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Effects of crop rotation and fertilization on catalase activity in a soil of the southeastern United States

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Summary Catalase activity of a loamy sand under a 3-year crop rotation in the southeastern U.S.A. was monitored. Corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and soybean [*Glycine max* (L.) Merr.] were the summer crops in the rotation. Winter wheat (*Triticum aestivum* L.) was planted after corn, and soybean was followed by a winter fallow period. Cotton was followed by a mixture of common vetch (*Vicia sativa* L.) and crimson clover (*Trifolium incarnatum* Gibelli & Belli) which was eventually plow-incorporated as a green manure. Highest mean catalase activities were recorded in soil under the wheat, soybean, and winter legume crops; lowest activities were found in soil bearing corn and cotton, and during the winter fallow period. The fertilization regime influenced soil catalase activity independently of the crop. Soil deficient in any of the major elements showed low enzyme activity. Highest activity was found in soil fertilized with P and K, and with N supplied by a winter legume crop. Addition of supplementary mineral nitrogen to this regime reduced catalase activity. Elimination of the winter legume crop from an otherwise complete fertilization regime resulted in a drastic reduction in enzyme activity. In soil receiving a complete fertilization regime there was a close correlation between soil catalase and xylanase activities. A similar correlation between these two enzymes was not found in soil receiving incomplete fertilization.

Introduction

Catalase (H_2O_2: H_2O_2 oxidoreductase, E.C. 1, 11, 1.6) is widely associated with the tissues of animals, higher plants, and aerobic micro-organisms. Activities of catalase and various other enzymes in soils have been correlated with such soil variables as particle size, carbon content, nitrogen content, numbers of micro-organisms, and fertility[1,2,3,4,5,7]. In a previous study[9], we showed that catalase activity of a cultivated soil in Alabama was correlated with bacterial and fungal counts, cation exchange capacity, dehydrogenase activity, and cotton yield. As part of a continuing study of soil enzyme activities in relation to soil properties and crop yields, we present here the results of an investigation into the catalase activity in an Alabama soil under a rotation of corn, cotton, and soybean. In addition, data are presented on the effects of fertilization and winter cover crops on soil catalase activity.

Methods

Soil samples were collected from plots in a rotation system on the Agronomy farm of the Alabama Agricultural Experiment Station, Auburn, Alabama, U.S.A. The rotation, which includes plots

subjected to a variety of fertilization regimes, was established in 1914. The fertilization regimes have remained constant for the past 15 years. The soil is a loamy sand with < 1% (w/w) oxidizable organic matter and a cation exchange capacity of 2 to 5 meq/100 g soil.

The field was divided into three tiers each having either corn ('Florida 200 A'), soybean ('Bragg'), or cotton ('Auburn 56') in a 3-year-rotation scheme. Winter wheat ('Georgia 1121') followed corn as a winter cover crop and soybean was followed by a fallow period. A mixture of common vetch and crimson clover ('Autauga') was planted after cotton as a winter cover, and was plowed under as a green manure the following spring. Corn and wheat stubbles were plowed under soon after crop harvest; soybean and cotton plants were chopped after crop harvest but were not soil-incorporated.

The tiers consisted of six-row plots (6.1 by 29.1 m), each plot representing a different fertilization regime. Six regimes in each tier were selected for investigation (Table 1). Crop sequences and fertilization schedules are presented in Fig. 1. Each fertilization regime in each tier was represented by four, 7-m-long subplots from which the soil samples were collected at approximately 2- to 3-month intervals throughout the growing season beginning in March, 1970.

Soil samples were collected from the root zone every 0.5 m along the two center rows of each subplot using a 2.5-cm-diam soil probe to a depth of 18 cm. Soil cores from individual subplots were combined, passed through a 1-mm-mesh screen, and air-dried at 25°C. Samples were stored in darkness at 4°C until assayed.

Catalase activity was determined polarographically using a Model 53, Biological Oxygen Monitor (Yellow Springs Instrument Co., Yellow Springs, Ohio) equipped with a Clark oxygen electrode, as described previously[9]. In brief, the procedure consisted of adding a low concentration (0.02% v/v) of H_2O_2 to a suspension of the soil sample in air-saturated water at 30 ± 0.5°C. The production of oxygen in the reaction mixture over the first 1 to 2 min was recorded and the tangent of the angle θ formed by the recorder trace with the horizontal axis was calculated. The value of tan θ represented relative enzymatic activity of the sample. A tan θ value of 1.0 was approximately equivalent to 0.34 international units of enzyme activity. All determinations were performed in quadruplicate.

Data were analyzed following standard procedures. Except where otherwise stated, indicated differences were significant at the 5% or lower level of probability.

Table 1. Fertilization regimes employed in a 3-year rotation program with corn, soybean, cotton and wheat

Fertilizer treatment*	Winter legume**	Factor studied
NPK	+	Mineral and legume N
NPK	–	Mineral N
PK	+	Legume N
PK	–	No N
NK	+	No P
NP	+	No K

* Lime was applied to all plots at 0.36 MT/ha following soil-test recommendations: N was applied as NH_4NO_3 at (kg/ha): 135 to cotton, 67 to corn, 67 to wheat, 135 to corn on plots with NPK but no winter legume; P was applied at 224 kg P_2O_5/ha per 3-year rotation; minor elements were applied at (kg/ha): 5.6 cupric sulfate, 11.2 manganous sulfate, 1.1 sodium borate, 16.8 zinc sulfate, and 0.6 sodium molybdate. One-half of the mineral fertilizer was applied broadcast just prior to planting wheat and one-half just prior to planting cotton.

** + and – refer to the presence or absence, respectively, of a winter legume combination, consisting of common vetch and crimson clover, included in the 3-year rotation following cotton and before corn.

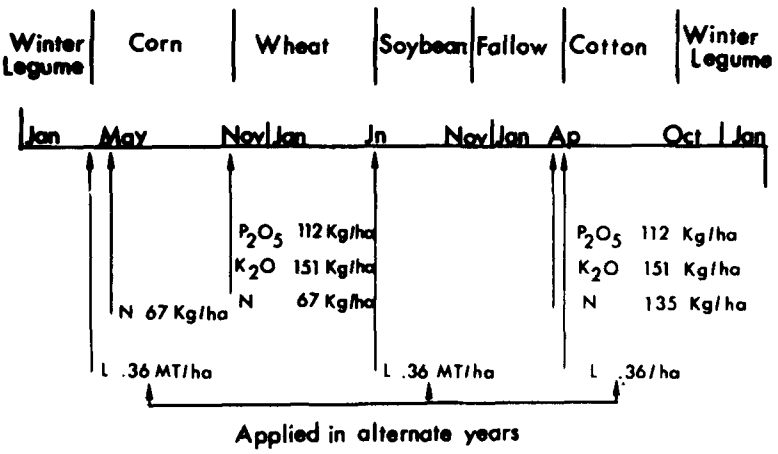

Fig. 1. Sequence of crops, and the quantities and times of application of fertilizers used in a 3-year rotation-fertility experiment at Auburn, Alabama.

Results and discussion

Statistical analyses showed that there was no significant interaction between ground cover (crop) and fertilization regime. This permits discussion of seasonal effects of crops independently of fertilization regime and *vice versa*.

Seasonal variation in soil catalase activity with ground cover

Catalase activity in soil under wheat (Fig. 2A) increased linearly from March through crop harvest in early May. Because of the recorded relationship between catalase activity and soil microbial populations[9], we interpret this increase as reflecting enhanced rhizosphere microbial populations accompanying the rapid development of the crop root system. Following wheat harvest, and the plowing under of the wheat stubble, the catalase activity continued to increase. The release of nutrients into the soil from decaying organic matter would increase microbial activity even further and could account for the continuing rise in enzyme activity.

Soybean was planted in early June, but soybean growth produced no change in the pre-established catalase activity level until a gradual decline in activity commenced after August. Soybeans were harvested in mid-November and the plant remains were allowed to decay on the soil surface without being incorporated. Failure to incorporate the plant debris, together with decreasing soil temperatures commencing in August[8], may explain why enzyme activity continued to decline through the ensuing winter months.

The pattern of catalase activity decline established through the winter fallow period was unafffected by the growth of cotton planted in late March and followed to harvest in September (Fig. 2B). Catalase activity after harvesting cotton through the planting of a mixed winter-legume crop was only about 30%

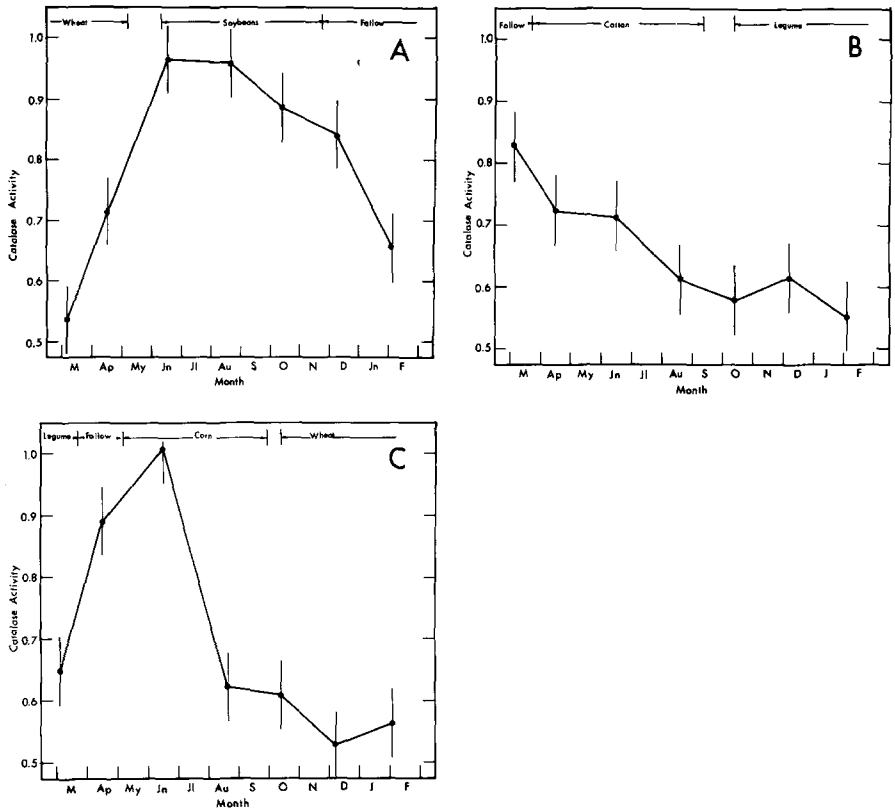

Fig. 2. Seasonal changes in catalase activity in soil from a 3-year rotation with soybeans (**A**), cotton (**B**), and corn (**C**) as the summer crops.

of the activity recorded at the beginning of the cotton growing season. The decline in activity associated with cotton culture may reflect the relatively low number of roots produced by cotton compared with root production by other crops in the rotation[8]. The absence of a significant increase in soil catalase activity following the harvest of cotton, as occurred following wheat harvest, was probably related to the fact that, unlike wheat stubble, cotton residues were left on the surface and not incorporated into the soil.

The seeds of the winter legume crop were drilled into the soil without prior soil preparation. Soil catalase activity increased sharply after the winter-legume crop was plowed under in mid-March (Fig. 2C). As with the soil incorporation of wheat subble (Fig. 2A), we interpret the rise in activity to reflect an increase in soil microbial activity due to decomposition of the plant debris with the release of nutrients.

The rise in catalase activity continued after corn was planted in early May, and the highest level of activity was recorded in mid June (Fig. 2C). This was followed 2 months later by a dramatic decrease in activity. Catalase activity showed little

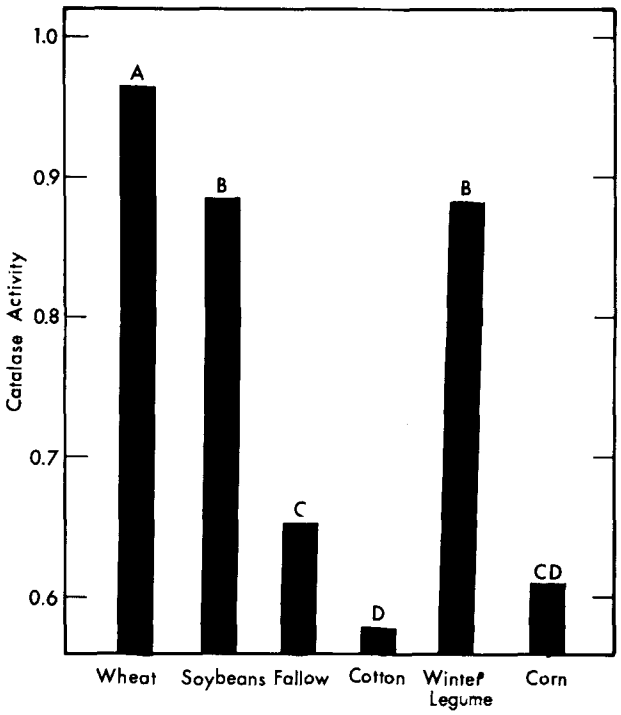

Fig. 3. Levels of soil catalase activity observed at the termination of each rotation component in a 3-year crop-rotation study.

subsequent change through the time of corn harvest and the early stages of growth of the following wheat crop. The decline in activity over the latter part of the corn-growth season contrasts markedly with the increase in enzyme activity of soil under wheat (Fig. 2A), the other grass species used in the rotation. These differences may be due to differences in the prevailing soil temperature and rainfall during the growing seasons of the two crops[8]; however, we believe that a more convincing interpretation may lie in reported differences in the composition of root exudates of the two species. The data of Vancura[12] working with wheat, and Matsumoto *et al.*[6] working with corn, showed that while the roots of both species exuded a full complement of free amino acids, there were clear differences in the nature of the carbohydrates released. Wheat released four oligosaccharides, and maltose, glactose, glucose, arabinose (fructose), xylose, ribose, and rhamnose; while corn exudates were shown to contain only stachyose, glucose, and fructose. This marked dissimilarity in the nature of exuded sugars could produce major differences in both the types and quantities of rhizosphere micro-organisms, which in turn could result in a change in soil catalase activity. Our earlier studies[9] showed significant correlations between total numbers of bacteria and fungi and soil catalase activity.

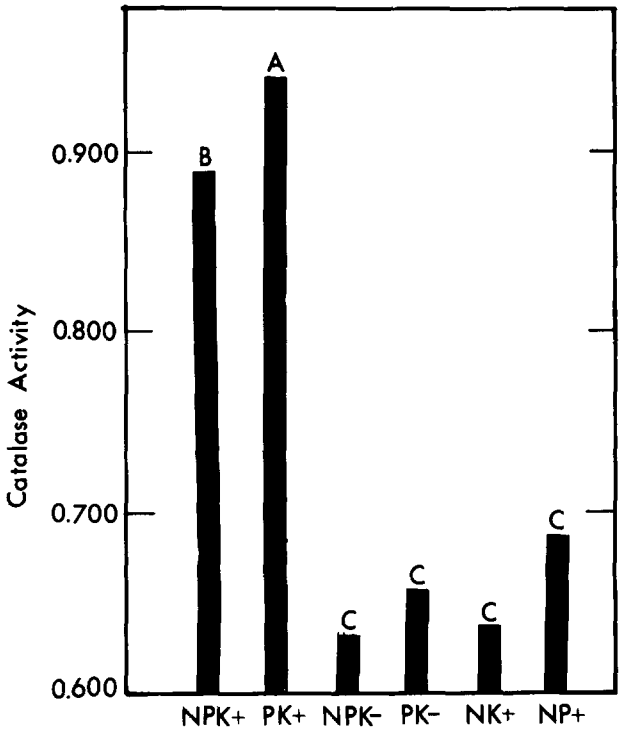

Fig. 4. Relation between catalase activity and fertilization regime in soils from a 3-year rotation study; + and − refer to the presence or absence of winter legumes in the fertilization regime. Bars topped with a common letter were not statistically different ($P = 0.05$).

Table 2. Linear correlation coefficients established for soil catalase activity in plots with different fertilization regimes in a study of a long-term rotation with corn, cotton, and soybean as the major crops*

Fertilization regime**	NPK+	PK+	PK−	NPK−	NK+	NP+
NPK+	1.000	0.709	0.704	0.710	0.718	0.769
PK+		1.000	0.764	0.840	0.657	0.449
PK−			1.000	0.831	0.422^{ns}	0.600
NPK−				1.000	0.664	0.544
NK+					1.000	0.651
NP+						1.000

* All coefficients were statistically significant ($P = 0.05$) except for the one with the superscript ns.
** + or − denote the presence or absence of winter legumes (common vetch + crimson clover) in the fertilization regime.

The final levels for catalase activity at the termination point of each rotation component are shown in Fig. 3. These values indicate that following growth of wheat, soybean, and winter legumes there was a high catalase-exhibiting soil microbial activity that was not found following the growth of cotton and corn, or following a winter-fallow period. Under the conditions of these experiments, we have previously shown a positive correlation between the yield of cotton and the catalase activity of the soil in which the cotton was grown[9]. The data we present here strongly suggest that rotation of cotton with a winter legume, or some other soil-catalase-inducing crop, would be desirable for maintenance of high crop yields.

Catalase activity and fertilization regime.

Fig. 4 summarizes the effects of the various fertilization regimes used in this study. Highest activities were recorded in soil from plots that had received P and K with winter legumes as the only nitrogen source (PK+). The addition of inorganic nitrogen to the regime (NPK +) resulted in a small but significant reduction in catalase activity. Elimination of a winter legume from an otherwise complete fertilization regime (NPK−) resulted in a dramatic reduction in catalase activity. Other regimes deficient in one or more of the fertilization components also showed reduced catalase activity. Because catalase activity is related to soil microbial activity[9], these results would be anticipated. These data again emphasize the value of a winter-legume cover crop. The decline in activity in response to mineral-nitrogen fertilization remains unexplained.

Correlation analyses (Table 2) showed that seasonal variations in soil catalase activity were unafffected by fertilization regime, *i.e.*, the changes in catalase activity for all plots and rotation components were 'in phase'. This suggests that the major component contributing to soil catalase activity variation was the rotation component and not the fertilization regime.

Table 3. Linear correlation coefficients established between values for catalase and xylanase activities in soil with different fertilization regimes from a long-term rotation with corn, cotton, and soybean as the major crops*

	Fertilization regime**					
	NPK+	PK+	PK−	NPK−	NK+	NP+
Correlation coefficient	0.573	0.544	0.509	0.689	0.234^{ns}	0.520

* All coefficients were statistically significant ($P = 0.05$) except for the one with the superscript ns.
** + or − denote the presence or absence of winter legumes (common vetch + crimson clover) in the fertilization regime.

In previous research with this same soil[8], xylanase activity was measured. Soil xylanase is primarily of microbial origin and is associated with the degradation of plant hemicelluloses[8,10,11]. Seasonal changes in xylanase activity in these soils were correlated with the corresponding catalase activity (Table 3). Calculated correlation coefficients indicated that, over time, there was a close correlation between the levels of these two enzymes in plots that received complete fertilization regimes (PK+, NPK+, NPK−). The correlations for the two activities in plots given incomplete fertilization were not so close, and activity levels for the two enzymes in P-deficient plots were unrelated. Both Sorensen[10,11] and Rodríguez-Kábana[8] have shown that xylanase activity in soil is directly related to soil fertility and is associated with a specialized microflora; catalase, on the other hand, is ubiquitous in aerobic organisms. It would be anticipated, therefore, that the shifts in soil microbial populations which occur in response to incomplete fertilization would affect the level of xylanase-producing organisms more than those exhibiting catalase activity.

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