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The role of "effective microorganisms" in the composting of banana (*Musa* ssp.) residues

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Abstract

"Effective microorganisms" (EM) are a poorly defined mixture of supposedly beneficial microorganisms that are claimed to enhance microbial turnover in compost and soil. In Costa Rica, EM are used to produce organic compost (bokashi) from banana residues (*Musa* ssp.). Given the scarcity of scientific data about the effects of EM on the mineralization of plant residues, this study aimed at investigating the effects of EM addition on the decomposition of banana residues during Bokashi production. To this end, the following non-EM treatments were compared to EM Bokashi: Bokashi produced with water (W), with molasses (M) as an EM additive, and with sterilized EM (EMst). Subsequently, the effects of the resulting Bokashi treatments on the growth of young banana plants were evaluated. Compared with non-EM controls, the effect of EM on the mineralization of banana material was negligible. Dry-matter losses of the composts with different EM treatments were similar, with about 78% over 5 weeks. Ergosterol concentration was highest in EM Bokashi (77 μg (g dry soil)$^{-1}$) and lowest in EMst Bokashi (29 μg (g dry soil)$^{-1}$). Microbial biomass carbon (C_{mic}) and microbial biomass nitrogen (N_{mic}) were both lowest in EM (C_{mic} = 3121 μg g^{-1}; N_{mic} = 449 μg g^{-1}), while C_{mic} was highest in Bokashi produced with molasses (3892 μg g^{-1}) and N_{mic} was highest in EMst (615 μg g^{-1}). Treatment effects on adenylate concentrations and adenylate energy charge were negligible. Application of all Bokashi variants to young banana plants significantly increased shoot growth under greenhouse conditions compared to plants grown in a control soil without amendments. However, these effects were similar for all Bokashi treatments, even if EM Bokashi increased the K concentrations in banana leaves significantly compared to Bokashi produced with EMst and the control. Bokashi produced with only molasses and EM Bokashi decreased the number of root nematodes under greenhouse conditions compared to the control. Overall, the results confirmed the expected influence of composting on the degradation of organic material and the effect of compost application on plant growth. Hower, under the conditions of this study, EM showed no special effects in this, except for increasing the K concentrations in the leaves of young banana plants.

Key words: bokashi / compost / ergosterol / organic fertilizer / microbial biomass

1 Introduction

"Effective microorganisms" (EM) refer to an undisclosed mixture of naturally occurring microorganisms that supposedly have beneficial properties in a wide range of applications (*Higa*, 2002). It was developed in the early 1980s by Tiruo Higa, Professor at the University of Ryukyu, Okinawa, Japan, and is sold as a commercial product. The exact composition of EM has never been disclosed by the manufacturer. Work of *Kyan* et al. (1999) showed that it predominantly contained populations of lactic acid bacteria, photosynthetic bacteria, yeasts, and *Actinomycetes.* According to *Daly* and *Steward* (1999), 1 mL of the EM concentrate contains a minimum of 10^5 viable organisms of the species *Streptomyces albus*, *Propionibacterium freudenreichil*, *Streptococcus lactis*, *Aspergillus oryzae*, *Mucor hiemalis*, *Saccharomyces cerevisiae*, and *Candida utilis*, in addition to an unspecified number of *Lactobacillus* sp., *Rhodopseudomonas* sp., and *Streptomyces griseus.* Application of EM supposedly leads to increases in the microbial biodiversity of soils which enhances their quality and the growth, yield, and quality of crops (*Higa* and *Parr*, 1994).

Bokashi is the Japanese term for "fermented" organic matter and is equivalent to compost used in traditional organic farming which is mostly prepared with the addition of EM. It can be prepared under complete anaerobic or aerobic conditions. The latter means that partial anaerobic conditions occur in the middle of the compost pile while the outer layers remain aerobic. The preparation of anaerobic Bokashi is made in closed vessels while the preparation of aerobic Bokashi is similar to traditional composting with additional usage of a cover such as a jute bag, straw mat, or similar material (*Kyan* et al., 1999). Enhanced decomposition of plant material after addition of EM during Bokashi production has been proposed as an innovative approach that allows the odorless breakdown of banana residues in as little as 3 weeks and the facilitation of a rapid recycling of plant nutrients (*Shintani* and *Tabora*, 2000). In addition to the undisclosed composition of EM ingredients, many of the described effects (*Higa* and *Parr*, 1994; *Wood* et al., 1998; *Xu* et al., 2000) are not conclusively proven (often due to insufficient control treatments) nor are the experimental conditions well documented. Very little is known about the underlying microbial processes such as time courses of the C and N turnover and of bacterial and fungal populations during the decomposition process. The potential causes of the reported large yield increases,

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improvements of banana health, and increases in secondary root growth of adult banana plants following the application of EM Bokashi in plantations (*Tabora* et al., 2000) are not known. Nevertheless, EM is used widely in agriculture production in Asian countries and for Bokashi production on banana farms in Cost Rica.

Therefore, the objective of this study was to fill the existing gaps of knowledge about the effectiveness of EM Bokashi on the compost quality and plant growth of bananas. The focus of this study was based on the hypothesis that application of EM increases mineralization processes of Bokashi through an enhanced microbial colonization and activity. It was further hypothesized that EM Bokashi increases growth of young banana plants. To test these hypotheses, Bokashi of banana residues was produced with daily applications of EM and compared to three other non-EM treatments (water, molasses, and sterilized EM) which allowed the differences between the effects of added living organisms and pure substrate effects to be distinguished. The produced Bokashi variants were then applied to banana plants in a pot experiment.

2 Materials and methods

2.1 Study site

All experiments were conducted at EARTH University (Escuela de Agricultura de la Región Trópico Húmedo) in Costa Rica which is located between 83° and 84° W and at 10° N, 50 m asl. At this site, air temperatures range from 20°C to 30°C, with a mean relative humidity of 80% and annual rainfall of approximately 3200 mm (*EARTH*, 2003).

2.2 Compost production

For the so called "activation" of EM following the product description, 2 L of EM-stock solution were diluted in a closed plastic barrel with 2 L of molasses and 60 L of tap water (1:1:30). After 7 d, this mixture was applied to compost piles (treatment EM). This solution contained 13.1 g L^{-1} dissolved organic carbon (C_{org}), 244 mg L^{-1} nitrogen (N), 7.0 mg L^{-1} phosphorus (P), and 1.3 g L^{-1} potassium (K; Tab. 1).

Besides this EM treatment, three control treatments were included in this study: A fixed amount of the diluted EM solution was heat-sterilized for 30 min at 121°C in an autoclave (treatment EMst). Nutrient concentrations in EMst, after 7 d of fermentation followed by sterilization, were higher than in EM and pure molasses (Tab. 1). The latter was prepared under the same conditions as EM but without effective microorganisms at a molasses-to-water ratio of 1:30 (treatment M). This solution contained more dissolved organic carbon and P than EM (Tab. 1). The fourth treatment consisted of pure tap water (treatment W). These four Bokashi variants, with five replications each, were produced by daily, early morning, applications of 31 mL of each treatment solution using a spray bottle to achieve uniform surface coverage of the compost piles. All twenty Bokashi heaps, originally consisting of 120 kg of fresh banana material (chopped fruits and stalks), were covered with approximately 10 kg sawdust and arranged in a randomized block design on a concrete platform protected by a roof.

Table 1: Total dissolved organic carbon (C_{org}), total nitrogen (N), phosphorus (P), and potassium (K) concentrations in the solution of effective microorganisms activated during 7 d of fermentation with molasses and water at a ratio 1:1:30 (EM), in the sterilized solution of activated EM (EMst) and in molasses (M), produced like EM but without the addition of effective microorganisms.

Treatment	C_{org} (g L^{-1})	N (mg L^{-1})	P (mg L^{-1})	K (g L^{-1})
EM	13.1	244	7.0	1.3
EMst	14.1	272	9.3	1.5
M	13.7	246	7.8	1.3

Temperatures in the middle of the compost heaps were measured at 7:00 a.m., 11:30 a.m., and 5:00 p.m. every day with a digital thermometer inserted to 20 cm depth. On eight occasions, the total weight of each heap was determined with a tension spring balance. For this, each heap was scooped in a big plastic barrel and subsequently turned. Representative samples, consisting of mixed equal amounts of the outer and inner compost layers, were taken on these eight occasions for analysis of their nutrient and ergosterol concentrations. Microbial biomass carbon (C_{mic}) and microbial biomass nitrogen (N_{mic}) as well as ATP concentrations were only measured at the end of the experiment to determine differences in microbial colonization and activity at the end of the composting period.

2.3 Pot experiment

Three-week-old banana plants of *Musa acuminata.* (AAA) cv. "Giant Cavendish"/"William" were planted in 22 L plastic pots filled with a mixture of 16 L of a typical planting soil (containing 2.49% C_{org}) and 6 L of the four Bokashi variants (EM = produced with activated EM; EMst = produced with sterilized activated EM; M = produced with molasses; W = produced with water). Each treatment, replicated six times, comprised one plant per pot. The plants were placed in a randomized block design in the greenhouse and rearranged every 4 d within blocks.

Over a period of 3 months, shoot growth (height and diameter) was measured at weekly intervals with a measuring tape. Finally, total plants were harvested, and shoot and root fresh weight was determined. For this, roots were separated from the adhering soil by carefully washing with tap water over a sieve to prevent losses of fine roots. After cleaning roots and leaves with demineralized water, they were dried at 65°C to weight constancy, ground, and analyzed for their total N, P, and K contents.

2.4 Analytical measurements

2.4.1 Chemical and biological analyses of the compost and plant samples

The ash content in the compost materials was determined gravimetrically after heating at 550°C for 12 h in a muffle

oven. Organic-matter content was calculated as the difference between dry matter (24 h at 105°C) and ash content. Total N was analyzed using an FP-328 N-analyser (LECO, St Joseph, Mi, USA). For P and K analysis, dry matter was determined after drying at 105°C for 24 h. Following combustion at 550°C in a muffle oven, the ash was dissolved in 20 mL HCl (32%) and filled up to 100 mL after 12 h in the dark with bi-distilled water. Subsequently, total P was measured by spectrophotometry (U-2000 spectrophotometer, Hitachi, Tokyo, Japan) using the vanadate-molybdate-method (*Gericke* and *Kurmies*, 1952). Potassium was measured using a flame photometer (Laboratory Instrument 543, Massachusetts, Lexington, USA).

For nematode counts, aliquots of 250 g root samples from the banana plants of the greenhouse trial were washed and then crushed for 10 s in a mixer filled with water. After sieving the roots at 425 μm, 75 μm, and 45 μm, roots were filtered for more then 24 h over a funnel (Bearmann funnel method; *Decker*, 1969). Subsequently, an aliquot of 5 mL was taken and subsamples analyzed using a microscope under which the nematodes were counted.

2.4.2 Microbial analyses of the compost samples

Compost samples were frozen and stored at –18°C from sampling until analysis. Ergosterol as an indicator of fungal biomass in the soils was determined according to *Djajakirana* et al. (1996). Two grams of wet compost were extracted with 100 mL of ethanol by 30 min oscillation shaking at 250 rev min^{-1} and then filtered (Whatman GF/A, UK). The extract was dried at 40°C in a rotary evaporator and subsequently dissolved in 9 mL of methanol (3 × 3 mL). After filtration (0.45 μm cellulose-acetate filter, Sartorius AG, Göttingen, Germany), ergosterol concentrations were measured by reversed-phase HPLC analysis at 26°C.

Microbial biomass C and N were estimated using the chloroform-fumigation-extraction method (CFE; *Brookes* et al., 1985; *Vance* et al., 1987) modified for compost samples (*Joergensen* et al., 1996). After pre-extracting 25 g of unsieved wet compost with 200 mL of 0.05 M K_2SO_4 on a horizontal shaker (200 rev min^{-1}; 30 min) and manual removal of larvae, the samples were filtered (Schleicher & Schuell 595½, Dassel, Germany). A subsample of 10 g of remaining compost material was immediately fumigated for 24 h at 25°C with ethanol-free $CHCl_3$. Fumigated and nonfumigated samples were extracted with 100 mL 0.5 M K_2SO_4, filtered as before and C_{mic} and N_{mic} calculated (*Wu* et al. 1990; *Brookes* et al., 1985; *Joergensen* and *Mueller*, 1996).

The detection of adenine nucleotides (ATP, ADP, and AMP) was carried out according to *Dyckmans* and *Raubuch* (1997) using 4 g of wet compost. The adenylate energy charge (AEC) was calculated as

$$AEC = (0.5\ ADP + ATP) / (AMP + ADP + ATP).$$

2.5 Statistical analysis

All results were tested for normal distribution of residuals using the Kolmogorov-Smirnov test. Compost variants were compared using a GLM-repeated-measures ANOVA with the treatments as between-subject factors. Variables were regarded as innersubject factors, and means were separated using Tukey's honestly significant difference ($HSD_{0.05}$). Temperature measurements of the composts, plant growth (both with time as a covariant), and plant samples were compared using the GLM-univariate ANOVA, and means were separated using Tukey's $HSD_{0.05}$. All statistical analyses were performed with SPSS 11.5 (*Backhaus* et al., 2003).

3 Results

3.1 Bokashi characteristics

All compost variants reached temperatures between 47°C and 48°C after one day and up to 50°C on the second day of composting. After the second turning of the heaps (8th day of composting), temperatures reached 34°C–41°C. Following a second thermophilic phase from the 15th to the 28th day, the cooling or maturing phase started, and the temperatures equaled ambient temperatures with no further temperature changes. After 12 d, compost temperatures were significantly different between EMst being the warmest and EM being the coolest variant. The periodically measured sudden temperature drops coincided with the turning of the compost piles (Fig. 1).

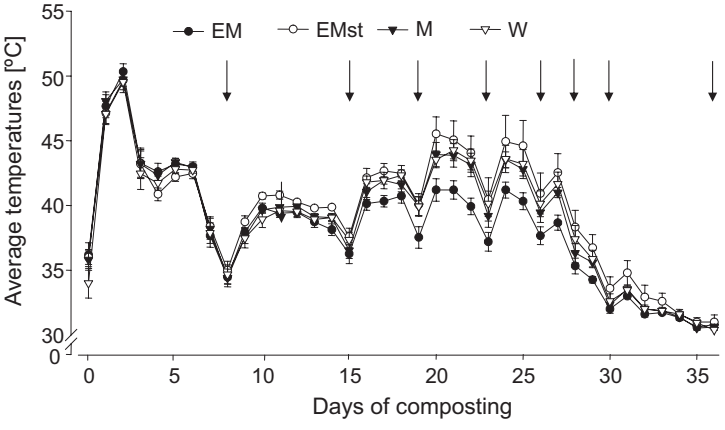

Figure 1: Average temperatures of Bokashi (compost) produced with daily application of the four variants EM = effective microorganisms, EMst = sterilized EM, M = molasses, and W = water. Temperature measurements were conducted at 7 a.m., 11:30 a.m., 5 p.m. each day. Vertical bars represent ± one standard error of the mean. Vertical arrows indicate the eight occasions when samples were taken and the compost heaps turned.

Table 2: Nutrient concentrations, expressed on a dry-matter basis, of the initial banana material and the four different Bokashi variants at the end of composting (EM = produced with effective microorganisms; EMst = produced with sterilized EM; M = produced with molasses; W = produced with water). Means with different letters in each column are significantly different at $p < 0.05$ (Tukey's HSD).

	C_{org}	N	P	K	C : N
Treatment	(%)				
Initial material	46.9	0.89	0.21	4.1	53.0
EM	28.5[a]	1.53	0.25	6.6	18.6
EMst	43.6[b]	1.71	0.25	6.5	25.6
M	24.6[a]	1.62	0.25	6.4	15.3
W	48.6[b]	1.67	0.27	6.6	29.0

During 35 d of composting, all variants lost between 76.5% and 78.3% of their initial fresh weight. The dry-matter content of all Bokashi treatments decreased from 32 kg to approximately 11 kg during the first 15 d of composting. Afterwards, dry matter slowly declined to a final value of approximately 7 kg (data not shown). The C_{org} concentrations were similar for all treatments ranging from 44% to 50% during composting, until they dropped to 24.6% and 28.5% in Bokashi produced with molasses and with EM during the last week (Tab. 2). However, statistical analysis indicated that EM Bokashi contained significantly lower C_{org} concentrations than Bokashi produced with EMst and water. In EM Bokashi, the N concentration increased from 0.89% to finally 1.53%, whereas it increased to 1.6%–1.7% in the other treatments. The C : N ratios dropped from initially 53.0 to 29.0 and 25.6 for Bokashi produced with water and with sterilized EM, and to 15.3 and 18.6 for Bokashi produced with molasses and EM, respectively. Phosphorus increased only slightly in all treatments, with initial concentrations of 0.21% and final concentrations of 0.25%–0.27%, whereas K concentrations increased from initially 4.1% to finally 6.4% to 6.6% (Table 2). No significant differences were found for N, P, and K concentrations.

After 15 d of composting, the ergosterol concentration reached its peak in all treatments with the highest concentrations in Bokashi produced with water (105.3 μg g^{-1}) and the lowest in EM (68.0 μg g^{-1}; Fig. 2). At the end of the composting process, this had changed, and the highest ergosterol concentration was found in EM Bokashi (77.0 μg g^{-1}), while the other variants had dropped to 29.6 μ g^{-1} (EMst), 31.2 μg g^{-1} (W), and 36.6 μg g^{-1} (M). Microbial-biomass C and N_{mic} were both lowest in EM (C_{mic} = 3121 μg g^{-1}; N_{mic} = 449 μg g^{-1}), while C_{mic} was highest in Bokashi produced with molasses (3892 μg g^{-1}) and N_{mic} was highest in EMst (615 μg g^{-1}; Tab. 3). However, these differences were not statistically significant. The C_{mic} : N_{mic} ratio ranged from 5.9 to 7.7, with its lowest ratio in Bokashi produced with EMst and the highest in Bokashi produced with molasses. The ergosterol–to–biomass C ratio was highest in EM followed by molasses (Tab. 3). Adenylate (ATP, ADP, and AMP) concentrations were lowest in EMst and highest in the molasses treatment. The adenylate energy charge (AEC) was very similar in all treatments (Tab. 3).

3.2 Pot experiment

Banana-shoot growth was similar in all treatments during the first 3 weeks of the experiment. After 4 weeks, the growth rates of all Bokashi treatments started to be higher than that of the unamended control and between the 11th week and harvest time, this difference was significant (data not shown). Young banana plants grown in the control soil produced significantly less shoot biomass (leaves and pseudo stem) than plants grown with Bokashi, but significantly more root mass (Tab. 4). Leaf analyses showed significantly higher P concentrations in banana grown in the Bokashi treatments than in control plants, whereas no significant differences were found for N concentrations (Tab. 4). Potassium concentrations were significantly higher in banana leaves grown in the EM-Bokashi treatment compared to those of EMst Bokashi and of the control (Tab. 4).

Root-borne nematodes were most frequent in the control treatment, however, only differences between control on the one hand and EM and M on the other hand were significant (Fig. 3).

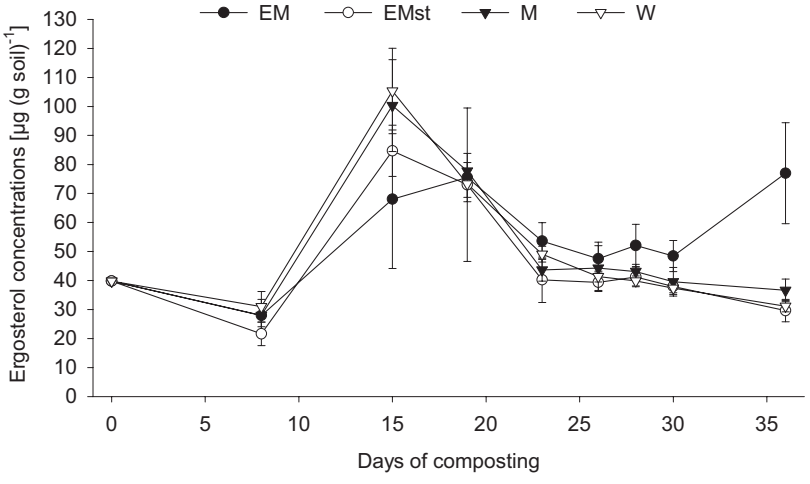

Figure 2: Ergosterol concentrations during the composting process of the following four Bokashi (compost) variants: EM = produced with effective microorganisms, EMst = produced with sterilized EM, M = produced with molasses, and W = produced with water. Vertical bars represent ± one standard error of the mean.

Table 3: Concentrations of microbial biomass C (C_{mic}), microbial biomass N (N_{mic}), ergosterol, adenylates (ATP, ADP, AMP), and AEC as well as the ratios of C_{mic} : N_{mic} and ergosterol to C_{mic} at the last day of composting (36th day; EM = produced with effective microorganisms; EMst = produced with sterilized EM; M = produced with molasses; W = produced with water). Means with different letters in each column are significantly different at $p < 0.05$ (Tukey's HSD).

	C_{mic}	N_{mic}	Ergosterol	C_{mic} : N_{mic}	Ergosterol (%) to C_{mic} (%)	AMP	ADP	ATP	AEC
Treatment	(μg g^{-1})					(nmol g^{-1})			
EM	3121	449	77.0^{a}	6.7	2.47^{a}	8.5	5.4	15.7	0.62
EMst	3636	615	29.6^{b}	5.9	0.81^{b}	8.0	3.7	13.6	0.61
M	3892	505	36.6^{b}	7.7	0.94^{b}	9.6	6.2	19.3	0.64
W	3846	576	31.2^{b}	7.0	0.81^{b}	8.5	4.9	15.1	0.62

Table 4: Dry matter (DM; 105°C) of shoots and roots and nutrient concentrations (all three expressed on a dry-matter basis) of young banana plants grown over 3 months under greenhouse conditions with application of four Bokashi variants (EM = produced with effective microorganisms; EMst = produced with sterilized EM; M = produced with molasses; W = produced with water) compared to a control without Bokashi application. Means with different letters in each column are significantly different at the level $p < 0.05$ (Tukey's HSD).

			Leaf nutrient concentrations		
	Shoot DM	Root DM	N	P	K
Treatment	(g)		(mg g^{-1})		
EM	1142^{b}	304^{a}	25.9	1.51^{b}	48.7^{c}
EMst	1250^{b}	326^{a}	26.2	1.56^{b}	42.7^{b}
M	1246^{b}	363^{a}	26.1	1.53^{b}	45.5^{bc}
W	1182^{b}	321^{a}	26.0	1.56^{b}	43.5^{bc}
Control	772^{a}	430^{b}	25.8	1.22^{a}	19.6^{a}

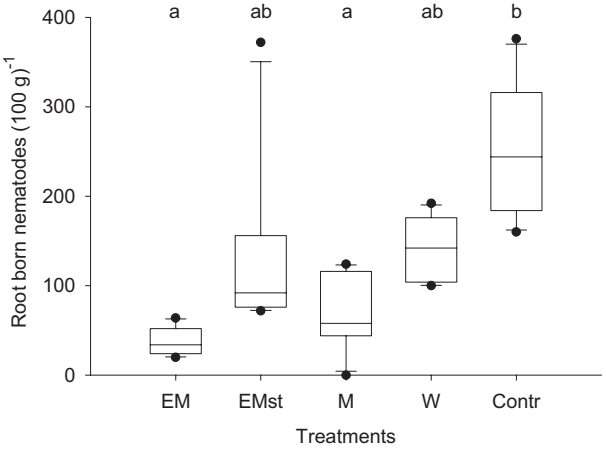

Figure 3: Nematode counts from root samples grown in the following Bokashi (compost) treatments: EM = produced with effective microorganisms, EMst = produced with sterilized EM, M = produced with molasses, W = produced with water, and Contr = control without Bokashi application. The vertical box plots show the median and the 25% and 75% percentiles as vertical boxes and 10% and 90% percentiles as error bars. Minimum and maximum values are indicated by black dots. The means with different letters are significantly different at $p < 0.05$ (Tukey's HSD).

4 Discussion

The measured temperatures in the compost piles were typical for the often reported mesophilic, thermophilic, and cooling phases in organic-matter decomposition (*Chefetz* et al., 1996; *Butler* et al., 2001). However, the higher temperatures in the Bokashi produced with sterilized EM might be explained by the nutrient contents of the 31 mL applied EM solution (Tab. 1). At the first day of application, EMst contained 30 mg higher organic C, 9 mg higher N, 0.07 mg higher P, and 5 mg higher K contents compared to the activated EM. These additional nutrients can be used by the naturally occurring microorganisms for their reproduction, increasing their activity and may thus have led to the higher temperatures observed.

The observed high losses of fresh weight and dry matter during the first 15 d of composting might be the result of the high liquid losses and respiration processes. Consequently, a decrease in C concentration and an increase in the ash concentration over time would have been expected as reported by *Daly* and *Stewart* (1999) and *Chefetz* et al. (1996). Their results are in contrast to the more or less stable concentrations of organic C measured during this study. Our constant C concentrations should therefore be interpreted with caution. However, in composts or other organic fertilizers C : N ratios > 20 will normally lead to a temporary immobilization of N through microorganisms and thus cause N deficiency in plants (*Akhtar*, 2000; *Lloyed* et al., 2002). Therefore, Bokashi produced with molasses and with EM, having C: N ratios < 20, seems to be a better plant-growth promoter than a Bokashi produced with water and sterilized EM, both of which had C : N ratios > 20.

Except for K, the nutrient concentrations of all Bokashi variants were in the range of those measured in aerobically composted or fermented composts made of different organic materials (*Bruns*, 1996; *Körner* and *Ritzkowski*, 1999). When compared to the values reported by *Körner* and *Ritzkowski* (1999), the up to 22-fold higher K concentrations measured in our Bokashi variants were probably due to the high K concen-

trations in the raw banana material used for compost production (*Ultra Jr.* et al., 2005). No significant effect of living EM on mineralization processes was found in this study. This might be due to the relatively small amount of EM applied that was unlikely to dominate the microbial-community structure in the compost piles. In contrast, *Schloss* et al. (2003) observed effects of EM on mineralization processes in the treatment of waste water. Similarly, *VanderGheynst* and *Lei* (2003) reported an influence of mixing and aeration on the microbial-community structure of a rice straw– and dairy manure–based compost. Assuming that the quality of Bokashi depends on the predomination of lactic acid fermentation, which is decreased through increased aeration (*Yamada* and *Xu*, 2000), it cannot be completely ruled out that the high frequency of turning the compost piles practiced in our study might have hampered the enhancement of mineralization through the inoculum. The different material and slightly different composting technique, using saw dust as a cover for the compost heaps instead of jute bags or straw mats, might have led to differences in the chemical and microbiological parameters characterizing the Bokashi produced in the present study as compared to the one described by *Yamada* and *Xu* (2000). On the other hand, our results are in line with observations by *Goto* and *Muramoto* (1995). They compared EM Bokashi from the EM company with ordinary Bokashi made by farmers without using EM and did not find any differences between the two composts.

The measured ergosterol concentrations, which were highest on the 15th day of composting in all variants, are in contrast to findings reported in the literature, where the final community structure of composts is dominated by fungi (*Hellmann* et al., 1997; *Mondini* et al., 2002). Only EM Bokashi showed an increase in fungi colonization on the final date of measurement. Microbial C concentrations in Bokashi of about 3.1 to 3.9 mg g^{-1} on the final day of composting (36th day) are almost 3-fold higher than those measured by *Hellmann* et al. (1997) at the same time of composting, but comparable to the C_{mic} concentrations of about 3 mg g^{-1} in a 9-week-old biowaste compost (*Gattinger* et al., 2004). This leads to the assumption that the microbial population inside the Bokashi piles was growing faster than in a traditional compost and would thus confirm earlier findings that Bokashi composts are ready for usage in a very short time (*Kyan* et al., 1999). In EM Bokashi, in contrast, the significant lower temperatures with significantly higher ergosterol and lower C_{mic} concentrations at the end of composting compared to the other variants indicates a change in the microbial-community structure towards one dominated by fungi. This is in contrast to the first hypothesis that mineralization was enhanced through increased microbial colonization after the addition of EM.

Even though the AEC indicates dormant cells or microorganisms in a stationary phase as found for *in vitro* cultures (AEC < 0.8; *Brookes* et al., 1983), the ATP concentrations in all Bokashi variants were almost 7 to 10 times higher (6.9–9.8 μg g^{-1}) than those measured by *Tseng* et al. (1995) after 10 d of composting. This indicates a higher microbial activity in the Bokashi variants in our study, which was probably due to the 7%–12% higher water content (67%–72%) inside the Bokashi piles. The dormant cells (AEC < 0.8) with large ATP concentrations might have been in a state of "metabolic alertness" (*De Nobili* et al., 2000), which allows them to rapidly increase their metabolism again if necessary, such as for capturing nutrients suddenly available after turning the compost piles.

The usual increase in plant growth through compost application was also found in this study for all Bokashi variants compared to the control (*De Brito Alvarez* et al., 1995). Significantly more root mass was measured in the control without any Bokashi application, probably due to the poor nutrient status of the substrate that forced plants to develop more, longer, and more active roots scavenging for nutrients (*Xu*, 2000). Hence, it could not be confirmed that Bokashi produced with EM promotes plant growth more than any of the other Bokashi variants.

The ability of Bokashi to suppress nematodes might be partly due to sterilizing compounds produced by lactic acid bacteria that suppress harmful microorganisms, such as *Fusarium* and nematodes (*Kyan* et al., 1999). Nematode-controlling effects may also arise from the chemical composition of the organic additives and the type of microorganisms that develop during the decomposition process (*Rodriguez-Kabana* et al., 1987). Organic amendments may also control nematodes by direct toxicity (*Akhtar* and *Alam*, 1993). However, our data did not confirm nematode-controlling effects through the addition of EM such as reported by *Tabora* et al. (2000). All Bokashi variants tended to reduce nematode infestation, even if the observed effects were statistically significant only for EM and M. This may lead to the assumption that the reduced numbers of nematodes might be due to a more general Bokashi effect.

5 Conclusions

Specific effects of EM could only be observed for the temperature development during composting and fungi population indicated by ergosterol concentrations at the last sampling date. This study did not provide evidence of EM-induced enhanced mineralization nor increased colonization of composts with added microorganisms. In the EM treatment rather the opposite was observed with a fungi-dominated microbial population at the end of composting. The results provide evidence that the plant growth–promoting effect of added EM Bokashi was based on the applied organic substrate rather than the addition of EM. Thus, under the conditions of this study (EM-application rates, frequency of compost-pile turning), effects of EM on the decomposition process and growth of young banana plants were minor at most. Further research under different composting conditions, with higher treatment rates, an additional N source, and optimized banana-growing conditions would be needed to verify our results.

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References

Akhtar, M. (2000): Approaches to biological control of nematode pests by natural products and enemies. *J. Crop Prod.* 3, 367–395.

Akhtar, M., Alam, M. M. (1993): Utilization of waste materials in nematode control: a review. *Bioresour. Technol.* 45, 1–7.

Backhaus, K., Erichson, B., Plinke, W., Weiber, R. (2003): Multivariate Analysemethoden – eine anwendungsorientierte Einführung. Springer-Verlag, Berlin, Germany.

Brookes, P. C., Tate, K. R., Jenkinson, D. S. (1983): The adenylate energy charge of the soil microbial biomass. *Soil. Biol. Biochem.* 15, 9–16.

Brookes, P. C., Landman, A., Pruden, G., Jenkinson, D. S. (1985): Chloroform fumigation extraction and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass in soil. *Soil Biol. Biochem.* 17, 837–842.

Bruns, C. (1996): Suppressive Effekte von Komposten aus der getrennten Sammlung organischer Abfälle und von Rindermistkompost gegenüber bodenbürtigen Schaderregern. PhD thesis, Organic Agriculture (Ökologischer Landbau), University of Kassel.

Butler, T. A., Sikora, L. J., Steinhilber, P. M., Douglass, L. W. (2001): Compost age and sample storage effects on maturity indicators of biosolids compost. *J. Environ. Quality* 30, 2141–2148.

Chefetz, B., Hatcher, P. G., Hadar, Y., Chen, Y. (1996): Chemical and biological characterization of organic matter during composting of municipal solid waste. *J. Environ. Quality* 25, 776–785.

Daly, M. J., Stewart, D. P. C. (1999): Influence of "effective microorganisms" (EM) on vegetable production and carbon mineralization – a preliminary investigation. *J. Sustain. Agric.* 14, 15–25.

De Brito Alvarez, M. A., Gagné, S., Antoun, H. (1995): Effect of compost on rhizosphere microflora of the tomato and on the incidence of plant growth-promoting rhizobacteria. *Appl. Environ. Microbiol.* 61, 194–199.

De Nobili, M., Contin, M., Mondini, C., Brookes, P. C. (2000): Soil microbial biomass is triggered into activity by trace amounts of substrate. *Soil Biol. Biochem.* 33, 1163–1170.

Decker, H. (1969): Phytonematologie. VEB Deutscher Landwirtschaftsverlag, Berlin, Germany.

Djajakirana, G., Joergensen, R. G., Meyer, B. (1996): Ergosterol and microbial biomass relationship in soil. *Biol. Fert. Soils* 22, 299–304.

Dyckmans, J., Raubuch, M. (1997): A modification of a method to determine adenosine nucleotides in forest organic layers and mineral soils by ion-paired reversed-phase high-performance liquid chromatography. *J. Microbiol. Methods* 30, 1–20.

EARTH (Escuela de Agricultura de la Región Tropical Húmeda) (2003): www.earth.ac.cr. Date of access: May 13, 2003.

Gattinger, A., Bausenwein, U., Bruns, C. (2004): Microbial biomass and activity in composts of different composition and age. *J. Plant Nutr. Soil Sci.* 167, 556–561.

Gericke, S., Kurmies, B. (1952): Die kolorimetrische Phosphorsäurebestimmung mit Ammonium-Vanadat-Molybdat und ihre Anwendung in der Pflanzenanalyse. *Z. Pflanzenernähr. Düng. Bodenk.* 59, 235–247.

Goto, I., Muramoto, J. (1995): Chemical properties of EM bokashi, in Nishio, M.: Microbial fertilizer in Japan 11. Proceedings of the Japanese Society of Soil Science and Plant Nutrition 41, p. 158.

Goto, I., Muramoto, J. (1995): Chemical properties of EM bokashi., in Nishio, M. (1996): Microbial fertilizer in Japan, 11. Proceedings of the Japanese Society of Soil Science and Plant Nutrition 41, p. 158; www.eprida.com/hydro/ecoss/background/microbefert.pdf. Date of access: November 15, 2006.

Hellmann, B., Zelles, L., Palojarvi, A., Bai, Q. Y. (1997): Emission of climate-relevant trace gases and succession of microbial communities during open-window composting. *Appl. Environ. Microbiol.* 63, 1011–1018.

Higa, T. (2002): Eine Revolution zur Rettung der Erde: mit effektiven Mikroorganismen (EM) die Probleme unserer Welt lösen. OLV (Organischer Landbau Verlag), Kevelaer, Germany.

Higa, T., Parr, J. F. (1994): Beneficial and effective microorganisms for a sustainable agriculture and environment. INFRC (International Nature Farming Research Center), Atami, Japan.

Joergensen, R. G., Mueller, T. (1996): The fumigation-extraction method to estimate soil microbial biomass: calibration of the k_{EN} value. *Soil Biol. Biochem.* 28, 33–37.

Joergensen, R. G., Meyer, B., Roden, A., Wittke, B. (1996): Microbial activity and biomass in mixture treatments of soil and biogenic municipal refuse compost. *Biol. Fertil. Soils* 23, 43–49.

Körner, I., Ritzkowski, M. (1999): Charakteristik von Bioabfällen und Komposten, in Körner, I., Ritzkowski, M., Stegmann, R.: Neue Techniken zur Kompostierung, Verwertung auf landwirtschaftlichen Flächen, Teilvorhaben 10.b Teil 1 und Teil 2, Band II. Umweltbundesamt Hamburg, Abfallwirtschaft und Altlastensanierung des BMBF, pp. 73–95.

Kyan, T., Shintani, M., Kanda, S., Sakurai, M., Ohashi, H., Fujisawa, A., Pongdit, S. (1999): Kyusei nature farming and the technology of effective microorganisms, guidelines for practical use. Sangakkara, R. (ed.), APNAN (Asia Pacific Natural Agriculture Network, Bangkok, Thailand) and INFRC (International Nature Farming Research Center), Atami, Japan.

Lloyed, J. E., Herms, D. A. H., Stinner, B. R., Hoitink, H. A. J. (2002): Comparing composted yard trimmings and ground wood as mulches. *Bio Cycle* 43, 52–56.

Mondini, C., Contin, M., Leita, L., De Nobili, M. (2002): Response of microbial biomass to air-drying and rewetting in soils and compost. *Geoderma* 105, 111–124.

Rodriguez-Kabana, R., Morgan-Jones, G., Chet, I. (1987): Biological control of nematodes: soil amendments and microbial antagonists. *Plant Soil* 100, 237–247.

Schloss, P. D., Hay, A. G., Wilson, D. B., Walker, L. P. (2003): Molecular assessment of inoculum efficacy and process reproducibility in composting using ARISA. *Transactions of the ASAE* 46, 919–927.

Shintani, M., Tabora, P. (2000): Organic fertilizer: managing banana residues with effective microorganism (EM), in Alföldi, T., Lockeretz, W., Niggli, U.: IFOAM 2000 – The world grows organic. Proceedings 13th International IFOAM Scientific Conference, Basel, 28–31 August 2000. Vdf Hochschulverlag AG an der ETH Zurich, Switzerland, p. 269.

Tabora, P., Shintani, M., Elango, F. (2000): Banana researches in Costa Rica (Central America) with Effective Microorganisms. www.emro.jp./english/library/abstracts/0006banana.html. Date of access: June 25, 2003.

Tseng, D. Y., Chalmers, J. J., Tuovinen, O. H., Hoitink, H. A. J. (1995): Characterization of a bench-scale system for studying the biodegradation of organic solid wastes. *Biotechnology* 11, 443–451.

Ultra Jr., V. U., Mendoza, D. M., Briones, A. M. (2005): Chemical changes under aerobic composting and nutrient supplying potential of banana residue compost. *Ren. Agric. Food Sci.* 20, 113–125.

Vance, E. D., Brookes, P. C., Jenkinson, D. S. (1987): An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19, 703–707.

VanderGheynst, J. S., Lei, F. (2003): Microbial community structure dynamics during aerated and mixed composting. *Transactions of the ASAE* 46, 577–584.

Wood, M. T., Miles, R., Tabora, P. (1998): EM-fermented plant extract and EM5 for controlling pickleworm (*Ciaphania nitidalis*) in organic cucumber, in Foguelman, D., Lockeretz, W. (eds.): IFOAM 98 – Organic agriculture the credible solution for the XXIst Century. Proceedings of the 12th International IFOAM Scientific Conference, Mar del Plata/ Argentinien, November 1998, IFOAM c/o Ökozentrum Imsbach, Tholey-Theley, Germany, pp. 219–224.

Wu, J., Joergensen, R. G., Pommerening, B., Chaussod, R., Brookes, P. C. (1990): Measurement of microbial biomass C by fumigation extraction – an automated procedure. *Soil Biol. Biochem.* 22, 1167–1169.

Xu, H. L. (2000): Effects of a microbial inoculant and organic fertilizers on the growth, photosynthesis and yield of sweet corn. *J. Crop Prod.* 3, 183–214.

Xu, H. L., Wang, R., Mridha, M. (2000): Effects of organic fertilizers and a microbial inoculant on leaf photosynthesis and fruit yield and quality of tomato plants. *J. Crop Prod.* 3, 173–182.

Yamada, K., Xu, H. L. (2000): Properties and applications of an organic fertilizer inoculated with effective microorganisms. *J. Crop Prod.* 3, 255–268.

