Enzyme activity in soils: location and a possible role in microbial ecology. *Soil Biology & Biochemistry* **14,** 423–427.

Dick R. P., Myrold D. D. and Kerle E. A. (1988) Microbial biomass and soil enzyme activities in compacted and rehabilitated skid trail soils. *Soil Science Society of America Journal* **52,** 512–515.

Dick R. P. (1994) Soil enzyme activities as indicators of soil quality. In *Defining Soil Quality for a Sustainable Environment* (Doran *et al.*, Eds), pp. 107–124. Soil Science Society of America Special Publication, Madison.

Eivazi F. and Tabatabai M. A. (1988) Glucosidases and galactosidases in soils. *Soil Biology & Biochemistry* **20,** 601–606.

Foster R. C. (1994) Microorganisms and soil aggregates. In *Soil Biota, Management in Sustainable Farming Systems* (C. E. Pankhurst *et al.*, Eds), pp. 144–155. CSIRO Information Services, East Melbourne.

Frankenberger W. T. and Dick W. A. (1983) Relationship between enzyme activities and microbial growth and activity indices in soil. *Soil Science Society of America Journal* **47,** 945–951.

Frankenberger W. T. and Tabatabai M. A. (1980a) Amidase activity in soils: I. Method of assay. *Soil Science Society of America Journal* **44,** 282–288.

Frankenberger W. T. and Tabatabai M. A. (1980b) Amidase activity in soils: II. Kinetic parameters. *Soil Science Society of America Journal* **44,** 532–536.

Frankenberger W. T. and Tabatabai M. A. (1981) Amidase activity in soils: III. Stability and distribution. *Soil Science Society of America Journal* **45,** 334–338.

Frankenberger W. T. and Tabatabai M. A. (1991a) L-asparaginase activity of soils. *Biology and Fertility of Soils* **11,** 6–12.

Frankenberger W. T. and Tabatabai M. A. (1991b) Factors affecting L-asparaginase activity in soils. *Biology and Fertility of Soils* **11,** 1–5.

Gupta V. V. S. R. and Germida J. M. (1988) Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. *Soil Biology & Biochemistry* **20,** 777–786.

Hayano K. and Katami A. (1977) Extraction of β-glucosidase activity from pea field soil. *Soil Biology & Biochemistry* **9,** 349–351.

Hayano K. and Tubaki K. (1985) Origin and properties of β-glucosidase activity of tomato-field soil. *Soil Biology & Biochemistry* **17,** 553–557.

Herzenberg L. A. (1959) Introduction of β-galactosidase in a cryptic strain of *Escherichia coli*. *Biochimica. Biophysica Acta* **31,** 525–534.

Jackson R. W. and DeMoss J. A. (1965) Effects of toluene on *Escherichia coli*. *Journal of Bacteriology* **90,** 1420–1425.

Jenkinson D. S. and Powlson D. S. (1976) The effect of biocidal treatments on metabolism in soil-V. A method for measuring soil biomass. *Soil Biology & Biochemistry* **8,** 209–213.

Lähdesmäki P. and Piispanen R. (1992) Soil enzymology: Role of protective colloid systems in the preservation of exoenzyme activities in soil. *Soil Biology & Biochemistry* **24,** 1173–1177.

Levinthal C., Signer E. R. and Fetherhof K. (1962) Reactivation and hybridization of reduced alkaline phosphatase. *Proceedings of the National Academy of Science U.S.A.* **48,** 193–198.

Martens D. A., Johanson J. B. and Frankenberger W. T. (1992) Production and persistence of soil enzymes with repeated addition of organic residue. *Soil Science* **153,** 53–61.

Miller M. and Dick R. P. (1995) Dynamics of soil C and microbial biomass on whole soil and aggregates in two cropping systems differing in C-input. *Applied Soil Ecology*. In press.

Mouraret M. (1965) *Contribution à l'Etude de l'Activité des Enyzme du Sol*. ORSTOM, Paris.

Reganold J. P. (1988) Comparison of soil properties as influenced by organic and conventional farming systems. *American Journal of Alternative Agriculture* **3,** 144–155.

Skujins J. (1976) Extracellular enzymes in soil. *CRC Critical Reviews in Microbiology* **4,** 383–421.

Tate R. L. III (1987) Soil enzymes and organic matter transformations. In *Soil Organic Matter; Biological and Ecological Effects*, pp. 69–94. Wiley-Interscience Publications, New York.

Weetal H. H. (1975) Immobilized enzymes and their application in the food and beverage industry. *Process Biochemistry* **10,** 3–24.