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PRODUCTION AND PERSISTENCE OF SOIL ENZYMES WITH REPEATED ADDITION OF ORGANIC RESIDUES

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Soil enzymes mediate biochemical transformations involving organic residue decomposition and nutrient cycling in soil. A field study was conducted to determine the activity and persistence of soil enzymes with repeated additions of different organic residues. The activities of 10 soil enzymes involved in carbon, nitrogen, phosphorus, and sulfur cycling were assayed in an Arlington coarse-loamy soil which had received 100 Mg ha^{-1} of either poultry manure, sewage sludge, barley straw, (*Hordeum vulgare*) or fresh alfalfa (*Medicago sativa*) over a 31-month time period (25 Mg ha^{-1} × 4 additions). The enzyme activity assayed in the amended soil was increased by an average of 2- to 4-fold by incorporation of the four organic amendments when compared with the unamended soil (tillage alone) during the 31-month study. In general, the addition of organic amendments greatly increased the enzyme activity during the first year of the experiment, but subsequent additions failed to sustain high enzyme activity in the amended soil. The straw amendment was the most effective amendment enhancing the soil enzyme activity for all the enzymes assayed except for urease. In contrast to other reported studies, soil enzyme activities were not inhibited by sewage sludge addition in this investigation. The increased level of enzyme activity in the organic-amended soil may be a reflection of the increased protective sites within the soil as a result of enhanced humus content.

Decomposition of plant or animal residues in soil releases essential nutrients such as nitrogen, phosphorus, and sulfur required for both plant and microbial growth. Nutrient cycling in soils involves biochemical, chemical and physicochemical reactions. The various biochemical processes are mediated by soil enzymes which are derived from microorganisms, plant roots, and soil animals (Tabatabai 1982).

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Incorporation of organic materials in soil promotes microbial and soil enzyme activity (Balasubramanian et al. 1972; Zantua and Bremner 1976; Nannipieri et al. 1983). Skujins (1976) reported that the total enzyme activity of a soil depends on the level of extracellular enzymes present, the amount of active enzymes within dead cells and associated cell fragments, and the level of activity associated with living cells. Nannipieri et al. (1978) suggested that the increase and decrease in soil enzyme activity in a glucose-amended soil follows a pattern similar to the microbial biomass. Sparling et al. (1981) reported that enzyme activity in a glucose-amended soil increased with increased levels of glucose applied.

Often the level of soil enzyme activity increases with increasing soil organic matter content (Speir 1977). This may be related to the population dynamics of the soil microbiota (Speir and Ross 1976; Speir 1977). Enzymes directly contributed by the organic amendment may also influence the soil enzyme activity in soil. Many of these enzymes are important in the formation of recalcitrant organic molecules that contribute to the chemical stability of the soil ecosystem. The effects of simple carbohydrates added to soil on the production and persistence of specific soil enzymes have been previously reported. However, little is known on soil catalysis involved in C, N, P, and S cycling upon long-term field conditions in response to added complex organic amendments such as plant materials, animal waste, or sewage sludge.

The objective of this study was to monitor the levels of activity of soil enzymes in response to repeated applications of plant residues and waste products under field conditions over several years. Soil enzyme activities were compared with changes in soil structure upon the application of organic amendments. The organic materials as a source of enzymes added to soil were also assessed.

MATERIALS AND METHODS

Field Plots

Replicated (5×) field plots (2 m × 2 m) amended with poultry manure (pH, 8.8; C/N,

5.3), sewage sludge (pH, 6.9; C/N, 5.0) (Riverside, CA), barley straw (pH, 6.4; C/N, 48.5), and green alfalfa (pH, 6.1; C/N, 7.0) were established at the Citrus Research Center, Agricultural Experiment Station, Riverside, CA. Organic applications (25 Mg ha^{-1} each; dry weight) were made in April 1987, February 1988, October 1988, and July 1989 to an Arlington coarse-loamy, mixed, thermic Haplic Durixeralf (pH 7.9, 670 g sand, 250 g clay kg^{-1} soil; 10 g organic C kg^{-1} soil; 1.1 g N kg^{-1} soil). These amendments were mechanically incorporated with a single pass of a tractor mounted rotary-tiller into the upper 15 cm of soil and sprinkler irrigated [10 cm water 24 h^{-1}; electrical conductivity (EC), 0.67 dS m^{-2}; sodium adsorption ratio (SAR), 1.3 $(mmol/L)^{1/2}$; pH, 7.1] weekly. The plots were maintained in a fallow state by frequent hand cultivation to remove the effects that growing vegetation may have had on the soil enzyme activity or the soil physical parameters measured. The Arlington soil was sampled before the addition of the organic materials and then sampled on a monthly basis with the exception of April 1987 which was sampled 10 days after the organic incorporation. Soil samples were sieved (2-mm) in a field-moist state and either analyzed immediately or stored at 4°C before the analysis.

Soil Physical Parameters

The soil physical parameters including water infiltration rates, bulk density, soil-moisture percentage, aggregate stability, and soil respiration rates were determined in the Arlington soil as described by Martens and Frankenberger (1990a). Organic C was determined by a modified Mebius procedure described by Nelson and Sommers (1982), and total N was determined by the permanganate method of Bremner and Mulvaney (1982).

Enzyme Assays

The activities of various soil enzymes were based on the release and quantitative determination of the product in the reaction mixture when soil samples were incubated with their respective substrate and buffer solutions. The enzyme assays were performed as follows using 1 g of field-moist soil (air-dry basis) in 50 mL Erlenmeyer flasks.

Phosphomonoesterases

Soil samples (1.0 g), 4 ml of modified universal buffer (MUB; pH 6.5 for acid phosphatase or pH 11.0 for alkaline phosphatase) and 1 ml p-nitrophenyl phosphate (0.025 *M*) were mixed and incubated at 37°C for 1 h (Tabatabai 1982). The flasks were treated with 1 ml 0.5 *M* $CaCl_2$, 4 ml of 0.5 *M* NaOH, and filtered. The yellow color intensity was measured with a Turner Spectrophotometer Model 350 (Turner Associates, Palo Alto, CA) at 410 nm.

Arylsulfatase

All experimental conditions were identical to those described for the phosphomonoesterase assay except that 4 ml of 0.5 *M* acetate buffer (pH 5.8) was used, and 1 ml p-nitrophenyl sulfate (0.025 *M*) was employed as the substrate (Tabatabai 1982).

N-Acetyl-β-Glucosaminidase, β-Glucosidase, β-Galactosidase

All experimental conditions were identical to those described for the phosphomonoesterase assay except that 4 ml of 0.1 *M* acetate buffer (pH 5.0) was used and 1 ml of p-nitrophenyl-*N*-acetyl-β-D-glucosaminide, p-nitrophenyl-β-glucopyranoside, and p-nitrophenyl-β-galactopyranoside were used as substrates for *N*-acetyl-β-glucosaminidase, β-glucosidase and β-galactosidase activities, respectively (Batistic et al. 1980).

Invertase

The reaction mixture consisted of soil (1.0 g), 5 ml 0.17 *M* MUB (pH 5.0) and 2 ml (10%) sucrose solution and was incubated at 37°C for 24 h. The reducing sugars produced were then determined as described by Frankenberger and Johanson (1983).

Dehydrogenase

Distilled water (1 ml) and 1 ml of 2,3,5-triphenyltetrazolium chloride (3%) were added to the soil sample (1.0 g) and incubated at 37°C for 24 h. The soil solution was filtered through a cotton plug and washed with enough methanol to remove the reddish color from the plug and diluted to 25 ml. The red color intensity was measured at 485 nm (Tabatabai 1982).

Amidase and Urease

The reaction mixture consisted of soil (1.0 g), 2 ml 0.1 *M* THAM-sulfuric acid buffer (pH 8.5) and 1 ml 0.1 *M* formamide or 1 ml 0.2 *M* urea and incubated for 2 h. The ammonium formed

during incubation was determined as described by Frankenberger and Tabatabai (1980).

The correlation analysis used here was run in accordance with SAS procedures (SAS Institute Inc. 1985).

RESULTS

The initial state (before organic additions) of the Arlington coarse-loamy soil was in continuous fallow from August 1984 to April 1987 with disk harrow tillage to control weed species. The addition of organic amendments and weekly irrigation during this 31-month study had a positive influence on the physical properties of the soil. Table 1 shows an increase in soil aggregate stability, infiltration rates, soil respiration, moisture percentage, and organic matter content and a decrease in bulk density in the organic-amended soil when compared with the unamended check plot. The treatment means, standard error of the mean, and the LSDs for the four organic treatments and the unamended check are presented in Table 1.

TABLE 1

Mean values of the effects of organic amendments on soil parameters over a 31 month period (14 sampling periods)

Soil parameters[b]	Amendments[a]					
	Poultry manure	Sewage sludge	Straw	Alfalfa	Check	LSD (0.05)
Aggregate stability, %	36.40	37.26	49.46	42.48	31.19	9.90
	(4.60)	(4.96)	(5.59)	(5.16)	(3.98)	
Bulk density, g cm^{-3}	1.37	1.31	1.31	1.36	1.46	0.09
	(0.03)	(0.32)	(0.03)	(0.03)	(0.03)	
Cumulative infiltration, mm 240 min^{-1}	43.60	50.63	58.30	56.72	37.12	14.00
	(2.43)	(4.35)	(5.82)	(5.32)	(5.32)	
Moisture content, %	11.60	12.99	14.20	11.60	10.93	2.41
	(0.90)	(0.92)	(0.94)	(0.77)	(0.69)	
CO_2 evolution, μg CO_2 g^{-1} soil day^{-1}	14.20	21.70	17.09	12.30	3.90	5.83
	(7.25)	(10.86)	(7.49)	(8.08)	(1.44)	
Organic matter content, %	1.56	1.84	1.37	1.16	1.02	0.17
	(0.07)	(0.08)	(0.06)	(0.03)	(0.04)	
Acid phosphatase	187.61	196.69	252.23	204.46	119.85	63.97
	(15.17)	(10.74)	(40.30)	(21.15)	(11.81)	
Alkaline phosphatase	588.92	479.92	539.85	493.46	295.31	155.08
	(66.06)	(57.23)	(56.46)	(48.29)	(43.23)	
Sulfatase	83.35	71.00	92.38	74.85	44.85	27.77
	(8.55)	(9.35)	(12.88)	(10.16)	(7.21)	
N-Acetyl-β-glucosaminidase	81.40	69.08	113.31	91.23	44.39	26.65
	(8.87)	(4.00)	(16.06)	(8.84)	(3.59)	
β-Glucosidase	231.85	203.69	345.23	268.61	162.31	84.40
	(23.50)	(18.36)	(44.65)	(31.02)	(24.61)	
β-Galactosidase	61.82	51.38	68.38	59.38	36.31	15.59
	(4.25)	(5.51)	(6.76)	(6.63)	(3.71)	
Invertase	284.31	279.92	391.00	306.77	153.54	173.16
	(68.20)	(68.17)	(65.96)	(67.48)	(23.07)	
Dehydrogenase	10.19	8.86	10.89	10.33	4.98	1.38
	(0.76)	(0.33)	(0.44)	(0.78)	(0.32)	
Amidase	38.15	36.31	42.08	35.77	15.61	20.26
	(4.00)	(3.27)	(3.54)	(6.29)	(1.79)	
Urease	45.23	39.54	41.18	32.46	10.74	27.67
	(11.49)	(11.80)	(10.31)	(10.01)	(0.79)	

[a] Values in parentheses indicate standard error of the mean.

[b] The units of activity of acid phosphatase, alkaline phosphatase, arylsulfatase, *N*-acetyl-β-glucosaminidase, β-glucosidase and β-galactosidase are reported as mg p-nitrophenol released kg^{-1} soil h^{-1}. Amidase and urease activity is expressed as mg NH_4-N released kg^{-1} soil h^{-1}. Invertase activity is expressed as mg reducing sugars released kg^{-1} soil h^{-1}. Dehydrogenase activity is expressed as mg formazan released kg^{-1} soil 24 h^{-1}.

Phosphomonoesterases

Organic phosphorus (P) in soil can comprise 30–70% of the total P content. Hydrolysis of these organic P compounds is essential for uptake by plants and microorganisms (Skujins 1976). The addition of the four organic amendments maintained high levels of acid phosphatase activity in the soil during this long-term study (Fig. 1). All amendments significantly increased acid phosphatase activity compared with the control soil (Table 1). However, the increased acid phosphatase activity did not correspond to the additive sum of the enzyme activity assayed in the amendments (Table 2) plus the soil itself.

The four organic amendments also significantly increased alkaline phosphatase activity during the 31-month study when compared with the unamended plots (Table 1). In contrast to the acid phosphatase activity, alkaline phosphatase activity fluctuated dramatically with time (Fig. 2). Alkaline phosphatase activity was stimulated more so during the first two organic applications than with the third or fourth additions.

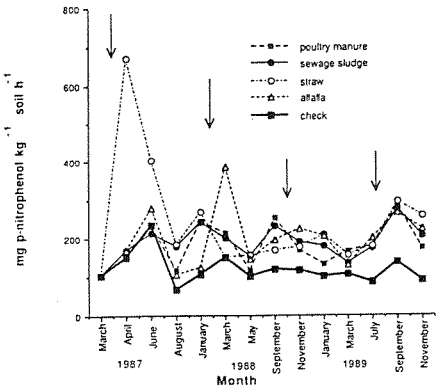

FIG. 1. Influence of organic amendments on soil acid phosphatase activity. $LSD_{0.05}$ = 63.97. Arrows indicate addition of organic amendments.

Arylsulfatase

A large fraction (40–70%) of the total sulfur (S) content in soil may be present as ester sulfate. Arylsulfatases play an important role in the mineralization of S (Skujins 1976; Tabatabai 1982). Sulfatase activity was substantially increased with each of the four organic additions, and generally the first application gave rise to the highest increase in activity (Fig. 3).

N-Acetyl-β-Glucosaminidase, β-Glucosidase, β-Galactosidase, Invertase and Dehydrogenase

The addition of the four organic amendments increased the activity of the carbon-cycling enzymes during this 31-month study (Figs. 4–8). The mean *N*-acetyl-β-glucosaminidase activity was significantly increased by all the amendments except sewage sludge when compared with the unamended plot (Table 1). The increase in *N*-acetyl-β-glucosaminidase activity was more pronounced with the first two additions (Fig. 4). The plant residues contained markedly higher levels of *N*-acetyl-β-glucosaminidase activity compared with the poultry manure or sewage sludge amendments (Table 2). *N*-Acetyl-β-glucosaminidase activity in the organic

TABLE 2

Enzyme activity of organic amendments[a]

Enzyme	Amendment			
	Poultry manure	Sewage sludge	Straw	Alfalfa
Acid phosphatase	8010	2020	1200	5600
Alkaline phosphatase	7760	1880	900	1250
Arylsulfatase	780	360	500	280
N-Acetyl-β-glucosaminidase	367	42	2250	2667
β-Glucosidase	300	167	22665	21000
β-Galactosidase	250	217	7835	2335
Invertase	150	131	605	300
Dehydrogenase	750	13	1125	2250
Amidase	322	1368	1030	5175
Urease	821	640	800	690

[a] Units of enzyme activity are the same as listed in Table 1.

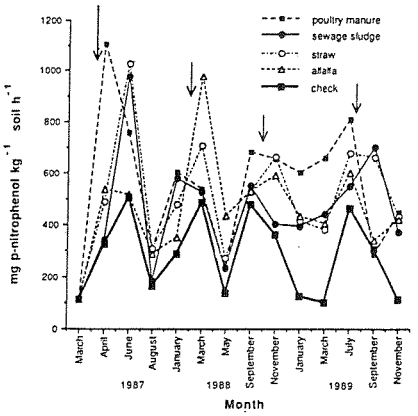

FIG. 2. Influence of organic amendments on soil alkaline phosphatase activity. $LSD_{0.05}$ = 155.08. Arrows indicate addition of organic amendments.

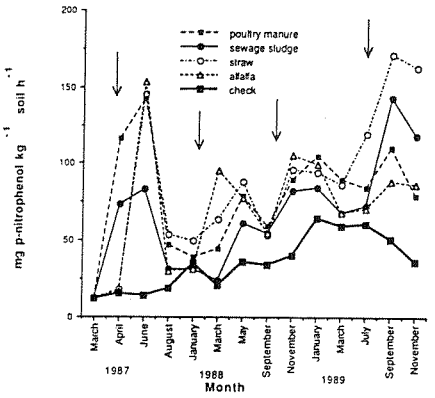

FIG. 3. Influence of organic amendments on soil arylsulfatase activity. $LSD_{0.05}$ = 27.77. Arrows indicate addition of organic amendments.

amendments was found to be correlated (r = 0.96**) with their respective aminosaccharide content (Martens and Frankenberger 1990b).

β-Glucosidase activity in soil was significantly increased by the addition of plant materials when compared with the poultry manure and sewage sludge or the unamended soil (Table 1). The plant materials contained extremely high levels of β-glucosidase activity (135×) compared with the poultry manure or sewage sludge amendments (Table 2). Increased soil β-glucosidase activity with the first two organic additions may reflect the addition of large amounts of the enzyme present in plant residues, in addition to stimulating microbial production, but the third and fourth additions did not promote this marked increase in activity (Fig. 5).

β-Galactosidase activity assayed in the Arlington soil during this field study was significantly increased by all of the amendments except for the sewage sludge treatment when compared with the unamended soil (Table 1; Fig. 6). The sewage sludge and poultry manure contained comparable levels of both β-glucosidase and β-galactosidase activity. In contrast, the plant materials exhibited 2.9× (straw) to 9.0× (alfalfa) more β-glucosidase activity when compared with their respective β-galactosidase activity (Table 2).

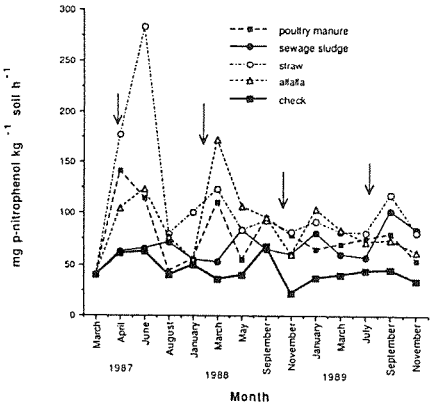

FIG. 4. Influence of organic amendments on soil N-acetyl-β-glucosaminidase activity. $LSD_{0.05}$ = 26.65. Arrows indicate addition of organic amendments.

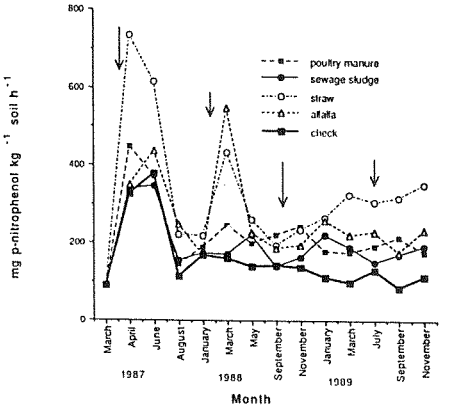

FIG. 5. Influence of organic amendments on soil β-glucosidase activity. $LSD_{0.05}$ = 84.4. Arrows indicate addition of organic amendments.

Invertase activity in soil was significantly increased by the straw amendment when compared with the other treatments during the initial phase of this study. Increased invertase activity was noted 5 months after the organic additions particularly after the second or third application (Fig. 7).

Dehydrogenase activity was significantly increased upon incorporation of the organic amendments when compared with the unamended plots (Table 1). A significant increase in soil dehydrogenase activity with all four organic amendments was noted even though the sewage sludge was extremely low in dehydrogenase activity (Fig. 8).

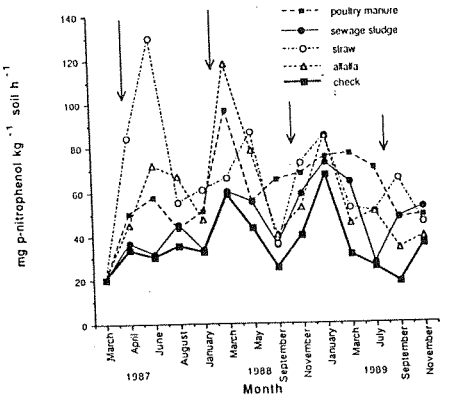

FIG. 6. Influence of organic amendments on soil β-galactosidase activity. $LSD_{0.05}$ = 15.59. Arrows indicate addition of organic amendments.

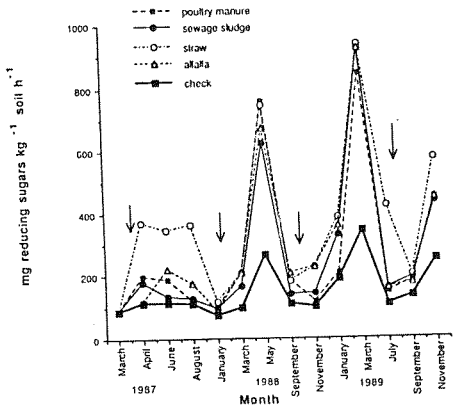

FIG. 7. Influence of organic amendments on soil invertase activity. $LSD_{0.05}$ = 173.16. Arrows indicate addition of organic amendments.

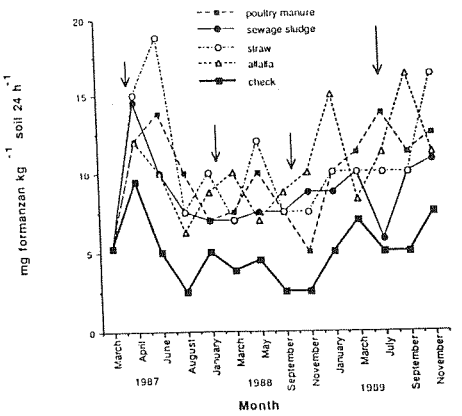

FIG. 8. Influence of organic amendments on soil dehydrogenase activity. $LSD_{0.05}$ = 1.38. Arrows indicate addition of organic amendments.

Amidase and Urease

Amidase and urease hydrolyze C-N bonds other than peptide bonds in linear amides. Both enzymes are widely distributed in nature and have an important role in regulation of the hydrolysis of naturally occurring amides (Frankenberger and Tabatabai 1982; Tabatabai 1982).

Amidase activity in soil was substantially increased upon the addition of the organic materials and all treatments; except in the alfalfa amended soil, there was significantly increased amidase activity during the 31-month study when compared with the unamended plots (Table 1). The peak of amidase activity was noted after the second application of the organic amendments (Fig. 9). Alfalfa had approximately four times the amidase activity of the next most active amendment (sewage sludge), but this activity was not additive upon incorporation into soil (Table 2; Fig. 9).

The mean activity of soil urease during this 31-month study was significantly increased by all the organic additions with the exception of the alfalfa treatment when compared with the unamended plots (Table 1). Urease activity in soil was greatly increased upon addition of the first two amendments (Fig. 10). The peak of urease activity was noted 1 month after the application of barley straw at the initiation of this study.

DISCUSSION

Soil is a dynamic living system where all biochemical activities proceed through enzymatic

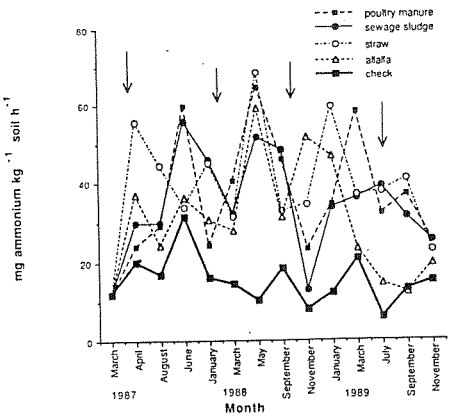

FIG. 9. Influence of organic amendments on soil amidase activity. $LSD_{0.05}$ = 20.26. Arrows indicate addition of organic amendments.

processes (Tabatabai 1982). Many of the enzymes added to soils by decaying microbial tissues and by plant and animal residues are partially degraded by proteases with the remainder being incorporated into soil humus (McLaren 1975).

The activity of soil enzymes may be inhibited by addition of certain organic amendments. Perucci et al. (1984) found that the addition of organic residues (tomato, maize, and wheat straw) inhibited several soil enzymes assayed during a laboratory study. Other researchers (Frankenberger et al. 1983; Bonmati et al. 1985) have reported that the presence of heavy metals in sewage sludge were responsible for the inhibitory effects when amended soil was assayed for urease and phosphatase activity. In this study, the sewage sludge amendment was consistently the least effective amendment in stimulating soil enzyme activity which may have been due to the metals present.

Soil enzyme activities may also be increased by the addition of organic materials (Nannipieri et al. 1983; Zantua and Bremner 1976; Balasubramanian et al. 1972). This increased activity has generally been attributed to the increased microbial biomass although additional evidence has shown that plant materials and sludges may directly contribute enzymes to soil (Zantua and Bremner 1976; Frankenberger and Tabatabai 1980; Frankenberger et al. 1983). Microorganisms associated with organic residues may also contribute to the enzyme pool in soil (Johnson 1957). Although the incorporation of poultry manure, sewage sludge, straw, and alfalfa into the Arlington soil resulted in increased soil enzyme activity for all the enzymes assayed, this increase was not additive. This increase in activity may be due to the incorporation and protection of the enzyme fraction upon increasing the soil humus content.

The soil utilized in this study had not received any organic inputs for almost three years (August 1984–April 1987). The low residual enzyme activity reflects the fallow conditions. Tateno (1988) reported that enzyme reactions in soil are often limited by the substrate supply and not the enzyme itself. In most cases, a rapid increase in enzyme activity was noted upon the first addition of the organic amendments (April, 1987). This suggests a trigger molecule or a promoter could have been released by the decay of the organic amendments that stimulated soil organisms to secrete high levels of enzymes. This promotion of soil enzyme activity has been suggested by Burns (1982). This would explain the large increases noted for the first year but not explain the less dramatic response upon subsequent organic additions.

If the increased enzyme production, presumably by microbes is stimulated by a promoter molecule(s), then perhaps a feedback mechanism is also present to terminate the production of enzymes in a situation where adequate energy sources are available. This may explain why organic applications incorporated during the latter time period failed to increase the enzyme activity. In a soil receiving constant or regular organic additions, the process of promotion and

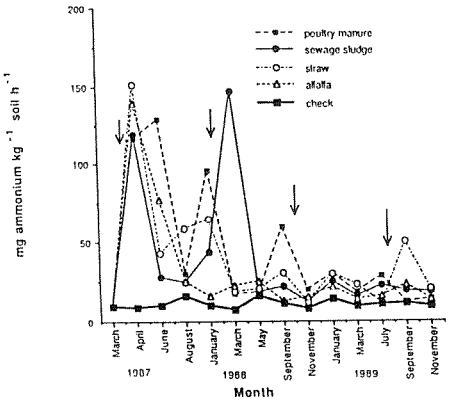

FIG. 10. Influence of organic amendments on soil urease activity. $LSD_{0.05}$ = 27.67. Arrows indicate addition of organic amendments.

TABLE 3

Correlation matrix (r-values) between soil enzyme activities and soil physical parameters (31 months)

Parameters and enzyme activity	Parameter				
	Aggregate stability	Bulk density	Cumulative infiltration	CO_2 evolution	Organic matter
			r values		
Bulk density	0.25				
Cumulative infiltration	0.17	−0.42*			
CO_2 evolution	0.06	0.12	0.17		
Organic matter	−0.06	−0.45**	0.24	0.16	
			Enzyme activity		
Acid phosphatase	0.02	−0.42***	0.66***	−0.07	0.17
Alkaline phosphate	0.09	−0.36*	0.52**	−0.04	0.27
Arylsulfatase	0.45**	−0.46**	0.47**	−0.06	0.30*
N-Acetyl-β-glucosaminidase	−0.03	−0.48***	0.61***	0.16	0.21
β-Glucosidase	−0.24	−0.60***	0.63***	0.09	0.14
β-Galactosidase	0.05	−0.24	0.35	0.26	0.20
Invertase	0.25*	−0.01	0.12	0.27	0.06
Dehydrogenase	0.14	−0.59***	−0.51**	−0.10	0.35*
Amidase	0.09	−0.09	0.17	0.26	0.08
Urease	−0.44***	−0.47***	0.26	0.09	0.25

*, **, *** Indicates significance at the 5, 1, and 0.1% level, respectively.

suppression may be balanced resulting in a relative constant level of enzyme activity. This hypothesis is supported by other researchers who have observed only minor fluctuations in enzyme activity in actual cropping situations from season to season (Ramirez-Martinez and McLaren 1966; Kiss et al. 1975).

Organic amendments or green manures applied to soil have long been employed to enhance favorable soil conditions (Khaleel et al. 1981; MacRae and Mehuys 1985; Boyle et al. 1989; Martens and Frankenberger 1990a). The mechanism by which organic amendments improve the physical structure of soil is not well understood, although the effects of organic additions are universally recognized (Jenny 1980). It has been shown that the addition of organic materials to sterile soil results in little or no structural improvement indicating a biotic contribution (Chesters et al. 1957). Speir (1976) and Speir and Ross (1976) suggested that the organic residue-decomposing organisms were major contributors to the soil enzyme activity. Thus a relationship between soil enzyme activities and soil structural improvements may exist.

Simple correlation coefficients between the soil physical parameters and enzyme activities are shown in Table 3. The activities of the carbon-cycling enzymes (*N*-acetyl-β-glucosaminidase, β-glucosidase and dehydrogenase) were significantly related to decreased bulk density and increased water infiltration rates. Only arylsulfatase, invertase, and urease were significantly correlated with aggregate stability.

To conclude, the addition of organic amendments stimulated enzyme activity in the Arlington soil, particularly after the first application. Continued application did not further promote the soil enzyme activities. Soil enzyme activity increased as the soil structure and productivity improved upon the addition of organic amendments. The enhanced levels of soil enzyme activity upon addition of these organic amendments promoted the recycling of nutrients in the soil ecosystem.

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