Influence of compost and liquid bioferment on the chemical and biological characteristics of soil cultivated with banana (*Musa* spp. L.)

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Abstract

The effects of compost and liquid biofertiliser on the chemical and biological characteristics of soil cultivated with banana (*Musa* spp. L.) cv. 'Grand Naine' were investigated. Four treatments (liquid bioferment (LBF), compost (COM), liquid bioferment + compost (LBF + COM) and unamended (CONTROL)) were applied to banana plants in the field in a completely randomised design in the crop cycles of 2005-2006 and 2007-2008. The physico-chemical characteristics, enzymatic activity and microbial diversity were determined prior to the bioferment addition and 140 and 280 days after application (DAP). The microbial groups were determined (i.e., bacteria, fungi, actinomycetes). Next, cellulolytic, proteolytic, starch hydrolysing, asymbiotic N_2-fixing and phosphorous-solubilising microorganisms were determined. The application of LBF + COM increased organic matter 1.39 times, increased compost 1.33 times and increased LBF 1.16 times at 140 DAP. The greatest microbial populations were found in soil amended with LBF + COM at 140 DAP. The highest esterase and invertase enzyme activities were found in soil amended with LBF+COM at 140 DAP. The highest xylanase activity was found in soil amended with compost at 280 DAP. These results showed that the addition of compost and liquid bioferment improves soil fertility and stimulates microbial activity and their capacity to metabolise complex organic molecules.

Keywords: enzyme activities, cellulolytic microorganisms, proteolytic microorganisms, starch hydrolysing microorganisms, asymbiotic N_2 fixing microorganisms, phosphorous solubilising microorganisms.

1. Introduction

Restoring and sustaining the soil fertility of banana fields (*Musa* spp. L.) has become an important topic in recent years (Bouman *et al*., 1998). The international network for the improvement of banana and platano (INIBAP), the network research and development of banana and plantain of Latin America and the Caribbean (MUSALAC) and the regional fund for agricultural technology (FONTAGRO) have developed the project "Technological innovations in the management of health and quality of banana soils in Latin America and the Caribbean". The aim of this joint project was to restore the productivity of banana plantations by improving the physical, chemical and biological characteristics of soil. Various strategies were suggested and tested to achieve this objective. It was suggested that the addition of organic matter (OM) in different forms might restore soil fertility and promote sustainable agricultural practices. It is well known that applying OM to soil improves soil structure, aeration and water retention. Organic matter also serves as a C-substrate for soil macrofauna and microorganisms. After its mineralisation, OM provides nutrients for crops and might produce a number of organic substances that increase soil fertility and plant development. Despite the well-known fact that OM application improves soil fertility, this practise is not often used in the cultivation of bananas.

Organic matter decomposition is catalysed by enzymes, which play an important role in biogeochemical cycles (Wittmann *et al*., 2004). Enzyme activity in soil is used as an indicator of changes in the quality and fertility of soil (Qu *et al*., 2011) and is studied when a factor, such as organic material application, might affect soil microbial activity and nutrient cycling (Alotaibi and Schoenau, 2011). The objective of this work was to study the effect of adding two different forms of organic material, i.e., compost and liquid bioferment, on the chemical and biological characteristics of soil cultivated with the banana cv. "Grand Naine".

2. Materials and Methods

2.1. Location of the study site

The study was conducted in a banana plantation located in Tapachula, Chiapas, Mexico (92º 22' 26.96" W; 14º 50' 36.82" N; 34 masl). The field had been cultivated with bananas (Musa AAA Cavendish cv "Grand Naine") in a conventional way (using fertilisers and chemical pesticides) for more than 20 years.

2.2. Compost production

Leaves, pseudostems, rachis and fruits of banana plants were stacked in approximately 1000-kg piles and covered with black plastic to reduce water loss and avoid the invasion of flies and rodents. The compost was aerated by being manually turned over every 7 days until stabilisation (12 weeks). The characteristics of the compost with a water content of 600 g kg^{-1} were on a dry weight basis as follows: organic carbon: 222 g kg^{-1}, total nitrogen: 20 g kg^{-1}, phosphorus as P$_2$O$_5$: 11g kg^{-1} and potassium as K$_2$O: 13.38 g kg^{-1}.

2.3. Liquid bioferment production

Liquid bioferment was prepared by mixing 50 kg fresh cattle manure, 20 kg sugarcane molasses, 4 kg ash, 2 l microbial inoculant to accelerate the fermentation (see below) and tap water until reaching a final volume of 200 l. The mixture was anaerobically fermented in a hermetically sealed tank for 21 days. At the end of fermentation, 1 l of the promoting growth plant microorganisms (see below) and 1 l of phos-

phate-solubilising fungi (see below) were added and the mixture was left for 48 h. The liquid bioferment average composition (w v^{-1}) was as follows: total nitrogen: 1.5%, P_2O_5: 0.98% and K_2O: 1.04%.

2.4. Production of fermentation microorganisms

A vial of yeast isolated from '*pozol*', a traditional beverage from Chiapas (México), was resuspended in a 1:l sterile Sabouraud broth culture medium at pH 6.5. The vessel was maintained at 28 °C with agitation at 200 rpm for 24 h (for a final cell concentration of 10^9 ml^{-1}). Further details of the isolation method can be found in Alcántara-Hernández *et al*. (2010).

2.5. Production of plant growth promoting microorganisms

A vial of a free-living N_2 fixing bacteria isolated from the banana soil was resuspended in a 1-l sterile culture medium (nutrient broth) at pH 7.0. The vessel was maintained at 28 °C with agitation at 200 rpm for 12 h. The final cell concentration was 10^{11} colony former units (CFU) ml^{-1}.

2.6. Treatments

In 2005, four treatments were applied to newly planted banana plants (1,000 m^2 with 180 plants per plot^{-1}) in triplicate using a randomised block design. In treatment 1, 20 kg dry compost was applied once to the base of each banana plant (considered the COM treatment). In treatment 2, 10 kg dry compost was applied once, while 2:l liquid bioferment (dilution 1:2) was sprayed six times on the leaves and soil surrounding each banana pseudostem every three days (considered the LBF+COM treatment). In treatment 3, 4 l of liquid bioferment (dilution 1:2) was sprayed six times on the

leaves and soil every three days (considered the LBF treatment). In treatment 4, 340 g urea was applied around each banana plant (considered the CONTROL treatment). Compost and liquid bioferment were applied at a rate of 160 g N plant^{-1}. The same treatments were applied in the 2007 crop cycle.

2.7. Soil samples

In the crop cycles 2005-2006 and 2007-2008, soil samples were taken from the 0-30-cm depth layer just after the bananas were planted (August) but before the treatments were applied and 140 (January) and 280 (June) days after application of the treatments. In the first sampling, 10 soil samples were taken systematically (zigzag) from the 0-30-cm depth layer in each plot. In the second and third samplings, 10 soil samples were taken from the area around 10 randomly selected plants in each of the three plots of each of the four treatments. In both cases, one kilogramme of soil was collected. The banana plants were also sampled at the second and third samplings. The banana roots were separated from the soil and used to quantify the associated microorganisms. The soil was sieved at <2 mm. Part of the soil was characterised as described in Ruíz-Valdiviezo *et al*. (2010), while another portion was stored at -30 °C until it was used for the enzymatic analysis.

2.8. Phosphate-solubilising fungi

Phosphate-solubilising fungi isolated from banana soil was grown in Roux bottles using potato dextrose agar (PDA). After inoculation with 1 ml spore suspension (10^6 spores ml^{-1}), bottles were incubated at 32 °C until sporulation (7 days). The spores were collected in a Ringer's solution and diluted to 1 l in sterilised water (the final spore concentration was 10^8 spores ml^{-1}).

2.9. Quantification of microorganisms associated with the banana roots

Ten grammes of roots were suspended in 100 ml sterile water and stirred under aseptic conditions at 100 rpm for 30 min. Serial dilutions up to 10^5 were made of the supernatant and 100 µl aliquots of the last dilution were inoculated in different culture media. Aerobic mesophilic bacteria were quantified (colony forming units, CFU) in a nutrient medium (pH 7.0). The nutrient medium was incubated at 37° C for 48 h. The fungi were quantified in potato-dextrose agar (pH 5.6) after incubation at 37 °C for 5 days. Actinomycetes were quantified in a Czapeck-Dox-Agar medium (pH 7.3) after incubation at 37 °C for 5 days.

2.10. Substrate utilisation profile and soil enzymatic activity

Microbial cellulolytic, amylolytic, asymbiotic nitrogen fixation and phosphate solubilising capacities were determined as described by Hankin and Anagnostakis (1977), Castro *et al.* (1993), McKinley (2001) and Alam *et al.* (2002), respectively. The proteolytic capacity was enumerated using a gelatin agar (consisting of nutrient agar [Difco Laboratories, Detroit, MI] and 3% [w v^{-1}] gelatin [Sigma, St. Louis, MO]) as the differential medium.

Invertase activity was determined by the Hofmann method (Kokalis-Burelle *et al.*, 1994), esterase activity was determined by hydrolysis of fluorescein diacetate (FDA), as described by Kokalis-Burelle *et al.* (1994) and the xylanase activity was measured using the procedure described by Bailey *et al.* (1992), using birch glucoronoxilan as a substrate.

2.11. Statistical analysis

Organic matter; N, P, K, Ca and Mg content; pH; electrolytic conductivity (EC); the number of different microorganism groups; and esterase, invertase and xylanase activities were subjected to one-way analysis of variance using PROC GLM (SAS Institute Inc., 1989) to test for significant differences between treatments with the Tukey's Studentised Range test. All data presented were the mean of three different plots, i.e., $n = 3$.

3. Results

3.1. Physico-chemical characteristics of the soil
Application of compost or compost+fertiliser increased SOM compared with the control soil and soil amended with fertiliser after 0, 140 and 280 days (Fig. 1a). The electrolytic conductivity and the total P, K, Ca and Mg content increased when compost or compost+fertiliser was added to soil compared with the control soil and soil amended with fertiliser after 140 and 280 days (Figures 1b, c, d, e, f, g, h). The effect, however, was more prominent after 140 days than after 280 days. The measured soil characteristics were significantly affected by treatment (control, compost, fertiliser and compost+fertiliser), time (0, 140 and 280 days) and the interaction between them, except for the interaction between treatment and time on soil total N (*p <0.05*) (Table 1).
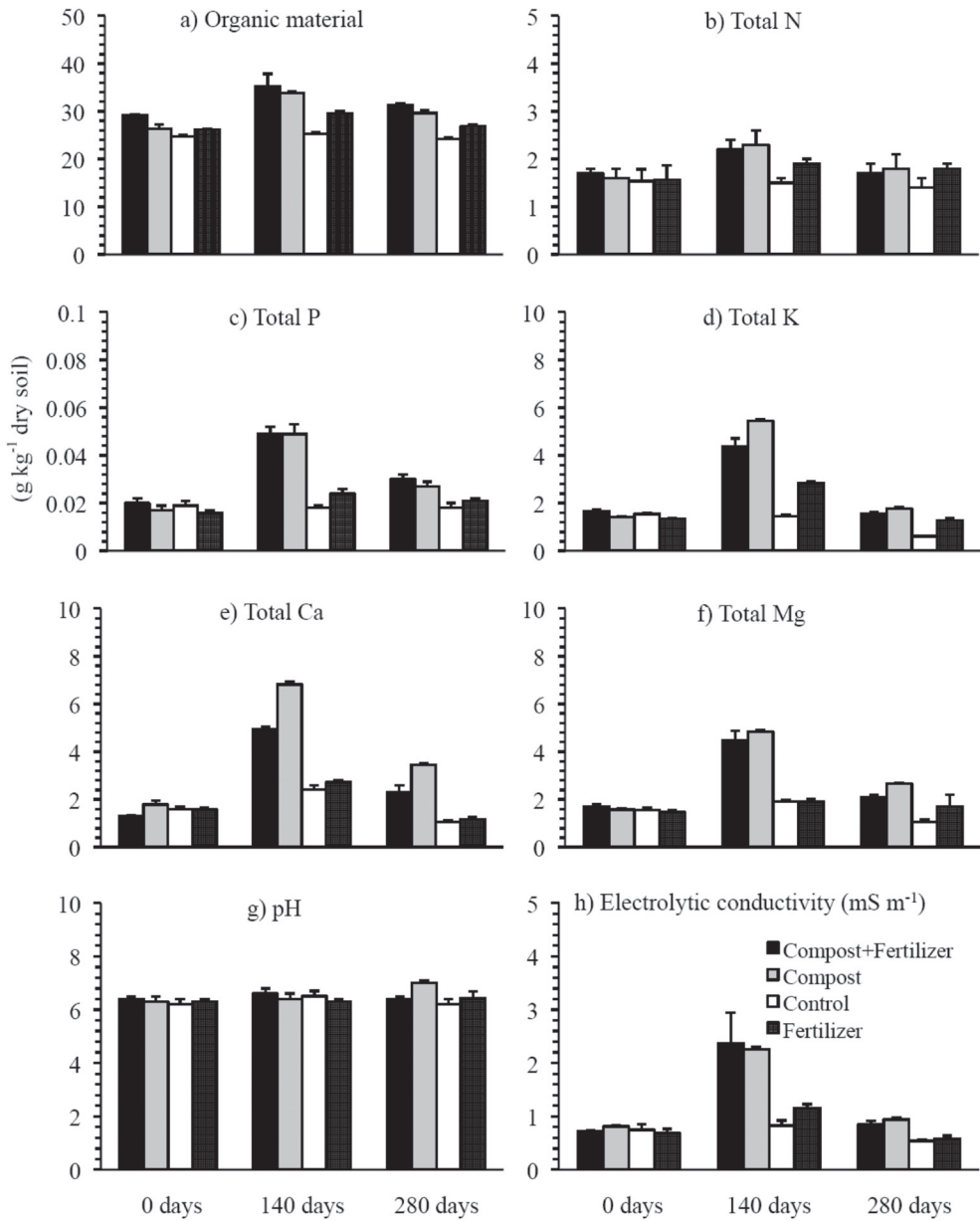
Fig. 1

Figure 1. a) Organic matter content, b) total N, c) total P, d) total K, e) total Ca, f) total Mg (g kg⁻¹ dry soil), g) pH and h) electrolytic conductivity (mS m⁻¹) in soil cultivated with banana (*Musa* spp. L.) amended with compost, fertilizer or compost + fertilizer, or left unamended after 0, 140 and 280 days.

Table 1. Characteristics of unamended and amended soils cultivated with the banana clone "Grand Naine".

Soil characteristics	Treatment		Time		Treatment´Time	
	F value	P value	F value	P value	F value	P value
Organic mater	123.90	<0.0001	85.32	<0.0001	10.67	<0.0001
Total N	7.40	0.0011	10.57	0.0005	2.01	0.1037
Total P	105.71	<0.0001	191.14	<0.0001	43.79	<0.0001
Total K	368.33	<0.0001	1348.88	<0.0001	183.12	<0.0001
Total Ca	594.40	<0.0001	1370.94	<0.0001	173.60	<0.0001
Total Mg	127.96	<0.0001	251.36	<0.0001	48.42	<0.0001
pH	4.46	<0.0001	4.71	<0.0001	4.97	<0.0001
Electrolytic conductivity	31.95	<0.0001	109.83	<0.0001	15.42	<0.0001
Microbial organisms						
Aerobic mesophilic	47.10	<0.0001	17.26	<0.0001	12.99	<0.0001
Actinomycetes	26.54	<0.0001	30.37	<0.0001	23.93	<0.0001
Fungi	3.11	<0.0001	0.28	<0.0001	5.77	<0.0001
Cellulolytic	4.67	0.0074	10.00	0.0004	7.98	<0.0001
Amylolytic	10.46	<0.0001	18.64	<0.0001	7.71	<0.0001
Proteolytic	39.05	<0.0001	99.78	<0.0001	19.56	<0.0001
Phosphate solubilising	48.19	<0.0001	27.96	<0.0001	28.33	<0.0001
N_2 fixing	291.40	<0.0001	996.77	<0.0001	106.79	<0.0001
Enzyme activity						
Esterase	154.41	<0.0001	176.86	<0.0001	54.72	<0.0001
Invertase	58.82	<0.0001	145.06	<0.0001	22.22	<0.0001
Xylanase	121.27	<0.0001	181.07	<0.0001	34.29	<0.0001

3.2. Microbial population

The number of aerobic mesophilic microorganisms increased when compost was added to soil compared with the other treatments at 140 and 280 days (Figure 2a). The number of actinomycetes also increased in the compost-amended soil but only after 140 days (Figure 2b). The effect of the different treatments on the other microorganisms measured was less clear, although the number of microorganisms increased generally after 140 days and less so after 280 days compared with the number at day 0 (Figures 2c, d, e, f, g).

The number of free-living N_2 fixing bacteria increased in all treatments after 140 and 280 days, but the effect was more prominent after 140 days than after 280 days (Figure 2h). The measured soil microorganisms were significantly affected by treatment, time and the interaction between them ($p < 0.01$) (Table 1).
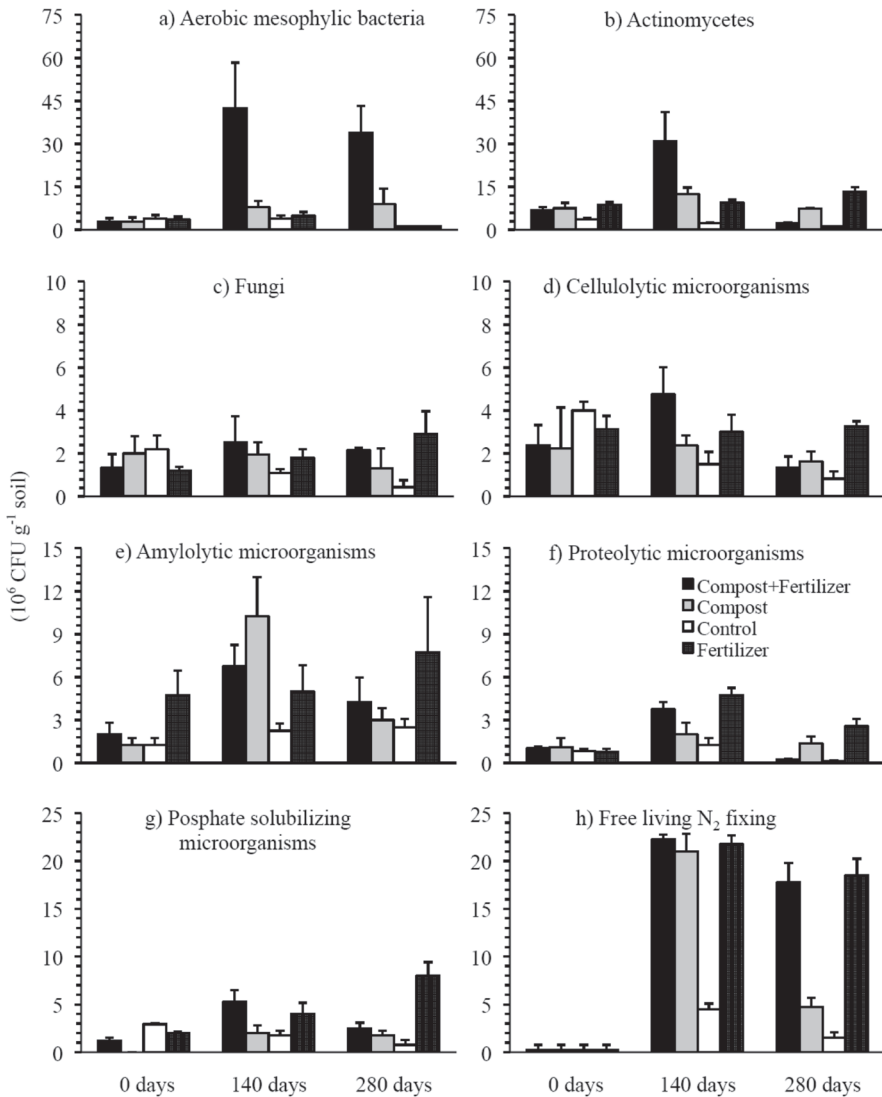

Figure 2. a) Number of aerobic mesophylic bacteria, b) Actinomycetes, c) fungi, d) cellulolytic microorganisms, e) amylolytic microorganisms, f) proteolytic microorganisms, g) phosphate solubilising microorganisms and h) free living N_2 microorganisms (106 cell forming units (CFU) g^{-1} dry soil) in soil cultivated with banana (*Musa* spp. L.) amended with compost, fertilizer or compost + fertilizer, or left unamended after 0, 140 and 280 days.

3.3. Enzyme activities

The activity of esterase increased when compost and compost + fertiliser were added to the soil compared with the unamended soil and soil amended with fertiliser after 140 and 280 days, with the greatest effect being found in the former (Figure 3). The activity of invertase also increased when compost and compost + fertiliser were added to soil compared with the unamended soil and soil amended with fertiliser but only after 140 days. The enzyme activity was significantly affected by treatment, time and the interaction between them ($p< 0.0001$) (Table 1).
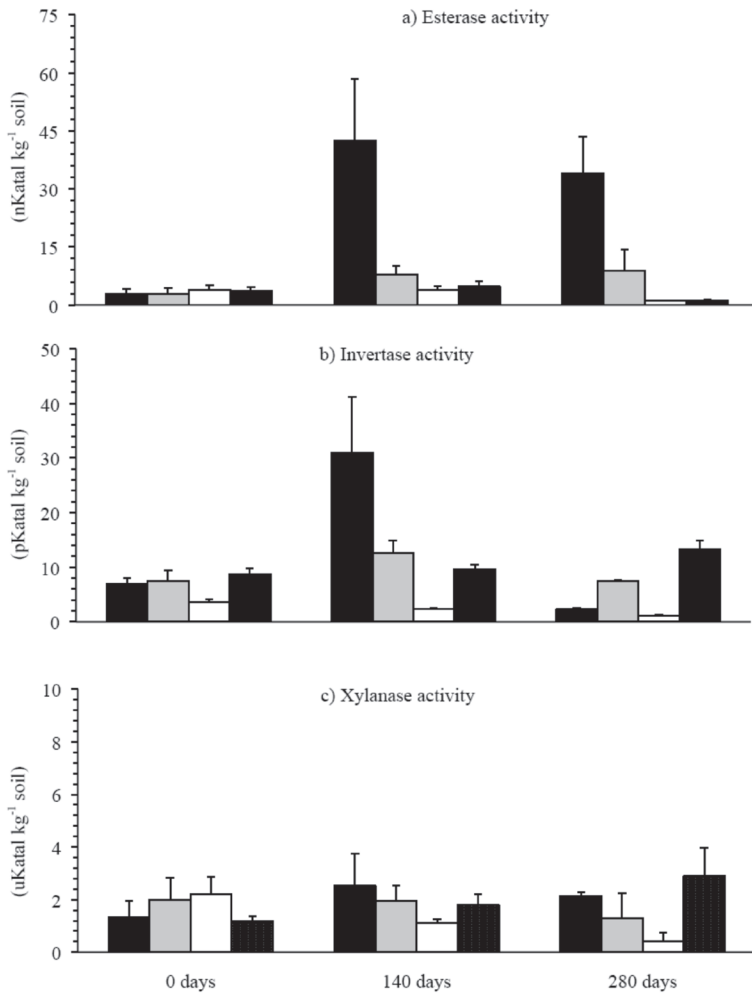

Figure 3. a) Esterase (nKatal kg^{-1} dry soil), b) invertase (pKatal kg^{-1} dry soil), and c) xylanase activity (μKatal kg^{-1} dry soil) in soil cultivated with banana (*Musa* spp. L.) amended with compost, fertilizer or compost + fertilizer, or left unamended after 0, 140 and 280 days. Legends to the Figure can be found in Figure 2.

4. Discussion

The increase in N, K, P, Ca and Mg content in soils is closely related to the accumulation of OM (Table 1). Similar results have been reported in soils amended with different forms of organic wastes, i.e., compost derived from sewage sludge mixed with cotton gin waste and/or olive mill wastewater, urban organic waste, domestic organic waste, green waste (leaf falls, tree pruning, garden clippings and other bulky cellulose and lignin-rich materials) and cattle manure (Ros *et al.*, 2006). McClintock and Diop (2005) reported that the addition of compost increased soil C content from 2.8 kg ha^{-1} to 5.0 kg ha^{-1}, as well as the total amount of K, Mg and P. That the amended soil had more available nutrients 140 days after application (DAP) than after 250 days indicated that most of added organic material was mineralised after 140 days (Table 1). This result indicates that as time passed, the mineralisation of the added OM slowed down and fewer nutrients were released. Fabrizio *et al.* (2009) reported similar results. These researchers found that 150 days after the application of compost, the labile carbon pool was virtually exhausted.

The decrease in easily decomposable organic material (labile carbon fraction) over time was confirmed by the microbial population and enzyme activity in soil (Figures 2 and 3). Even with the limitations of the tests used (Larkin, 2003), the addition of OM increased the microbial biomass in the rhizosphere of banana cv. "Grand Naine". The increase in microorganisms when OM was added to the soil has been reported often (Peacock *et al.*, 2001). The composition of the added organic material is also known to affect the structure of the microbial population (Marschner *et al.*, 2003). Additionally, the cultivated plants affect the composition and activity of the soil microbial community in the rhizosphere (Wardle *et al.*, 2004). The opposite view, that such microorganisms as my-

corrhizal fungi determine the plant community, has also been proposed (van der Heijden *et al.*, 1998).

The addition of OM might prolong the presence of microbiota in the rhizosphere of the banana cv. "Grand Naine". Marschner *et al.* (2003) stated that the application of easily degradable material favoured the growth of microorganisms that prefer environments rich in nutrients (copiotrophos) over organisms adapted to conditions of limited nutrient availability (oligotrophos). Additionally, the incorporation of crop residues favours cellulolytic microorganisms compared with those that cannot degrade cellulose (Debosz *et al.*, 1999). The increase in microbial biomass, as indicated by an increase in esterase activity, was due to the addition of OM, as no such increase was found in the unamended control treatment (Kokalis-Burelle *et al.* 1994). Similar results were reported when untreated OM or compost were applied to soil (Kandeler *et al.*, 1999; Pérez-Piqueres *et al.*, 2006). The application of compost or the stabilised liquid bioferment increased the availability of precursors for cell metabolism. However, the organic material applied consists mostly of complex organic molecules, as the easily degraded substrate was mineralised during composting and fermentation. As such, the increase and duplication of the microbial biomass was low. The addition of compost and liquid bioferment will change both the amount and composition of the soil microbial biomass. Compost and liquid bioferment also contain microorganisms, which might change the composition of the soil microorganisms.

The activities of invertase, which hydrolyses sucrose into glucose and fructose and xylanases, which hydrolyse hemicellulose, both increased. Similar results were reported by Kandeler *et al.* (1999), who studied the dynamics of invertases and xylanases in soil amended with corn stubble. Kandeler *et al.* (1999) found that the activity of invertases increased more than that of xylanases. The characteristics of the OM

applied also affected the increase in enzyme activity, as Kokalis-Burelle *et al*. (1994) found that invertase activity doubled in soil amended with pine bark compared with soil amended with composted bark.

5. Conclusions

It was concluded that the addition of fermented OM (compost or liquid) increased soil microbial activity and stimulated the production of enzymes degrading organic material. The increased activity of invertases, xylanases and esterases will degrade the added organic material more rapidly, thereby releasing nutrients that are available to the cultivated banana plants or other soil habitants.

Acknowledgments

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