SCIENTIFIC PAPERS
OF THE UNIVERSITY OF PARDUBICE
Series A
Faculty of Chemical Technology
14 (2008)

MICROBIAL NUMBERS AND ENZYMATIC ACTIVITIES IN AGRICULTURAL SOILS AND THEIR VARIATIONS DURING STORAGE

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Received March 15, 2008

The manipulation of soil microbial communities using soil and crop management practices is a basic strategy in the development of stable agricultural systems. Such systems are based, in part, on the efficient management of soil microorganisms to improve soil quality. Microbial numbers and enzymatic activities in agricultural soils that differed in fertilization practice, method and intensity of tillage, and crop rotation were investigated. Agricultural soils showed significant differences in some of physical-chemical properties (phosphorus content, available Ca) between the two cropping systems tested. Microbial counts as well as enzyme activities of the site with crop rotation were higher than in the soil under many years of continuous banana cultivation. The decrease in microbial activity of tested soils after the storage for 30 and 60 days at room temperature indicates that the selected temperature is not suitable for storage, even for the short periods.

Introduction

Soil is a living dynamic, non-renewable resource, and its conditions influence food production, environmental efficiency and global balance [1-3]. Soil quality is closely linked to organic matter content and the activity of beneficial soil organisms. Bacteria and fungi, among soil organisms, actively participate in organic matter decomposition liberating chemical nutrients and furthering plant growth. Microorganism numbers vary in and between different soil types and conditions, with bacteria being the most numerous. Bacterial counts in different soils ranged from 4×10^6 to 2×10^9 g^{-1} dry soil [4].

In addition to plants, soil microorganisms are one of the most important sources of soil enzymes. Soil enzymes are both mediators and catalysts of important soil functions and have been used to measure the influence of natural processes and anthropogenic activities on soil quality. Function of some enzymes is associated with the microbes themselves, such as dehydrogenase activity of which the main localization is the plasma membrane of bacteria or mitochondrial membranes of fungi. Other enzymes are synthesized and secreted extracellularly by bacteria or fungi (e.g. phosphatases, urease, cellulases, and pectinases). Microbially-secreted enzymes may take part in the soil matrix as extracellular enzymes, also called abiontic. Soil enzyme production as a result of microbial metabolism is a sensitive indicator of soil microbial activity, thus factors influencing the latter will certainly exert control over the former [5,6].

A study of the quality of selected agricultural soils has formed part of a broader survey of these soils performed by the Department of Civil Engineering, Pontifícia Universidade Católica do Rio de Janeiro, Brazil; in this study, storage of the soils for periods up to several weeks was sometimes necessary. It was, therefore, desirable to determine whether changes in microbial counts and enzyme activities occurred during the storage of these soils and whether such changes were important in relation to other factors affecting the levels of these indicators in the field. There is little information on the effects of storage conditions on soil microbial activity. However, the most commonly used method to store soils for microbiological analysis is to place them in cold or frozen storage [7,8]. Anderson [9] showed that the storage temperature has a great influence on the survival of microbial biomass in soil as analyzed by substrate induced respiration. Storage for 70 days at +22 °C resulted in 39 % loss of biomass, while storage at +2 °C for 70 days only gave a loss of 18 %.

Our objective in the present investigation was to determine microbial numbers and enzymatic activities in selected agricultural soils and evaluate the effect of storage on microbial activity of these soils.

Material and Methods

Sampling Locations

Two cropping systems (P1, P2) located in the municipality Bom Jardim (Rio de Janeiro, Brazil) that differed in fertilization practice, method and intensity of tillage, and crop rotation were included in this study. P1 was a farming system of full fertility for 15 years used for banana-growing with application of mineral fertilizers (NPK) every two years. P2 was a farming system of conventional tillage and yam-corn-bean rotation with annual applications of mineral fertilizers (NPK) and herbicides Roundup® or Gramoxone®.

Sampling Procedure

Subsurface bulk soil samples (10-30 cm depth) were collected along transects established for each plot at the Bom Jardim sites. A minimum of three soil cores was collected along each transect and stored in closed plastic bags at 15-20 °C. Soils were processed within 3 days of collection and then after 30 and 60 days of storage at room temperature.

Microbial Counts

One gram of each soil sample was added to 9 ml of sterile distilled water. The tubes were stoppered and the contents thoroughly vortexed. After homogenization, this solution was decimally diluted (10^{-1} to 10^{-5}) and 100 µl of the resulting solutions plated on Tryptone Soya Agar (TSA, Oxoid, England) culture medium. After incubation at 30 °C, for up to 7 days, the colony forming units (CFU) were counted. Each dilution of soil sample was analysed in triplicates.

Soil Enzyme Analysis

The soil microbial activity expressed as fluorescein diacetate (FDA) hydrolysis was determined following the method of Adam and Duncan [10]. FDA is a general substrate for several hydrolytic enzymes including esterases, lipases and certain proteases. FDA hydrolytic activity was detected spectrophotometrically by measuring the product of hydrolysis (fluorescein). The assay consisted of suspending 2.0 g of soil in 15 ml 60 mM potassium phosphate buffer (pH 7.6). Stock solution (0.2 ml 1000 µg FDA ml^{-1}) was added to start the reaction. Blanks were prepared without the addition of the FDA substrate along with a suitable

number of sample replicates. The assay mixture was then placed in an orbital incubator (100 rpm) at 30 °C for 20 min. The assay was terminated by extraction with 15 ml of chloroform/methanol (2:1 v/v) followed by centrifugation at 2000 rpm for approximately 3 min. The supernatant from each sample was then filtered (Whatman, No. 2) into 50 ml conical flasks and the filtrates measured at 490 nm on a spectrophotometer (Spectronic UNICAM, USA). The concentration of fluorescein released during the assay was calculated using the calibration graph produced from 0 to 10 μg fluorescein ml^{-1} standards, which were prepared from a 20 μg fluorescein ml^{-1} standard solution. The 0 μg ml^{-1} fluorescein standard was used to zero the spectrophotometer before each set of blanks and samples were read.

2,3,5-Triphenyltetrazolium chloride (TTC)-dehydrogenase activity was used to estimate respiratory activity for viable microorganisms. Thirty grams of soil was placed in a 50 ml conical flask, and 0.3 g $CaCO_3$ was added. The flasks were stoppered and the contents shaken by hand. Then 4.0 g of this mixture was placed in a test tube (each soil was tested in triplicates) and 1 ml of 3 % TTC stock solution and 1.5 ml of sterile distilled water was added. The tubes were stoppered and vortexed for 3 minutes. A small amount of liquid was present at the surface of the soil. The soil samples were placed in incubator for 24 h at 37 °C in the dark. The 1,3,5-triphenylformazan (TPF) product was extracted with 10 ml of methanol. The tubes were stoppered and the contents thoroughly vortexed. The supernatant from each sample was then filtered (Whatman, No. 1) into 50 ml conical flasks. The whole extraction procedure was repeated until the supernatant was transparent. The filtrates were measured at 485 nm on a spectrophotometer (Spectronic UNICAM, USA). The concentration of TPF released during the assay was calculated using the calibration graph produced from 0 to 20 μg TPF ml^{-1} standards, which were prepared from a 1000 μg TPF ml^{-1} standard solution. The 0 μg ml^{-1} TPF standard was used to zero the spectrophotometer before each set of samples were read.

All values for enzymatic activities were reported on a dry soil basis. After drying the soil at 105 °C for 24 h, soil moisture was determined for each sample.

Statistical Analysis

Microsoft Excel 2003 was used in the statistical processing of the data (ANOVA, Bonferoni post-test). The statistical significance was set at 0.05.

Results and Discussion

The soils selected for this study had different physical structures and pH values, and contained different plant species, nutrient concentrations and organic matter (see Table I). The highest phosphorus content was found in the soil taken from crop rotation system which has been utilized for several yearly crops (yam, corn and bean) and is periodically fertilized (NPK). The soil effects on the number of CFU of total bacteria and fungi were also higher in the crop rotation soil; in comparison to the soil taken from the banana field (see Table II).

Table I Selected chemical and physical characteristics of soils at each crop management site

Study site	P1	P2
Soil type	Yellow argisol	Cambisol
Moisture, %	25.3	20.6
Clay, %	22.2	20.1
Silt, %	6.7	24.7
Sand, %	70.6	53.1
pH_{H2O} *	5.17 ± 0.11	5.48 ± 0.16
Total N, %	1.5	1.7
Organic C, g/100 g *	1.25 ± 0.12	1.36 ± 0.24
P, g/100 g *	0.6 ± 0.10	2.3 ± 0.31
Ca, mg/100 g *	186.3 ± 8.8	214.4 ± 5.3
Mg, mg/100 g *	24.1 ± 1.3	22.4 ± 2.9

* – mean values ± SD
P1 – farming system used for banana-growing
P2 – farming system with yam-corn-bean rotation

The number of total bacteria, actinomycetes and fungi in soils is enhanced by chemical fertilizers or manure [11,12]. Belay *et al.* [13] reported direct effect of NPK on the number of bacteria, actinomycetes and fungi in a soil under maize cultivation. In addition, the interactions between plants species, soil and microbial communities were reported in literature [4,13,14]. Therefore, the results in this study suggest that the differences in microbial biomass between tested soils might be attributed to the effects of chemical fertilization and crop rotation.

Soil enzymes representative of main nutrient cycles and of microbial biomass were selected to characterize the microbial activity. Dehydrogenase is present in all microorganisms. Therefore it can be considered as an accurate

indicator of the microbial oxidative activity in the soil and should have a direct relationship to total viable microorganisms [2,15]. FDA hydrolysis was used as a general indicator of soil hydrolytic activity, as it measured the activities of proteases, lipases, and esterases that are all capable of cleaving fluorogenic FDA [16]. FDA hydrolysis, like dehydrogenase activity, is regarded by some as a reliable measure of total microbial activity although, unlike dehydrogenases, these enzymes can function outside of the cell and form stable complexes with soil colloids [17].

Table II Bacterial populations and microbial enzyme activity in soils at each crop management site

Study site	P1			P2		
Length of storage, days	0	30	60	0	30	60
Bacterial counts, CFU 10^5 g^{-1} dry soil*	36.0 ± 0.8	33.7 ± 0.9	24.0 ±0.8	62.0 ± 0.8	64.0 ± 0.8	28.7 ± 0.5
FDA hydrolysis, μg product g^{-1} dry soil*	306.2 ± 0.3	124.4 ± 0.5	100.8 ± 0.7	463.3 ± 0.7	142.3 ± 0.7	80.9 ± 0.4
Dehydrogenase, μg product g^{-1} dry soil*	114.7 ± 0.6	91.1 ± 0.8	61.1 ± 0.9	204.8 ± 0.5	208.6 ± 0.8	163.4 ± 0.7

* – mean values ± SD
P1 – farming system used for banana-growing
P2 – farming system with yam-corn-bean rotation

The results obtained in the present work point out that soil enzymatic activities play an integrative role between physico-chemical and microbial properties. In our study, microbial activity based on FDA hydrolytic and dehydrogenase enzymes indicated that soil under crop rotation system was metabolically more active than soil under banana cultivation (see Table II). The increased microbial activity observed in the soil under more intensive management may reflect a greater availability of substrates that support such activity. Improvements in soil microbial biomass and numbers as a result of the use of fertilizers together with crop rotation have been reported by several researchers [13,18,19].

The microbial properties of analysed agricultural soils revealed that the microbial activity was highest in soils sampled fresh from the field. The influence of storage periods of 30 and 60 days on microbial counts and enzymatic activity is shown in Table II. In both soils, the study revealed significant difference ($P < 0.05$) in the numbers of culturable bacteria after the storage for 60 days at room temperature. Both FDA hydrolytic and dehydrogenase activities showed signi-

ficant decrease of values ($P < 0.05$) within the whole storage period. This agrees with the findings of other authors who concluded that the long-term storage of soils at room temperature was not satisfactory for the preservation of general microbial activity [8,9,20].

In conclusion, it is apparent from the present work that the chemical and microbiological quality of a soil along with the crop production can be enhanced by crop rotation and appropriate use of NPK fertilizers. The positive interaction and additional benefits accrued by crop rotation and fertilizer application emphasize the importance of integrated crop and nutrient management systems to the maintenance and improvement of the quality and productive capacity of soils.

Acknowledgements

The author is grateful to Prof. Denise Maria Mano Pessoa of Department of Civil Engeneering, Pontifícia Universidade Católica do Rio de Janeiro, for the supply of soil and for advice and discussion during the course of the work. The study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (Research Intention No. 0021627502).

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