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# Effect of compost and slow-release fertilizers addition on soil biochemistry and yield of maize (*Zea mays* L.) in Oaxaca, Mexico

## Efecto de la adición de compost y fertilizantes de liberación lenta en la bioquímica del suelo y el rendimiento de maíz (*Zea mays* L.) en Oaxaca, México

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### ABSTRACT

The effect of Bokashi (B, a fermented compost), slow-release fertilizers (SRFs) and their combined application on mycorrhizal colonization (MC), soil invertase, cellulase, acid (AcP) and alkaline (AIP) phosphatases activities and maize (*Zea mays* L.) yield was investigated in terrace (TS) and valley (VS) soils in Oaxaca, Mexico. A complete randomized design, seven fertilizer treatments and four replications were used: unamended control (C); conventional fertilization (90-46-00 NPK) (CF); B; SRF1 (Multigro 6®, 21-14-10 NPK); SRF2 (Multigro 3®, 24-05-14 NPK); B+SRF1; B+SRF2. Highest root colonization percentage: CF in VS, and SRF2 in TS. Highest extraradical mycelium length: B, B+SRF1, CF in VS, and B+SRF1 in TS. In both soils, B increased the spore number. Highest AcP activity: B, SRF2 in VS, and B+SRF1, B+SRF2 in TS. Highest AIP activity: B+SRF1, CF in VS, and C in TS. Highest invertase activity: B+SRF1, SRF2, CF in VS, and B in TS. Grain yield only increased with B in VS. The significant interaction soil type × fertilizer treatment for the majority of the biological soil properties analyzed suggests that MC and soil enzyme activity response to fertilization was influenced by soil type. Bokashi, alone or combined with SRFs improves biological soil fertility in maize fields.

### RESUMEN

Se determinó el efecto de bocashi (B, compost fermentado) y fertilizantes de liberación lenta (SRFs) sobre la colonización micorrízica (MC), la actividad de invertasa, celulasa, fosfatasa ácida (AcP) y alcalina (AIP) y el rendimiento de maíz en suelos de terraza (TS) y valle (VS) en Oaxaca, México. Se utilizó un diseño completamente al azar con siete tratamientos y cuatro repeticiones: control (C) sin fertilización; fertilización convencional (CF) (90-46-00 NPK); B; SRF1 (Multigro 6®, 21-14-10 NPK); SRF2 (Multigro 3®, 24-05-14 NPK); B+SRF1; B+SRF2. El porcentaje más alto de colonización micorrízica fue con CF en VS, y con SRF2 en TS. La mayor longitud de micelio extraradical se registró con B, B+SRF1 y CF en VS, y con B+SRF1 en TS. En ambos suelos, B incrementó el número de esporas. La actividad más alta de AcP fue con B y SRF2 en VS, y con B+SRF1 y B+SRF2 en TS. La actividad más alta de AIP se detectó con B+SRF1 y CF en VS y con C en TS. La actividad más alta de invertasa se encontró con B+SRF1, SRF2 y CF en VS y con B en TS. El rendimiento de grano incrementó solamente con B en VS. La interacción significativa tipo de suelo × tratamiento de fertilización, para la mayoría de las propiedades biológicas analizadas,

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**Keywords**

bokashi • cellulase • invertase  
• mycorrhizal colonization •  
phosphatases • *Zea mays* L.

**Palabras clave**

bocashi • celulasa • invertasa •  
colonización micorrízica • fosfatasas  
• *Zea mays* L.

sugiere que la respuesta de la colonización micorrízica y de la actividad enzimática a la fertilización, estuvo determinada por el tipo de suelo. Bocashi sola o combinada con SRFs puede mejorar la fertilidad biológica del suelo en cultivos de maíz.

**INTRODUCTION**

Many researchers have studied the effect of fertilization on soil fertility by investigating soil enzymatic activity and soil microbial biomass (26, 33). The measurement of biochemical soil properties such as soil hydrolases (phosphatase, invertase, cellulase, protease, urease) activity provides an early indication of changes in soil fertility, as they are related to the mineralization of nutrients such as P, C and N (55).

Phosphatases catalyze the hydrolysis of both organic phosphate esters and anhydrides of phosphoric acid into different forms of inorganic P, which are assimilable by plants. Acid phosphatases are secreted by various fungi, including *Aspergillus niger* (22). Invertase catalyzes the hydrolysis of sucrose to D-glucose and D-fructose, and plays a critical role in releasing low-molecular-weight sugars that are important energy sources for microorganisms (27). Invertase has been reported in bacteria (59), yeast (6) and filamentous fungi, such as *Aspergillus ochraceus* (19) and *Aspergillus niger* (44). Cellulases are a group of enzymes that catalyze the degradation of cellulose, the most abundant polysaccharide in plant cell walls (11). Cellulolytic bacteria belonging to the genus of *Cellulomonas*, *Clostridium*, *Bacillus*, *Thermomonospora*, *Ruminococcus*, *Bacteriodes*, *Erwinia*, *Acetovibrio*, *Microbispora*, and *Streptomyces* can effectively produce cellulases (47).

Arbuscular mycorrhizal (AM) fungi represent an important component of the soil microbial community and can significantly affect plant growth and soil stability (16). Hydrolytic enzymes seem to be involved in the penetration and development of AM fungi in plant roots. Mycorrhizal fungi hyphae secrete acid and alkaline phosphatases into the rhizosphere (49). Extracellular invertases play a crucial role in the AM symbiosis (48). Some AM fungi are also capable of secreting cellulase (53).

In most developing countries, the cost of mineral fertilizers is often limiting for small-scale, resource poor farmers (50). It is therefore imperative that other sustainable alternatives for soil fertility management are researched so as to ensure improved crop production and, consequently, improved food security. Integrated management based on slow-release fertilizers (SRFs) and organic fertilizers is one such alternative. One of the most promising organically based soil nutrient management practices is the

use of bokashi (B). Bokashi composting uses a selected group of microorganisms to ferment organic waste (21). As a compost, B is used to improve the soil organic matter content (7). The addition of organic matter raises the chemical, physical and biological soil fertility (28). Mineral fertilizers, on the other hand, enhance the decomposition of soil organic matter, which leads to degradation of the soil structure. They also reduce the colonization of plant roots with AM and inhibit symbiotic N fixation by rhizobia due to high N fertilization (8). Therefore, they must be used in conjunction with strategies that are designed to manage and maintain soil organic matter. Some research has reported the favorable effects of compost on AM (32) and soil enzyme activities (38).

Slow-release fertilizers are commonly fertilizers coated with many different materials, such as wax resin (17), natural rubber, polyvinyl chloride and polylactic acid (20). The granules contain primarily NPK nutrients in a form which either a) delays its availability for plant uptake and use after application, or b) is available to the plant significantly longer than a reference 'rapidly available nutrient fertilizer' (29). If the use of slow nutrient-release fertilizers meets the requirements of individual plants, the plants can then utilize nutrients from these fertilizers more effectively, which in turn decrease crop costs. One application can cover several applications of highly soluble mineral fertilizers (17).

Maize (*Zea mays* L.) is one of the most important crops worldwide and sustainable agricultural systems for this crop are urgently required (44, 63). In the state of Oaxaca, Mexico, inappropriate soil management practices have led to the degradation and deterioration of the soils, and consequently, the yield of maize has decreased as a result of low soil fertility levels (23). Vergara-Sánchez *et al.* (2005) reported that 80% of maize cropping in Oaxaca state is performed in hillside soils (terrace) with steep slopes > 30%, and only 20% in plains soils (valley).

The objective of this study was to evaluate the effect of B compost and SRFs application on the mycorrhizal colonization, soil invertase, cellulase, acid and alkaline phosphatases activities and yield of maize in terrace and valley soils in Nochixtlán District of Oaxaca, Mexico.

## MATERIALS AND METHODS

The study was conducted over the 2009 spring-summer growing cycle under rainfed conditions in terrace and valley soils in Nochixtlán District (17°31' N y 97° 17' O), state of Oaxaca, Mexico. The average annual rainfall was 580 mm, average annual temperatures range from 12°C to 18°C and the climate is temperate at an altitude of 2095 m (54). First, we determined the soil properties of the study sites. The valley soil has a clay loam texture with pH 8.20 (1/2 ratio in water), with bulk density of 1.02 Mg m<sup>-3</sup>; 1.37 % organic carbon (Walkley and Black); 0.11% total N (micro- Kjeldahl); 7.04 mg kg<sup>-1</sup> P-Olsen; 0.35, 22.45 and 4.85 cmol<sub>c</sub> kg<sup>-1</sup> exchangeable K, Ca and Mg (atomic absorption spectrometry). The terrace soil has a clay loam texture with pH 8.50 (1/2 ratio in water), with bulk density of 1.16 Mg m<sup>-3</sup>; 0.41% organic carbon

(Walkley and Black); 0.03% total N (micro-Kjeldahl); 10.76 mg kg<sup>-1</sup> P-Olsen; 0.23, 21.25 and 3.85 cmol<sub>c</sub> kg<sup>-1</sup> exchangeable K, Ca and Mg (atomic absorption spectrometry).

The assays were independently conducted in both soil types; a complete randomized design with seven fertilizer treatments and four replications was used. The seven fertilizer treatments were:

- unamended control (C).
- conventional fertilization (CF) with highly soluble mineral fertilizers (90- 46- 00 NPK), di-ammonium phosphate (18-46-00) 100 kg ha<sup>-1</sup>, ammonium sulphate (20- 00-00) 125 kg ha<sup>-1</sup>, urea (46-00-00) 100 kg ha<sup>-1</sup>.
- B compost, 10 Mg ha<sup>-1</sup>.
- coated slow-release fertilizer (SRF1) [Multigro 6®, 21-14-10 NPK of Haifa Chemicals, Haifa, Israel] 328.57 kg ha<sup>-1</sup> + urea 45.65 kg ha<sup>-1</sup>.
- coated slow-release fertilizer (SRF2) [Multigro 3®, 24-05-14 NPK of Haifa Chemicals, Haifa, Israel] 375 kg ha<sup>-1</sup> + triple super phosphate 59.78 kg ha<sup>-1</sup>.
- B compost 4 Mg ha<sup>-1</sup> + SRF1 328.57 kg ha<sup>-1</sup> + urea 45.65 kg ha<sup>-1</sup> (B+SRF1).
- B compost 4 Mg ha<sup>-1</sup> + SRF2 375 kg ha<sup>-1</sup> + triple super phosphate 59.78 kg ha<sup>-1</sup> (B+SRF2).

The CF was consistent with the standard practices of local farmers. The B compost dose in treatments 6 and 7 is reduced because the Leopardo® hybrid is high yield and early variety (51) and high quantities of available N can extend its productive cycle. According to the owners of the land, both plots have been cropped in rainfed conditions for more than 30 years. The terrace plot is cropped with wheat-barley-maize rotation, but the valley plot only with maize. All the crops are managed in accordance with the technical recommendations of the Campo Agrícola Experimental Mixteca Oaxaqueña from the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (24).

The size of the experimental plot was 22.4 m<sup>2</sup> with three useful rows and two rows used to minimize edge effect; length of 7.0 m per row and 140 plants per plot. B compost was prepared with cattle manure, wheat straw, "piloncillo" (powdered brown sugar), coal, ash and soil.

The mean B compost composition was 18.46% organic carbon, 1.44% total N, 2.49% total P, 2.46% total K; 8.13 pH (42). Land was prepared mechanically with a disk plough in March and a harrow plough 15 days prior to sowing. The crops were sowed on June 15, 2009 at a density of 50,000 plants ha<sup>-1</sup>, "Leopardo®" (Asgrow- Monsanto, St. Louis, Missouri, USA) maize seed was used. The seeds were pre-treated with Furadan® (carbofuran, a systemic acaricide, insecticide and nematicide is the active ingredient in Furadan) for grub control.

The application of the fertilizer and B compost treatments were done eight days post-emergence, incorporated 5 cm around the stem of each plant at a soil depth of 15 cm. At 40 days Gramoxone® and Banvel® were applied for weed control. Paraquat is the active ingredient in Gramoxone®, and is the most highly acutely toxic herbicide

to be marketed over the last 60 years. Nonetheless, it is one of the most widely used herbicides in the world, and in most countries where it is registered it can be used without restriction (60). Dicamba, a benzoic acid herbicide used on various crops to control a broad spectrum of woody plants and broad leaf weeds is the active ingredient in Banvel®. All three cited pesticides are authorized products by Mexican regulation (9).

The maize was harvested on December 15, 2009. On December 8, 2009, one composed sample of rhizospheric soil and roots (10-15 cm from the stem, 0-20 cm soil depth) of five mounds of maize was collected at each replication treatment; the subsamples were randomly chosen. A portion of the soil from each sample was stored in polyethylene bags and refrigerated (4-6°C) for the analysis of mycorrhizal parameters. Following Zornoza *et al.* (2006), a second portion from the soil of each sample was air-dried and sieved (< 2 mm) for the determination of soil enzyme activity. The maize roots were recovered from the soil samples, thoroughly washed, dried (60°C 48 h) and stored in plastic bags.

To determine the percentage of mycorrhizal colonization, the dry roots were re-soaked in tap water (3-4 h). The extent of mycorrhizal colonization was determined after clearing and trypan blue staining by the visual analysis (43) of 25-30 root segments mounted on slides using a compound microscope (40×) (14). In soil samples, the quantification of extraradical mycelium (ERM) length was carried out using the semisolid gel technique described by Baath and Söderström (4,5). Recovery and counting of spores from soil samples was carried out using the wet sieving and decanting method (13) followed by centrifugation in a sucrose solution at 50% and observation under a stereoscopic microscope (40×).

Acid and alkaline phosphatase activity was determined according to Tabatabai and Bremner (52). The soil sample was incubated with a substrate containing *p*-nitrophenyl phosphate; the amount of *p*-nitrophenol liberated during enzymatic hydrolysis was measured by spectrophotometry. Invertase activity was determined with sucrose as the substrate. The soil sample was incubated at 37°C for 5 h. The liberated reduced sugars were determined with the method described by Nelson (1944). The same incubation conditions were used in determining carboxymethylcellulase activity, with the exception being that the substrate was carboxymethylcellulose and the incubation time was 24 h (41).

Maize ears were harvested at physiological maturity from the central part of the experimental units in order to obtain grain yield.

Data was submitted to the two-way analysis of variance (ANOVA) to analyze effects of both factors: fertilizer treatments and soil type and the interaction fertilizer treatment × soil type. The means were separated with Tukey test (0.05%). The data was transformed before analysis with the arcCos procedure in order to meet the requirement of normal distribution. The statistical procedures were conducted using the JMP 7.0® software package (JMP 7.0 for windows 7).

## RESULTS AND DISCUSSION

The root colonization percentage ranged from 15 to 58% in valley soil and, from 7 to 45% in terrace soil. The highest value of this variable was with CF in valley soil, and with SRF2 in terrace soil (table 1, page 187). Similarly, Gryndler *et al.* (2005) reported that the addition of mineral N and P increased maize root length colonized by the mycorrhizal fungi. Contrarily, Guillemin *et al.* (1995) found that in a P-deficient soil, mycorrhizal colonization in pineapple roots was not modified by phosphate fertilization.

The ERM length ranged from 4.15 to 12.08 m g<sup>-1</sup> in dry soil in valley soil and, from 2.29 to 8.65 m g<sup>-1</sup> in dry soil in terrace soil. The highest value of this parameter was found with B, B+SRF1 and CF in valley soil and with B+SRF1 in terrace soil (table 1, page 187). Spore number ranged from 275 to 1412 per 100 g of soil in valley soil and, from 356 to 837 per 100 g of soil in terrace soil. In both soils the addition of B compost increased the spore number (table 1, page 187).

In general, the addition of B compost, alone or combined with SRF, promoted the growth of ERM length and spore number in both soils. These results coincide with those reported in previous studies, which have shown that organic fertilization may increase sporulation of some AM fungi species (3), mycorrhizal roots and mycelium length (57). Álvarez-Solís *et al.* (2010) found that the percentage of colonization of mycorrhizal was 1.3 times higher with B than without compost in corn fields under rainfed conditions.

Maize is an obligatory mycorrhizal species and its roots are readily colonized by many non-host-specific AM fungi (31). Gryndler *et al.* (16) expressed that relatively low amounts of organic matter applied to soil can affect both plant growth and development of AM fungi, measured as colonized root length and hyphal growth. Humic substances, such as fulvic acids that result from the decomposition of organic fertilizers, adsorb free cations from the soil solution and may favor the physiological functions of the fungal mycelia (absorption and transport) (15).

Acid phosphatase activity ranged from 21.92 µg p-NF g<sup>-1</sup> soil h<sup>-1</sup> to 88.70 µg p-NF g<sup>-1</sup> soil h<sup>-1</sup> in valley soil and, from 23.18 µg p-NF g<sup>-1</sup> soil h<sup>-1</sup> to 36.90 µg p-NF g<sup>-1</sup> soil h<sup>-1</sup> in terrace soil. The highest acid phosphatase activity was registered with B and SRF2 in valley soil and with B+SRF1 and B+SRF2 in terrace soil (table 1, page 187).

Alkaline phosphatase activity ranged from 126.98 µg p-NF g<sup>-1</sup> soil h<sup>-1</sup> to 178.63 µg p-NF g<sup>-1</sup> soil h<sup>-1</sup> in valley soil and, from 32.25 µg p-NF g<sup>-1</sup> soil h<sup>-1</sup> to 84.75 µg p-NF g<sup>-1</sup> soil h<sup>-1</sup> in terrace soil. The highest alkaline phosphatase activity was found with B+SRF1 and CF in valley soil and with C in terrace soil.

Mineral fertilizers do not directly inhibit enzyme activity, but rather, repress its production (37). Martens *et al.* (1992) also reported that the addition of organic matter maintained high levels of phosphatase activity in soil during a long-term study. Álvarez-Solís *et al.* (2010) indicated a positive effect in the activity of both phosphatases with the application of compost in corn fields under rainfed conditions. Martínez-Gallegos *et al.* (2012) reported that the simultaneous addition of mineral SRFs and compost enhance the phosphatase activity in the rhizosphere of *Agave angustifolia* Haw.

**Table 1.** Mean value ( $\pm$  standard error) of hydrolytic soil enzyme activity in the rhizosphere of maize (*Zea mays* L.) as affected by fertilizer treatment and soil type in Nochixtlán District, Oaxaca, Mexico.**Tabla 1.** Valores promedio ( $\pm$  error estándar) de la actividad de enzimas hidrolíticas en la rizósfera de maíz (*Zea mays* L.) como respuesta a los tratamientos de fertilización y al tipo de suelo en el Distrito de Nochixtlán, Oaxaca, México.

Fertilizer treatment	Acid phosphatase µg p-NF g <sup>-1</sup> soil h <sup>-1</sup>	Alkaline phosphatase µg p-NF g <sup>-1</sup> soil h <sup>-1</sup>	Invertase µg of glucose g <sup>-1</sup> dry soil 5 h <sup>-1</sup>	Cellulase µg of glucose g <sup>-1</sup> dry soil 24 h <sup>-1</sup>
	Valley soil			
C	21.92 $\pm$ 1.21 D g	133.02 $\pm$ 2.83 C c	3.46 $\pm$ 0.14B e	0.04 $\pm$ 0.00A a
CF	46.46 $\pm$ 3.13 B b	168.99 $\pm$ 3.26 A a	4.86 $\pm$ 0.29A d	0.10 $\pm$ 0.00A a
B	88.70 $\pm$ 1.81 A a	164.18 $\pm$ 2.55AB ab	3.66 $\pm$ 0.24B e	0.08 $\pm$ 0.00A a
SRF1	42.49 $\pm$ 1.66 B bc	144.05 $\pm$ 11.53BC bc	4.18 $\pm$ 0.07AB de	0.10 $\pm$ 0.01A a
SRF2	86.80 $\pm$ 1.60 A a	162.83 $\pm$ 4.68 AB ab	4.88 $\pm$ 0.12A d	0.07 $\pm$ 0.00A a
B+SRF1	41.55 $\pm$ 1.75 B bc	178.63 $\pm$ 2.40A a	5.17 $\pm$ 0.43A d	0.05 $\pm$ 0.00A a
B+SRF2	31.53 $\pm$ 0.56 C def	126.98 $\pm$ 2.42C c	4.27 $\pm$ 0.22AB de	0.05 $\pm$ 0.00A a
<i>p</i>	<0.0001	<0.0001	<0.0001	>0.466
	Terrace soil			
C	25.73 $\pm$ 0.03B fg	84.75 $\pm$ 0.96A d	11.53 $\pm$ 0.18BC b	0.03 $\pm$ 0.00A a
CF	25.70 $\pm$ 0.00B fg	58.40 $\pm$ 0.66C ef	11.90 $\pm$ 0.13B ab	0.03 $\pm$ 0.00A a
B	23.18 $\pm$ 1.29B g	47.66 $\pm$ 1.97DE fg	12.72 $\pm$ 0.20A a	0.03 $\pm$ 0.00A a
SRF1	24.17 $\pm$ 1.26B fg	38.26 $\pm$ 1.07EF fg	11.50 $\pm$ 0.14BC b	0.03 $\pm$ 0.00A a
SRF2	27.05 $\pm$ 1.96B efg	74.10 $\pm$ 4.06B de	11.11 $\pm$ 0.04C b	0.07 $\pm$ 0.00A a
B+SRF1	36.90 $\pm$ 0.82A cd	32.25 $\pm$ 1.80F g	11.03 $\pm$ 0.26C b	0.02 $\pm$ 0.00A a
B+SRF2	33.66 $\pm$ 0.97A de	55.51 $\pm$ 2.89CD ef	9.92 $\pm$ 0.05D c	0.02 $\pm$ 0.00A a
<i>p</i>	<0.0001	<0.0001	<0.0001	>0.523
soil type $\times$ fertilizer effect <i>p</i>	<0.0001	<0.0001	<0.0001	>0.446

B, bokashi; C, unamended control; CF, conventional fertilization; SRF1, slow-release fertilizer 1 (Multigro 6®, 21-14-10 NPK); SRF2, slow-release fertilizer 2 (Multigro 3®, 24-05-14 NPK).

Numbers in a column followed by different uppercase letters indicate fertilizer treatment effect and numbers in a column followed by different lowercase letters indicate soil type  $\times$  fertilizer effect at 0.05-probability levels based on Tukey test.

B, bocashi; C, control sin fertilización; CF, fertilización convencional; SRF1, fertilizante de liberación lenta 1 (Multigro 6®, 21-14-10 NPK); SRF2, fertilizante de liberación lenta 2 (Multigro 3®, 24-05-14 NPK).

Los números en una columna seguidos por letras mayúsculas diferentes indican el efecto de los tratamientos de fertilización y los números en una columna seguidos por letras minúsculas diferentes indican el efecto de la interacción tipo de suelo  $\times$  fertilizante con base en la prueba de Tukey a una probabilidad de 0,05.



Organic matter is a source of nutrients for microorganisms and as the organic matter content in soils increases so does the potential nutrient supply (39). N from fertilization stimulates phosphatase activity, since that phosphatase requires substantial N investment, and so adding N to soil increase phosphatase production (34).

Invertase activity ranged from 3.46  $\mu\text{g}$  of glucose  $\text{g}^{-1}$  dry soil  $5 \text{ h}^{-1}$  to 5.17  $\mu\text{g}$  of glucose  $\text{g}^{-1}$  dry soil  $5 \text{ h}^{-1}$  in valley soil and, from 9.92  $\mu\text{g}$  of glucose  $\text{g}^{-1}$  dry soil  $5 \text{ h}^{-1}$  to 12.72  $\mu\text{g}$  of glucose  $\text{g}^{-1}$  dry soil  $5 \text{ h}^{-1}$  in terrace soil. The highest invertase activity was found with B+SRF1, SRF2 and CF in valley soil and with B in terrace soil. Adriano *et al.* (2012) also reported a higher invertase activity in soils cultivated with banana (*Musa* spp. L.) cv. 'Grand Naine' amended with liquid bioferment+compost compared with the unamended soil and soil amended with mineral fertilizer. Saha *et al.* (2008) reported the highest invertase activity in treatments that received organic manure along with the recommended fertilizer under rainfed soybean-wheat rotation.

Cellulase activity ranged from 0.04  $\mu\text{g}$  of glucose  $\text{g}^{-1}$  dry soil  $24 \text{ h}^{-1}$  to 2.05  $\mu\text{g}$  of glucose  $\text{g}^{-1}$  dry soil  $24 \text{ h}^{-1}$  in valley soil and, from 0.02  $\mu\text{g}$  of glucose  $\text{g}^{-1}$  dry soil  $24 \text{ h}^{-1}$  to 0.07  $\mu\text{g}$  of glucose  $\text{g}^{-1}$  dry soil  $24 \text{ h}^{-1}$  in terrace soil. There was not a significant change in the activity of this enzyme among fertilizer treatments in both soils.

Others studies have indicated that manure application significantly increased cellulase activity under rainfed soybean-wheat rotation (46). Kautz *et al.* (2004) reported that cellulase activity increased markedly with straw and green manure treatment, but increased only slightly with mineral fertilization. Bokashi is a compost obtained in short time (2-4 weeks), and fibrous residues (ligno-cellulosic) are the main component (42). The mineralization of these components is only partial and it is assumed (there is no published data) that the final product has a considerable proportion of cellulose.

Martens *et al.* (1992) showed that organic residues generally caused a significant increase in activities of key enzymes involved in the C, N, P, and S cycles 30 days after the first application, probably due to decomposing organic compounds releasing a trigger molecule or promoter that stimulates the production of hydrolytic enzymes. Organic manures supply many available nutrients that can accelerate the multiplication of microorganisms and enzyme production, and then increase soil organisms and enzymes activities (38).

The nutrients from the decomposition and transformation of organic manure can be maintained in an available form in soil, and thus can support plant growth and development needs. High levels of soil enzyme activity accelerated the mineralization of organic matter in soil releasing more essential nutrients such as N, P and S, required for better plant growth and high yields (61).

Significant effects were found for the interaction soil type  $\times$  fertilizer treatment for all of the biological soil properties analyzed except for cellulase activity (table 1, page 187).

The highest value of root colonization percentage was detected in valley soil with CF, the highest values of ERM length and spore number were found in valley soil with B. The highest value of alkaline phosphatase activity was detected in valley soil with B+SRF1 and CF; the highest value of acid phosphatase activity was registered in valley soil with B and SRF2; and, the highest value of invertase activity was found in terrace soil with B (table 1, page 187). These results suggest that the response of the majority of biological soil properties analyzed to the fertilization was influenced by the soil type.

The high soil organic carbon content in valley soil, compared with terrace soil, may have promoted an increase in the soil microbial biomass and their activity. Moreover, many extracellular enzymes may become stabilized through association with humic acids as a result of an increase in the humus content derived from the addition of B compost (38).

In valley soil, grain yield ranged from 6.72 to 11.44 Mg ha<sup>-1</sup> and increased with the addition of B (table 1, page 187). In terrace soil, grain yield ranged from 2.46 to 6.87 Mg ha<sup>-1</sup>, the lowest grain yield was registered with C (table 1, page 187). There were significant differences between the fertilizer treatments in each soil, but there was no significant interaction between soil type × fertilizer treatments.

According to Álvarez-Solís *et al.* (2010) the yield response to compost in valley soil may be partly due to the contribution of N and P through mineralization, which stimulates soil acid and alkaline phosphatase activity and colonization of mycorrhizal fungi (table 1, page 187). The doses of B compost (B vs B+SRF1 or B+SRF2) had significant effects, although these varied by soil type. In valley soil, the acid phosphatase activity, spore number, ERM and grain yield were higher with B, the higher dose of compost. In terrace soil, only invertase activity was higher with B.

The greatest effects of the addition of compost for all measured variables, with the exception of the invertase activity, were registered in valley soil (table 1, page 187; table 2, page 190).

In many countries there is great interest in changing agricultural management technologies based in the intensive use of agrochemicals. With respect to plant nutrition, organic matter can substitute, partially or totally, the use of mineral fertilizers.

In Mexico, many public offices, research centers and private organizations impulse these changes, with B compost as one of the products being used as an alternative, e.g. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (25), El Colegio de la Frontera Sur (12), Universidad de La Cañada (56), Colegio Superior para la Educación Integral Intercultural de Oaxaca (10) and Instituto Politécnico Nacional (this paper).

**Table 2.** Mean value ( $\pm$  standard error) of mycorrhizal colonization and maize (*Zea mays* L.) yield as affected by fertilizer treatment and soil type in Nochixtlán District, Oaxaca, Mexico.

**Tabla 2.** Valores promedio ( $\pm$  error estándar) de la colonización micorrizica y del rendimiento de maíz (*Zea mays* L.) como respuesta a los tratamientos de fertilización y al tipo de suelo en el Distrito de Nochixtlán, Oaxaca, México.

Fertilizer treatment	Root colonization %	Extraradical mycelium length m g <sup>-1</sup> dry soil	Spore number 100 g <sup>-1</sup> soil	Grain yield Mg ha <sup>-1</sup>
Valley soil				
C	15.95 $\pm$ 0.53E d	4.15 $\pm$ 0.15C ef	756.25 $\pm$ 58.07C cd	6.72 $\pm$ 0.86C bcd
CF	58.45 $\pm$ 0.80A a	10.53 $\pm$ 0.46A ab	981.25 $\pm$ 21.34B b	9.81 $\pm$ 0.36B ab
B	47.45 $\pm$ 1.30B b	12.08 $\pm$ 0.78A a	1412.50 $\pm$ 38.86A a	11.44 $\pm$ 0.44A a
SRF1	28.20 $\pm$ 0.96D c	6.77 $\pm$ 0.46B cd	768.75 $\pm$ 25.76C cd	7.31 $\pm$ 1.52BC abc
SRF2	31.00 $\pm$ 0.62D c	6.78 $\pm$ 0.52B cd	418.75 $\pm$ 38.69D fg	8.94 $\pm$ 0.80B ab
B+SRF1	44.60 $\pm$ 0.52BC b	10.23 $\pm$ 0.65A ab	868.75 $\pm$ 50.38BC bc	7.86 $\pm$ 1.07BC abc
B+SRF2	41.35 $\pm$ 0.74C b	5.50 $\pm$ 0.37BC de	275.00 $\pm$ 17.67D g	7.87 $\pm$ 1.52BC abc
<i>p</i>	<0.0001	<0.0001	<0.0001	<0.004
Terrace soil				
C	7.40 $\pm$ 0.22E d	2.29 $\pm$ 0.26D f	356.25 $\pm$ 27.71E fg	2.46 $\pm$ 0.55B d
CF	12.45 $\pm$ 0.81D d	3.85 $\pm$ 0.26C ef	450.00 $\pm$ 10.20CDE ef	6.38 $\pm$ 0.79A bcd
B	33.20 $\pm$ 0.58B c	7.09 $\pm$ 0.38B cd	837.50 $\pm$ 26.02A bc	6.75 $\pm$ 1.08A bcd
SRF1	16.30 $\pm$ 0.44C d	3.33 $\pm$ 0.17CD f	368.75 $\pm$ 29.53DE fg	6.87 $\pm$ 0.72A bc
SRF2	45.00 $\pm$ 0.49A b	3.54 $\pm$ 0.26CD ef	500.00 $\pm$ 22.82BC ef	6.26 $\pm$ 0.50A bcd
B+SRF1	34.65 $\pm$ 0.40B c	8.65 $\pm$ 0.35A bc	612.50 $\pm$ 36.08B de	6.67 $\pm$ 1.08A bcd
B+SRF2	16.95 $\pm$ 0.49C d	3.23 $\pm$ 0.35CD f	487.50 $\pm$ 29.75CD ef	5.21 $\pm$ 0.41AB cd
<i>p</i>	<0.0001	<0.0001	<0.0001	<0.024
soil type $\times$ fertilizer effect <i>p</i>	<0.0001	<0.0001	<0.0001	>0.089

B, bokashi; C, unamended control; CF, conventional fertilization; SRF1, slow-release fertilizer 1 (Multigro 6 $\oplus$ , 21-14-10 NPK); SRF2, slow-release fertilizer 2 (Multigro 3 $\oplus$ , 24-05-14 NPK).

Numbers in a column followed by different uppercase letters indicate fertilizer treatment effect and numbers in a column followed by different lowercase letters indicate soil type  $\times$  fertilizer effect at 0.05-probability levels based on Tukey test.

B, bocashi; C, control sin fertilización; CF, fertilización convencional; SRF1, fertilizante de liberación lenta 1 (Multigro 6 $\oplus$ , 21-14-10 NPK); SRF2, fertilizante de liberación lenta 2 (Multigro 3 $\oplus$ , 24-05-14 NPK).

Los números en una columna seguidos por letras mayúsculas diferentes indican el efecto de los tratamientos de fertilización y los números en una columna seguidos por letras minúsculas diferentes indican el efecto de la interacción tipo de suelo  $\times$  fertilizante con base en la prueba de Tukey a una probabilidad de 0,05.

## CONCLUSIONS

This study has demonstrated that the type of fertilizers applied to soil could greatly affect the arbuscular mycorrhizal fungi colonization and hydrolytic enzyme activity, which are considered to be sensitive indicators of soil health. Since it is generally accepted that soil functioning and the maintenance of soil fertility depends on the activity of soil microorganisms.

We conclude that the application of bokashi, alone or combined with slow-release fertilizers, may significantly improve the biological soil fertility in maize fields. The response of mycorrhizal colonization and soil enzyme activity to the fertilizer treatments however was influenced by the soil type. The addition of bokashi increased the grain yield in only one of the two soils studied. We suggest that the fertilizer treatments need to be further broadened in order to be as inclusive as possible.

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