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Changes On Soil Microbiota Induced By The Use Of Commercial Products And The Incorporation Of Plant Materials

Mudanças na microbiota do solo causada pelo uso de produtos comerciais e incorporação de materiais vegetais

Darlan F. Borges[1]*, Geovane de A. Nogueira[1], Gisellya de A. Cruz[1], Shamyra G. de A. Silva[1], Washington L. da Silva[2], Márcia M. de Q. Ambrósio[1]

[1]Department of Agronomic and Forestry Sciences, Universidade Federal Rural do Semi-Árido, Mossoró, RN, Brazil. [2]Department of Plant Pathology and Ecology, The Connecticut Agricultural Experiment Station, New Haven, CT, U.S.A.

ABSTRACT - Growers have adopted monoculture to maintain the high melon (*Cucumis melo* L.) production demand in the Northeastern region of Brazil. This cultivating practice culminates in up to three crop cycles per year being used in the same growing area. The main objective of this study was to evaluate if the incorporation of plant material used with polyethylene mulch and or in association with commercial soil amendment products can help to condition an environment that is beneficial to soil microbial communities. Two identical greenhouse experiments were conducted using a completely randomized design with seven treatments and seven replications. The treatments were: (C) – Control, (M) - polyethylene mulch, (C+M) - incorporation of *Crotalaria juncea* L. + polyethylene mulch, (P+M) - incorporation of *Pennisetum glaucum* L. + polyethylene mulch, (M+CS) - polyethylene mulch + (Compost-Aid® + Soil-Set®, trade names of products produced by Alltech Crop Science), (C+M+CS) - incorporation of *C. juncea* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®), and (P+M+CS) - incorporation of *P. glaucum* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®). To quantify the target soil microbiota (fungi, bacteria, sporulating bacteria, and actinomycetes), isolations were attempted on selective culture media specific for each group of microorganisms. The incorporation of *P. glaucum* together with the use of polyethylene mulch and commercial products (Compost-Aid® and Soil-Set®), (P+M+CS), increased the total fungal population by 183%, total bacteria by 55%, sporulating bacteria by 21%, and actinomycetes by 146% in relation to the control treatment.

Keywords: Soil microorganisms. Mulching. Cover crop. Biological control. *Cucumis melo* L.

RESUMO - Os produtores há anos adotam a monocultura para manter a demanda de produção de melão alto (*Cucumis melo* L.) na região Nordeste do Brasil. Esta estratégia de cultivo é usada em até três ciclos por ano. O objetivo principal deste estudo foi avaliar se a incorporação de material vegetal utilizado com mulch de polietileno e/ou em associação com produtos corretivos de solo pode condicionar um ambiente benéfico para as comunidades microbianas do solo. Dois experimentos idênticos em casa de vegetação foram conduzidos em um delineamento inteiramente casualizado com sete tratamentos e sete repetições. Os tratamentos foram: (C) – Controle, (M) - cobertura de polietileno, (C+M) - incorporação de *Crotalaria juncea* L. + cobertura de polietileno, (P+M) - incorporação de *Pennisetum glaucum* L. + cobertura de polietileno, (M+CS) - cobertura de polietileno + (Compost-Aid® + Soil-Set®, nomes comerciais dos produtos produzidos pela Alltech Crop Science), (C+M+CS) - incorporação de *C. juncea* L. + cobertura de polietileno + (Compost-Aid® + Soil-Set®) e (P+M+CS) - incorporação de *P. glaucum* L. + cobertura de polietileno + (Compost-Aid® + Soil-Set®). Para quantificar a microbiota do solo (fungos, bactérias, bactérias esporulantes e actinomicetos), foram realizados isolamentos em meios de cultura seletivos para cada grupo de microrganismos. Conclui-se que, coletivamente, a incorporação de *P. glaucum* juntamente com o uso de mulch de polietileno e os produtos comerciais (Compost-Aid® e Soil-Set®), aumentou a população total de fungos em 183%, bactérias totais em 55%, bactérias esporulantes em 21% e actinomicetos em 146% em relação ao tratamento controle.

Palavras-chave: Microrganismos do solo. Mulching. Cultura de cobertura. Controle biológico. *Cucumis melo* L.

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***Corresponding author:**
<darlanborges@unifucamp.edu.br>

INTRODUCTION

Agriculture is a vital industry in Brazil and the country ranks as the 10th largest producer and 3rd largest exporter of melon (*Cucumis melo* L.) in the world Food and Agriculture Organization of the United Nations (FAO, 2021). According to Instituto Brasileiro de Geografia e Estatística (IBGE, 2021), 96% of the Brazilian melon production is concentrated in the semiarid region of Brazil, including the states of Rio Grande do Norte(mainly in the Mossoró city microregion), Ceará, Bahia, and Pernambuco. Monoculture has been widely used to keep up with the high production demand and growers usually reach three melon production cycles in a single year. All the agricultural practices associated with the high production pressure (*e.g.*, high use of agrochemicals) and monoculture may impact the soil microbiota abundance, diversity, and species richness. In addition, it interferes with plant growth and with the severity of

diseases caused by soilborne pathogens (HUANG et al., 2013).

Monoculture tends to be unsustainable as the lack of genetic diversity in the crop planted contributes to the selection and rapid raise of pathogens that compromise crop yield (SHEN et al., 2018). Furthermore, this practice promotes changes in the soil microbial community that may culminate in a high incidence of soilborne diseases (MUELLER et al., 2016). There is a close link between the increase in the incidence of soilborne pathogens and the loss of beneficial microbial groups for plants and in the composition of the soil microbiota (SHEN et al., 2018). Agreeable in the scientific community, high diversity and appropriate composition of soil microbiota foster soil health and mitigates the upsurge of plant pathogens populations (LING et al., 2011).

Different techniques are used to manage soilborne diseases, such as chemical and biological control and the use of natural products - all these agricultural practices can be used alone or concomitantly (SALES JÚNIOR et al., 2017). In fact, the use of soil solarization associated with the incorporation of plant materials raises the soil temperature higher than when each technique is used alone, which has been shown as a promising practice in controlling soilborne pathogens (ROCHA; CARNEIRO, 2016). Thus, polyethylene mulch, which is already used in melon cultivation in the Northeast of Brazil for controlling weeds, if modified to be used without holes before transplanting (such as solarization treatment) may be an alternative approach for disease control. Polyethylene mulch can be applied in combination with other techniques (*e.g.,* incorporation of vegetal material) to raise the soil temperature to levels detrimental to the development of soilborne plant pathogens (WONG; AMBRÓSIO; SOUZA, 2011). Polyethylene mulch can also capture volatile and non-volatile substances, released from the soil and organic matter, that can potentially affect the soil microflora. However, practices to raise soil temperature need to be taken with caution as such high temperature can be detrimental to the beneficial soil microorganisms that are important for agriculture (NASCIMENTO et al., 2016a).

The most abundant groups of soil microbes are bacteria and fungi, which are regulators of various biological, chemical, and physical processes in the soil (MATTOS, 2015). These microbes promote soil health and consequently plant growth by catalyzing unique and indispensable transformations in soil formation, soil biogenesis, organic matter decomposition, toxins degradation, and biogeochemical cycling (SHEN et al., 2018). The composition of the soil microbial community is influenced by several factors, such as temperature, moisture, soil aeration, organic substrates, and nutrient availability (NASCIMENTO et al., 2016a). These factors are likely to be affected when soilborne pathogen management strategies are performed.

In order to minimize these disturbances in the soil, commercial products have been used such as Compost-Aid® (*Lactobacillus plantarum* - 1.25 x 10^8 UFC g^{-1}; *Bacillus subtilis* - 1.25 x 10^8 UFC g^{-1}; *Enterococcus faecium* - 1.25 x 10^8 UFC g^{-1}), which is composed of microorganisms beneficial to the soil, and Soil-Set® (Sulfur – 45.51 g L^{-1}; Zinc – 39.36 g L^{-1}; Copper – 24.60 g L^{-1}; Iron – 19.68 g L^{-1}; Manganese – 9.84 g L^{-1}) as a source of micronutrients, which together have already shown positive results in the control of *Pratylenchus brachyurus* and *Meloidogyne javanica* (MIAMOTO et al., 2017). Moreover, the application of Compost-Aid® alone was shown to inhibit 100% and 98.57% of the growth of the fungi *Macrophomia phaseolina* and *Sclerotium rolfsii*, respectively (NASCIMENTO et al., 2016b). There is a lack of studies designed to investigate how soil microbial communities respond to the concomitant use of polyethylene mulch with the incorporation of plant materials added to commercial products to control soilborne pathogens.

Thus, the goal of this study was to investigate if the techniques used for raising soil temperature, to manage root rot pathogens, impact the communities of the soil microbes: actinomycetes, sporulating bacteria, total bacteria, and total fungi. To address our research questions, we incorporated plant materials (crotalaria, *Crotalaria juncea* L., and millet, *Pennisetum glaucum* L.) with polyethylene mulch, used alone or concomitantly with commercial products (Compost-Aid® + Soil-Set®). Then, we evaluated the development of target soil microbial communities under those systems.

MATERIALS AND METHODS

Experiment setup

The experiments were conducted twice (the second trial was set up 30 days after the first trial was finished) in a greenhouse located in the city of Mossoró, in the state of Rio Grande do Norte, Brazil (5° 11' 17" South, 37° 20' 39" West). We used 14 L plastic pots of 0.28 m in diameter. The soil used in the experiments were collected from an area extensively cultivated with melon plants, up to three crop cycles per year in the same field. The soil has the following chemical characteristics: pH(H_2O)=6.10, P(mg dm^{-3})=101.0, sum of bases (SB) (cmolc dm^{-3})=2.99, K^+(mg dm^{-3})=85.1, Mg^{+2}(cmolc dm^{-3})=0.50, Al^{+3}(cmolc dm^{-3})=0.0, cation exchange capacity (CEC) (cmolc dm^{-3})=3.65, O.M=3.56 (g Kg^{-1}), and base saturation (V%)=82.0. The same treatments and soil were used in both trials.

Experimental design

A completely randomized design with seven treatments and seven replications was used. The treatments were: (C) - Control (pots were not covered with polyethylene mulch nor with vegetal material), (M) - polyethylene mulch (pots were covered with black-white polyethylene mulch but not with vegetal material), (C+M) - incorporation of *C. juncea* L. +

polyethylene mulch, (P+M) - incorporation of *P. glaucum* L. + polyethylene mulch, (M+CS) - polyethylene mulch + (Compost-Aid® + Soil-Set®, trade names of products produced by Alltech Crop Science), (C+M+CS) - incorporation of *C. juncea* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®), and (P+M+CS) - incorporation of *P. glaucum* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®).

Plant cultivation

Seeds of hybrid yellow melon GOLDEX TOPSEED were sown in trays with substrate for 12 days, then seedlings were individually transplanted in pots. Exactly 17 days before transplanting, the vegetal materials (*C. juncea* L. and *P. glaucum* L.) were incorporated in the first 10 cm of the soil and applied at a rate of 4 kg/m² of plant material per pot (AMBRÓSIO, 2003). The pots were covered with polyethylene mulch and kept covered for 15 days. On the fifteenth day, holes were drilled on the polyethylene mulch to remove toxic gases and to lower the soil temperature to condition the soil for the melon seedlings that were transplanted two days later.

Throughout the experiment, plants were watered by drip irrigation and fertigation was conducted according to soil analysis to meet the crop needs (CAVALCANTI et al., 2008). In treatments (M+CS), (C+M+CS), and (P+M+CS), Compost-Aid® (*Lactobacillus plantarum* - 1.25 x 10^8 UFC g^{-1}; *Bacillus subtilis* - 1.25 x 10^8 UFC g^{-1}; *Enterococcus faecium* - 1.25 x 10^8 UFC g^{-1}) and Soil-Set® (Sulfur – 45.51 g L^{-1}; Zinc – 39.36 g L^{-1}; Copper – 24.60 g L^{-1}; Iron – 19.68 g L^{-1}; Manganese – 9.84 g L^{-1}) were applied once at one day after transplanting, according to the manufacturer's recommendations, at the dosage of 3 kg ha^{-1} and 2 L ha^{-1}, respectively. Those two products were applied twice again, at seven and 14 days after transplanting, at the concentrations of 2 kg ha^{-1} (Compost-Aid®) and 1.5 L ha^{-1} (Soil-Set®) - considering a population of 12,500 plants ha^{-1} and one plant per pot. The maximum temperature of the soil in each pot was measured by a mercury thermometer and the maximum temperature and humidity of the air were measured by a digital hygro-thermometer, daily at 1.00 p.m.

Microbiota evaluation

Before filling up the pots with soil to set up the experiments, three soil samples were randomly collected from the bulk homogenized soil. Then the single samples were combined into a composite sample of 300 g per experiment. Two other soil samples were collected in each pot (a sample per pot each time), one on the day the mulch was drilled (Pre-planting) and another at harvest (60 days post-transplanting). Soil samples were kept in transparent plastic bags and stored at 10 °C to perform microbial community evaluations.

In order to quantify the target soil microbiota (fungi, bacteria, sporulating bacteria, and actinomycetes), isolations were attempted on selective culture media specific for each group of microorganisms. For total fungi counting, we used Martin's medium (K_2HPO_4 – 1.00 g; $MgSO_4.7H_2O$ - 0.50 g; peptone – 5.00 g; dextrose – 10.00 g; rose bengal - 0.03 g; agar – 16.00 g; distilled water - 1,000 mL) (MARTIN, 1950) plus 0.05 g L^{-1} of tetracycline. For total and sporulating bacteria, the agar nutrient medium was used (nutrient agar - 23.00 g; distilled water - 1,000 mL). For actinomycetes, we used the culture medium starch casein (starch - 10.00 g; casein - 0.30 g; KNO_3 - 2.00 g; NaCl – 2.00 g; K_2HPO_4 - 2 g; 0.05 g; $MgSO_4.7H_2O$ - 0.01 g; agar – 16.00 g; distilled water - 1,000 mL) (CUNHA et al., 2014).

Soil microorganism isolations were performed by using the serial dilution technique. One gram of soil was taken from each sample and placed in test tubes containing 9 mL of sterile distilled water. Each tube was homogenized in a vortex tube shaker and serial dilutions were performed by a factor of 10 (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}). For each dilution point, 100 µL of the solution was collected and placed individually on a 9.0 cm diameter Petri dish, containing the specific selective media for the corresponding microbe group analyzed, and dispersed with a Drigalski spatula. For the analysis of sporulating bacteria, the samples were kept for 20 minutes in a water bath at 80 °C, prior to placing the sample aliquots in Petri dishes, to kill the non-sporulating bacteria (BETTIOL, 2007). Three plates were plated per sample per dilution point and after counting, the values were converted to colony forming units per gram of soil (CFU g^{-1}). Only the dilution points that had 20 to 200 colonies per plate were considered for the calculations because of colonies saturation that occurs when too many microbial colonies grow together in a Petri dish, which inhibits the growth of other colonies and underestimates the results (TORTORA; CASE; FUNKE, 2016). All plates were inverted and kept in a biochemical oxygen demand (BOD) incubator for six days at 28 ± 2 °C. The quantification of microbial communities by the plate count method was chosen because this technique has the advantage of providing the quantification of viable microbe cells (TORTORA; CASE; FUNKE, 2016). All steps involved in the experiments are depicted in Figure 1.

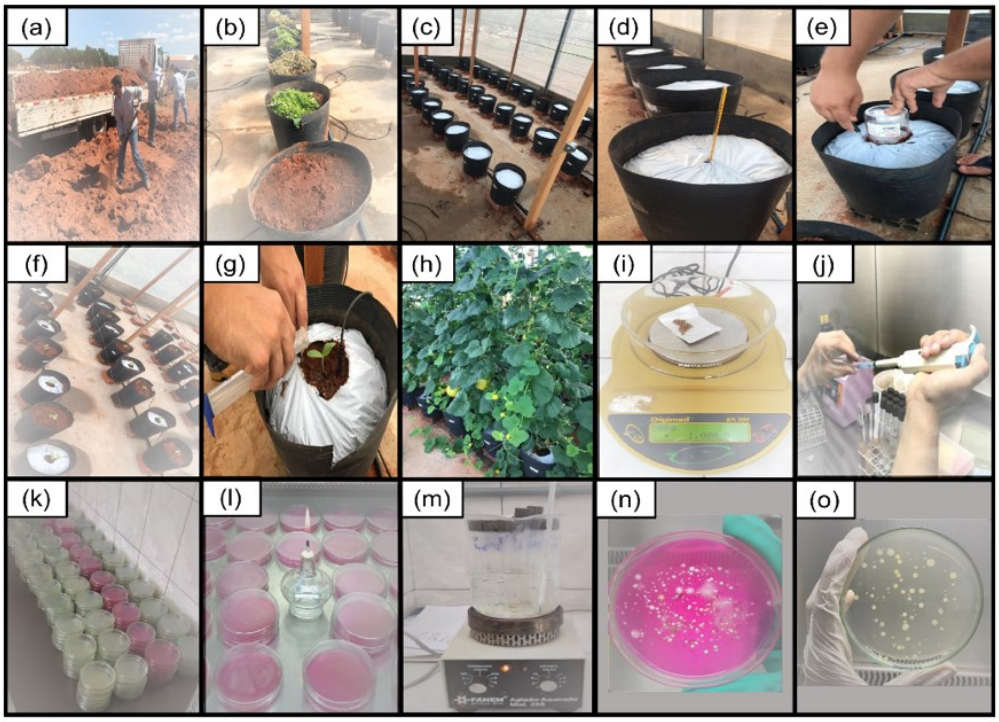

Figure 1. Steps depicting the implementation, conduction, and evaluation of the experiments. A - Collection of soil (with a long history of natural infestation by soilborne pathogens) to be used in the experiments. B – Incorporation of plant material. C - Pots covered with polyethylene mulch during the 15-days soil treatment period (solarization). D – Recording the soil temperature. E – Drilling holes on the polyethylene mulch. F – Seedlings planting. G – Application of Compost-Aid® + Soil-Set®. H – Plants at 45 days after transplanting. I – Soil weighing for dilution. J – Serial dilution step. K – Petri dishes with each specific culture media. L – Plates containing Martin's medium. M – Analysis of sporulating bacteria, the samples were kept for 20 minutes in a water bath at 80 °C. N – Count of fungal colony forming units. O – Count of bacterial colony forming units.

Statistical analysis

The results of the population quantification for total fungi, total bacteria, sporulating bacteria, and total actinomycete were analyzed by the non-parametric method, the Kruskal Wallis test. All statistical analyzes and graph plotting were performed in R version 3.1.1 (R CORE TEAM, 2019).

RESULTS AND DISCUSSION

The maximum soil temperature in all treatments was higher than the greenhouse air temperature in both experiments (Figure 2). However, at the end of experiment 2 – starting at 41 days after, the holes were punctured in the polyethylene mulch - both temperatures, air, and maximum soil temperature, had similar measurements (Figure 2B). Throughout the experiment period, in both experiments, the maximum soil temperature did not exceed 41 °C and it didn't go below 32 ^{o}C; except in the treatments: (C+M), (P+M), (C+M+CS), and (P+M+CS), which at seven days in the first experiment the maximum soil temperature reached 42 ^{o}C (Figure 2).

The temperature was measured every day during the course of the experiments in the hottest hour of the day for the region, between 12 and 1 p.m., and it did not vary significantly among treatments in both experiments over time. It did follow the air temperature trend - when the air temperature dropped so did the maximum temperature in each treatment (Figure 2), unlike the results obtained by Nascimento et al. (2016a), which showed that in the period from 10 to 18 days after transplanting the melon seedlings the treatment with vegetable cover + polyethylene mulch had the highest soil temperature. However, the same did not occur in the period from 30 to 46 days after the transplant, the soil temperature dropped during that treatment. We did not observe this effect in our experiments.

The relative air humidity was similar until day 41 in both experiments, then it became higher in experiment 1 in comparison to experiment 2 (Figure 3). Humidity interferes with the soil microbiota population, because high soil humidity means low availability of oxygen for microbial development (SOUTO et al., 2008), and that depends on the characteristics of the soil and the metabolic requirements of each class of microorganisms. In our study, the largest populations of the soil microbes evaluated were identified in the post-harvest in the second experiment, in which the air humidity measured throughout the experiment reached the lowest levels after day 41 (Figure 3).

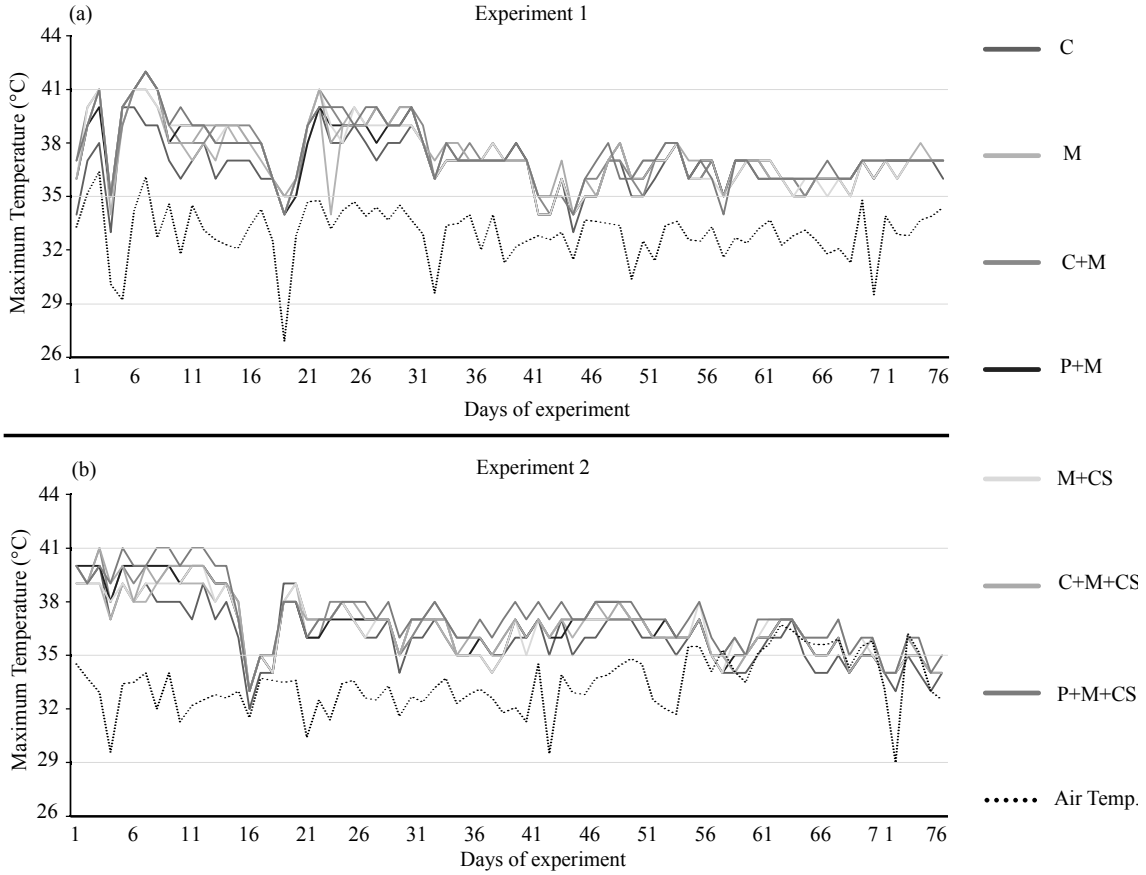

Figure 2. Maximum soil temperature measured in experiment 1 (A) and in experiment 2 (B). (C) - Control (pots were not covered with polyethylene mulch nor with vegetal material), (M) - polyethylene mulch (pots were covered with black-white polyethylene mulch but not with vegetal material), (C+M) - incorporation of *C. juncea* L. + polyethylene mulch, (P+M) - incorporation of *P. glaucum* L. + polyethylene mulch, (M+CS) - polyethylene mulch + (Compost-Aid® + Soil-Set®, trade names of products produced by Alltech Crop Science), (C+M+CS) - incorporation of *C. juncea* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®), and (P+M+CS) - incorporation of *P. glaucum* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®).

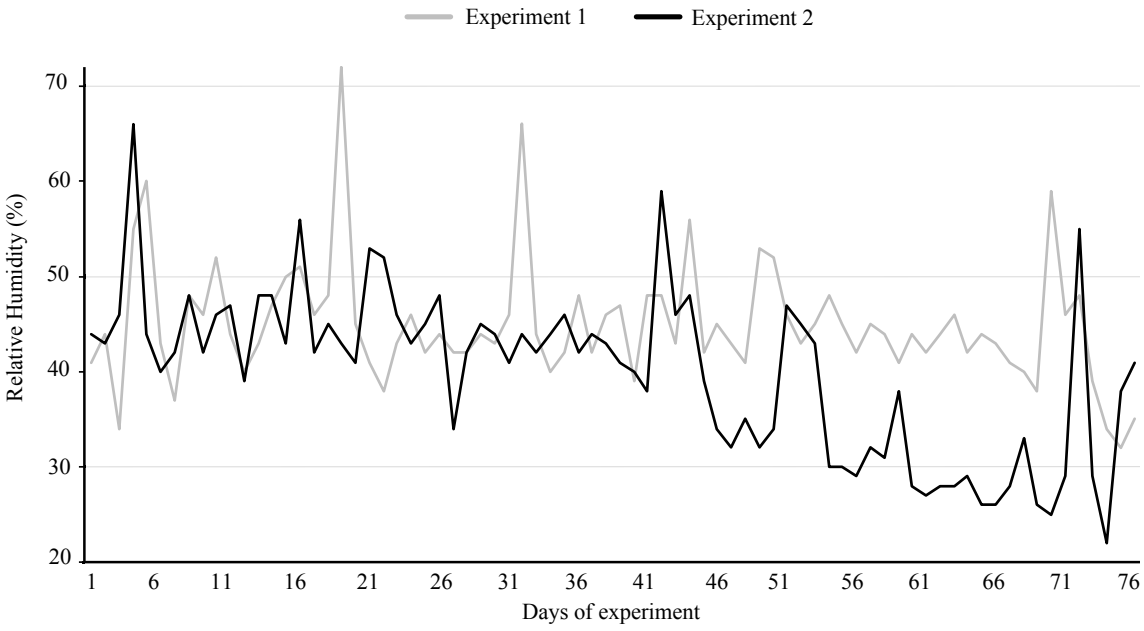

Figure 3. Relative humidity inside the greenhouse for the duration of experiments 1 and 2.

The initial fungal population was similar in the first and second experiments, $5.90x10^3$ and $5.10x10^3$ CFUs g^{-1}, respectively (Table 1). After drilling a hole in the polyethylene mulch (pre-planting), the (P+M) treatment in the first experiment had the largest total fungal population ($8.31x10^3$ CFUs g^{-1}) in comparison to all other treatments. Interestingly, at the end of the cycle (post-harvest), the treatment (C+M) in experiment 1, which had the largest total fungi population ($14.20x10^3$ CFUs g^{-1}), was precisely the one with the lowest population at pre-planting ($4.36x10^3$ CFUs g^{-1}). Additionally, the total fungi population was statistically higher in (C+M) than in (C) treatment; but the (C+M) total fungi population was not statistically different from the ones in (P+M) and (C+M+CS) treatments. In the second experiment after drilling a hole in the polyethylene mulch (pre-planting), the (C+M+CS) treatment had the largest total fungal population; however, it did not differ statistically from the other treatments: (C), (C+M), (P+M), and (M+CS). At harvest, the treatment (P+M+CS) was statistically higher than the control treatment (C), it had the highest fungal population increase among all treatments, reaching $26.10x10^3$ CFUs, which corresponds to over 86% increase of the total fungi population from the pre-planting period to the end of the cycle in that treatment. It also had the lowest population count at pre-planting ($3.44x10^3$ CFUs). In the first experiment, the treatment (P+M+CS) was not statistically different, but it increased 68% of the population in post-harvest compared to the treatment (C). In the second experiment, in addition to this result being statistically different, this increase in the population was even greater, reaching an increase of 297% of the treatment (P+M+CS), compared to the treatment (C).

Table 1. Number of colonies forming units (CFUs) of total fungi in different treatments.

Treatments	Total fungi					
	Experiment 1			Experiment 2		
	Pre-mulch	Pre-planting	Post-harvest	Pre-mulch	Pre-planting	Post-harvest
	----------10^3 of the number of CFUs g^{-1}----------					
C	5.90 a	6.07 abc	5.95 a	5.10 a	6.41 c	6.58 a
M	5.90 a	6.48 bc	7.78 a	5.10 a	4.51 ab	21.80 ab
C+M	5.90 a	4.36 a	14.20 b	5.10 a	6.03 bc	7.36 ab
P+M	5.90 a	8.31 c	14.00 ab	5.10 a	5.85 abc	16.70 ab
M+CS	5.90 a	7.25 c	5.91 a	5.10 a	5.66 abc	13.10 ab
C+M+CS	5.90 a	4.60 ab	12.20 ab	5.10 a	6.70 c	15.20 ab
P+M+CS	5.90 a	6.23 abc	10.00 a	5.10 a	3.44 a	26.10 b

(C)- Control; (M)- polyethylene mulch; (C+M)- incorporation of *Crotalaria juncea* L. + polyethylene mulch; (P+M)- incorporation of *Pennisetum glaucum* L. + polyethylene mulch; (M+CS)- polyethylene mulch + (Compost-Aid® + Soil-Set®); (C+M+CS)- incorporation of *C. juncea* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®); (P+M+CS)- incorporation of *P. glaucum* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®). Means followed by the same letter in the same column do not differ by the Kruskal Wallis test ($p<0.05$).

Vegetation cover has been shown to increase the abundance and species richness of soil microbiota (MORENO et al., 2009). Although we did not directly attempt to quantify the microbiota species richness in our study, our results corroborate Moreno et al. (2009), the treatments where *C. juncea* L. and *P. glaucum* L. were incorporated into the soil significantly increased soil fungal abundance. When combined with Compost-Aid® and Soil-Set® products, the incorporation of vegetal material yielded even more expressive results, especially the treatment (P+M+CS), in which the population increased by 68% and 297% in relation to treatment (C) in the first and second experiments, respectively (Table 1). On the other hand, studies have shown that the population of fungi is not affected by the incorporation of green manure into the soil for the cultivation of lettuce when compared with the chemical fertilization (crop recommended mineral fertilization) (OLIVEIRA et al., 2012). The Compost-Aid® is used to accelerate the decomposition of organic materials, promote microbiological activation and improve the balance of the soil microbiota. On the other hand, Soil-Set® promotes greater balance and it hinders the appearance of effects caused by environmental stresses.

The initial total bacteria population was $3.63x10^5$ CFUs g^{-1} in the first and $5.63x10^5$ CFUs g^{-1} in the second experiment (Table 2). During the first experiment, the treatment that reached the largest total bacteria population was (P+M) in pre-planting, which differed statistically from the (C), (M), and (C+M) treatments. Although no statistical significance was observed among the treatments in post-harvest evaluations, in the second experiment the largest increase in total bacteria population in pre-planting evaluations was observed in the (C+M+CS) treatment ($9.30x10^5$ CFUs g^{-1}), and the population count was 67% superior to (C) treatment. In post-harvest, the (P+M+CS) treatment had the highest total bacteria population among the treatments; however, it was only statistically different from

the (C+M+CS) treatment. In post-harvest, the treatment (P+M+CS), when compared to control, showed a 10% drop in the population in the first experiment. On the other hand, in the second experiment, the same treatment increased the total bacteria by 119%.

Table 2. Number of colonies forming units (CFUs) of total bacteria in different treatments.

Treatments	Total bacteria					
	Experiment 1			Experiment 2		
	Pre-mulch	Pre-planting	Post-harvest	Pre-mulch	Pre-planting	Post-harvest
	----------10^5 of the number of CFUs g^{-1}----------					
C	3.63 a	3.71 a	7.64 a	5.63 a	3.10 a	6.95 ab
M	3.63 a	4.40 ab	6.79 a	5.63 a	5.36 ab	8.16 b
C+M	3.63 a	4.20 a	6.77 a	5.63 a	7.83 bc	7.92 b
P+M	3.63 a	22.50 c	9.78 a	5.63 a	6.70 bc	8.13 b
M+CS	3.63 a	6.69 bc	7.46 a	5.63 a	3.50 a	6.81 ab
C+M+CS	3.63 a	6.33 bc	7.34 a	5.63 a	9.30 c	5.29 a
P+M+CS	3.63 a	9.40 c	6.90 a	5.63 a	6.05 bc	15.20 b

(C) control; (M)- polyethylene mulch; (C+M)- incorporation of *Crotalaria juncea* L. + polyethylene mulch; (P+M)- incorporation of *Pennisetum glaucum* L. + polyethylene mulch; (M+CS)- polyethylene mulch + (Compost-Aid® + Soil-Set®); (C+M+CS)- incorporation of *Crotalaria juncea* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®); (P+M+CS)- incorporation of *Pennisetum glaucum* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®). Means followed by the same letter in the same column do not differ by Kruskal Wallis test ($p<0.05$).

The initial population of sporulating bacteria was $5.20x10^4$ CFUs g^{-1} in the first experiment, while in the second experiment it was much higher: the population started at $29.70x10^4$ CFUs g^{-1} (Table 3). There was no statistical difference between treatments in pre-planting and in post-harvest evaluations in the first experiment, the only statistical difference was between treatments (C) and (M) in post-harvest. In the second experiment after drilling a hole in the polyethylene mulch (pre-planting), the (C+M+CS) treatment had the largest sporulating bacteria population, but it did not differ statically from (P+M+CS), (P+M), and (C+M) treatments. At harvest, the treatment with the largest population was (P+M+CS), with 57.10 $x10^4$ CFUs g^{-1}. Interestingly, (C+M+CS) had the smallest population count in post-harvest, but it had the highest population count in the pre-planting evaluations, a drastic reduction in the population count from 24.40 $x10^4$ to 8.45 $x10^4$ CFUs g^{-1}. The population of sporulate bacteria followed the same pattern as the total bacteria, where there was a drop in the population in the first experiment, on the treatment (P+M+CS) of 17%, but in the second experiment increased by 58%, both in comparison to treatment (C).

Table 3. Number of colonies forming units (CFUs) of sporulating bacteria in different treatments.

Treatments	Sporulating bacteria					
	Experiment 1			Experiment 2		
	Pre-mulch	Pre-planting	Post-harvest	Pre-mulch	Pre-planting	Post -harvest
	----------10^4 of the number of CFUs g^{-1}----------					
C	5.20 a	15.90 a	14.90 b	29.70 a	6.10 a	36.20 bc
M	5.20 a	15.90 a	7.25 a	29.70 a	8.26 ab	29.90 bc
C+M	5.20 a	9.81 a	12.10 ab	29.70 a	11.90 bc	20.00 ab
P+M	5.20 a	14.30 a	10.10 ab	29.70 a	14.70 c	32.00 bc
M+CS	5.20 a	13.40 a	9.78 ab	29.70 a	5.69 a	17.90 ab
C+M+CS	5.20 a	12.20 a	12.30 ab	29.70 a	24.40 c	8.45 a
P+M+CS	5.20 a	14.70 a	12.30 ab	29.70 a	15.10 c	57.10 c

©-(C) control; (M)- polyethylene mulch; (C+M)- incorporation of *Crotalaria juncea* L. + polyethylene mulch; (P+M)- incorporation of *Pennisetum glaucum* L. + polyethylene mulch; (M+CS)- polyethylene mulch + (Compost-Aid® + Soil-Set®); (C+M+CS)- incorporation of *Crotalaria juncea* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®); (P+M+CS)- incorporation of *Pennisetum glaucum* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®). Means followed by the same letter in the same column do not differ by the Kruskal Wallis test ($p<0.05$).

Changes in the microbial community interfere directly with biological and biochemical processes in the soil, in agricultural productivity, and in the sustainability of agro-ecosystems – acting as an indicator of soil degradation (MATSUOKA; MENDES; LOUREIRO, 2003). Covering the soil with polyethylene mulch + spontaneous vegetation offers great water retention in the system, which increases humidity that in turn may favor the growth of bacteria in the soil, as low humidity is known to restrict the movement and replication of bacterium cells (BERNARDES; SANTOS, 2007). This was exactly what we observed in this study. The treatment (P+M+CS) in the evaluation of sporulating bacteria, only in the second experiment, achieved an increase of 58% of the population in comparison to the control treatment. At the time of pre-planting to total bacteria, evaluations done right after drilling holes in the polyethylene mulch reviewed that most treatments involving polyethylene mulch and the incorporation of plant material showed significantly higher microbe population count than the control treatment (Table 2). That did not happen in the post-harvest evaluations in our experiments.

The initial population count of actinomycetes was $12.70x10^4$ CFUs g^{-1} in the first and $16.00x10^4$ CFUs g^{-1} in the second experiment (Table 4). Treatment (P+M) had the largest population count among all treatments in the pre-planting and post-harvest evaluations in experiment 1. In the second experiment, the (C+M+CS) treatment had the highest population in pre-planting evaluations, while in post-harvest evaluations (P+M+CS) treatment had the highest actinomycetes total population count. Even though there was no significant difference in both experiments in comparison to treatment (C), in the first experiment treatments such as (P+M), (C+M), and (P+M+CS) showed an increase in population actinomycetes of 122, 68, and 49%, respectively. In the second experiment, in post-harvest evaluation, the treatment (P+M+CS) showed the greatest increase among all treatments, compared to treatment (C), there was an increase of 242% in the population of actinomycetes.

Table 4. Number of colonies forming units (CFUs) of total actinomycete in different treatments.

Treatments	Total actinomycete					
	Experiment 1			Experiment 2		
	Pre-mulch	Pre-planting	Post-harvest	Pre-mulch	Pre-planting	Post -harvest
	----------10^4 of the number of CFUs g^{-1}----------					
C	12.70 a	5.45 a	15.60 bc	16.00 a	19.10 a	17.10 ab
M	12.70 a	12.80 b	5.68 a	16.00 a	37.10 ab	13.40 ab
C+M	12.70 a	8.27 ab	26.20 bc	16.00 a	42.90 ab	7.07 a
P+M	12.70 a	48.00 c	34.70 c	16.00 a	48.90 ab	21.00 b
M+CS	12.70 a	10.10 ab	10.10 ab	16.00 a	42.00 ab	15.30 ab
C+M+CS	12.70 a	7.19 ab	9.37 ab	16.00 a	63.70 b	4.56 a
P+M+CS	12.70 a	11.10 b	23.20 bc	16.00 a	51.50 ab	58.40 b

(C)- control; (M)- polyethylene mulch; (C+M)- incorporation of *Crotalaria juncea* L. + polyethylene mulch; (P+M)- incorporation of *Pennisetum glaucum* L. + polyethylene mulch; (M+CS)- polyethylene mulch + (Compost-Aid® + Soil-Set®); (C+M+CS)- incorporation of *Crotalaria juncea* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®); (P+M+CS)- incorporation of *Pennisetum glaucum* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®). Means followed by the same letter in the same column do not differ by the Kruskal Wallis test ($p<0.05$).

Studies evaluating population and microbial activity in agroecological production system indicate that there is a significant effect of the crop developmental stage on the soil microbiota - higher actinomycetes counts were consistently found in post-harvest than before and during the planting stage (FERREIRA; STONE; MARTIN-DIDONET, 2017). However, in our study, the period that showed the highest actinomycetes counts was pre-planting. It is possible that this stage presents greater plant material decomposition and better soil conditions for the development of actinomycetes, given the treatments carried out. That is a plausible hypothesis, since different cultivation systems cause distinct changes in soil microbiological attributes, prompting different effects on soil health and plant development (FERREIRA; STONE; MARTIN-DIDONET, 2017).

A correct management of the soil and cultural remains support the soil microbial population, benefiting groups of specific microorganisms such as actinomycetes, bacteria, and fungi (HUNGRIA et al., 1994). In fact, at 60 days after vegetal material incorporation in the soil, *C. juncea* L. and *P. glaucum* L. were shown to stimulate the highest number of fungi and actinomycetes propagules, significantly higher than the control (no incorporation of plant material) (BOTELHO et al., 2007). We observed the same trend in the treatments where *P. glaucum* L. was incorporated. Noteworthy, this effect was amplified when Compost-Aid® and Soil-Set® products were applied concomitantly with *P. glaucum* L., there was an increase in the population count of the microbes

evaluated. This enhancement effect may occur because these products are known for stimulating the decomposition of organic matter.

CONCLUSION

The incorporation of vegetal materials, *P. glaucum* or *C. juncea*, accompanying with polyethylene mulch when added the soil amendment composts, Compost-Aid® and Soil-Set® increased the population of all soil microbes evaluated in this study (actinomycetes, sporulating bacteria, total bacteria and total fungi), more expressive in the second experiment. The treatment that showed the best results was (P+M+CS), in average of the results obtained from the two experiments in comparison to the control treatment (C), increased in the total fungi population in 183%, total bacteria in 55%, sporulating bacteria in 21%, and actinomycetes in 146%. Therefore, the use of those composts combined is recommended for increase of soil beneficial microbes that will potentially alleviate the detrimental effects of the intensive melon production activities. This was the first step of a promising study, and it should be repeated in field conditions for the direct application of the results. By fostering the establishment and conservation of soil-dweller communities, we can harness great benefits for all crops and food supply. Also, this much-needed novel approach has a great potential to be used as biofungicide to control root rot diseases in intensive melon production areas.

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