

**Division - Soil Processes and Properties** | Commission - Soil Biology

# Microbial Communities in Soil Cultivated with Muskmelon under Different Management Systems

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**ABSTRACT:** Microorganisms have a fundamental importance in agricultural ecosystems and may be influenced by several factors, including soil management. The aim of this study was to evaluate the effects of cropping systems and soil covers on the microbial community in soil cultivated with muskmelon (Cucumis melo L.). The experiment was conducted in a randomized complete block design with four replications. The treatments were arranged in a split plot design with cropping systems (no-tillage and conventional tillage systems) assigned to the whole plot treatments, while a group of seven soil covers (sunn hemp; millet; sunn hemp + millet; corn + brachiaria; spontaneous vegetation; bare soil; and spontaneous vegetation + polyethylene film) defined the subplot treatments. Total bacteria, sporulating bacteria, fluorescent Pseudomonas sp., and total fungi were quantified at six different times (in fallow soil, at planting of green manures in the soil, when transplanting muskmelon seedlings, and 20, 40, and 60 days after transplanting [DAT]). To determine the quantity of microorganisms, the plate count method was used, with a specific culture medium for the groups. The cultivation of sunn hemp associated with no-tillage at transplanting of muskmelon showed a greater quantity of colony forming units (CFUs) of total bacteria compared to the conventional tillage system. In most treatments, conventional tillage showed greater amounts of sporulating bacteria in relation to no-tillage at the time of transplanting muskmelon and at 40 DAT. The tillage systems and soil cover did not change the total amount of fungi and fluorescent Pseudomonas sp.

**Keywords:** Cucumis melo L., plant cover, soil microorganisms.

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#### INTRODUCTION

Brazil is one of the main global producers of muskmelon, and the Northeast part of the country is responsible for approximately 95 % of the total fruit production (IBGE, 2013). Despite the high technology used on muskmelon crops, there is little information on the effects of these soil management practices on the environment.

Soil microorganisms are fundamental for agricultural ecosystems and are involved in important processes and biogeochemical cycles. Growth-promoting bacteria of plants, among them sporulating bacteria such as *Bacillus* spp. and fluorescent *Pseudomonas* sp., have major agricultural importance since, in addition to favoring plant development, they may act as biological control agents for plant diseases. Several researchers have reported on the importance of these bacteria for development of different crops and induction of resistance to and control of phytopathogens that attack both the shoots and root system of the plant (Teixeira et al., 2005; Araújo and Marchesi, 2009).

The composition of the soil microbial community is influenced by several factors, such as temperature, moisture, soil aeration, nutrient availability, and organic substrates. Physical and chemical factors, in turn, may be modified by the management system, due to how the residues of previous crops are deposited and the degree of soil disturbance (Vargas and Scholles, 2000).

Soil tillage methods, such as the no-tillage and the conventional tillage system, may change edaphic conditions and, consequently, influence the biological processes of the soil (Alvarez et al., 1995; Vargas et al., 2004). Some studies report an increase in soil microbial biomass in the no-tillage system in relation to the conventional tillage system, and they associate these results to the fact that the no-tillage system induces an increase in living and dead organic matter content, considering that the former is constituted by microorganisms that are part of the active portion of organic matter and are responsible for the decomposition, mineralization, and immobilization of animal and plant residues (Santos et al., 2005; Nascimento et al., 2009).

The use of plant soil cover has been considered one of the main alternatives for conservation and improvement of the agricultural environment, reducing soil degradation, especially in regard to organic matter, since this practice improves the physical, chemical, and biological properties of the soil (Eiras and Coelho, 2012).

Furthermore, soil cover with plant residue in the no-tillage system may offer lower heating and soil thermal amplitude, which, in a tropical climate with high environmental temperature, favors crop development (Resende et al., 2005; Coelho et al., 2013) and offers favorable conditions in terms of water, oxygen, and substrate availability, favoring the soil microbial community (Moreira and Siqueira, 2002). However, the response of the microbial community to the tillage and soil cover systems depends on the species used and the edaphic and climatic conditions (Gama-Rodrigues et al., 2005; Cunha et al., 2014).

Some researchers reported the influence of the no-tillage system on increasing the soil microbial community. Cunha et al. (2014) reported that soil cover with plant residue in the no-tillage system reduced heating of the soil in relation to treatments without soil cover, offering better conditions for development of total fungi, bacteria, and actinomycetes. Carneiro et al. (2004), upon studying the biological indicators associated with the phosphorus cycle on *Cerrado* (Brazilian tropical savanna) soils with no-tillage and conventional tillage systems, also observed that the no-tillage system offers a more favorable environment for the occurrence of total fungi and bacteria, as well as phosphate solubilizers, at a depth of 0.00-0.05 m.

Several methods are used to evaluate the soil microbial community, and plate count is the technique most used to determine the size of a bacterial population since this method measures the number of viable cells. Along with this methodology, the serial dilution



method is used to assure that the number of colonies on the plate remains within the desired range (Tortora et al., 2012).

Few studies report the effects of tillage systems and soil cover on the microbial community (total bacteria, sporulating bacteria, fluorescent *Pseudomonas* sp., and total fungi) in muskmelon production. The hypothesis of this study was that the no-tillage system for muskmelons and plant covers increase the soil microbial community. The objective of this study was to evaluate the effects of tillage systems and plant cover on the microbial community of soil cultivated with muskmelon.

### **MATERIALS AND METHODS**

The experiment was conducted at the Famosa Farm, located at 4° 52′ 4.13″ South latitude and 37° 20′ 16.94″ West longitude, between the municipalities of Tibau, RN, and Icapuí, CE, Brazil. The climate of the region, according to the Köppen classification system, is BSh, warm and dry, with mean annual rainfall around 700 mm; mean air temperature is 26.7 °C and relative humidity is 68.9 %. The rainy period in the region is from February to June, with low probability of rain between August and December (Alvares et al., 2014).

The experimental area had been fallow for 3 years and had a history of the muskmelon crop and diseases caused by pathogens that soilborne pathogens. Before sowing the species intended for soil cover, all the vegetation of the area was removed, except in the experimental plots with treatments in which spontaneous vegetation was maintained. At the time of implementation of the experiment, the soil in the experimental area had the following chemical properties in the 0.00-0.20 m layer: pH(H<sub>2</sub>O) 7.40; P 60 mg dm<sup>-3</sup>; K 28.83 mg dm<sup>-3</sup>; SB 3.07 cmol<sub>c</sub> dm<sup>-3</sup>; Mg<sup>2+</sup> 0.64 cmol<sub>c</sub> dm<sup>-3</sup>; Al<sup>3+</sup> 0.0 cmol<sub>c</sub> dm<sup>-3</sup>; CEC 4.29 cmol<sub>c</sub> dm<sup>-3</sup>; organic matter (OM) 0.65 dag kg<sup>-1</sup>; and V 71.63 %. Physical analysis showed the following composition: 881 g kg<sup>-1</sup> of sand; 53 g kg<sup>-1</sup> of silt; and 66 g kg<sup>-1</sup> of clay.

A randomized block experimental design was used, with four replications. The treatments were arranged in a split-plot design, and the plots consisted of two soil tillage systems: the conventional tillage system (incorporation of cover plants through harrowing) and the no-tillage system (desiccation of cover plants using herbicide, with the plant residue remaining on the soil surface). For the split-plots, seven soil cover strategies were used (sunn hemp; millet; sunn hemp + millet; corn + brachiaria; spontaneous vegetation; bare soil; and spontaneous vegetation + polyethylene film). All conventional tillage treatments had polyethylene film, since this material is normally used for commercial production of muskmelon in Rio Grande do Norte state, Brazil, as it increases moisture retention, maintains constant soil temperature, and helps in weed control (Sampaio and Araújo, 2001). Each experimental unit consisted of three 6-m-length rows of muskmelon at a spacing of 2.0 m, and muskmelon plants were 0.3 m away from each other in the rows.

The species were planted in the conventional tillage and no-tillage plots so as to obtain the straw during the rainy period. Planting was on April 18, 2011 (for the treatments with corn + brachiaria) and on May 15, 2011 (for the other crops). The difference in the planting time of green fertilizer crops (brachiaria, sunn hemp, millet, and corn) is due to the fact that the crops have different development cycles, and corn and brachiaria are the species with the longest cycles.

In the case of the corn + brachiaria treatments, the corn was sown in a double row, with the brachiaria grass placed between the rows (intercropping). Sowing and fertilization for planting brachiaria were conducted together with corn in the plant row, using a planter/fertilizer device. Fertilization at planting was NPK - 6-24-12 (350 kg ha<sup>-1</sup>) and topdressed fertilization was through fertigation, using urea (200 kg ha<sup>-1</sup>), according to the result of soil chemical analysis, following the recommendations of Cavalcanti (1998).



After harvesting the corn, the brachiaria grass was allowed to grow freely up to September 2011, when the first desiccation was conducted with the use of 1.90 kg ha<sup>-1</sup> of glyphosate herbicide, 20 days before transplanting the muskmelon.

In the plots with conventional tillage, the soil was plowed and then harrowed twice. After desiccation of green manures and one week before transplanting the muskmelon seedlings, the materials were incorporated in the soil. In the no-tillage system, there was no soil turnover, and the desiccated materials were kept on the soil surface.

Muskmelon seedlings from the Goldex hybrid were transplanted; then, agricultural textile tunnels (nonwoven fabric) were installed over the rows, which were maintained up to 25 days after the transplanting, for the purpose of protecting the crop from attack by pests, especially white fly (*Bemisia argentifolii*) and leafminer (*Liriomyza* spp.). After this period, fungicides and insecticides were spray applied as needed, based on observations conducted *in loco*, with use of cyromazine, thiamethoxam, and acetamiprid.

The muskmelon was irrigated after transplanting the seedlings using a drip irrigation system, with emitters with a flow rate of  $1.7 \, L \, h^{-1}$  spaced at a distance of  $0.4 \, m$ , with the daily watering rate determined by full restitution of crop evapotranspiration, estimated by the Penmam-Motheith equation (Allen et al., 2006) and the crop coefficient (Kc), as recommended by the United Nations Food and Agriculture Organization (FAO, 2015).

Quantification of the soil microbial community (total bacteria, sporulating bacteria, fluorescent *Pseudomonas* sp., and total fungi) was conducted based on collection of three simple soil samples from the 0.00-0.10 m layer, with the aid of an auger. The samples were homogenized to create one compound sample per treatment; the auger was washed with water and disinfested with 70 % alcohol at every replication within and across the treatments. The soil collections were conducted at six different times: in fallow soil; at planting of green manures in the soil; when transplanting muskmelon seedlings; and 20, 40, and 60 days after transplanting the seedlings (DAT).

The collected samples were sieved through a 2.0 mm mesh; the soil that was retained to the sieves was removed, and the sieves were washed with water and disinfected with 70 % alcohol for every replication, within and across the treatments. The samples were packed in plastic bags, transported, and then refrigerated at 10 °C. Serial dilution and the method of plating on a specific medium were applied to quantify each group of microorganisms. For that purpose, 1 g of soil was taken from each sample, which was placed in test tubes with 9 mL of distilled and sterilized water. From this soil/water suspension, serial dilutions were conducted up to  $10^{-5}$ , which were homogenized to collect a  $100~\mu\text{L}$  aliquot of each dilution, placed on 9.0 cm diameter Petri dishes on the culture media, and then, they were distributed with a Drigalski spatula on the surface of the solidified culture medium. The plates were inverted and kept in a biochemical oxygen demand (BOD) chamber at a temperature of  $28\pm2$  °C for five days.

In order to count total fungi, a Martin medium was used ( $KH_2PO_4 - 1.0$  g;  $MgSO_4.7H_2O - 0.5$  g; peptone - 5.0 g; dextrose - 10.0 g; rose bengal - 0.03 g; agar - 16.0 g; distilled water - 1000 mL) (Martin, 1950). For total and sporulating bacteria, a nutrient agar medium was used (nutrient agar - 23.0 g; distilled water - 1000 mL) and, for the *P. fluorescens* count, the King B base medium was used (Himedia) (proteose peptone # 3 - 20.0 g;  $MgSO_4.7H_2O - 1.5$  g;  $K_2HPO_4 - 1.5$  g; agar - 20.0 g; distilled water - 1000 mL) (King et al., 1954).

The plate counting method was chosen due to the significant advantage of quantifying viable cells. For each dilution evaluated, three plates were used, and the dilutions considered for calculation were only the ones that had from 20 to 200 colonies per plate (Tortora et al., 2012). For sporulating bacteria, the procedure used was similar to the one described above; however, the soil suspension obtained was placed on bain-marie at 80 °C for 20 min, before plating on the culture medium, for the purpose of selecting these bacteria (Bettiol, 1995).



The percentage of sporulating bacteria was calculated in relation to total bacteria in order to verify the treatment that most influences the sporulating communities.

For the fluorescent *Pseudomonas* sp. bacteria, the colonies that were fluorescent under the light were considered as part of the fluorescent group, with wavelength close to ultraviolet. The values obtained were converted into colony forming units per gram (CFU  $g^{-1}$ ).

Soil temperature was recorded with copper-constantan thermocouple sensors wrapped on polyethylene foam to avoid oxidation of the thermocouple, at a depth of 0.05 m. The data were collected every 10 min and stored on Campbell CR 1000 dataloggers. From the data, soil temperature variation was verified throughout the day for each treatment (Figure 1).

The data obtained were subjected to analysis of variance through the F test at 5 % probability, and the means were compared according to Tukey's test at 5 % probability. The statistical program SAEG (2007) was used. The error suppositions of the analyses of variances were met.

### **RESULTS AND DISCUSSION**

The maximum temperature of the soil with the muskmelon showed the highest variation among treatments in the no-tillage system, and the treatments with millet and spontaneous vegetation cover were the ones that offered the lowest temperature along the crop cycle (Figure 1) due to greater shading of the soil in relation to the soil with no plant cover. Torres et al. (2006) also verified that millet in the no-tillage system showed low temperatures and thermal amplitudes of the soil in rotation with corn or soybean, certainly due to the large amount of dry matter produced by this material (Jimenez et al., 2008), which has slower decomposition and keeps the soil covered for a longer period of time. Within the period from 10 to 18 days after transplanting (DAT) the seedlings, the spontaneous vegetation + polyethylene film treatment in the conventional tillage system was among the treatments with the highest temperatures; however, in the period from 30 to 46 days after transplanting, this did not occur, due to shading of muskmelon.

In most evaluation times, the number of colony forming units (CFUs) of total bacteria in the soil was not influenced by the tillage and plant cover system, except for the third time (transplanting of muskmelon seedlings) (Table 1).

The reason for a difference among the treatments only during this time may be related to the fact that this was the first sample collection after desiccation of the materials, where there was a greater amount of straw on the soil and, consequently, a greater amount of organic matter. This was also verified by Cardoso et al. (2013) in a study verifying the effects of green manure on microbial activity of the soil with the soybean crop. Another hypothesis would be due to the irrigations, since they started at the time of transplanting the seedlings. Microbial activity is increased by favorable moisture conditions (Moreira and Siqueira, 2006).

In the conventional tillage system, the soil cropped under spontaneous vegetation + polyethylene film showed a higher number of bacteria in relation to the soil with no vegetation. This is possibly due to the presence of the polyethylene film, which offered higher moisture levels (Tosta et al., 2010), favoring the growth and development of this group of microorganisms in the soil. In addition, the diversity of species in spontaneous vegetation may favor some groups of soil microorganisms (Almeida et al., 2014). These authors observed a larger number of CFUs of bacteria in a treatment consisting of a combination of gramineae, leguminosae, and brassica, and related these results to the superior environmental and nutritional conditions offered by the diversity of plants to the soil microbiota,



favoring its multiplication in the area. In the experiment, the weeds identified were verdolaga (*Portulaca oleraceae* L.), slender amaranth (*Amaranthus spinosus* L.), desert horsepurslane (*Triantema portucastrum* L.), green carpet-weed (*Mollugo verticillata* L.), scarlet starglory [*Merremia aegyptia* (L.) Urban.], dayflowers (*Commelina benghalensis* L.), and alexandergrass [*Digitaria bicornis* (Lam.) Roemer & Schultes]. This diversity also

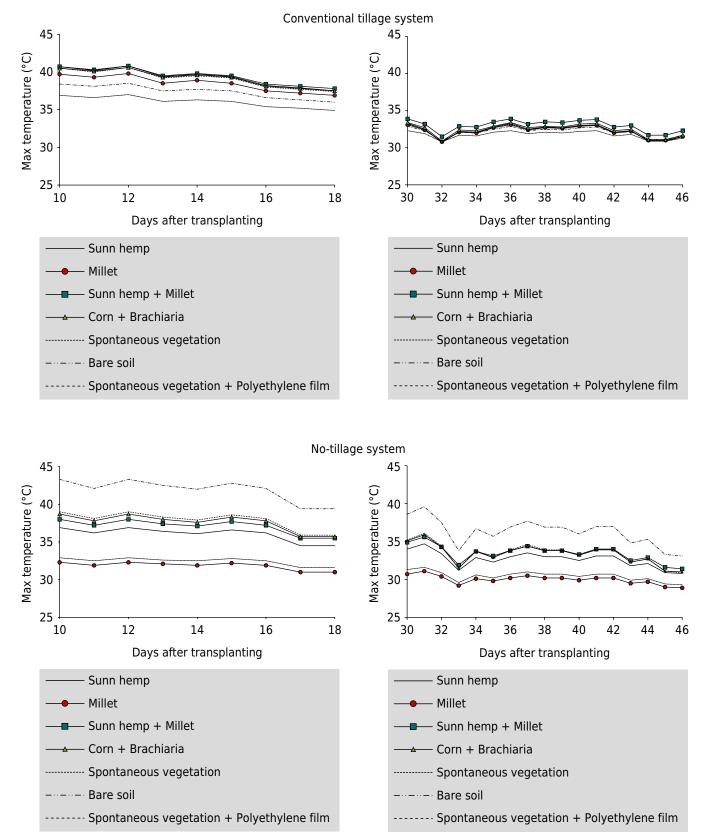


Figure 1. Maximum (Max) daily soil temperatures during the muskmelon cycle according to cropping systems and soil covers.



results in different decomposition times of the plant material, due to variations in the carbon/nitrogen (C/N) ratio of each species. This may favor the soil microbial community upon releasing substances that promote plant growth, in addition to the fact that this community is antagonistic to pathogens.

Sunn hemp associated with the no-tillage system offered a greater amount of CFUs of total bacteria in relation to the same treatment in the conventional tillage system (Table 1). Sunn hemp is one of the species most used as green manure, mainly due to the fact that it fixes atmospheric N when in symbiosis with diazotrophic bacteria (Dourado et al., 2001). This leads to a high content of this nutrient in the soil, which is highly important for the growth of the bacteria (Tortora et al., 2012).

The no-tillage system of millet, corn + brachiaria, and spontaneous vegetation + polyethylene film on the straw reduced the number of total bacteria in relation to the conventional tillage system (Table 1). According to Moreira and Siqueira (2006), organic matter and the rhizosphere effect are due to plant cover of the soil, which has a major influence on soil organisms. The soil with no plant cover tends to have less organic matter and a smaller community and biological diversity. Variation in groups of soil microorganisms was also verified by Cunha et al. (2014) in a study on the effects of the no-tillage and the conventional tillage systems associated with weed management on the soil microbial community with the pepper crop. Sanomiya and Nahas (2003) also reported that the management system, fertilization, and the crop affect the density of soil microorganisms.

**Table 1.** Number of colony forming units (CFUs) of total bacteria throughout the muskmelon cycle in different management systems and soil sampling times.

	Total bacteria					
Management system	Fallow area	Upon planting of green manure crops	Upon transplanting of muskmelon	20 DAT	40 DAT	60 DAT
	—————————————————————————————————————					
			Conventional t	illage		
Sunn Hemp	23.6±3.4 <sup>ns</sup>	16.5±2.2 <sup>ns</sup>	8.4±7.6 Bab	$9.81 \pm 5.7^{ns}$	$13.6 \pm 4.0^{ns}$	$8.0\pm2.1^{ns}$
Millet	17.8±5.4 <sup>ns</sup>	$11.8 \pm 1.0^{ns}$	9.7±6.4 Aab	5.1±1.6 <sup>ns</sup>	19.9±18.7 <sup>ns</sup>	$12.6 \pm 1.2^{ns}$
Sunn Hemp + Millet	23.0±6.2 <sup>ns</sup>	16.4±2.1 <sup>ns</sup>	9.0±4.6 Aab	$19.5 \pm 20.9^{ns}$	$12.5 \pm 6.7^{ns}$	16.7±20.6 <sup>ns</sup>
Corn + Brachiaria	21.4±5.3 <sup>ns</sup>	13.8±2.4 <sup>ns</sup>	10.6±6.3 Aab	16.5±12.6 <sup>ns</sup>	13.3±2.6 <sup>ns</sup>	$13.5 \pm 6.2^{ns}$
Spontaneous vegetation	$16.7 \pm 6.4^{ns}$	$9.5 \pm 2.7^{ns}$	8.4±1.4 Aab	7.8±5.9 <sup>ns</sup>	17.2±9.4 <sup>ns</sup>	$12.1 \pm 9.7^{ns}$
Soil with no vegetation	21.7±9.3 <sup>ns</sup>	12.5±1.6 <sup>ns</sup>	6.2±11.7 Ab	$10.9 \pm 10.6^{ns}$	$9.7 \pm 3.7^{ns}$	$10.7 \pm 3.5^{ns}$
Spontaneous vegetation + Polyethylene film	12.0±2.6 <sup>ns</sup>	11.4±2.0 <sup>ns</sup>	11.1±7.0 Aa	8.4±2.6 <sup>ns</sup>	14.4±1.9 <sup>ns</sup>	11.9±2.8 <sup>ns</sup>
			No-tillage	1		
Sunn Hemp	9.9±1.7 <sup>ns</sup>	10.2±4.5 <sup>ns</sup>	11.6±9.8 Aa	24.1±22.5 <sup>ns</sup>	16.4±0.4 <sup>ns</sup>	28.2±20.7 <sup>ns</sup>
Millet	15.5±4.0 <sup>ns</sup>	10.1±1.7 <sup>ns</sup>	6.7±8.2 Bb	22.9±13.1 <sup>ns</sup>	19.1±12.2 <sup>ns</sup>	11.8±3.7 <sup>ns</sup>
Sunn Hemp + Millet	13.9± 3.6 <sup>ns</sup>	7.0±1.3 <sup>ns</sup>	7.9±1.4 Aab	10.3±7.5 <sup>ns</sup>	18.0±13.2 <sup>ns</sup>	11.3±4.6 <sup>ns</sup>
Corn + Brachiaria	14.7±2.2 <sup>ns</sup>	7.5±2.6 <sup>ns</sup>	7.4±2.1 Bb	11.6±3.1 <sup>ns</sup>	27.7 ±15.6 <sup>ns</sup>	10.1±4.0 <sup>ns</sup>
Spontaneous vegetation	$9.4 \pm 1.0^{ns}$	6.6±1.6 <sup>ns</sup>	8.9±1.3 Aab	22.6±17.9 <sup>ns</sup>	21.6±13.5 <sup>ns</sup>	21.0±30.0 <sup>ns</sup>
Soil with no vegetation	$13.7 \pm 6.8^{ns}$	6.9±1.2 <sup>ns</sup>	7.8±1.8 Aab	15.2±10.9 <sup>ns</sup>	$12.3 \pm 7.2^{ns}$	13.6±4.9 <sup>ns</sup>
Spontaneous vegetation + Polyethylene film	12.1± 4.2 <sup>ns</sup>	18.7±0.8 <sup>ns</sup>	5.7±12.6 Bb	25.4± 14.0 <sup>ns</sup>	18.7±12.4 <sup>ns</sup>	7.2±2.5 <sup>ns</sup>
CV (%)	30.8	61.1	24.4	85.4	59.4	90.5

In the columns, the means followed by the same lowercase letter do not differ from each other within each tillage system, and the means followed by the same uppercase letter do not differ across the systems according to Tukey's test at 5 % probability. <sup>ns</sup>: not significant at 5 % probability. DAT: days after transplanting; CV: coefficient of variation.



Although some *Pseudomonas* species and sporulating bacteria are phytopathogens, other are related to beneficial effects on plants, such as the fluorescent *Pseudomonas* sp. and some sporulating bacteria, which are considered to be biocontroller bacteria and plant growth-promoting bacteria (BPCPs) (Araújo and Marchesi, 2009; Ferreira et al., 2009). These bacteria may promote the growth of plants using different mechanisms, among them, the production of plant hormones; however, few studies report the influence of agricultural practices on the populations of sporulating bacteria and fluorescent *Pseudomonas* sp. In this study, tillage and crop management practices changed the amount of sporulating bacteria only in the third and fifth evaluations (Table 2).

The millet, sunn hemp + millet, spontaneous vegetation, and spontaneous vegetation + polyethylene film treatments differed in relation to the tillage systems; however, the conventional tillage system showed a higher number of CFUs of sporulating bacteria in relation to the no-tillage system (Table 2). The millet treatment within the conventional tillage system offered a larger amount of CFUs of sporulating bacteria in relation to millet + brachiaria and soil with no vegetation (Table 2). The other treatments showed no difference in relation to each other.

At the fifth time of evaluation (40 DAT), differences occur between the systems for the millet, sunn hemp + millet, soil with no vegetation, and spontaneous vegetation + film treatments, and, as observed at the third time of evaluation, most of the treatments of the conventional tillage system offered a greater number of sporulating bacteria. Within this same system for the sunn hemp + millet treatment, the number of CFUs of sporulating bacteria was higher in relation to the soil with no vegetation. For the no-tillage system, the corn + brachiaria treatment offered a larger number of sporulating bacteria in relation to spontaneous vegetation (Table 2).

**Table 2.** Number of colony forming units (CFUs) of sporulating bacteria in the no-tillage and in the conventional tillage systems in different management systems and soil sampling times.

	Sporulating bacteria							
Management system	Fallow area	Upon planting of green manures	Upon transplanting of muskmelon	20 DAT	40 DAT	60 DAT		
		- 10⁵ of the number	of CFUs g <sup>-1</sup> ± 10 <sup>5</sup> c	of the standar	d deviation —			
			Conventional tilla	age				
Sunn Hemp	$3.1\pm0.7^{ns}$	$3.7 \pm 1.3^{ns}$	5.1±1.1 Aab	$4.1 \pm 1.6^{ns}$	4.2±1.2 Aab	$3.9 \pm 1.3^{ns}$		
Millet	$4.0\pm1.3^{ns}$	$3.4 \pm 1.1^{ns}$	7.1±2.1 Aa	$3.5 \pm 1.2^{ns}$	4.5±0.9 Aab	$2.9 \pm 1.1^{ns}$		
Sunn Hemp + Millet	5.3±1.6 <sup>ns</sup>	$3.2 \pm 0.9^{ns}$	5.8±1.7 Aab	$3.3 \pm 1.4^{ns}$	5.1±1.1 Aa	$4.0 \pm 0.9^{ns}$		
Corn + Brachiaria	4.3±0.7 <sup>ns</sup>	$3.7 \pm 1.4^{ns}$	4.2±1.0 Ab	$3.3 \pm 1.0^{ns}$	4.1±1.4 Aab	$3.8 \pm 0.7^{ns}$		
Spontaneous vegetation	$4.2 \pm 1.0^{ns}$	$4.1\pm0.5^{ns}$	5.2±1.0 Aab	$3.3 \pm 1.4^{ns}$	3.4±1.0 Aab	$3.6 \pm 1.7^{ns}$		
Soil with no vegetation	$4.5 \pm 1.8^{ns}$	2.8±1.2 <sup>ns</sup>	4.3±1.0 Ab	$2.6 \pm 1.2^{ns}$	1.9±0.4 Bb	$2.3 \pm 0.3^{ns}$		
Spontaneous vegetation + Polyethylene film	3.5±0.5 <sup>ns</sup>	4.1±1.2 <sup>ns</sup>	5.6±0.9 Aab	4.6±2.0 <sup>ns</sup>	4.9±0.6 Aab	3.6±1.4 <sup>ns</sup>		
			No-tillage					
Sunn Hemp	$3.0\pm0.4^{ns}$	4.3±0.8 <sup>ns</sup>	3.7±1.0 Aab	$2.8 \pm 0.8^{ns}$	2.8±0.9 Ab	$3.0 \pm 1.0^{ns}$		
Millet	3.2±1.9 <sup>ns</sup>	$2.4\pm0.8^{ns}$	3.2±0.5 Bab	$2.3 \pm 1.0^{ns}$	3.0±0.8 Bab	$4.3 \pm 2.7^{ns}$		
Sunn Hemp + Millet	4.5±2.5 <sup>ns</sup>	$3.5 \pm 1.2^{ns}$	2.2±0.7 Bb	$2.7 \pm 0.7^{ns}$	3.3±0.7 Bab	$2.5 \pm 0.4^{ns}$		
Corn + Brachiaria	$3.3 \pm 1.7^{ns}$	4.1±0.2 <sup>ns</sup>	3.8±1.0 Aab	$3.3 \pm 1.2^{ns}$	5.0±1.1 Aa	$2.5 \pm 0.4^{ns}$		
Spontaneous vegetation	$3.4 \pm 1.2^{ns}$	4.7±2.1 <sup>ns</sup>	3.6±1.0 Bab	$2.9 \pm 0.6^{ns}$	2.7±1.8 Ab	$4.0 \pm 1.7^{ns}$		
Soil with no vegetation	$3.0\pm0.9^{ns}$	3.9±1.4 <sup>ns</sup>	5.1±1.2 Aa	$2.8 \pm 0.4^{ns}$	4.5±1.4 Aab	$2.8 \pm 0.6^{ns}$		
Spontaneous vegetation + Polyethylene film	3.5±0.7 <sup>ns</sup>	6.3±2.9 <sup>ns</sup>	3.8±0.4 Bab	3.2±0.7 <sup>ns</sup>	2.8±1.1 Bb	2.8±1.1 <sup>ns</sup>		
CV (%)	36.5	35.3	24.3	35.8	25.4	38.1		

In the columns, the means followed by the same lowercase letter do not differ from each other within each tillage system, and the means followed by the same uppercase letter do not differ across the systems according to Tukey's test at 5% probability. <sup>ns</sup>: not significant at 5 % probability. DAT: days after transplanting; CV: coefficient of variation.



When they studied the frequency of sporulating bacteria of the *Bacillus* spp. type on soils of different irrigated rice cropping systems in Cachoeirinha, Rio Grande do Sul, Brazil, Fritz et al. (2010) observed that there was no significant difference among the tillage systems analyzed. They also observed that the frequency of *Bacillus* spp. varied across the different phases of the rice crop in the periods sampled. However, when Ceja-Navarro et al. (2010) conducted a phylogenetic and multivariate analysis to determine the effects of different agricultural practices on the microbial community of the soil, they identified different groups of bacteria, among them, the phylogenetic group Bacillales, of which the *Bacillus* genus is part. These researchers observed a greater quantity of this microorganism in the residue retention system containing crop residues (wheat and corn) retained on the soil in relation to all the other treatments, among them, the treatment to reduce residues (treatment associated with soil with no vegetation). This is explained by the presence of organic matter in the no-tillage system with crop residues, as opposed to the treatment with no vegetation, which had no organic matter.

By analyzing the percentage of sporulating bacteria in relation to total bacteria (Table 3), it can be observed that, in the conventional tillage system, millet was the material that offered a higher percentage of sporulating bacteria in relation to total bacteria (SBTB) at two soil sampling evaluation times, although, at the end of the crop cycle (60 DAT), the highest percentage was observed in the sunn hemp treatment. It is noteworthy that these treatments are among the ones that caused the highest maximal soil temperatures, especially from 10 to 18 DAT (Figure 1). It could also be concluded for this management system that the period in which the highest percentage of SBTB occurred, for all treatments, was during transplanting of the muskmelon, the time at which irrigation started. This fact was also observed by Fritz et al. (2010); these researchers concluded that irrigation was the main factor that contributed to variation in the frequency of sporulating bacteria of the *Bacillus* sp. type in the tillage systems.

**Table 3.** Sporulating bacteria in relation to total bacteria in the no-tillage and in the conventional tillage systems in different management systems and soil sampling times.

	Sporulating bacteria in relation to the total bacteria						
Management system	Fallow area	Upon planting of green manures	Upon transplanting of muskmelon	20 DAT <sup>(1)</sup>	40 DAT	60 DAT	
			%				
			Conventional tilla	ge			
Sunn Hemp	13.1	22.4	60.7	41.7	30.8	48.7	
Millet	22.4	28.8	73.1	68.6	22.6	23.0	
Sunn Hemp + Millet	23.0	19.5	64.4	16.9	40.8	23.9	
Corn + Brachiaria	20.0	26.8	39.6	20.0	30.8	28.1	
Spontaneous vegetation	25.1	43.1	61.9	42.3	19.7	29.7	
Soil with no vegetation	20.7	22.4	69.3	23.8	19.5	21.4	
Spontaneous vegetation + Polyethylene film	29.1	35.9	50.4	54.7	34.0	30.2	
			No-tillage				
Sunn Hemp	30.3	42.1	31.8	11.6	17.0	10.6	
Millet	20.6	23.7	47.7	10.0	15.7	36.4	
Sunn Hemp + Millet	32.3	50.0	27.8	26.2	18.3	22.1	
Corn + Brachiaria	22.4	54.6	51.3	28.4	18.0	24.7	
Spontaneous vegetation	36.1	71.2	40.4	12.8	12.5	19.0	
Soil with no vegetation	21.8	56.5	65.3	18.4	36.5	20.5	
Spontaneous vegetation + Polyethylene film	28.9	33.6	66.6	12.5	14.9	38.8	

<sup>(1)</sup> DAT: days after transplanting.



Regarding the no-tillage system (Table 3), it could be observed that the treatments that offered higher percentages of SBTB in most of the times evaluated were the spontaneous vegetation and spontaneous vegetation + polyethylene film treatments, considering that the latter showed the highest temperature at 60 DAT. Although the spontaneous vegetation + polyethylene film treatment was not the one that caused the highest soil temperature, it was among the treatments that caused greater increase in soil temperature (Figure 1). Sporulating bacteria are usually associated with unfavorable conditions for microbial growth, such as high temperatures, since they have endospores, which are spores that are resistant to adverse conditions. Another hypothesis is that the spontaneous vegetation plants may have stimulated the development of this group of bacteria.

No statistical differences were observed in the number of CFUs of fluorescent *Pseudomonas* sp. within and across the tillage systems (Table 4). This may be due to the short period of time in which the soil was exposed to organic matter, since this is a specific group of bacteria in which the radicular exudates are determinant factors for the structure of this group of microorganisms (Ferreira et al., 2009). A similar result was found by Ferreira et al. (2009) upon studying the diversity of fluorescent *Pseudomonas* sp. in different soil management and crop rotation systems, observing that, in regard to the soil management system, the number of isolates obtained on the no-tillage system was very similar to the number obtained in the conventional tillage system, 51 and 49 % respectively.

**Table 4.** Number of colony forming units (CFUs) of fluorescent *Pseudomonas* sp. in the no-tillage and in the conventional tillage systems in different management systems and soil sampling times

	Fluorescent Pseudomonas sp.							
Management system	Fallow area	Upon planting of green manures	Upon the transplanting of muskmelon	20 DAT	40 DAT	60 DAT		
		— 10 <sup>2</sup> of the nun	nber of CFUs g <sup>-1</sup> ± 1	0 <sup>2</sup> of the standa	rd deviation —			
			Conventional	tillage				
Sunn Hemp	5.3±3.7 <sup>ns</sup>	2.4±1.1 <sup>ns</sup>	$3.0 \pm 1.8^{ns}$	$1.6 \pm 1.3^{ns}$	$5.9 \pm 6.9^{ns}$	4.5±4.3 <sup>ns</sup>		
Millet	5.6±2.7 <sup>ns</sup>	2.4±2.2 <sup>ns</sup>	$0.8 \pm 0.5^{ns}$	$1.7\!\pm\!1.7^{\text{ns}}$	3.5±1.5 <sup>ns</sup>	2.0±2.0 <sup>ns</sup>		
Sunn Hemp + Millet	12.0±10.9 <sup>ns</sup>	3.1±3.2 <sup>ns</sup>	$0.8 \pm 0.6^{ns}$	$1.6 \pm 1.2^{ns}$	$1.8 \pm 0.8^{ns}$	2.5±2.4 <sup>ns</sup>		
Corn + Brachiaria	11.3±2.8 <sup>ns</sup>	4.8±1.5 <sup>ns</sup>	1.0±0.2 <sup>ns</sup>	8.3±5.9 <sup>ns</sup>	$7.6 \pm 6.5^{ns}$	$3.0 \pm 1.4^{ns}$		
Spontaneous vegetation	12.3±1.7 <sup>ns</sup>	2.9±3.8 <sup>ns</sup>	$3.0 \pm 1.9^{ns}$	5.6±3.8 <sup>ns</sup>	9.5±4.9 <sup>ns</sup>	$1.6 \pm 0.9^{ns}$		
Soil with no vegetation	5.7±6.2 <sup>ns</sup>	$0.9 \pm 0.4^{ns}$	$1.4 \pm 1.6^{ns}$	4.8±5.3 <sup>ns</sup>	4.8±4.2 <sup>ns</sup>	$0.7 \pm 0.4^{ns}$		
Spontaneous vegetation + Polyethylene film	10.0±4.0 <sup>ns</sup>	2.4±1.6 <sup>ns</sup>	0.6±0.3 <sup>ns</sup>	3.5±4.1 <sup>ns</sup>	8.2±5.2 <sup>ns</sup>	1.9±1.5 <sup>ns</sup>		
		No-tillage						
Sunn Hemp	2.9±1.5 <sup>ns</sup>	$1.8 \pm 1.0^{ns}$	1.8±1.8 <sup>ns</sup>	2.5±3.6 <sup>ns</sup>	4.1±2.8 <sup>ns</sup>	$0.8 \pm 0.6^{ns}$		
Millet	7.0±4.0 <sup>ns</sup>	1.2±0.1 <sup>ns</sup>	1.4±1.0 <sup>ns</sup>	4.9±3.5 <sup>ns</sup>	16.0±18.3 <sup>ns</sup>	$0.9 \pm 0.4^{ns}$		
Sunn Hemp + Millet	8.6±8.0 <sup>ns</sup>	$1.1 \pm 0.9^{ns}$	0.8±0.3 <sup>ns</sup>	3.6±2.4 <sup>ns</sup>	5.5±4.4 <sup>ns</sup>	$1.5 \pm 0.8^{ns}$		
Corn + Brachiaria	14.0±7.6 <sup>ns</sup>	1.5±0.5 <sup>ns</sup>	3.1±3.9 <sup>ns</sup>	2.8±1.6 <sup>ns</sup>	6.1±4.5 <sup>ns</sup>	$1.9 \pm 0.8^{ns}$		
Spontaneous vegetation	3.6±3.8 <sup>ns</sup>	3.2±1.3 <sup>ns</sup>	1.1±0.5 <sup>ns</sup>	6.5±6.5 <sup>ns</sup>	5.6±5.6 <sup>ns</sup>	4.0±3.7 <sup>ns</sup>		
Soil with no vegetation	8.6±7.6 <sup>ns</sup>	3.2±4.7 <sup>ns</sup>	$0.5\pm0.2^{ns}$	4.5±2.0 <sup>ns</sup>	5.8±2.8 <sup>ns</sup>	1.6±0.9 <sup>ns</sup>		
Spontaneous vegetation + Polyethylene film	9.9±5.9 <sup>ns</sup>	2.3±37.8 <sup>ns</sup>	0.6±0.1 <sup>ns</sup>	4.8±4.2 <sup>ns</sup>	8.0±9.7 <sup>ns</sup>	2.2±2.9 <sup>ns</sup>		
CV (%)	75.7	266.4	105.9	96.0	123.50	87.44		

<sup>&</sup>lt;sup>ns</sup>: not significant at 5 % probability. DAT: days after transplanting; CV: coefficient of variation.



In contrast with this study, Ceja-Navarro et al. (2010) observed that the Pseudomonadales group, represented mainly by fluorescent *Pseudomonas* sp., was favored mainly by treatments in which crop residues were maintained, both in the conventional and the no-tillage systems. However, these microorganisms were negatively affected by the no-vegetation treatment.

No statistical difference was observed in the number of CFUs of total fungi, within and across the tillage systems (Table 5). Fungi show a higher sensitivity to physical disturbance of the soil in relation to bacteria since the rupture of hyphae may occur, harming development of the fungus population (Calderón et al., 2001; Hossain and Sugiyama, 2011); however, in the no-tillage system, this phenomenon occurs with less intensity, due to the lack of soil disturbance. Therefore, one of the expectations was to find differences in the number of fungal CFUs in the no-tillage system in relation to the conventional tillage system. This was likely not observed due to the short period in which the cover plants were used (no-tillage system), since microbial activity in this soil is best observed in medium- and long-term evaluations (Costa and Rava, 2003; Neves et al., 2009).

Evaluating the effect of soil and crop management on the activity of microorganisms, Castro et al. (1993) observed similar values between the no-tillage and the conventional tillage systems regarding fungus quantification, showing the lack of notable differences between the tillage systems on the fungal CFUs. When studying biological markers associated with the phosphorus cycle on Cerrado soils with no-tillage and conventional tillage systems, Carneiro et al. (2004) observed that the no-tillage system allowed the occurrence of greater total fungus populations (11.2  $\times$  10 $^4$  CFU g $^{-1}$  of soil) in relation to the conventional tillage system (8.2  $\times$  10 $^4$  CFU g $^{-1}$  of soil) in the 0.00-0.05 m soil layer.

**Table 5.** Number of colony forming units (CFUs) of fungi in the no-tillage and in the conventional tillage systems in different management systems and soil sampling times

	Total fungi					
Management system	Fallow area	Upon planting of green manures	Upon transplantingof muskmelon	20 DAT	40 DAT	60 DAT
		— 10 <sup>3</sup> of the numbe	r of CFUs $g^{-1} \pm 10^3$ of	the standard d	eviation —	
			Conventional tillage	ge		
Sunn Hemp	12.0±3.8 <sup>ns</sup>	2.5±0.4 <sup>ns</sup>	$6.4 \pm 1.2^{ns}$	4.6±2.1 <sup>ns</sup>	$6.7 \pm 1.8^{ns}$	6.0±1.3 <sup>ns</sup>
Millet	11.3±2.3 <sup>ns</sup>	2.9±1.5 <sup>ns</sup>	5.1±0.7 <sup>ns</sup>	3.2±2.1 <sup>ns</sup>	$7.7 \pm 2.5^{ns}$	6.4±2.8 <sup>ns</sup>
Sunn Hemp + Millet	9.0±3.4 <sup>ns</sup>	4.1±1.7 <sup>n.s</sup>	4.3±0.5 <sup>ns</sup>	4.2±2.0 <sup>ns</sup>	6.3±3.0 <sup>ns</sup>	5.8±2.5 <sup>ns</sup>
Corn + Brachiaria	9.1±1.3 <sup>ns</sup>	2.1±1.5 <sup>ns</sup>	$6.0\pm2.2^{ns}$	4.2±3.5 <sup>ns</sup>	9.5±2.2 <sup>ns</sup>	5.2±1.9 <sup>ns</sup>
Spontaneous vegetation	7.5±1.1 <sup>ns</sup>	2.2±0.9 <sup>ns</sup>	$5.4 \pm 1.9^{ns}$	4.9±6.5 <sup>ns</sup>	7.1±2.4 <sup>ns</sup>	6.6±1.9 <sup>ns</sup>
Soil with no vegetation	12.6±3.6 <sup>ns</sup>	$1.4\pm0.4^{ns}$	$4.7 \pm 1.0^{ns}$	3.4±3.1 <sup>ns</sup>	5.6±2.7 <sup>ns</sup>	$4.0\pm0.3^{ns}$
Spontaneous vegetation + Polyethylene film	11.8±4.7 <sup>ns</sup>	1.8±0.4 <sup>ns</sup>	5.7±1.3 <sup>ns</sup>	7.8±7.9 <sup>ns</sup>	7.6±2.2 <sup>ns</sup>	5.5±2.1 <sup>ns</sup>
			No-tillage			
Sunn Hemp	10.6±4.2 <sup>ns</sup>	9.2±3.9 <sup>ns</sup>	7.1±2.0 <sup>ns</sup>	4.3±1.1 <sup>ns</sup>	7.1±4.6 <sup>ns</sup>	5.3±1.4 <sup>ns</sup>
Millet	7.4±1.8 <sup>ns</sup>	7.1±3.0 <sup>ns</sup>	$5.4 \pm 1.0^{ns}$	$3.4 \pm 1.5^{ns}$	4.2±0.7 <sup>ns</sup>	6.5±2.1 <sup>ns</sup>
Sunn Hemp + Millet	8.2±4.0 <sup>ns</sup>	5.3±1.0 <sup>ns</sup>	$4.1 \pm 1.9^{ns}$	4.3±0.9 <sup>ns</sup>	5.3±2.4 <sup>ns</sup>	9.2±8.6 <sup>ns</sup>
Corn + Brachiaria	9.9±4.4 <sup>ns</sup>	7.7±4.9 <sup>ns</sup>	10.5±6.6 <sup>ns</sup>	6.8±1.9 <sup>ns</sup>	8.1±3.6 <sup>ns</sup>	8.0±2.6 <sup>ns</sup>
Spontaneous vegetation	$10.7 \pm 6.2^{ns}$	10.3±5.1 <sup>ns</sup>	5.4±1.6 <sup>ns</sup>	5.0±3.3 <sup>ns</sup>	4.3±1.5 <sup>ns</sup>	$6.2 \pm 6.4^{ns}$
Soil with no vegetation	9.9±2.3 <sup>ns</sup>	$6.0 \pm 1.9^{ns}$	6.3±1.3 <sup>ns</sup>	$3.1 \pm 0.7^{ns}$	$4.0 \pm 0.9^{ns}$	$10.3 \pm 4.0^{ns}$
Spontaneous vegetation + Polyethylene film	10.5±2.6 <sup>ns</sup>	8.0±2.4 <sup>ns</sup>	6.7±1.9 <sup>ns</sup>	5.4±2.0 <sup>ns</sup>	7.4±2.8 <sup>ns</sup>	10.0±5.8 <sup>ns</sup>
CV (%)	33.9	52.2	36.6	69.8	39.1	55.7

<sup>&</sup>lt;sup>ns</sup>: not significant at 5 % probability. DAT: days after transplanting; CV: coefficient of variation.



The no-tillage system and pulp cover on fungal biomass and the structure of the microbial community during decomposition of sugarcane material in Indonesia were studied by Miura et al. (2012), and unlike the results found in our study, they observed that the fungal biomass was twice as large in the no-tillage system and 2.5 times as large with the pulp cover in relation to the conventional tillage system (no soil cover), certainly due to the material used (sugarcane pulp) for plant cover, which reduced the soil temperature and thermal amplitude, offering favorable conditions for development of fungi. In that paper, the mean temperature in the treatments with the no-tillage system was around 29 °C, while in this paper, it was 35.3 °C (Figure 1). Both mild temperatures, between 20 and 40 °C and soil moisture are factors that favor the development of mesophilic fungi, which are predominant in the soil (Stamford et al., 2005).

Whenever there are changes to the system, such as management practices, application of agricultural chemicals, different substrate availabilities, and variation in moisture and soil temperature, among others, certain groups of microorganisms in the soil will be favored or inhibited (Stamford et al., 2005). Each change in the system corresponds to renovation of selection pressure, favoring some microbiota components, eliminating others, and rearranging the balance among the populations (Venâncio, 2002).

Although this study did not observe that the no-tillage system showed a higher population of microorganisms across all the treatments evaluated, for Colozzi-Filho et al. (2001), no-tillage practices and crop rotation change the soil biota and its activity, offering a beneficial effect to the soil microbial community. However, it is noteworthy that, in this study, evaluations of the microbial population were conducted according to the muskmelon cycle, and this period may have not been sufficient to observe a significant change. Costa and Rava (2003) also emphasize that, according to their results, the influence of microbial activity on the soil may best be observed in medium- and long-term evaluations. This was also reported by Neves et al. (2009), who found that the systems with no soil disturbance, during the first year, showed lower microbiological indexes (total organic carbon and microbial biomass carbon) and that they improved in the following years. These authors also point out that the systems evaluated are recent and that the microbial biomass may still be adapting to the new soil management conditions.

## **CONCLUSIONS**

Sunn hemp associated with the no-tillage system during transplanting of muskmelon offered a higher number of CFUs of total bacteria in relation to the same treatment in the conventional tillage system.

Conventional tillage system, in most treatments, showed larger numbers of sporulating bacteria in relation to the no-tillage system during transplanting of muskmelon and at 40 DAT.

Tillage and soil cover system did not change the amount of total fungi and fluorescent *Pseudomonas* sp.

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