ISSN 0370-5404
TRABAJO YA PUBLICADO

Rev. Ind. y Agríc. de Tucumán
Tomo 90 (1): 61-68; 2013

Effect of sugar cane trash blanketing on the development of microorganisms of agronomic and environmental interest

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ABSTRACT

The global sugar industry is progressively moving away from pre-harvest burning to a green-cane harvesting system. It is well known that when harvest residue is returned to the soil, nutrients and organic matter increase and soil structure is improved. However, the effect of trash blanketing on the development of different soil microorganisms has not been evaluated in Tucumán, Argentina. Hence, the aim of this study was to evaluate changes in microbial populations, especially those of agronomic and environmental interest, which occur under two management situations: with and without trash blanketing. Tests were performed at Finca San Genaro, located in eastern Tucumán (Dpto. Leales), using LCP 85-384 sugar cane variety at fourth ratoon age. This means that treatments started four years before sampling. During the 2011/2012 crop cycle, in June, July, November 2011 and May 2012, soil and different tissue samples from sugar cane roots and stems were microbiologically analyzed. Microorganisms were counted using different culture media: LB for mesophilic aerobic bacteria, PGA for fungi and yeasts, CA for *Pseudomonas* sp., and different N-free semisolid media for micro aerobic nitrogen fixing bacteria. It was observed that trash blanketing increased the number of yeast, fungus and *Pseudomonas* sp. populations in soil samples during high temperature seasons. Some of these isolated fungi showed ligninolytic activity and some *Pseudomonas* genus bacteria were able to solubilize phosphorus, thus indicating that these microorganisms may be involved in residue decomposition. Interestingly, trash blanketing also increased the number of nitrogen fixing bacteria associated with the plant root and stem tissues from June to February. The further development of trash degrading microorganisms and a better colonization of sugar cane tissues by nitrogen fixing bacteria could improve sugar cane crop growth and development.

Key words: sugar cane field, trash blanket, microbial communities.

RESUMEN

Efecto del residuo agrícola de la cosecha en verde de la caña de azúcar en el desarrollo de microorganismos de importancia agrícola y ambiental

En la actualidad, la industria azucarera mundial tiende a reemplazar la quema del cañaveral previo a la cosecha, por el sistema de caña verde. Trabajos demuestran que cuando el residuo agrícola de cosecha (RAC) regresa al suelo, aporta nutrientes, materia orgánica y mejora su estructura. Sin embargo, el efecto del RAC en el desarrollo de microorganismos del suelo aún no ha sido evaluado en Tucumán, R. Argentina. Por esta razón, el objetivo de este trabajo fue evaluar los cambios que ocurren en el desarrollo de microorganismos de importancia agrícola y ambiental, en dos situaciones de manejo del suelo: con y sin mantenimiento de cobertura con RAC. Los ensayos se realizaron en el Dpto. Leales, Tucumán, utilizando la variedad LCP 85-384 en la edad de soca 4; es decir, los tratamientos se establecieron cuatro años antes del muestreo. Para el análisis microbiológico, se tomaron muestras de suelo y de diferentes tejidos durante los meses de junio, julio, noviembre 2011 y mayo 2012. El recuento de microorganismos se realizó utilizando diferentes medios de cultivo: LB para aerobios mesófilos totales, APG para hongos y levaduras, AC para *Pseudomonas* sp. y medios de cultivo semisólidos libres de N_2 para bacterias microaeróbicas fijadoras de nitrógeno. En forma general, se observó que la cobertura con RAC aumentó el número de hongos, levaduras y *Pseudomonas* sp. durante las épocas con temperaturas más altas. Algunos hongos presentaron actividad ligninolítica y algunas *Pseudomonas* sp. fueron capaces de solubilizar fósforo, lo que indica que estos microorganismos podrían estar involucrados en la descomposición del residuo. Fue interesante observar que la cobertura con RAC también incrementó el número de bacterias fijadoras de nitrógeno asociadas a raíces y tallos, de junio a febrero. El mayor desarrollo de microorganismos degradadores de materia orgánica y una mejor colonización de los tejidos por bacterias fijadoras de nitrógeno podrían mejorar el crecimiento y desarrollo del cañaveral.

Palabras clave: cañaveral, cobertura, comunidades microbianas.

Paper presented at the XXVIII Congress of the International Society of Sugar Cane Technologists, Sao Paulo, Brazil, 24-27 June 2013 and published here with the agreement of the Society.

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INTRODUCTION

Sugar cane has high agricultural and economic importance in Tucumán, Argentina, and 217.000 ha are currently planted with this crop (Romero *et al*., 2009). However, it is well known that continuous sugar cane planting in fields and the burning of crop residues reduce concentrations of soil organic matter, which results in soil structural degradation. In an attempt to improve soil health, the practice of pre-harvest burning is being gradually replaced with green-cane harvesting practices all around the world. During green sugar cane harvesting, 7 to 30 tons of dry matter, depending on the variety and mainly on field productivity level, remains on the field. Residue management implies preserving it on the soil as mulching (trash blanketing), mixing it with the most superficial soil layers, or removing it mechanically (Digonzelli *et al*., 2007; Digonzelli *et al*., 2009). It has been demonstrated that when harvest residue is returned to the soil, contents of nutrients and organic matter increase and soil structure is improved. This management system allows the return of an important quantity of vegetable residues to the soil, favoring nutrient recycling, reducing both water and wind erosion, diminishing soil water evaporation, increasing infiltration and allowing a better conservation of soil moisture, while also reducing soil temperature in topsoil profile and favoring meso and microflora proliferation (Braunack and Ainslie, 2001; Scandaliaris *et al*., 2002; Thorburn *et al*., 2004; Digonzelli *et al*., 2007; Núñez and Spaans, 2007; Romero *et al*., 2007; Sanzano *et al*., 2009; Digonzelli *et al*., 2011a; Digonzelli *et al*., 2011b).

A sensitive indicator of soil organic matter dynamics is microbial biomass, because it changes relatively rapidly with C supply alterations and differences are detected before they can be measured by total organic matter content (Gregorich *et al*., 1997; Sparling, 1997). However, soil quality is strongly influenced by microorganism mediated processes, and measurement of microbial biomass gives no indication of microbial activity. Considering that until now there have not been any local studies showing the effect of sugar cane trash blanketing on the development of different soil microorganisms, this study aimed to evaluate changes in microbial populations, especially those of agronomic and environmental interest, which are produced under two different situations: sugar cane management with and without trash blanketing.

MATERIALS AND METHODS

The trials were conducted in a commercial field located at San Genaro (Dept. Leales), Tucumán, Argentina (27º 14'18" LS and 65º 12' 57" LW). This site is in the dry-subhumid saline depressed plain region, characterized by an annual mean temperature of 19ºC and a mean annual rainfall of 750 mm to 800 mm. There is also a moderate water deficit from August to October, which is a climatic limitation for sugarcane growing. Soils are non saline typic Haplustol, with silty loam texture in surface and clay loam at deeper levels, moderately well drained.

The evaluated variety was the main cultivar in Tucumán, LCP 85-384, which is planted in 76.65% of the whole sugarcane area (Ostengo *et al*., 2012). The trial was planted in 2007 and kept under conventional agronomic management practices: post-emergence herbicides were used for weed control, as well as nitrogen (urea) for fertilization (applied at a 115 kg/ha rate), and irrigation was not supplied. Evaluated treatments at the site included: i) harvest residues left on the soil surface, and ii) removal of residues with a fork. To ensure that soil quality was influenced by trash blanket, plots were kept under green cane system four years before sampling. Therefore, microbiological analyses were performed during the 2011/2012 crop cycle in June, July, November 2011 and May 2012, on samples of cane at its fourth ratoon age. The experimental design was completely randomized with four replications. Experimental plots consisted of five 1.6 m rows which were 10 m long.

Each treatment was sampled with four replicates that were processed separately. Soil and sugar cane root and stem tissue samples were collected from different spots in each row. For the analysis of soil and tissue microbial communities, viable plate counts on selective media (Alef and Nannipieri, 1995) were used to estimate populations of culturable bacteria, fungi and yeasts. Microorganisms were counted using different solid culture media: Luria Bertani (LB) for mesophilic aerobic bacteria, potato glucose agar (PGA) for fungi and yeasts, and cetrimide agar (CA) for *Pseudomonas* sp. Different semisolid culture media were used for enumeration and isolation of micro aerobic nitrogen fixing bacteria: N2 free malate (NFb) for *Azospirillum* sp., LGI-P for *Gluconoacetobacter* sp. and *Pantoea* sp., and JMV for *Burkholderia* sp. Some bacteria that grew in CA solid medium were selected to evaluate phosphate solubilization activity, by plating them on NBRIP medium (Nautiyal, 1999). Positive results were indicated by the formation of a clear halo around the colonies. For the isolation of microorganisms from sugar cane trash, residues were cut into 2 cm long fragments that were washed three times with sterile distilled water, and others that were sterilized with EtOH 70% (v/v) during 1 min, and then with NaClO 3% (v/v) during 3 min. After drying fragments with sterile papers, they were placed on Petri dishes containing PGA solid medium, supplemented with guaiacol 0.1% (v/v) to test ligninolytic activity. Brown dark halos surrounding fungi and yeast colonies indicated ligninolytic activity. Different fungi with ligninolytic activities were used as positive controls.

Results were analyzed with the Statistix program (Analytical Software, 1996). The LSD test was used to determine the arithmetic mean (significance level: 0.05) and the analysis of variance test (ANOVA) was performed to evaluate data dispersion with respect to the mean value.

RESULTS AND DISCUSSION

In general, we observed that when harvest residues were left on soil surface, changes in microbial population were induced depending on the season of the year. During cold weather months, trash blanket reduced the number of soil microorganisms, especially mesophilic aerobic bacteria, fungi and yeasts (Figure 1).

This could be explained by the fact that during cold weather months, trash blanket keeps soil temperature lower as compared to bare soil (Chapman *et al*., 2001; Digonzelli *et al*., 2009; Morandini *et al*., 2009; Digonzelli *et al*., 2011b). A different situation was observed in November. In this case, when soil temperatures tended to be equal, trash blanket increased the number of fungi, yeasts and *Pseudomonas* genus bacteria (Figure 1). These microorganisms were probably involved in residue decomposition. In June, after sugar cane harvest, stalk samples were taken from different plots to analyze the content of different endophytic microorganism populations, especially those having nitrogen fixing activities (Figure 2).

As observed in Figure 2, when residues were preserved on the soil as a cover, stalk endophytic colonization by nitrogen fixing bacteria was favored, in contrast to what happened with stalks collected from fields without a trash blanket. These results are of fundamental importance, if we consider that sugar cane crops have high nitrogen requirements and that endophytic nitrogen fixing microorganisms provide plants with a great proportion of the nitrogen they need to grow and develop (Boddey *et al*., 2003).

Similar results were observed when comparing nitrogen fixing microorganism populations found in sugar cane stems from fields with and without trash blanket (Figure 3).

Finally, we analyzed the effect of residue preservation on the development of different rhizosphere microorganisms associated with roots of sugar cane plants growing on soils with and without trash blanket (Figure 4).

In June, we observed that plant roots in fields under the trash blanket were colonized by a great number of fungi, yeasts and nitrogen fixing bacteria, in comparison with roots of sugarcane kept in bare soils. Although from July to November the number of rhizosphere microorganisms was not statistically different between treatments, we observed a significant increase in *Pseudomonas* genus bacteria, whose number remained high both in November and May. This result coincided with what was observed in soil samples analyzed in the same season of the year (Figure 1).

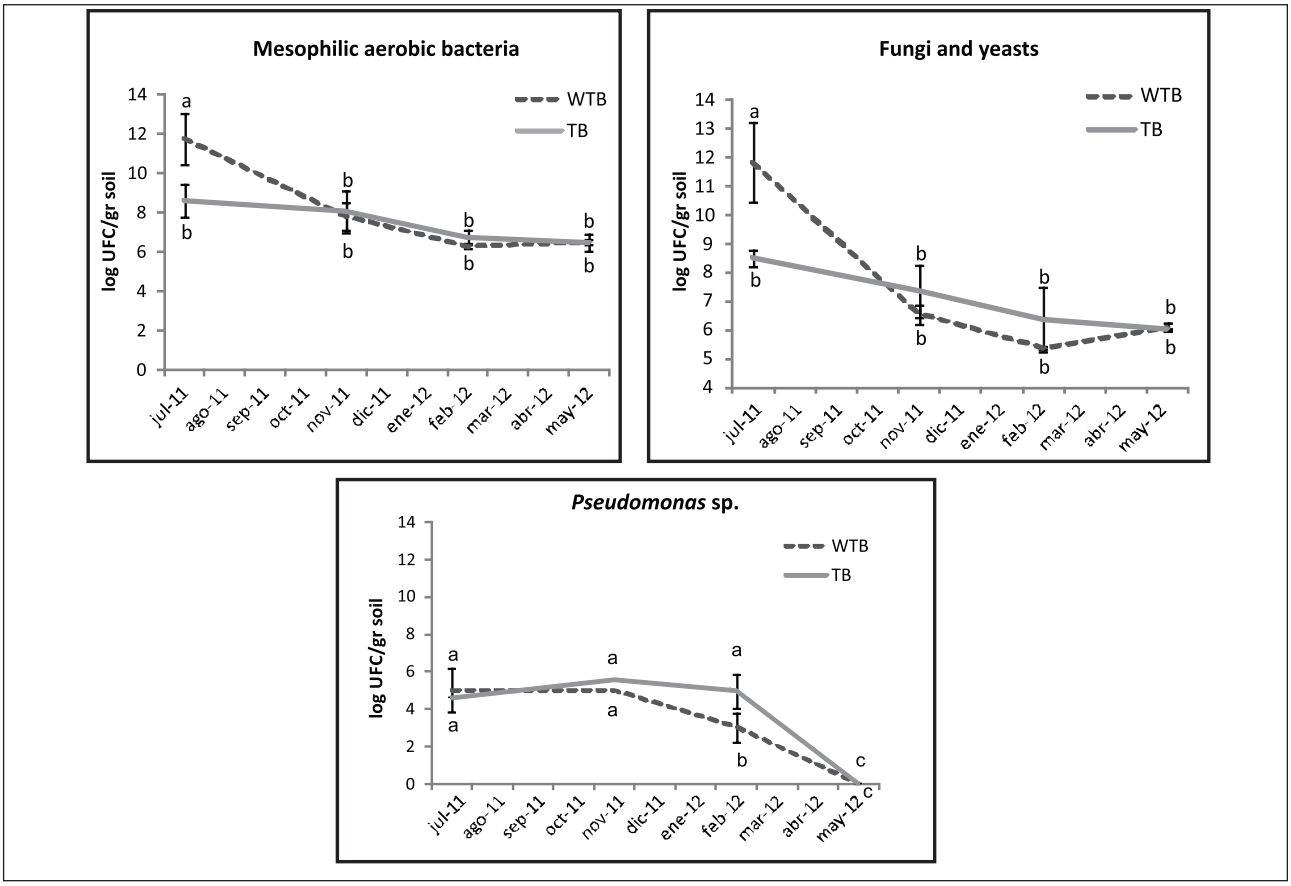

Figure 1. Count of culturable microorganisms in soil samples collected in different seasons. (- - -) and (——) lines represent the content of microorganisms in soil samples from fields without trash blanket (WTB), and from fields with trash blanket (TB), respectively. Mesophilic aerobic bacteria were counted on LB solid medium, fungi and yeasts on PGA solid medium, and *Pseudomonas* genus bacteria on CA solid medium.

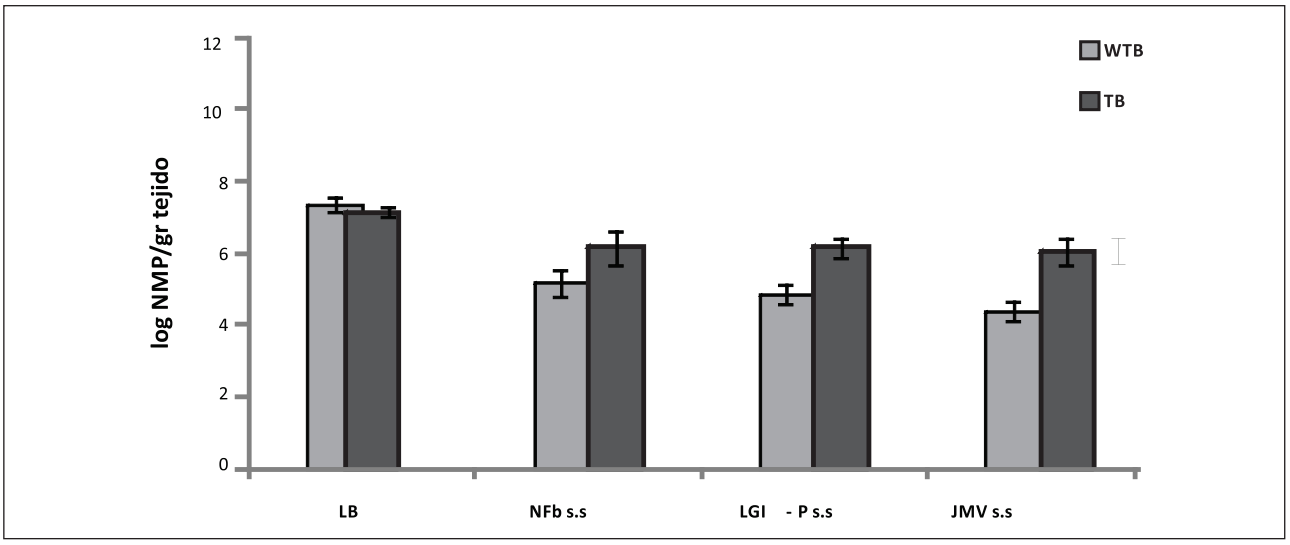

Figure 2. Count of different endophytic microorganisms in stalk samples collected in June 2011. Black and grey bars represent microorganism populations present in stalk samples from fields without trash blanket (WTB), and with trash blanket (TB), respectively. Mesophilic aerobic bacteria were counted on LB solid medium, and microaerobic nitrogen fixing bacteria were counted on different semisolid medium: *Azospirillum* sp. on NFb, *Gluconoacetobacter* and *Pantoea* on LGI-P, and *Burkholderia* sp. on JMV. Different letters indicate significant differences at $P \leq 0.05$.

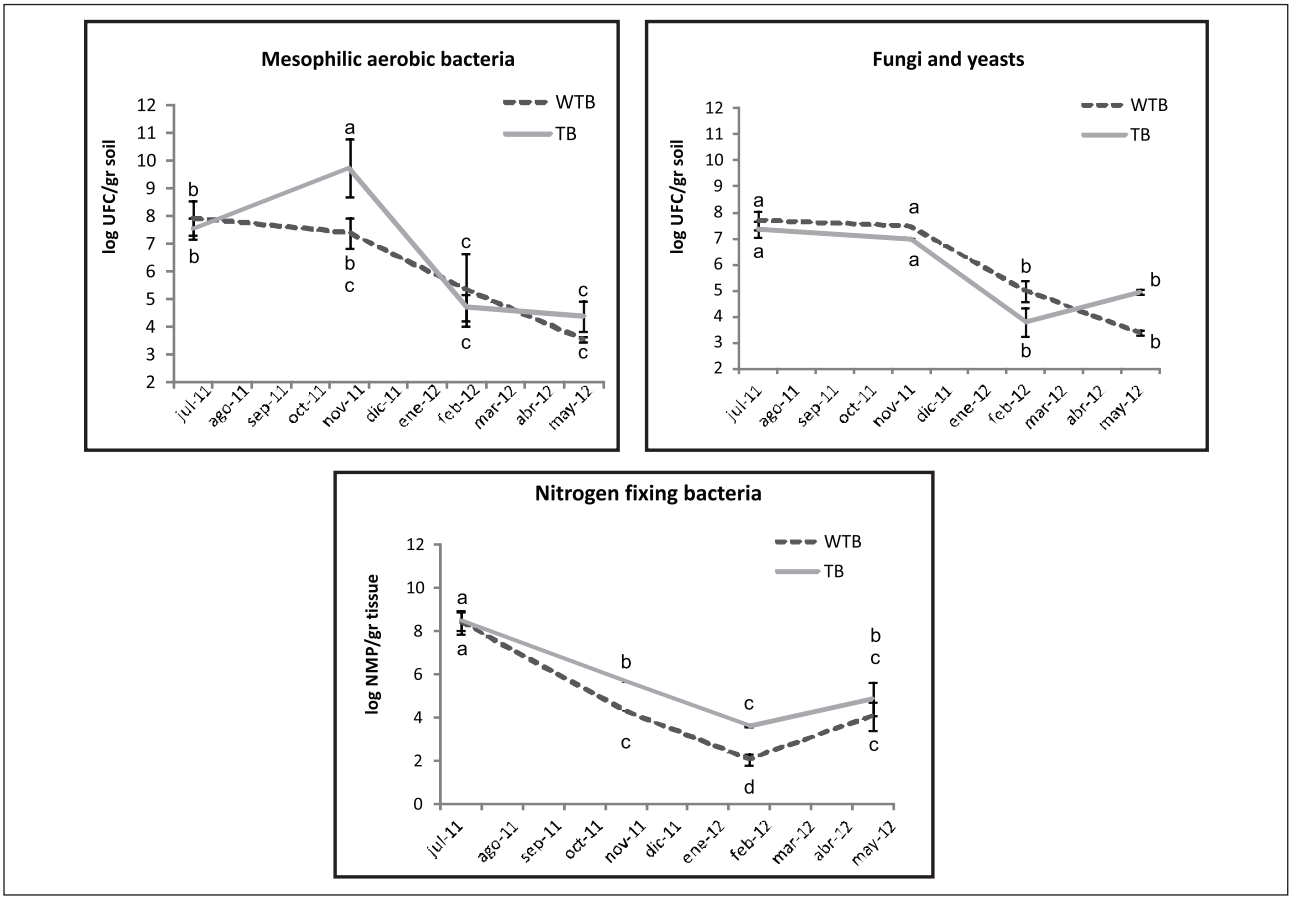

Figure 3. Count of culturable microorganisms on stem samples collected in different seasons. (- - -) and (——) lines represent microorganism contents in stem samples collected from fields without trash blanket (WTB), and fields with trash blanket (TB), respectively. Mesophilic aerobic bacteria were counted on LB solid medium, fungi and yeasts on PGA solid medium, and nitrogen fixing bacteria on NFb semisolid medium.

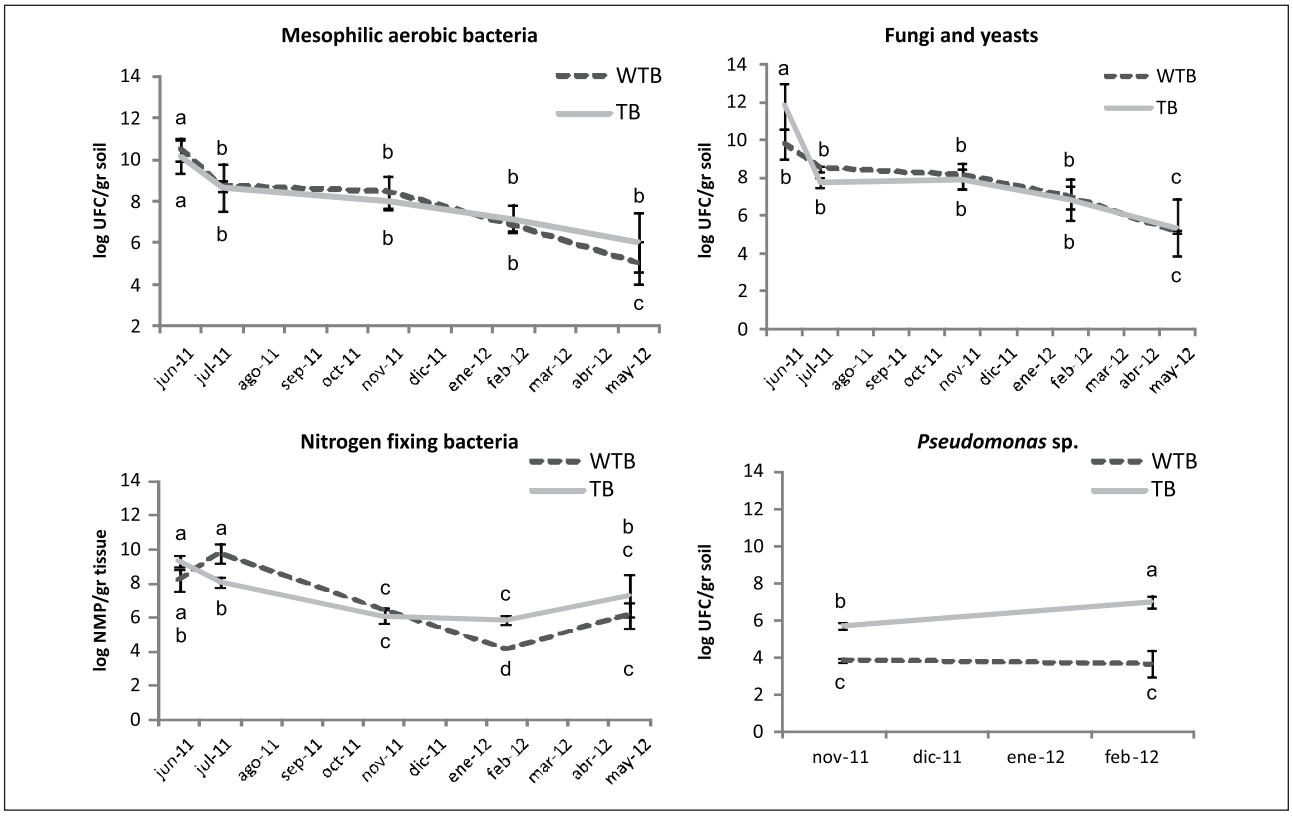

Figure 4. Count of rhizosphere microorganisms in samples collected in different seasons. (- - -) and (——) lines represent rhizosphere microorganism populations in sugar cane root samples collected from fields without trash blanket (WTB), and fields with trash blanket (TB), respectively. Mesophilic aerobic bacteria were counted on LB solid medium, fungi and yeasts on PGA solid medium, *Pseudomonas* sp. on CA solid medium, and nitrogen fixing bacteria on NFb semisolid medium.

Some bacteria that grew on CA solid medium were tested for their ability to solubilize phosphorus, by plating them on NBRIP medium. Five bacteria isolated from soil samples and three from stalk samples were able to solubilize phosphorus, as evidenced by clear halos surrounding the bacterial colonies (Figure 5). However, the ability to solubilize phosphorus depended on the origin of the strains: after seven days of incubation, isolates from soil samples had a better performance in solubilizing phosphate than those from stalks (Figure 5). This could be explained by the fact that endophytic bacteria live in a protected environment inside the host plant, and have all the nutrients they need for survival. Therefore, bacterial mechanisms for phosphate solubilization must be less efficient than in the rhizospheric environment. Although soil phosphate content was not statistically different between treatments, we observed that isolates from trash blanketed fields solubilized tricalcium phosphate better than isolates from soil of fields without residue cover.

Isolation of fungi and yeasts from sterile and non sterile sugar cane residues was performed on PGA solid medium (Figure 6). After seven days of incubation at 30ºC and under both sterile and non sterile conditions, we observed a very low diversity of fungi and yeasts, indicating that there are only a few species that were able to survive and which remained in sugar cane residues.

When analyzing trash composition, we observed that it consisted of approximately 35% cellulose, 40% hemicellulose and 25% lignin. Many microorganisms are known to be able to degrade and utilize cellulose and hemicellulose as carbon and energy sources, but only a much smaller group of filamentous fungi has the ability to break down lignin, the most recalcitrant component of plant cell walls (Sanchez, 2008). Considering this, representative fungi and yeasts observed above were plated on PGA solid medium supplemented with guaiacol 0.1 % (v/v), in order to analyze their ligninolytic activities. Three fungal strains with proven ligninolytic activities were used as positive controls. Only one of the tested strain isolates from the sterile trash sample (named as sterile trash strain 2) exhibited a small brown halo during the incubation period (Figure 7). Unlike control strains, the slow growth of this fungal strain could serve as an explanation for the small halo observed around the colony (Figure 7).

CONCLUSIONS

We conclude that when sugar cane harvest residues remain over the soil, they have strong effects on microbial communities in the soil and in different sugar cane tissues,

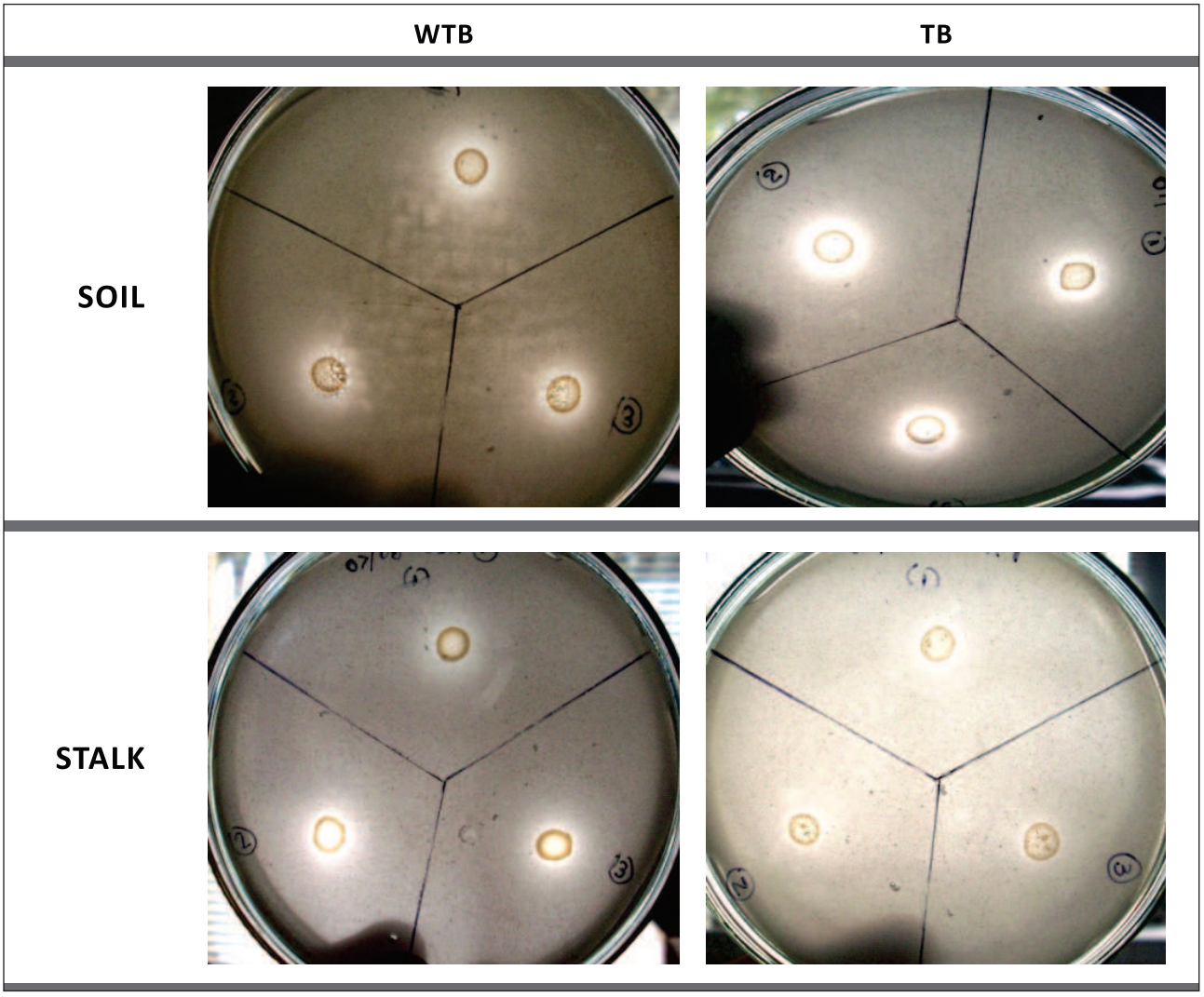

Figure 5. Phosphate solubilization activity of different strains isolated from soil and sugar cane samples collected in fields with (TB) and without (WTB) trash blanket.

but that is restricted to high temperature months. Our results indicate that trash blanket promotes the growth of soil and rhizosphere microorganisms, some belonging to the *Pseudomonas* genus, which have the ability to solubilize phosphorus. Trash blanket also increases the number of rhizosphere and endophytic nitrogen fixing bacteria capable of colonizing inner sugar cane stalk and stem tissues. A higher amount of nitrogen and available phosphorus could promote a better plant growth under nutrient-limited conditions.

The great number of soil fungi and yeasts recorded when residues were kept over the soil could serve as an explanation for the high residue decomposition rates observed during high temperature months. This hypothesis correlates with the ligninolytic activity observed in some fungal strain isolates from sterile trash fragments.

ACKNOWLEDGMENTS

We thank Dr. Carlos Gusils for providing unrestricted access to all of the laboratory reagents and equipment under his charge.

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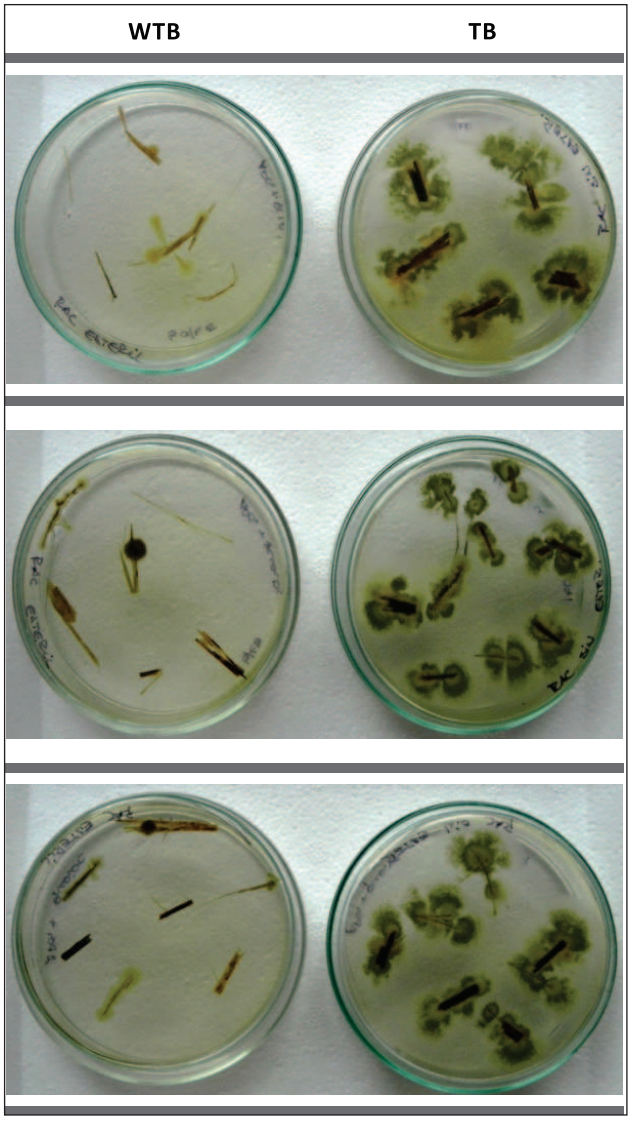

Figure 6. Fungus and yeast growth on PGA solid medium from sterile and non sterile trash fragments, after seven days of incubation at 30ºC.

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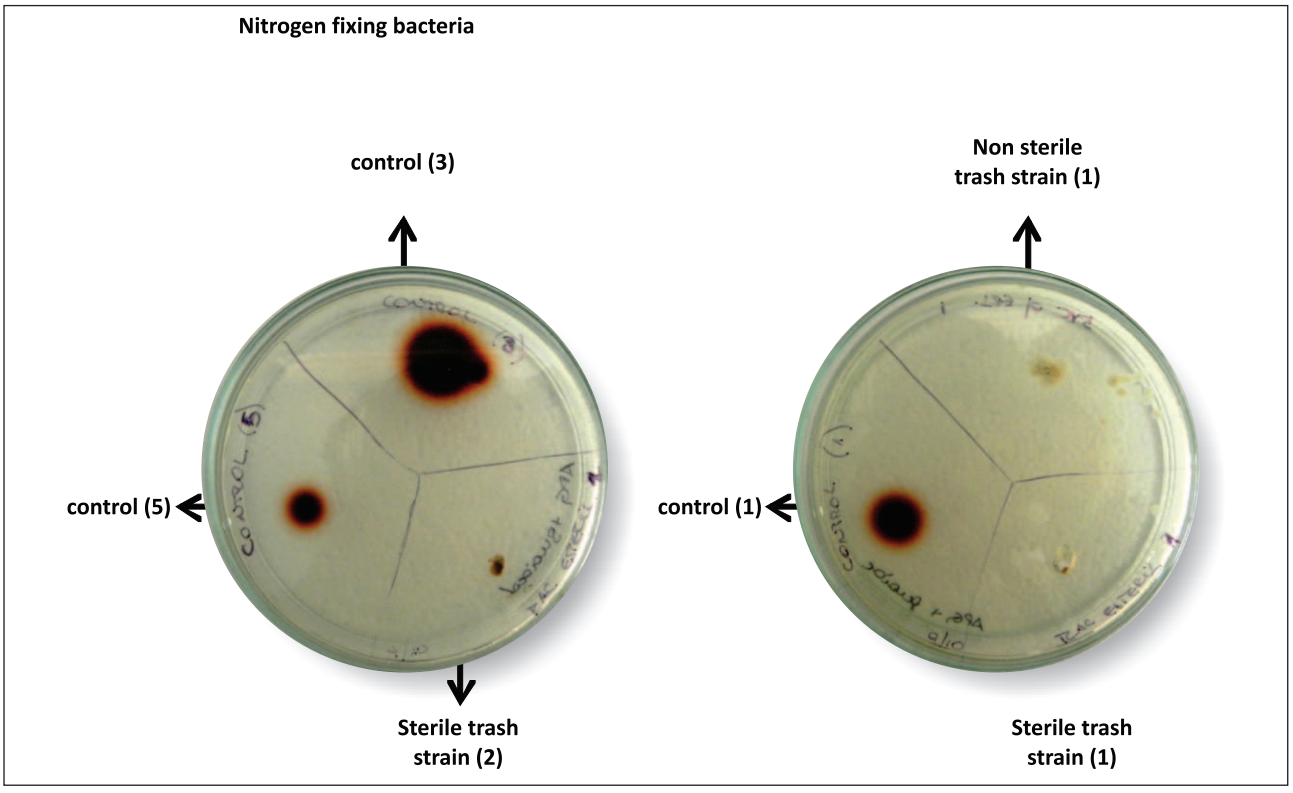

Figure 7. Ligninolytic activity of fungal strains isolated from sterile and non sterile trash fragments, after seven days of incubation at 30ºC. Tests were performed on PGA solid medium, supplemented with guaiacol 0.1% (v/v).

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