Journal of Agricultural Science; Vol. 14, No. 2; 2022
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Effects of *Trichoderma asperellum* BV10 and *Bacillus amyloliquefaciens* BV03 in *Meloidogyne incognita* Control Considering Three Different Management Systems

Camila Rebelatto Muniz[1], Jéssica Brasau da Silva[2], Caroline Sayuri Nishisaka[3,4], Josiane Barros Chiaramonte[2], Veridiana Cardozo Gonçalves Cantão[1], Hercules Diniz Campos[1] & Eduardo Souza Freire[1]

[1] College of Agronomy, University of Rio Verde, Rio Verde, Brazil

[2] Vittia Fertilizantes e Biológicos S.A., São Joaquim da Barra, Brazil

[3] Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, Brazil

[4] Laboratory of Environmental Microbiology, Embrapa Environment, Jaguariúna, Brazil

Correspondence: Eduardo Souza Freire, College of Agronomy, University of Rio Verde, Rio Verde, GO, Brazil. Tel: 55-64-981800126. E-mail: esfreire@unirv.edu.br

Received: November 11, 2021 Accepted: December 16, 2021 Online Published: January 15, 2022
doi:10.5539/jas.v14n2p48 URL: https://doi.org/10.5539/jas.v14n2p48

The research is financed by Dean of Research and Innovation of University of Rio Verde.

Abstract

Crop yield decrease is the main concern when a pathogen or plague is identified in an agriculture field. Thus, part of this issue can be attributed to plant-parasitic nematodes (PPNs), such as *Meloidogyne* species, due to, most of the time, the hard diagnosis, and non-specific symptoms. Its management is mainly based on chemical pesticides, followed by a few potential biological control agents, and the management system. Therefore, this study aimed to evaluate the effects of biological agents in *Meloidogyne incognita* control in different soil systems. For that, two biological products were chosen, *Trichoderma asperellum* BV10 and *Bacillus amyloliquefaciens* BV03, and soils were sampled from three different managements systems: (i) soybean no-tilled system at Goiás state, Brazil; (ii) forest soil at Goiás state, Brazil, and (iii) soybean conventional managed system at Mato Grosso do Sul state, Brazil. Biocontrol and growth promotion effects, volatile organic compounds (VOCs) and soil respiration were determined in vegetation house and laboratory, respectively. As a result, both BV10 and BV03 had significant nematode control activity, comparing to control treatment, in all the three soils systems. Plus, the number of immobile nematodes by potential VOCs had significant increase when BV03 was applied, while the application of BV10 agent raised the soil respiration rate. In conclusion, both biocontrol agents presented great efficiency in control *M. incognita*, with better performance of BV03. Lastly, more studies must be done to elucidate how the resident soil microbiome can influence on biocontrol agent establishment and performance, as well as the consequence of the application of biological products on soil microbiome network.

Keywords: *Meloidogyne* sp., plant-parasitic nematodes, biological control, conventional agriculture, no-till management

1. Introduction

Pathogens and pests are the major responsible of crop yield decrease in agriculture, resulting in economic losses at national and global level (Savary et al., 2019; Benttoumi et al., 2020). Part of this issue can be attributed to plant-parasitic nematodes (PPNs), microscopic roundworms, ranging from 250 µm to 12 mm in length, found in the soil (Kumar & Yadav, 2020). Most of the time, PPNs injuries are unnoticed due to the non-specific symptoms or even unseen damage expression (Kumar et al., 2020; Poveda et al., 2020). Thus, the late diagnosis can compromise plant development, and cause severe damages to the crop, such as distortion, reduction or enlargement of root structure and mass, respectively, what can cause reduction in plant vigor, nutrients uptake and even plant death (Kumar & Yadav, 2020). Among root-knot nematodes, *Meloidogyne* sp. is considered one of the most threat pest that affects many cultivars, such as cotton, soybean, rice, coffee, bell pepper, and tomato plantations (d'Errico et al., 2016; Xiang et al., 2017). Typically, species such as *M. incognita* and *M. javanica* are the most common studied species of PPN. The symptom caused by infection of *Meloidogyne* sp. is gall formation in infected radicular system, which consequently lead to wilting, stunting growth, dwarfism, and nutrients deficiency to the plant (Hawk, 2019; Mazzetti et al., 2019; Sasanelli et al., 2021). Its management is basically based on chemical pesticides such as fumigants and organophosphates (Benttoumi et al., 2020), as well as some potential biological control agents such as *Bacillus* and *Trichoderma* species (Chinheya et al., 2017; d'Errico et al., 2019; Pocurull et al., 2020).

The ascendance of biopesticide market in the past decade has increase the studies involving biological control in agriculture, enhancing the discovery of potential microorganism's species to combat soil borne pests and diseases (Ruiu, 2018; Ortiz & Sansinenea, 2021). It is a more sustainable alternative method of control comparing to chemical nematicides, avoiding human health harm and environment pollution (Abd-Elgawad & Askary, 2020). Plus, the application of a chemical pesticide aims to kill as many nematodes as possible, while the use of biopesticides intends to control nematodes populations and/or the damage caused by them, by introducing or manipulating an organism naturally antagonist to them (Poveda et al., 2020). Although there are several products to control PPNs, another important factor that influences on disease severity is the type of soil management (Atandi et al., 2017; Silva et al., 2018). It is known that most of the time, no-tillage system features higher index of organic matter compared to conventional management, what has directly influence on microbial activity and the improvement of soil structure, what can reduce the incidence of PPN on soil surface (Collange et al., 2011). Thus, comparing both systems, conventional management usually applies subsoil method to restore water and nutrient uptake of cropped plants, contributing to increase the incidence and spread of nematode communities throughout soil disturbance and residue supply on surface (Van Capelle et al., 2012; Zhang et al., 2019). Although it can happen most of the time depending on soil depth (Van Capelle et al., 2012), Timper and contributors (2021) showed that winter cover crops could enhance early season suppression of *Meloidogyne* sp. in conventional crop systems in any soil depth in some locations, but this control is more likely to follow the type of organism involved in the suppression. Certainly, many organisms could be part of the *Meloidogyne* sp. combat, such as bacteria and fungi communities (Timper et al., 2021), emphasizing the potential of soil microbiome to avoid PPN diseases. Eventually, *Bacillus amyloliquefaciens* and *Trichoderma asperellum* are commonly known to be applied in many biocontrol cases, such as controlling phytopathogenic fungi, bacteria, and nematodes (Harman et al., 2004; Lorito et al., 2010; Chowdhury et al., 2015; Rivera-Méndez et al., 2020). Thus, this study aimed to evaluate the effects of *Bacillus amyloliquefaciens* BV03 and *Trichoderma asperellum* BV10 in plant-parasitic nematode control, comparing different soils systems, forest (representing newly opened crop area), conventional managed and no- till systems.

2. Method

2.1 Site Descriptions and Soil Collection

Three different field sites were chosen to soil collection (0-20 cm) in Midwest Brazil; two sites (Forest and Soybean No-till Management) are in Rio Verde municipality (17°47′7.091″S and 50°57′53.528″W; 17°47′49.610″ S and 50°59′38.130″ W, respectively), in the state of Goiás (GO), Brazil. The third site (Soybean Conventional Management) is in Chapadão do Sul municipality (18°48′5.087″S and 2°36′26.208″W), in the state of Mato Grosso do Sul (MS), Brazil (Figure 1). Among the sampling sites, the forest is a conserved area without any type of management in its history, which was chosen to represent deforested areas for crop production, considered as first plating soil. However, soybean no-till management field is under this procedure for 10 years, using crop rotation (soybean/corn) with minimum soil disturbance and preservation of old crops remains; while soybean conventional management field is under this procedure for 3 years, planting soybean every year with soil tillage.

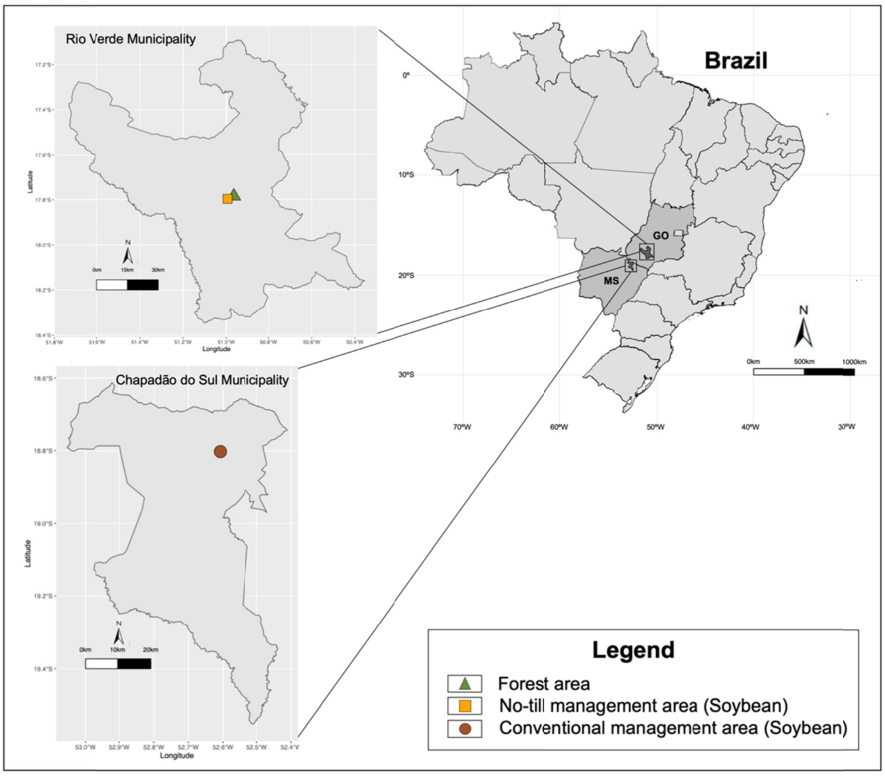

Figure 1. Sampling site locations for Forest, No-till management soybean area and Conventional management soybean area. Soil sampling cores were randomly chosen at each site of study during Brazilian summer season

2.2 Experiment Design and Treatments

Biocontrol agent efficiency test experiment was organized in randomized block design (RBD) in factorial arrangement 3x3, containing six replicates each treatment, totalizing 54 pots. All procedure was conducted in vegetation house at University of Rio Verde (UniRV), in Rio Verde municipality, GO, Brazil. The treatments consist in three soil managements (forest soil—representing newly opened agricultural areas, soybean conventional management and soybean no-till management soils), and two different commercial biocontrol agent application, the Tricho-Turbo® (*Trichoderma asperellum* BV10—Register number: 34018), and No-Nema® (*Bacillus amyloliquefaciens* BV03—Register number: 34518), plus the control treatment (deionized/sterilized water application).

To analyze the efficiency of both biocontrol agents as plant growth promoters and nematicide, under different managed soils, three seeds of Brasmax Bônus Impro-8579 Ipro soybean, which is susceptible to *M. incognita* nematode, were planted in 700 mL polythene pots. Before planting, seeds were microbiolized with BV10 (1 mL kg^{-1}), BV03 (2 mL kg^{-1}) or deionized water, according to each treatment. After nine days of seedling, the thinning of the plants was carried out, leaving just one plant per pot. The phytonematode inoculation occurred in the same day, by inoculate 5,000 *M. incognita* eggs per plant. Irrigation occurred daily by applying 65 mL of water once a day in each pot, with no other inoculation or fertilizer application during the time course. Furthermore, 30 days after the soil infestation by PPN, totalizing 40 days of experiment, it was evaluated the plants' aerial part fresh mass (g), root fresh mass (g), and the number of eggs per radicular system.

2.2.1 *Meloidogyne incognita* Inoculum

The inoculum of *M. incognita* was produced in *Lycopersicon lycopersicum* (tomato) roots cultivated in vegetation house using agricultural substrate. After 60 days of inoculation tomato roots showed galls and eggs masses, which provided the eggs extracted according to Hussey and Barker (1973) and modified by Bonetti and Ferraz (1981). Thus, after inoculum concentration adjustment using Peters' chamber (Southey, 1970), the resulted suspension was used as inoculum for the experiment.

2.2.2 Biocontrol Agent: *Trichoderma asperellum* BV10 and *Bacillus amyloliquefaciens* BV03

Both biocontrol agent was provided by Vittia Fertilizantes e Biológicos S.A (Vittia Group). The Tricho-Turbo® (Register number: 34018) is a microbiological fungicide and nematicide, with proven effect against diseases and pests such as *Rhizoctonia solani*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum* and *Pratylenchus brachyurus* (Vittia Group, 2021a). Besides that, No-Nema® (Register number: 34518) also has proven effectiveness as fungicide, but its application is mainly for PPNs, such as *M. javanica*, *M. incognita*, *Heterodera glycines*, and *Pratylenchus brachyurus* (Vittia Group, 2021b).

2.3 Volatile Organic Compounds Measurements

After the biocontrol agent efficiency test described in topic *2.2*, each treatment had its soil samples mixed separately and reused for possible volatile organic compounds (VOCs) analysis. For that, split Petri plates were used with 10 g of each treated soil, with further sealing and incubation at ±25 °C. After five days, it was added 500 second stage juveniles (J2) of *M. incognita*, with another sealing and incubation process. Finally, it was evaluated the mobility of J2 after 24, 48 and 72 hours after exposition to the treated soils using an inverted image microscope. This step took place at University of Rio Verde laboratory, which was completely randomized designed (CRD) in factorial arrangement of 3 × 3 + 1, with eight replicates, totalizing 80 Petri plates.

2.5 Microbial Activity Respirometry

The measurements were based on Anderson (1982) methodology, using completely randomized design (CRD) in factorial arrangement of 3 × 3 + 1, with three replicates, totalizing 30 bottles. For this, 200 g of treated soil from vegetation house experiment was incubated in hermetically sealed bottles, containing a recipient with 20 mL of NaOH 0.44 M solution, for the absorption of released CO_2. When the bottle was opened for CO_2 analysis, it was added 1 mL of 30% of $BaCl_2$ (to not mask the CO_2 consumed by microbiota when the bottle is opened), followed by titration with HCl 0.46 M and NaOH excess in presence of phenolphthalein (1 drop of phenolphthalein + 80 mL of 60% ethanol v/v, completing by 100 mL of ethanol). This experiment was conducted for 1 month and the evaluations were performed at intervals of 14 days, except for the first evaluation that occurred 24 hours after incubation. The CO_2 evolution is an important measurement of soil microbiota activity, facilitating the inference of "soil health", together with BioAS analysis (Section 2.6).

The quantification of released CO_2 was based in neutralization volumetry, where the oxide reacts with sodium chloride solution that is in excess. Thus, the non-reacted hydroxide is titrated with acid solution in the presence of acid-base indicator (phenolphthalein 1%). After that, the calculation for CO_2 quantification was based in Stotzky (1965), as the Equation (1).

$$CO_2\ (\text{mg } 100\ \text{g}^{-1}) = (\text{V blank} - \text{V sample}) \times \text{M(HCl)} \times \text{Eq g } CO_2 \tag{1}$$

where,

V blank = volum of HCl wasted on blank (mL); V sample = volum of HCl wasted in each sample (mL); M(HCl) = 0.48 mol L^{-1}; Eq g CO_2 = 6 (C-CO_2).

2.6 Soil Physical-Chemical and Enzyme Analysis

One composite sample of each treatment was sent to physical-chemical analysis and enzyme parameters using BioAS technology (Embrapa, 2020), at Embrapa Cerrado, Planaltina, DF, Brazil. Clay, silt, and sand contents were analyzed by NaOH dispersion and pH estimated in $CaCl_2$ solution. Micronutrients, such as copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn), were analyzed by NaOH dispersion, while macronutrients, such as phosphorus (P) and potassium (K), were analyzed by resin and mehlich 1 extractors, respectively. In addition, enzymatic activity of arylsulfatase and β-glucosidase were also quantified by the BioAS methodology (Tabatabai, 1994; Mendes et al., 2018), which allowed the estimation of biological quality index.

2.7 Data Analysis

The analysis of variance (ANOVA) was carried out to analyze the effects of biocontrol agents as plant growth promoters and nematicides in different soil managements. For that, traits such as aerial part and root fresh mass, VOCs in different time, nematodes total number and nematodes number per gram of soil were considered for analysis, Significant average differences were further investigated by Tukey's test ($P < 0.05$), considering the three types of soil used in this study. Parameters that allowed the application of ANOVA, normality of results and homogeneity of variants, were checked with the Shapiro Wilk and Bartlett tests. Multivariate analysis was based on principal components analysis (PCA) were applied to summarize the effect of each trait in samples and *Pearson* correlation coefficient was visualized by heatmap. All statistical analysis and graphs were created in RStudio, version 1.2.5033 (RStudio Team, 2020).

3. Results

3.1 Efficiency of Trichoderma asperellum BV10 and Bacillus amyloliquefaciens BV03 as Plant Growth Promoters and Nematostatic Effect in Different Soils Sources

Trichoderma asperellum BV10 showed higher weight of aerial part fresh mass in forest soil compared to control and *Bacillus amyloliquefaciens* BV03. The latter showed higher capacity as plant growth promoter considering soil from no-till management, presenting significant difference comparing to control and BV10. Considering soil effects, control treatment showed difference between plant development when compare forest, conventional management, and no-till management soils, with higher values of aerial part fresh mass in plants from forest soil. Thus, it is clear the effect of different soils on plant growth without any type of inoculation, and how BV10 and BV03 could balance these differences, with significant change in conventional manage soil when BV03 is applied (Table 1).

Plant root fresh mass from conventional managed soil had significant weight difference when received BV10 and BV03 treatments, compared to control. Plus, it also showed the lowest weight of root fresh mass in control and BV10 treatments when compare with other soils (Table 1).

Table 1. Aerial part and root fresh mass of soybean plants cultivated in vegetation house under treatment of water (control), *Trichoderma asperellum* BV10 and *Bacillus amyloliquefaciens* BV03 after 40 days of experiment

Soil management	Treatments		
	Control	BV10	BV03
Aerial part fresh mass (g)			
Forest	6.23^{Bb}	7.81^{Ab}	5.34^{Ba}
Conventional management	4.91^{Bc}	5.94^{Aab}	6.18^{Aa}
No-till management	7.38^{Aa}	6.86^{ABa}	6.31^{Ba}
Root fresh mass (g)			
Forest	11.26^{Aa}	11.7^{Aa}	9.76^{Aa}
Conventional management	6.99^{Bb}	8.55^{ABb}	9.72^{Aa}
No-till management	12.56^{Aa}	12.34^{Aa}	10.59^{Aa}

Note. BV10 = *Trichoderma asperellum*; BV03 = *Bacillus amyloliquefaciens*. Tukey's test was performed separately to compare the biocontrol agents within the same soil management (upper-case letters) and the effect of each one within different soil management (lower-case letters). Values with the same upper or lower-case letters were not significantly different ($P < 0.05$) between contrasted samples.

Nematode control of biological agents in different soils was measured by counting the *M. incognita* eggs number in plants radicular system at the end 40 days in vegetation house (Figure 2). Significant decrease in nematode eggs number was saw in application of BV10 and BV03 in control treatment, with lower number of nematodes eggs in BV03 than BV10 treatment. Conventional management, again, showed significant differences in *M. incognita* eggs when received BV10 and BV03, compared to each other and to control treatment. Plus, no-till management also had their nematode incidence decreased when biocontrol agent's application occurs, but there is no significant difference between BV10 and BV03 treatments (Figure 2).

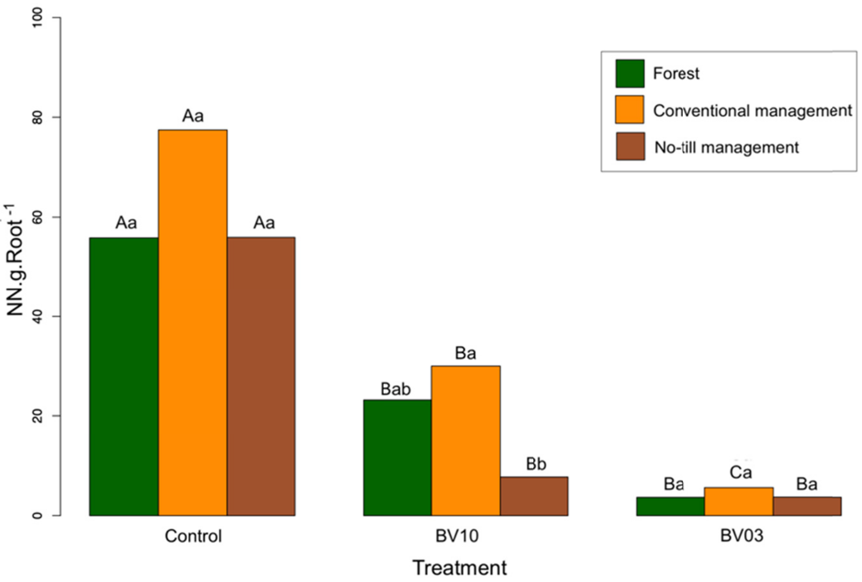

Figure 2. Bar chart of *M. incognita* eggs number per gram of root in the three different treatments and different soil managements

Note. NN g Root^{-1} = Nematode eggs number per gram of root; BV10 = *Trichoderma asperellum*; BV03 = *Bacillus amyloliquefaciens*. Tukey's test was performed separately to compare the biocontrol agents within the same soil management (upper-case letters) and the effect of each one within different soil management (lower-case letters). Values with the same upper or lower-case letters were not significantly different ($P < 0.05$) between contrasted samples.

3.2 Volatiles Organic Compounds (VOCs) Produced From Soils Treated With Biocontrol Agent, Trichoderma asperellum BV10 and Bacillus amyloliquefaciens BV03, During Meloidogyne incognita Control

Differences on VOCs production during Petri plate incubation of forest soil could be observed in the first 24 h, which BV03 treated forest soil had significant higher number of immobile nematodes compared to control and BV10 treatment (Table 2). Although the two agricultural soils did not show significant changes on nematode motility after 24 h of incubation, the results of VOCs production could be saw after 48 h, when the number of immobile nematodes in different soils and treatments had significant differences.

Table 2. Number of immobile nematodes after Petri plates incubation with different soil managements un- and treated with biological control agents

Soil managements	Treatments		
	Control	BV10	BV03
24 hours			
Control	1.00	1.00	1.00
Forest	0.92	0.87	0.96
Conventional management	0.96	1.00	1.00
No-till management	0.96	0.90	0.95
48 hours			
Control	1.00	1.00^{a}	1.00^{a}
Forest	0.96^{A}	0.93^{ABab}	0.86^{Bb}
Conventional management	0.90^{A}	0.88^{ABb}	0.80^{Bb}
No-till management	0.90^{A}	0.90^{Aab}	0.90^{Aab}
72 hours			
Control	0.98	0.98	0.98^{a}
Forest	0.86	0.90	0.83^{ab}
Conventional management	0.90^{A}	0.85^{A}	0.65^{Bb}
No-till management	0.88	0.86	0.88^{a}

Note. Tukey's test was performed separately, for each time of incubation, to compare the biocontrol agents within the same soil management (upper-case letters) and the effect of each biocontrol agent within different soil management (lower-case letters). Values with the same upper or lower-case letters were not significantly different ($P < 0.05$) between contrasted samples, and no letters indicate no statistics differences.

Forest and conventional managed soils treated with BV03 showed differences in nematode motility after 48h. The same occurs when considering 72h of incubation, significant differences could be noticed, with lower number of nematodes motility in conventional managed and forest soils. Plus, inside conventional management soil treatments, BV03 also showed to reduce nematodes motility, compared to BV10 and the control.

3.3 Soil Microbial Activity in Different Soils Under Trichoderma asperellum BV10 and Bacillus amyloliquefaciens BV03 Application

Among the three soils, in conventional managed soil BV10 and BV03 application got higher CO_2 release, comparing to its control (Figure 3B). The opposite occurs on no-tilled soil, where biocontrol agents did not overcome control treatment, and both, BV10 and BV03, had similar microbial respiration taxa (Figure 3C). Furthermore, forest soil showed higher CO_2 releasing when BV10 was applied, while BV03 showed similar behavior until day 1, decreasing the respiration drastically after this time point (Figure 3A).

Considering the treatments in different soil type, the respiration rate observed in treatment without inoculation (control) got the highest CO_2 measurement in no-tilled soil, followed by control and conventional managed soil. This result is certainly due to the levels of organic matter that no-tillage provides. Similarly, BV03 application also got the highest CO_2 measurement in no-tilled soil, followed by conventional managed and forest soils. Finally, when BV10 is applied, the lowest CO_2 emission was in forest soil, with medium and higher emission in conventional and no-tilled soils, respectively. These results showed that the biocontrol agents, besides the efficiency in nematodes control, also improved soil health.

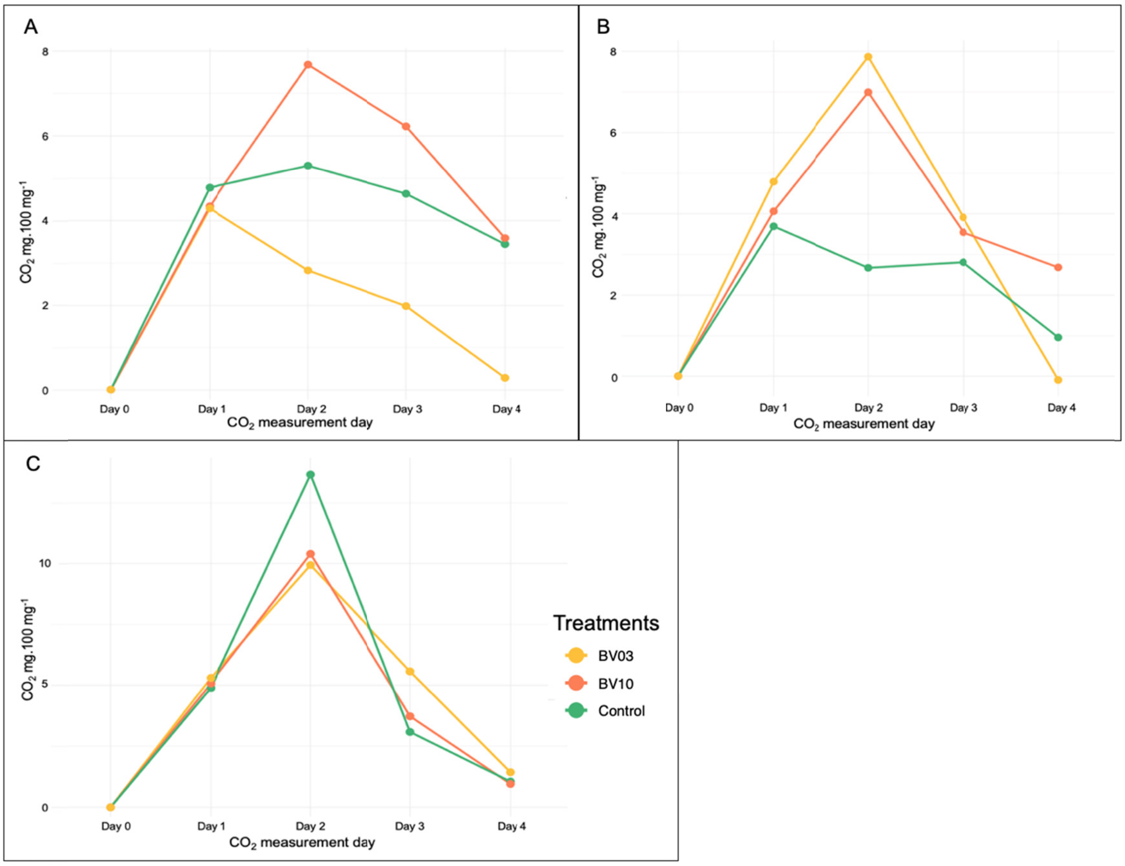

Figure 3. CO_2 measurements from different soil managements with application of two biocontrol agent. A. Forest soil; B. Conventional management soil; C. No-till management soil

Note. CO_2 measurements were taking in five time points, which is: day 0 (same day of experiment assembly), day 1 (1 day), day 2 (15 days), day 3 (30 days) and day 4 (45 days after experiment assembly)

3.4 Multivariate Analysis

The principal component analysis (Figure 4), forest soil (Figure 4A) showed negative correlation between application of both biocontrol agents, BV10 and BV03, and the number of nematodes per gram of roots. The opposite is observed in control treatment, which correlated positively with number nematodes, as well as with CO_2 collected at time 1. Soils treated with BV10 had positive correlation with aerial part fresh mass and volatiles (72h); while BV03 treated soils correlated with clay and β-glucosidase. The dimensions of Forest PCA had an explanation of 56% and 22.6% in "Dim1" and "Dim2", respectively. Plus, conventional managed soil (Figure 4B) showed, again, correlation between control treatment with number nematodes, followed by positive correlation between BV10 application and CO_2 production at time 4 and BV03 application with CO_2 production at time 1. For that, "Dim1" explained 60.7% of results, and "Dim2" 16.5%. Considering no-tilled soil (Figure 4C), control treatment correlated directly with number of nematodes and CO_2 at time 2, while BV10 treatment correlated positively with pH, and BV03 with CO_2 at times 1, 3 and 4, arylsulfatase, β-glucosidase, clay, and SOM content. The explanation for this PCA consisted in 56.4% in "Dim1", and 16.5% in "Dim2".

Correlating variables for each soil of study (Figure 5), in forest soil (Figure 5A) there was a negative correlation between nematode eggs number and clay, pH, β-glucosidase, and SOM (values of -0.80^{***}, -0.44^{*}, -0.66^{**}, and -0.92^{***}, respectively). However, in conventional soil (Figure 5B, upper triangle), there was positive correlation with the same previous variables (values of 0.63^{**}, 0.40^{*}, 0.47^{*}, and 0.70^{**}, respectively), and negative correlation with aerial and root part fresh masses, volatiles (24h), and microbial activity (CO_2 production) at times 1, 2 and 3 (values of -0.68^{**}, -0.61^{**}, -0.71^{***}, -0.74^{***}, -0.78^{***}, -0.80^{***}, respectively). Analogous to forest soil, no-tilled soil (Figure 5B, lower triangle), also presented negative correlation between nematodes number and clay, pH, β-glucosidase, and SOM, with addition of arylsulfatase and microbial activity at times 1 and 3 (values of -0.63^{**}, -0.42^{*}, -0.45^{*}, -0.62^{**}, -0.64^{**}, -0.84^{***}, and -0.72^{***}, respectively). A positive relationship was observed when compared nematodes number with aerial part fresh mass (value of 0.52*). All correlations cited were statistically significant according to *Pearson* correlation.

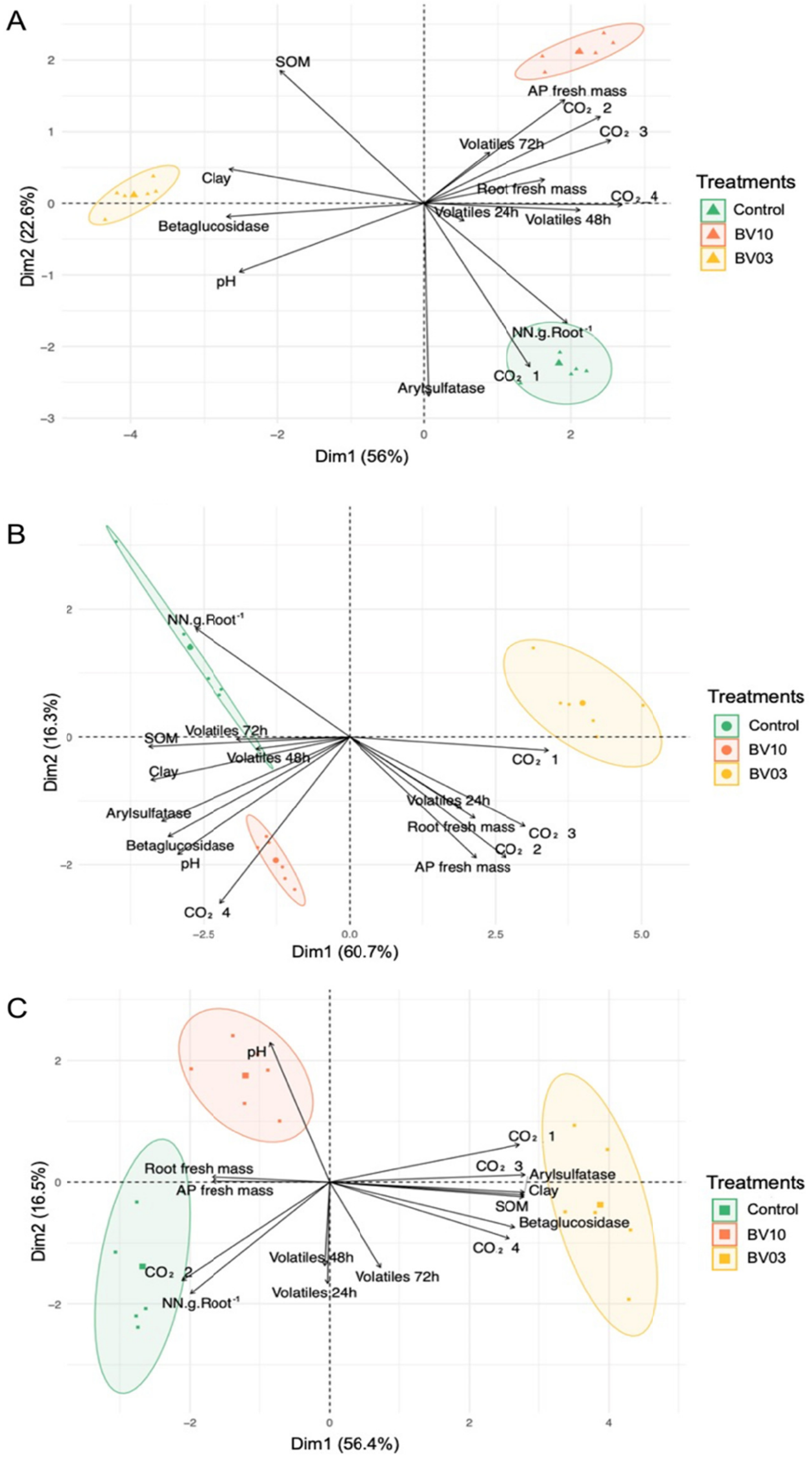

Figure 4. Principal components analysis (PCA) of different soil managements under two biocontrol agent effect and control. A. Forest soil; B. Conventional management soil; C. No-till management soil

Note. AP fresh mass = Aerial part fresh mass; NN g Root^{-1} = Nematode eggs number per gram of root; SOM = Soluble organic matter; CO_2 1 = CO_2 collection at time 1; CO_2 2 = CO_2 collection at time 2; CO_2 3 = CO_2 at time 3; CO_2 4 = CO_2 collection at time 4.

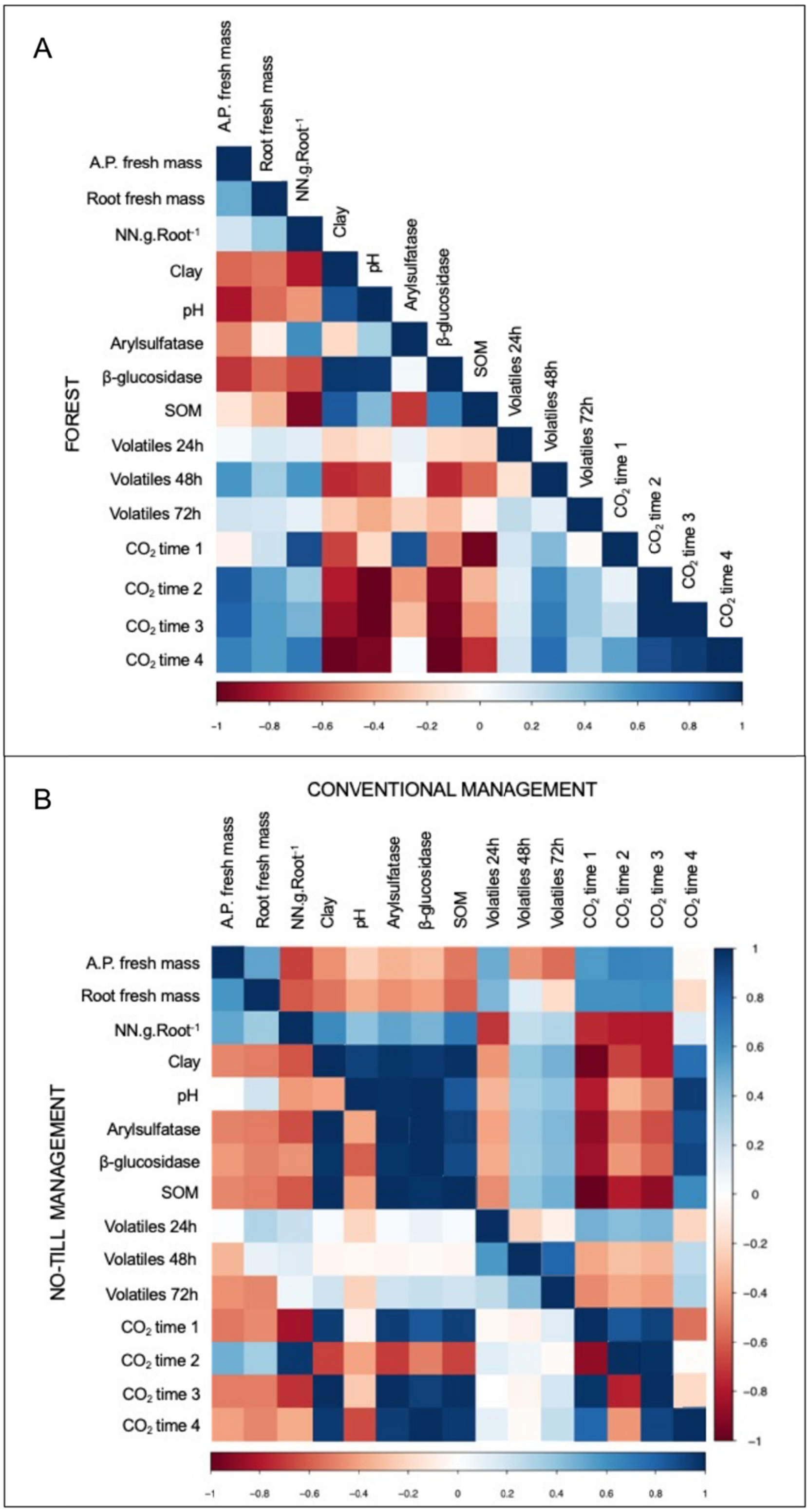

Figure 5. Heatmap showing the Pearson coefficient correlation of three different soil managements under two biocontrol agent effect and control. A. Forest soil; B. Conventional management soil (upper triangle) and No-till management soil (lower triangle)

Note. AP fresh mass = Aerial part fresh mass; NN g Root^{-1} = Nematode eggs number per gram of root; SOM = Soluble organic matter.

4. Discussion

The application of *Trichoderma asperellum* BV10 resulted in higher aerial part fresh mass in forest and conventional soils (Table 1). However, as biocontrol agent of *M. incognita*, it had different range according to soil management. No-till managed soil had the lowest number of nematodes eggs per gram of root, followed by forest soil and conventional managed soil (Figure 2). Plus, comparing BV10 treatment with control, there was a significant decrease on nematodes eggs number, in all soils. *Trichoderma* sp. (BV10 specie) is known to have antagonism effect on nematodes like *Meloidogyne* sp. (Lorito et al., 2010), one mechanism is the fungi hydrolytic enzymes that have biocontrol effects on eggs laying and hatching (Medeiros et al., 2017). Besides that, *Trichoderma* sp. can also induce systemic resistance against PPNs in a host plant (Medeiros et al., 2017; Pocurull et al., 2020). For instance, Martínez-Medina and collaborators (2017) showed systemic resistance in tomatoes against *M. incognita* infection by applying *Trichoderma* sp., which regulates both salicylic acid (SA) and jasmonic acid (JA) pathways through priming effect. Although those mechanisms could be the key to control *M. incognita*, as much it is known, volatiles organic compounds (VOCs) produced by *Trichoderma* sp. can also present nematode control effects (Bui & Desaeger, 2021). Thus, potential VOCs production from BV10 presented significant decrease on nematode mobility after 48 h of incubation (Table 2), in both forest and conventional managed soils, when it is added to soils.

By inoculating the *Bacillus amyloliquefaciens* BV03 in different soils, greater root fresh mass was observed in conventional managed soil (Table 1). Plus, a considerable decrease in nematode eggs was observed (Figure 2) compared to application of BV10 and the control. However, there was no difference between type of soils for BV03 bioinoculant. Hence, Rios et al. (2018) state that the *B. amyloliquefaciens* has been showing good results in control nematodes, such as the *Meloidogyne incognita* (Lobna & Zawam, 2010; Abdel-Salam et al., 2018). *Bacillus* spp. uses many mechanisms to fight parasitic nematodes, such as toxins (*e.g.*, Cry proteins), catabolic enzymes (*e.g.*, proteases, chitinases and glucanases), and other molecules, like peptide antibiotics (Lian et al., 2007; Abdel-Salam et al., 2018). Despite some researchers relate the VOCs production from *Bacillus* genre bacteria against other pathogenic bacteria and fungi (Kim et al., 2013; Raza et al., 2016), Bui and collaborators (2020) showed the potential of *Bacillus* sp. VOCs against nematodes from *Meloidogyne* sp. genre. Likewise, when BV03 was applied, fewer mobile nematodes were observed in forest and conventional managed soils, after 48h and 72h of incubation (Table 2).

Considering management systems, the presence of biocontrol agents influenced in microbial activity in forest and conventional managed soils (Figure 3). It is known that the microbiota participates in soils' physical and chemical processes, which are directly associated with crop development and management (Zhang et al., 2018). In this sense, the mineralization process of soil organic matters and the conversion of those fertilizers' compounds into readily available nutrients for plants stands out, mainly due to the enhancement microbial community's activity by the application of biocontrol agents. Furthermore, in forest soil, when compared different variables (Figure 4A), a positive correlation between control treatment and number of nematodes eggs per gram of root was observed. While the application of BV10 and BV03 negatively correlated with the same variable, indicating effectiveness of these microorganisms in nematodes controlling, and corroborating with above mentioned data. Plus, the application of BV10 in forest soils correlated positively with aerial part fresh mass (Figure 4A), which was significatively greater in this treatment than in control and BV03 (Table 1), corroborated by many authors (López-Bucio et al., 2015; Guzmán-Guzmán et al., 2019; Macena et al., 2020) due to the growth promotion action from *Trichoderma* species.

Like control treatment in forest soil, the control of conventional and no-till managed soil also correlated positively with nematode eggs number. Same happened in BV03 treatment from forest and no-till soil, both correlated positively with clay content and betaglucosidase (Figures 4A and 4C). However, the last one also correlated in a positive way with arylsulfatase, soluble organic matter, and microbial respiration at day 1, 3 and 4 (Figure 4C). Besides, the application of BV10 in conventional and no-till soils had different correlations, where the first one correlated positively with microbial respiration, and the second one with pH (Figure 4B and 4C). Thus, different relationships between biocontrol agent application and soil properties can be directly related to the soil management system, since it has directly influence on soil aggregation, nutrient availability, and resident microbial diversity (Fausto et al., 2018). To correlate the variables, negative correlation was observed between nematode number of eggs and clay, pH, betaglucosidase and SOM in forest soil (Figure 5A). Alike this, no-till system soil also showed negative correlation between nematode number of eggs and those variables, in addition of arylsulfatase, respiration in day 1, 3 and 4 (Figure 5B, lower triangle). On the other hand, distinct behavior between variables comparison is observed in conventional system (Figure 5B, upper triangle). Where nematode number of eggs is negative correlated with volatiles at 24 h measurement and microbial respiration at day 1, 2 and 3.

5. Conclusion

The control of *M. incognita* was effective when both biocontrol agents were applied. However, BV03 showed a better efficiency in decrease nematode number of eggs per gram of roots compared to BV10, in all three soils. In addition, in VOCs test, forest and conventional managed soils treated with BV03 showed fewer mobile nematodes after 48 and 72 hours, compared to control and BV10 application. Although there was a difference between BV10 and BV03, the first one also significantly decreased the nematode numbers of eggs per gram of roots but didn't present significative decrease on mobile nematodes compared to BV03. In agriculture, the main concern when a biocontrol agent is applied is how it will behave in front of different soil properties and management. Thus, even with good nematode control effect from both biocontrol agents, it is clear their different behavior when compared the three soil systems. For that, more studies need to be done to elucidate how the resident soil microbiome can influence on biocontrol agent establishment, as well as the consequence of the application of biological products on soil microbiome network.

Acknowledgements

To the Dean of Research and Innovation of University of Rio Verde for the granting of the research accomplished, and to the Vittia Group for the support.

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