

Efficacy of bioproducts on the microbial population and diversity of an agricultural soil in arid zones

Mirella Romero-Bastidas^{1,§}

Eslí Alexis Mayer-Félix¹

Pablo Misael Arce-Amézquita¹

Maurilia Rojas-Contreras¹

Carlos Rangel-Dávalos¹

José Saúl Hernández-Rubio¹

1 1 Universidad Autónoma de Baja California Sur. Carretera al sur km 5.5, colonia el Mezquitito, La Paz, Baja California Sur, México. CP. 23080. Tel. 612 1238800.

(e.mayer@uabcs.mx; parce@uabcs.mx; mrojas@uabcs.mx; crangel@uabcs.mx; jhrubio@uabcs.mx).

Autor para correspondencia: miromero@uabcs.mx

Abstract

Natural products are an alternative to the use of synthetic fertilizers. Nonetheless, their effect on microbial communities in arid soil is poorly known. To reveal the response of soil fungi, bacteria, and nematodes to organic amendments and beneficial microorganisms, plastic boxes were filled with a mixture of unsterilized agricultural soil and five bioproducts, such as *Sargassum* spp. dry matter, worm humus, worm humus leachate, *Trichoderma harzianum*, and *Bacillus amyloliquefaciens*; in addition, a treatment based on a synthetic fertilizer (T17), a fungicide/bactericide (copper), and water control were added. Each treatment was moistened with 1 L of sterile distilled water. At 0 and 30 days after the treatments, the variables of microbial population, relative abundance, and diversity of each type of microorganism were evaluated using the Shannon index. In most of the bioproducts, the microbial population decreased, but the diversity of species present increased, and although there were no significant differences between treatments, the treatments of humus and *Sargassum* spp. were recorded with the highest value in population and diversity. This study shows that not all bioproducts have a positive effect on the increase of microbiomes in the soil.

Keywords:

ecology, microbiome, microorganisms, richness.



Introduction

Natural products, extracted from microorganisms, plants or animals, have been used for centuries (Ranjha *et al.*, 2021). Processing these natural products for significant benefits has been the priority in all practical systems of biotechnology with the aim of achieving useful and safe production of sustained food (Ranjha *et al.*, 2022). This type of practice aligns with the fact that one of the main challenges faced by agriculture in the twenty-first century is to sustainably produce enough food, fiber, and biofuels to meet the needs of a rapidly growing population (FAO, 2017).

In addition to this, consumer demands are increasingly strict and specific, so modern agriculture is constantly changing and innovating strategies (Massaglia *et al.*, 2019). In recent decades, processes of transition and conversion from conventional agricultural production systems (monocultures, use of agrochemicals, among others) to agroecological production systems (agrobiodiversity, nutrient recycling, among others) have been promoted with the aim of promoting food security and sovereignty in accordance with the care of the environment (Cevallos *et al.*, 2019).

In this context, several studies have shown that the soil microbiota plays a key role in the optimal development of crops by influencing their yield and quality (Gazolla *et al.*, 2022). These microbial communities are primarily responsible for promoting and stabilizing carbon storage in the soil through the decomposition of organic matter through biogeochemical processes. Through this process, soil fertility, ecosystem function, and the productivity of cultivated plants improve (Zou *et al.*, 2017).

In addition, they act in the suppression of soil-borne diseases and the promotion of plant growth (Bardgett and van der Putten, 2014). Within the great diversity of the soil microbiome, fungi, bacteria, and nematodes are the most sensitive and rapid indicators of soil disturbance (Laasli *et al.*, 2022). It has long been known that one of the main problems in the destruction of soil microbiomes is the use of synthetic fertilizers employed to maximize yields in the short term and produce high yields in monocultures (Sangiorgio *et al.*, 2022), since these have generated negative consequences on terrestrial ecosystems, causing the loss of species through eutrophication and acidification, which impact ecological niches and their food chain (Suman *et al.*, 2022).

Therefore, the new challenge is to ensure healthy crop production approaches through alternative ecological strategies, such as the use of biological agents and natural products; however, although it has been reported that biological products benefit plant development and interact efficiently with the soil microbiota (Swaroop *et al.*, 2020), there are few studies that have investigated the effect of bioproducts on the abundance, composition, and diversity of these organisms in agricultural soils.

The hypothesis of the present study is that the different biological products will have a differential response on the soil microbiota, which can provide key information in production systems within sustainable agriculture. Therefore, this study aimed to evaluate the influence of bioproducts on the population and diversity of the microbiota of an agricultural soil in arid zones.

Materials and methods

Study area

The experiment was conducted in June 2022 in the Phytopathology Laboratory of the Autonomous University of Baja California Sur (UABCS, for its initialism in Spanish), located in the municipality of La Paz, in the state of Baja California Sur, Mexico (24° 06' 03" north latitude, 110° 18' 54" west longitude). This area is characterized by a semi-arid climate and its altitude ranged between 31 and 47 masl. The average annual rainfall was 275 mm and the average annual temperature was 23.8 °C. The soil texture is sandy loam, with a pH of 7.8, and poor in organic matter.

Soil sampling

Sampling was carried out randomly by obtaining nine soil subsamples (0-30 cm deep) in the experimental agricultural field of the UABCS, which had 0% vegetation cover. The subsamples obtained were homogeneously mixed to obtain a mother sample. These were stored in polyethylene bags and stored at room temperature (25 °C) until their subsequent analysis, which was 24 h after sampling.

Experimental design

This experiment was carried out *in vitro*, where 4 kg of soil collected from the agricultural field was deposited in 24 polystyrene trays (25 x 35 x 20 cm) previously disinfected with NaClO (1%) for 1 h. The corresponding treatments were previously added to each tray with soil in the established doses (Table 1) and then the soil was moistened with 1 L of sterile distilled water.

Table 1. Treatments established in the experiment.

Treatments	Manufacturer	Dose kg ⁻¹ of soil
Macroalga <i>Sargassum</i> spp.	UABCS	10 g
Worm humus	UABCS	10 g
Worm humus leachate	UABCS	10 ml
<i>Trichoderma harzianum</i>	Strain Th-A001	10 ml (1x10 ⁸ spores ml ⁻¹)
<i>Bacillus amyloliquefaciens</i>	Strain Ba-A001	10 ml (1x10 ⁹ CFU ml ⁻¹)
Synthetic fertilizer (NPK)	Vigoro [®]	g L ⁻¹
Fungicide/bactericide (copper hydroxide)	Cupravit-hidro [®]	1 ml L ⁻¹
Control (without treatment)	-	

The trays were established in a growth chamber under a photoperiod of 12-12 h (day/night), temperature 25-30 °C, and relative humidity of 60%. The treatments were irrigated weekly with sterile distilled water to maintain soil moisture. The experiment was established under a completely randomized design with three replications, where each replication corresponded to a plastic tray.

Soil microbial composition

The microbial population and diversity of the soil of each treatment (n= 24) was determined in each tray from the collection of five soil subsamples (0-10 cm deep) at random, before (0 days) and after the application of the treatments (30 days). The population of fungi and bacteria was determined with Lip#*a* and Ulea's (2018) method, by plate counting of colony-forming units (CFUs) in 1 g of soil through the method of serial dilutions of 1 x 10⁻³. Two hundred microliters were used for seeding.

The number of bacteria was quantified in nutrient agar (Bioxon) and that of fungi in potato-dextrose-agar (PDA) (Bioxon). They then went through an incubation period of 28 °C for 48 h in the case of bacteria and seven days in the case of fungi. From each sample, the isolates were purified for identification. The total nematode population was recorded using Baerman's (1917) funnel extraction method and 48 h later, the number of phytopathogenic, saprophytic or free-living specimens per 100 g of soil was counted.

Identification

The identification at the genus and species levels of bacterial and fungal isolates and the nematological specimens present in the soil was carried out in the Phytopathology Laboratory of the UABCS by means of light microscopy (40x) to determine the characteristics of each type of microorganism in relation to the shape, texture, and color of its morphology, which were compared

with taxonomic keys, such as Barnnet and Hunter's (1972) for fungi, Eisenback and Triantaphyllou's (2020) for nematodes, and Sher's (1966) for bacteria.

Statistical analysis

Data were obtained using a Kruskal-Wallis analysis with a significance level of 0.05. The diversity (richness) of each phylum was determined through relative abundance using formula of Muniappan and Muthukumar's (2014).

$$\text{Relative abundance(\%)} = \frac{\text{Number of samples with a particular genus}}{\text{Total number of samples evaluated}} \times 100$$

Likewise, the Shannon-Wiener index (H0) was also used through formula Shannon's (1948). Where: pi is the proportion of each taxon in the total population (P)

$$H0 = \frac{P \cdot Pi}{Pi}$$

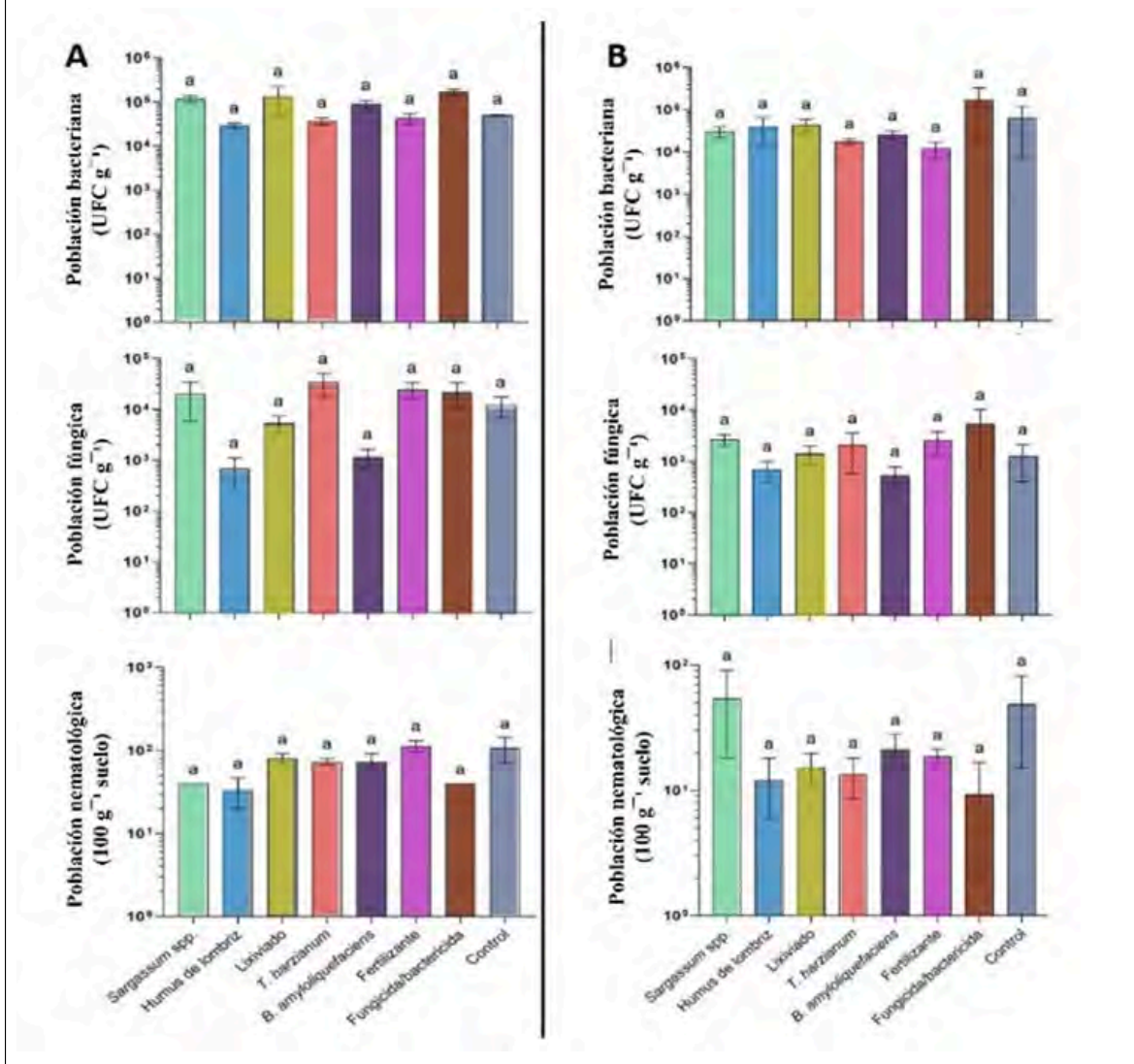
Results and discussion

Effect of bioproducts on the soil microbial population

The data obtained from the evaluation of the five bioproducts (*Sargassum* spp., worm humus, leachate, *T. harzianum*, *B. amyloliquefaciens*, T17 fertilizer and fungicide/bactericide, and the water control) on the microbial population associated with fungi, bacteria, and nematodes did not show significant differences ($p \geq 0.05$) between treatments at the beginning and end of the evaluation (Figure 1). In other words, in each evaluation time, the difference between populations was similar. Nonetheless, when comparing the population from day 0 to day 30, it was variable in most cases as it increased or decreased drastically.



Figure 1. Effect of bioproducts on the microbial population. A) initial population y B) final population.



In the bacterial population, it was observed that, in most treatments, the populations decreased after 30 days. This decrease in the population can be attributed to the fact that microorganisms enter a period of adaptation to the changes present in the soil, such as the introduction of substances derived from the natural products evaluated.

This is consistent with what was reported by Luo *et al.* (2020), who mention that soil microorganisms are highly sensitive to changes, hence their use as biological indicators of ecological degradation and restoration. In the case of bacteria, several studies have confirmed that they have a diverse metabolism that generally serves to adapt to various environments (Manfredini *et al.*, 2021).

Nevertheless, in some cases, such as the use of agrochemicals associated with fungicides and synthetic fertilizers, they generate serious and irreversible damage to microbial activity by reducing the action of enzymes and influencing OM mineralization, nitrification, denitrification, ammonification, redox reactions, and methanogenesis (Chaves-Bedoya *et al.*, 2013).

In this regard, although the results obtained did not present statistically significant differences between the treatments, in the soil ecology, they showed that on day 0, the largest population was shown by the fungicide/bactericide treatment with a total of 1.66×10^5 CFU g⁻¹ of soil; however,

this decreased on day 30, showing a population of 1.13×10^{-5} CFU g⁻¹ of soil, except for the control treatment, in which the population increased from 3.3×10^{-4} to 4.2×10^{-4} CFU g⁻¹ of soil.

For the fungal population, only in the humus treatment did it increase slightly from 6.8×10^{-2} to 7×10^{-2} CFU g⁻¹ of soil. In contrast, in the rest of the treatments, the population was low, as was the case of the *T. harzianum*-based treatment by showing an initial population of 3.4×10^{-4} CFU g⁻¹ of soil, but this decreased to 2×10^{-3} CFU g⁻¹ of soil after 30 days.

In the case of the nematode population, this only increased in the *Sargassum* spp. treatment, going from 13 nematodes per 100 g of soil to 54 nematodes at 0 and 30 days, respectively. These results show that, although biological and natural products have been shown to have beneficial effects on the soil, in some cases, soil microbiomes may have a negative response in their population.

Such is the case of the inoculation of antagonistic microorganisms, which can affect native microbiological populations. Likewise, Li and Wu (2018) pointed out that, within some agricultural management used to improve soil quality, not all of them can tend to improve the microbial population. This coincides with what was reported by Fatriana *et al.* (2020) when evaluating the effect of *Sargassum* spp. extract on corn growth and its response in the microbial population, where they verified the efficiency of *Sargassum* spp. in the vegetative development of the plant, but not in the increase of bacterial and fungal colonies.

This is contrary to what was pointed out by Russo and Beryln (1990), who report that marine macroalgae contain polysaccharides and alginates that activate the growth of fungi and bacteria in the rhizosphere. In this study, this response could only be verified in the case of the nematological population, which showed an increase.

Relative abundance of soil bacteria, fungi, and nematodes

Fungi

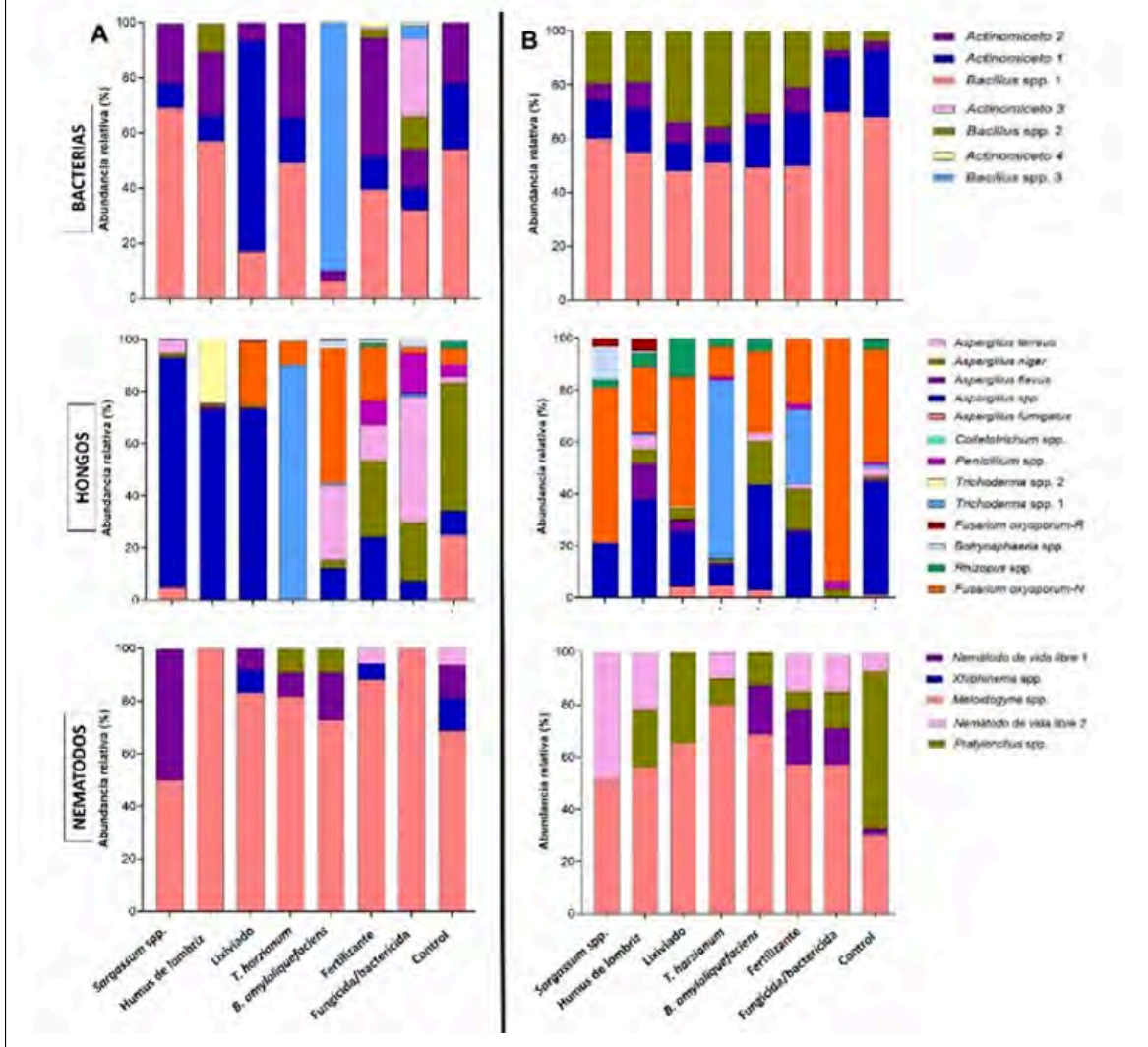
At the end of the 30 days of evaluation, the analysis of the soil microbiome revealed a total of 264 816 specimens per gram of soil, where 93.37% of this population corresponded to bacteria, 6.54% to fungi, and 0.09% to nematodes. These specimens were related to eight classes, nine orders, and 20 genera. Of the total fungal population obtained, it was observed that, on day 0 and 30, the order Eurotiales was predominant (100% relative abundance) as it was present in all the soil samples evaluated, followed by the order Hypocreales with 75 and 100% relative abundance on day 0 and 30, respectively.

The rest of the identified orders corresponded to Glomerellales, Mucorales, and Botryosphaerales. The fungi identified were associated with seven genera: *Aspergillus* (*A. fumigatus*, *A. flavus*, *A. niger*, *A. terreus* and an unknown species), *Trichoderma* (biotypes 1 and 2), *Fusarium oxysporum*, *Colletotrichum*, *Penicillium*, *Rhizopus* and *Botryosphaeria* (Figure 2).

Each treatment presented a variability in the diversity of genera present during the evaluation, where at day 0, the *Sargassum* spp., humus, leachate, and *T. harzianum* treatments presented a variability of five fungi with different relative abundance proportions, whereas *B. amyloliquefaciens* and fertilizer recorded eight fungi (Figure 2).



Figure 2. Relative abundance of bacteria, fungi, and nematodes. A) initial population y B) final population.



The fungicide/bactericidal treatment and the control showed the highest diversity with eight and 10 fungi in the samples evaluated. However, on day 30 of evaluation, all treatments presented an increase in the type of fungal genera present, except for the *B. amyloliquefaciens*-based and fungicide/bactericide treatments, where this diversity variable was decreased, whereas in the control, it was maintained. In both evaluation times, the fungi *Aspergillus*, *Trichoderma* and *Fusarium oxysporum* recorded more than 40% of relative abundance.

The response of the variability in the relative abundance of the identified genera to the action of the bioproducts may be associated with the type of compound of each treatment, which can modify the soil pH, fertility, and temperature. This coincides with what was reported by Delgado-Baquerizo *et al.* (2018), who evaluated ecological patterns in soil biodiversity and the relative abundance of ecological groups within a co-occurrence and found that factors, such as temperature, soil carbon, vegetation type, aridity, and pH, regulate the diversity of archaea, bacteria, and eukaryotes.

In the case of the fungicide/bactericide-based treatment, although it did not have a negative effect on the population, it did influence the diversity not only of the fungal communities but also of the bacteria by reducing the action of some species. This phenomenon could be associated with the action of the copper-based active ingredient on the morphology and metabolism of the fungus.

This response is consistent with what was reported by Golubeva *et al.* (2020) as they noted that an excess of copper ions causes cell damage due to their binding to functional groups, replacing cations, inducing oxidative stress, and affecting the membrane transport system. These changes within the fungal hyphae lead to reduced production of the fungal population. Regarding the fungi that showed the greatest abundance in the study (*Aspergillus*, *Trichoderma*, and *Fusarium oxysporum*), which belong to the division Deuteromycota, they are considered as one of the common genera, which have a diversity of species and biotypes that spread rapidly in the soil (Reverchon *et al.*, 2010).

In addition, they are considered to be one of the most efficient saprophytic microorganisms in the degradation of organic matter and play an important role in the carbon, nitrogen, and other soil nutrient cycles because they produce a wide range of lignocellulolytic enzymes (Dix and Webster, 1995). Nevertheless, their distribution and abundance may be conditioned by the different ecological niches.

In this regard, Zhao *et al.* (2019) evaluated the effect of natural restoration on fungal and bacterial communities in semi-arid areas of the southern Taihang Mountains and found that the fungal communities present in the soil corresponded to the Ascomycota, Basidiomycota, Chytridiomycota, Zygomycota and Glomeromycota types, with the Ascomycota and Basidiomycota groups being the most dominant with 57 to 81% of the fungal composition.

Likewise, the increase in types of genera within each treatment could be due to the selection of types of substrates to feed on that each microorganism in the soil has, which led to other types of fungi being activated after 30 days of evaluation and therefore maintaining a balance between the types of beneficial and pathogenic microorganisms, which helps in the regulation of niches, preventing the proliferation of plant pathogens, such as *Fusarium oxysporum*, *Colletotrichum* spp., and *Botryosphaeria* spp., from being greater.

Bacteria

Of the seven bacterial isolates, only two classes were observed, Bacillales (with three biotypes; *Bacillus* spp. 1, 2, and 3) and Actinomycetales; the latter of which is more predominant (57%) as it presents four biotypes (Actinomycetes 1, 2, 3, and 4). At the end of 30 days, *Bacillus* 3 and two actinomycetes (3 and 4) were inactivated with the treatments as they were not present in the samples analyzed (Figure 2). As in the case of fungi, each treatment presented a variability in bacterial diversity, where at day 0, the treatments of *Sargassum* spp., *T. harzianum*, *B. amyloliquefaciens*, and the control recorded a variability of three bacteria with different proportion of relative abundance, while humus and leachate presented four types of bacteria.

The fertilizer treatment and the fungicide/bactericide treatment presented greater diversity with six and seven isolated bacteria. On day 30 of evaluation, all treatments caused a decrease, showing a diversity of only four bacteria in the samples analyzed. The bacteria that predominated the most in both evaluation times were those classified as *Bacillus* 1 and actinomycetes 1 and 2, with a relative abundance greater than 50, 7 and 4%, respectively, after 30 days.

This response regarding the established abundance of bacteria at the end of the experiment may be associated with a regulation of these communities due to the type of substrate favorable as food, which leads to an interaction between them during their growth. This was reported by Yang *et al.* (2020) as they point out that there is a common exchange of information between bacteria and that individuals of this or different species compete or cooperate through their development in environments.

These results are of great relevance for the information generated since, based on these responses of the microorganisms to the different bioproducts, it is possible to have a greater knowledge of their biology. In this regard, Mulawarman *et al.* (2001) evaluated the effect of natural products, such as TerraPy, Magic Wet, and Chitosan, considered to be soil revitalizers, on the population density of soil fungi, bacteria, and nematodes and the stimulation of tomato plant growth for 15 days.

The results showed that the population of bacteria increased up to four times more but their diversity was not altered or decreased. In the type of bacteria identified, those of the genus *Bacillus* were higher, presenting 42% abundance. In the case of nematodes, saprophytic nematodes were higher compared to phytoparasitic nematodes. These results show the positive effect that natural products have on stimulating soil microbial activity, where potential antagonists reduce pathogen infestation and improve plant growth.

Nematodes

In the case of relative abundance in nematodes, the identified orders, such as Tylenchida and Dorylaimida, recorded two and one genus each, corresponding to *Meloidogyne* spp., *Pratylenchus* spp., and *Xiphinema* spp. In addition, the presence of two types of free-living nematodes was found (Figure 2). The relationship and interaction of these types of nematodes within an ecosystem is crucial for the maintenance of ecological niches.

This was noted by Laasli *et al.* (2022), who mention that the co-occurrence of phytoparasitic nematodes with free-living nematodes is a crucial part of soil diversity since these ecological relationships of coexistence of nematode species that share the same resource have potential uses for more effective biological control and the use of organic amendments to encourage disease suppression.

In addition, Villenave *et al.* (2009) point out that knowledge of the structure of the nematode community provides information related to the different processes carried out in the soil, the food web in it, and the state of stability of agroecosystems and their biodiversity. In addition, nematodes have been shown to improve the physical properties of the soil, in which they can promote the transformation of carbon and nitrogen.

In the case of phytoparasitic nematodes, they are considered polyphagous in nature, in particular the root-knot nematode (*Meloidogyne* spp.), which is capable of infecting a wide range of host plants (Saroj *et al.*, 2018). In addition, together with the dagger nematode (*Xiphinema* spp.) and the lesion nematode (*Pratylenchus* spp.), they are one of the most common groups in the soil and cause significant economic losses worldwide. This makes it extremely difficult to control them through crop rotation (Seid *et al.*, 2021).

At day 0, *Meloidogyne* spp. was found in 100% of the treatments with a minimum relative abundance of 80% and a maximum of 100%, followed by *Xiphinema* spp., present in only three of the eight treatments (37%), with a minimum and maximum relative abundance of 5 to 12%. In the case of *Pratylenchus* spp., its presence was recorded in only two treatments (25%), with an average abundance of 9%. Free-living nematodes (1 and 2) showed an abundance of 62 and 25%, respectively.

However, on day 30 of evaluation, the abundance of microorganisms varied significantly, where *Meloidogyne* spp. remained present in all treatments, but its relative abundance varied as its minimum value decreased to 30% and its maximum to 79%. The genus *Xiphinema* spp. was no longer present in any treatment, whereas *Pratylenchus* spp. was recorded in 87% of all treatments with a high relative abundance, presenting a minimum abundance of 7% and a maximum of 60%.

Likewise, in the case of free-living nematodes 1 and 2, they are present in 50 and 75% of the treatments, respectively, with a minimum relative abundance of 2 and 5% and a maximum of 21 and 49%. In the latter two organisms, it was observed that their population increased regardless of the application of bioproducts, which may be associated with their wide capacity to adapt to different types of substrates.

In this regard, several studies indicate that free-living nematodes can be present even in extreme environments due to their ecophysiological adaptation associated with their ability to switch between the stages of activity and 'anhydrobiosis' in wet seasons versus dry (extreme) seasons (Levi *et al.*, 2012).

Soil microbiological diversity

The microbial diversity index is shown in Table 2. The results show that the treatments evaluated have an influence on the diversity of microorganisms present in the soil and therefore, it is possible to consider that these substances can also affect the characteristics of the soil. This is consistent with what Doran (2002) reports, as he points out that the diversity and functions of soil determine its quality due to its ability to function within a given ecosystem to sustain organic production, maintain environmental quality, and promote plant health.

Table 2. Effect of bioproducts on the Shannon diversity index.

Treatments	0 days		30 days	
	Mean	Standard deviation	Mean	Standard deviation
<i>Sargassum</i> spp.	0.2127	0.3683	0.1423	0.1277
Worm humus	0.119	0.1094	0.111	0.1503
Leachate	0.2203	0.1101	0.1293	0.1219
<i>T. harzianum</i>	0.4983	0.3143	0.2743	0.2587
<i>B. amyloliquefaciens</i>	0.08433	0.07123	0.1173	0.1032
Fertilizer	0.6187	0.1487	0.445	0.2845
Fungicide/bactericide	0.097	0.168	0.03167	0.03814
Control	0.3893	0.3375	0.1183	0.117

It can be highlighted that, in the fertilizer-based treatment, diversity was higher in both evaluation times (0 and 30 days) compared to the rest of the treatments, followed by the *T. harzianum* treatment and the control treatment. In order of importance, *Sargassum* spp. and leachate followed with a lower diversity. In contrast, the treatments that significantly reduced the diversity index were *B. amyloliquefaciens*, fungicide/bactericide, and humus.

Conclusions

In summary, our results showed that the composition of the bacterial, fungal, and nematological microbiota present in the soil is influenced by the action of the bioproducts applied to the soil and these microbiomes presented a differential response to them. The reduction in population and the increase in microbial diversity were associated with the type of bioproduct and the ability of microorganisms to adapt to changes in the soil.

These results provide new evidence on changes in the relative abundance and diversity of the microbiome within ecological niches in arid soils, as they are modified by the application of natural products. This information is relevant in understanding the response of microbial communities and provides new perspectives for agricultural soil management.

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