AGRO PRODUCTIVIDAD

Bioactive Species Associated with Rambutan (*Nephelium lappaceum* L.) and their Influence on Soil Chemical and Microbiological Properties

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ABSTRACT

Objective: To know the influence of the bioactive plants *Ruta graveolens* L., *Ocimum basilicum* L., *Stachytarpheta jamaicensis* and *Chenopodium ambrosoides* L., on the chemical and microbiological properties of the soil associated with *Nephelium lappaceum* L.

Design/methodology/approach: Cuttings of *R. graveolens* L., *O. basilicum* L., *S. jamaicensis* (L.) Vahl and *C. ambrosoides* L. were collected and five treatments with six replications each were established, using a randomized complete block design. At the time of planting the medicinal plants and one year later, a soil sample was collected at a depth of 30 cm for physicochemical (UNACH-FCA) and microbiological analysis of the soil (MASTERLAB S. A. de C. V.) to record variables.

Results: The chemical analyses results showed changes in the content of most nutrients between sampling years, with the exception of N. Microorganism populations also exhibited contrasting values between the two sampling periods.

Limitations on study/implications: The answer may vary depending on plant density and time of year.

Findings/conclusions: The association of bioactive plants induces changes in soil nutrients, with year-to-year fluctuations. Furthermore, it promotes differential growth among the populations of beneficial and harmful microorganisms. Beneficial microorganisms increased, and the presence of pathogens in low populations did not result in plant damage. Beneficial microorganisms such as *Pseudomonas fluorescens* and *Bacillus* spp. were present with *S. jamaicensis*, and *Trichoderma* and *Aspergillus* with *R. graveolens* and *O. basilicum*, suggesting a dependence on exudates.

Keywords: rambutan-medicinal plant association; nutrients; rhizosphere; microorganisms; agroecology.

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INTRODUCTION

Nephelium lappaceum L. is a species native to Malaysia and Indonesia, introduced to Chiapas in the 1960s (Castillo-Vera *et al.*, 2017) as an alternative to traditional crops such as coffee (*Coffea arabica* L.), cacao (*Theobroma cacao* L.), banana (*Musa paradisiaca* L.), and mango (*Mangifera indica* L.). Currently, it covers more than 2,500 hectares under production (Osorio-Espinoza *et al.*, 2019) and has become an economically important crop in the Soconusco region (Avendaño-Arrazate *et al.*, 2011; Flores-Trejo *et al.*, 2016). However,

its productivity has declined, which has been attributed to various environmental and management-related factors.

Previous agronomic issues have been addressed through the use of agrochemicals belonging to various toxicological categories, which has led to ecological imbalances and adverse effects on human health (Ramírez-Montoya *et al.*, 2013), as well as increased production costs. As an alternative, agroecological systems have been implemented using medicinal plants such as *Ruta graveolens* L., *Ocimum basilicum* L., *Stachytarpheta jamaicensis*, and *Chenopodium ambrosioides* L. When associated with crops, these species provide benefits to the soil and, through various mechanisms of action, help reduce pests and diseases (Marroquín-Agreda *et al.*, 2019).

From a systemic perspective, polycultures have a positive impact on insect, fungal, and weed populations by enhancing biological balance and improving nutrient availability (Rodríguez-González *et al.*, 2008). The association of perennial crops with bioactive plants can generate various forms of interdependence, such as synergism, antagonism, symbiosis, allelopathy, or insect attraction within the agroecological system. These interactions are considered to influence nutrient and energy recycling, the replacement of external inputs, the increase of organic matter and soil biological activity, and the diversification of plant species and genetic resources (Osorio-Espinoza *et al.*, 2019). In this context, the objective of the present study was to associate four traditional bioactive plant species from the Soconusco region of Chiapas with a four-year-old *Nephelium lappaceum* L. crop, and to identify their influence on the chemical and microbiological properties of the soil.

MATERIALS AND METHODS

The research was conducted from June 25, 2021, to June 25, 2022, in a four-year-old agroecologically managed *Nephelium lappaceum* L. (rambutan) plot, located in the municipality of Metapa de Domínguez, Chiapas, at coordinates 14° 50' N and 92° 11' W, with a humid warm climate, an altitude of 100 m, an annual average temperature of 27 °C, and annual precipitation of 2,165 mm (García, 1973). The experimental area trees were selected for a height of 2 meters and non-dense crowns.

Plantation management. Vermicompost is incorporated once a year into the mid-drip zone of the trees, along with the foliar application of humic substances, and during the dry season, irrigation is carried out using micro-sprinklers. The average fruit yield ranges between 50 and 60 kg per tree.

Treatments and experimental design. Five treatments were established: 1) *Ruta graveolens* + *Nephelium lappaceum*, 2) *Ocimum basilicum* + *Nephelium lappaceum*, 3) *Stachytarpheta jamaicensis* + *Nephelium lappaceum*, 4) *Chenopodium ambrosoides* + *Nephelium lappaceum*, and 5) *Nephelium lappaceum* without medicinal plants (control), under a randomized complete block design. Each block represented a replication and consisted of five rambutan trees, with each tree considered an experimental unit. In each unit, four medicinal plants of a single species were associated according to the assigned treatment. They were planted in a cross shape in the mid-drip zone, for a total of 35 *Nephelium lappaceum* trees and 120 bioactive plants.

Experiment establishment

Bioactive plants were established around the selected trees; these were obtained from backyard gardens in Tapachula, Chiapas. The plants were propagated by cuttings and initially grown in Acrisol soil inside polyethylene bags. Transplanting to the field was carried out on June 25, 2021, when the plants reached an average height of 15 cm, placing them in the mid-drip zone. The planting area was delimited by tracing a circle using a plastic string and a stake. Then, four points were marked in a cross pattern, and holes of 30×30 cm were dug. From each hole, a soil sample was taken, and the spatula was sterilized by flaming with 96% ethyl alcohol. The soil sample was placed in a labeled transparent plastic bag and stored on ice in a plastic container. Physical, chemical, and microbiological analyses were performed on these soil samples.

Physicochemical and microbiological properties of the soil

These were analyzed at the beginning and end of the experiment. Soil color was determined using the Munsell Soil Color Chart (GretagMacbeth, 2000 edition, USA). Soil texture was determined using the method proposed by Bouyoucos (1962), based on Stokes' law. Textural classes were identified using the soil texture triangle (Gee and Bauder, 1986). The percentage of porosity (p%) was calculated using the formula proposed by Mandelbrot (1982):

$$Porosity\ (\%) = Rd - Bd\ /\ Bd \times 100$$

where $Rd =$ real density and $Bd =$ bulk density.

Bulk density (Bd) was determined using the cylinder method, and real density (Rd) was calculated by dividing the total weight of oven-dried soil by the volume occupied by the solids. Soil weight for both density determinations was measured using an analytical balance (Ohaus Adventure Pro AV4101, China). Soil reaction (pH) was measured with a potentiometer at a 1:2 ratio (soil: deionized water) (Thermo Orion model 230A+, USA), and electrical conductivity (E.C.) was measured using a conductivity meter (Thermo Orion 145A+, USA) at a 1:5 ratio (soil: deionized water). Organic matter percentage (O.M.) was determined using the Walkley and Black method (1934). Organic carbon (C) content in the organic residue was also measured. For both O.M. and C determinations, a magnetic stirrer with a lamp was used (Thermolyne Stir Light, USA).

The percentage of nitrogen (N) was determined using the micro Kjeldahl method (Kjeldahl, 1883), and phosphorus using the Olsen method (Olsen *et al.*, 1954) with a mechanical reciprocal shaker (Eberbach 6000, USA). Both determinations were carried out with the help of a spectrophotometer (Metach, model UV-6000 UV/VIS Spectrophotometer, China). Potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) were determined using the 1N CH_3COONH_3 extraction method at pH 7, quantified by atomic absorption with an analytical balance (Ohaus Adventure, China), a mechanical reciprocal shaker (Eberbach 6000, USA), and an atomic absorption spectrophotometer (GBC Scientific Equipment SensAA, Australia). Sulfur (S) and boron (B) were obtained using a shaker (Eberbach 6000, USA) and a spectrophotometer (Metach, model UV-6000 UV/VIS Spectrophotometer, China), using the turbidimetric and Azomethine H colorimetric methods, respectively. Cation exchange capacity (CEC) was determined using the sum of exchangeable cations method.

Microbiological analyses were carried out by counting colony-forming units (CFU) of bacteria, fungi, and actinomycetes at the beginning and end of the experiment through dilution and Petri dish plating, isolation for 144 hours, and identification of beneficial microorganisms in semi-selective media (Masterlab SA de CV). Fungi were quantified on PDA-AL medium, bacteria on PDA, King B, and Elmar media, and actinomycetes on ELMAR medium. Identification was performed by macroscopic and microscopic observation of morphology (actinomycetes and fungi), as well as staining and UV light exposure (bacteria).

For nematode detection, the Baermann funnel-sieving technique was used. Pathogenic bacteria (*Clavibacter* sp., *Ralstonia* sp., *Pseudomonas* sp., and *Erwinia* sp.) were identified through dilution and Petri dish counting on PDA, King B, and CTT culture media, with incubation at 28 °C. Additionally, worm cast counts were conducted in the treatments of each block every 15 days during the rainy season. Statistical analysis. Data were entered into a Microsoft Excel© spreadsheet, and an analysis of variance (ANOVA) was performed using R© software. Significant differences were analyzed with Tukey's test at $P \leq 0.05\%$.

RESULTS AND DISCUSSION

The soil showed a brown color with a clay loam texture. The bulk density (Bd) of the treatments ranged between 1.2 and 1.3 g mL^{-1}, and the particle density (Rd) between 1.7 and 1.9 g mL^{-1}. It showed low compaction and good moisture retention. The porosity percentage ranged from 31.11 to 33.25% (Table 1).

The chemical properties of the soil showed variations with statistically significant differences ($p \leq 0.05$) in the concentrations of some nutrients; however, a decrease in these nutrients was observed in the second analysis during 2022 (Figures 1 and 2).

The total nitrogen percentage showed little variation between treatments with medicinal plants associated with rambutan during 2021. In the sampling conducted during 2022, this nutrient increased in the soil where *O. basilicum*, *S. jamaicensis*, and *C. ambrosoides* were established, with the lowest value recorded where *R. graveolens* was associated. In contrast, the control treatment without medicinal plants showed the highest total nitrogen percentage in the soil. This same trend was observed in the assimilable nitrogen content.

The phosphorus content in the soil showed contrasting variations during the first sampling in 2021, and in 2022, the values were very similar between treatments.

K showed little variation during the first year of sampling. In this case, when *C. ambrosoides* was associated, the content was lower compared to the other treatments, but in 2022, the highest value was recorded. In the second year of sampling, the control treatment showed the lowest value for this nutrient.

In contrast, magnesium content increased in the soil where *S. jamaicensis* was established during 2021 and decreased in 2022. The same effect was observed in the control treatment.

Table 1. Physical properties of the soil in the association of different medicinal plants with *N. lappaceum* L in Soconusco, Chiapas.

Component	*R. greveolens*	*O. basilicum*	*S. jamaicensis*	*C. ambrosoides*	*N. lappaceum* (Testigo)	CV (%)**
% Sand (2021)*	43.2±3.0	38.2±0.8	38.7±0.5	37.8±0.0	36.5±1.8	7.4 (NS)
(2022)**	40.8±0.6 ab	36.8±0.6 c	41.2±1.0 a	38.2±0.0 bc	30.2±0.0 d	2.8
% Silt (2021)	24.6±4.9	29.2±1.2	24.8±0.9	28.3±0.0	26.0±2.9	17.4(NS)
(2022)	29.0±0.6	30.6±0.8	28.6±0.8	31.6±0.6	30.0±1.4	5.8 (NS)
% Clay (2021)	34.7±0.3	33.1±1.6	36.4±1.5	33.7±0.3	37.4±2.0	6.8 (NS)
(2022)	29.4±1.1	32.4±0.5	32.1±0.6	30.1±0.6	31.7±1.4	5.6 (NS)
Ad g mL^{-1} (2021)	1.2±0.03	1.2±0.03	1.3±0.01	1.2±0.01	1.2±0.03	3.7 (NS)
(2022)	1.2±0.01 ab	1.2±0.01 b	1.3±0.02 a	1.2±0.01 ab	1.2±0.006 b	2.0
Dr g mL^{-1} (2021)	1.8±0.03	1.8±0.02	1.9±0.01	1.8±0.02	1.9±0.05	3.1 (NS)
(2022)	1.9 ±0.03 a	1.7±0.02 b	1.8 ±0.04 ab	1.8±0.06 ab	1.8±0.02 ab	3.8
% Porosity (2021)	31.1±.3.0	31.6±2.4	31.5±0.2	31.9±4.0	33.2±0.4	13.7(NS)
(2022)	35.1±2.0	30.5±0.7	28.4±1.9	30.9±2.6	33.2±0.4	9.6
Color (2021)	7.5YR4/4 brown	7.5YR4/4 brown	7.5YR3/4 brown	7.5YR3/4 brown	7.5YR4/4 brown	
(2022)	7.5YR4/2 brown	7.5YR4/3 brown	7.5YR4/3 brown	7.5YR4/2 brown	7.5YR4/4 brown	

Clay-loam textura. Values are means of three replicates ± standard error. Different letters between lines indicate statistically significant differences ($p<0.05$). CV=Coefficient of Variation. * Start of study ** End of study.

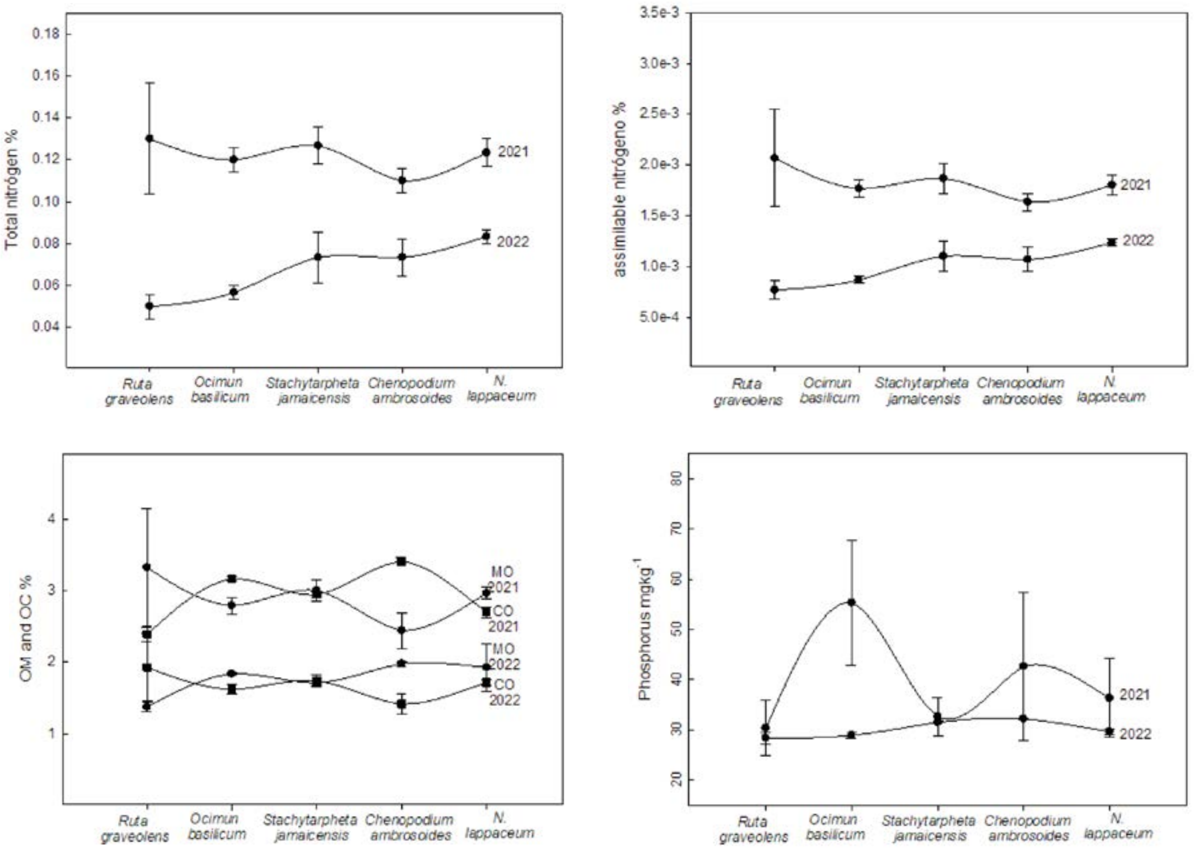

Figure 1. Total and assimilable nitrogen and phosphorus content of soils where *N. lappaceum* was associated with various medicinal plants in Soconusco, Chiapas. Values are means of four replicates ± standard error.

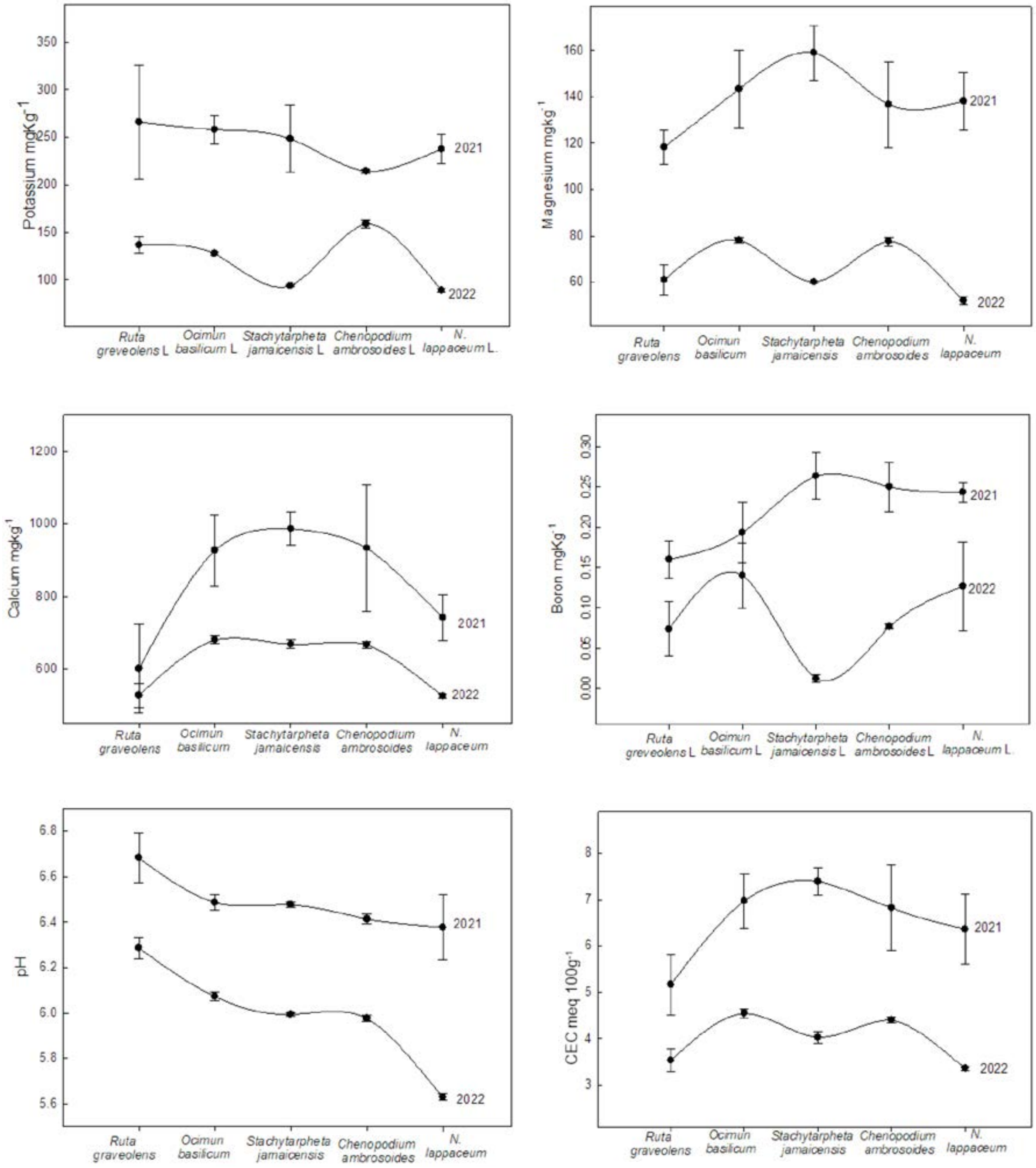

Figure 2. Potassium, magnesium, calcium, and boron content, pH, and cation exchange capacity (CEC) in a soil from Soconusco, Chiapas, planted with *N. lappaceum* L. and associated with medicinal plants. Values are averages of four replicates ± standard error.

However, with *C. ambrosoides* and *O. basilicum*, the magnesium content in the soil was low during 2021 and increased in 2022.

The calcium content in the soil of the control treatment and where *R. graveolens* was associated showed the lowest values compared to the other treatments during both years of sampling. In the treatments with the other medicinal plants associated with rambutan, the changes observed occurred between years.

The boron content in the soil increased during 2021 where *S. jamaicensis*, *C. ambrosoides*, and the control treatment were associated. In contrast, during 2022, the lowest value was found when *S. jamaicensis* was associated.

The microorganisms quantified in the soil, fungi and bacteria, also showed contrasting populations between the years of assessment. The differences in bacterial populations of *Pseudomonas fluorescens* and *Bacillus* spp. among the established species were variable. In the case of *P. fluorescens*, the highest increase occurred in the second year of evaluation, as was also the case with *Bacillus* spp. In the treatments where *R. graveolens*, *O. basilicum*, and *C. ambrosioides* were established, the amount of *P. fluorescens* was low, and in *S. jamaicensis* it was initially undetected but reached 100,000 CFU by the end. In the control treatment (*N. lappaceum* without medicinal plants), neither *P. fluorescens* nor *Bacillus* spp. were found. Among both bacteria, *Bacillus* spp. showed the highest populations (Figure 3a).

In the initial sampling, the soil where *R. graveolens* was established showed 426,667.00 CFUs, and in *O. basilicum*, 766,667.00 CFUs. These values increased in the second sampling to 419,333.00 and 3,640,000.00 CFUs, respectively. An increase of this microorganism was also recorded in the control treatment.

It is worth noting that the soil where *S. jamaiciensis* was established showed the highest amounts of both bacteria.

The presence of *Trichoderma* sp. and *Aspergillus* sp. exhibited contrasting variations between years and the species associated with rambutan. In the case of *Trichoderma*, the population increased in the soil where *R. graveolens* was established during the second year of sampling. The same trend was observed when *C. ambrosoides* was associated in 2022.

Aspergillus sp. appeared in both soil samplings when medicinal plants were associated with rambutan. In the case of the control treatment, no presence of *Aspergillus* sp. was recorded in the second sampling. The most abundant presence occurred in 2021 with *O. basilicum* and in 2022 with *S. jamaiciensis*.

Regarding aerobic and anaerobic bacteria, contrasting values were observed between the two samplings conducted. The highest increase in both types of bacteria occurred during the first sampling (2021) when the medicinal plants were not associated with the rambutan. During this same year, the presence of anaerobic bacteria predominated, whereas in the second year, aerobic bacteria were more abundant.

The highest population of both types of bacteria among the medicinal plants was found in *R. graveolens*, while the lowest was observed in *O. basilicum*. In contrast, actinomycetes decreased in *R. graveolens*, *O. basilicum*, and *S. jamaicensis* but increased in *C. ambrosoides* and *N. lappaceum*.

Fungi of the genus *Fusarium* sp. were abundant in 2022 in the treatments with bioactive plants, but not in *N. lappaceum*. Nematodes such as *Rotylenchulus* sp. were present during the first year of evaluation but were not detected in the second year. In 2022, *Aphelenchus* sp. was found in the treatments with *R. graveolens*, *S. jamaicensis*, and *N. lappaceum*.

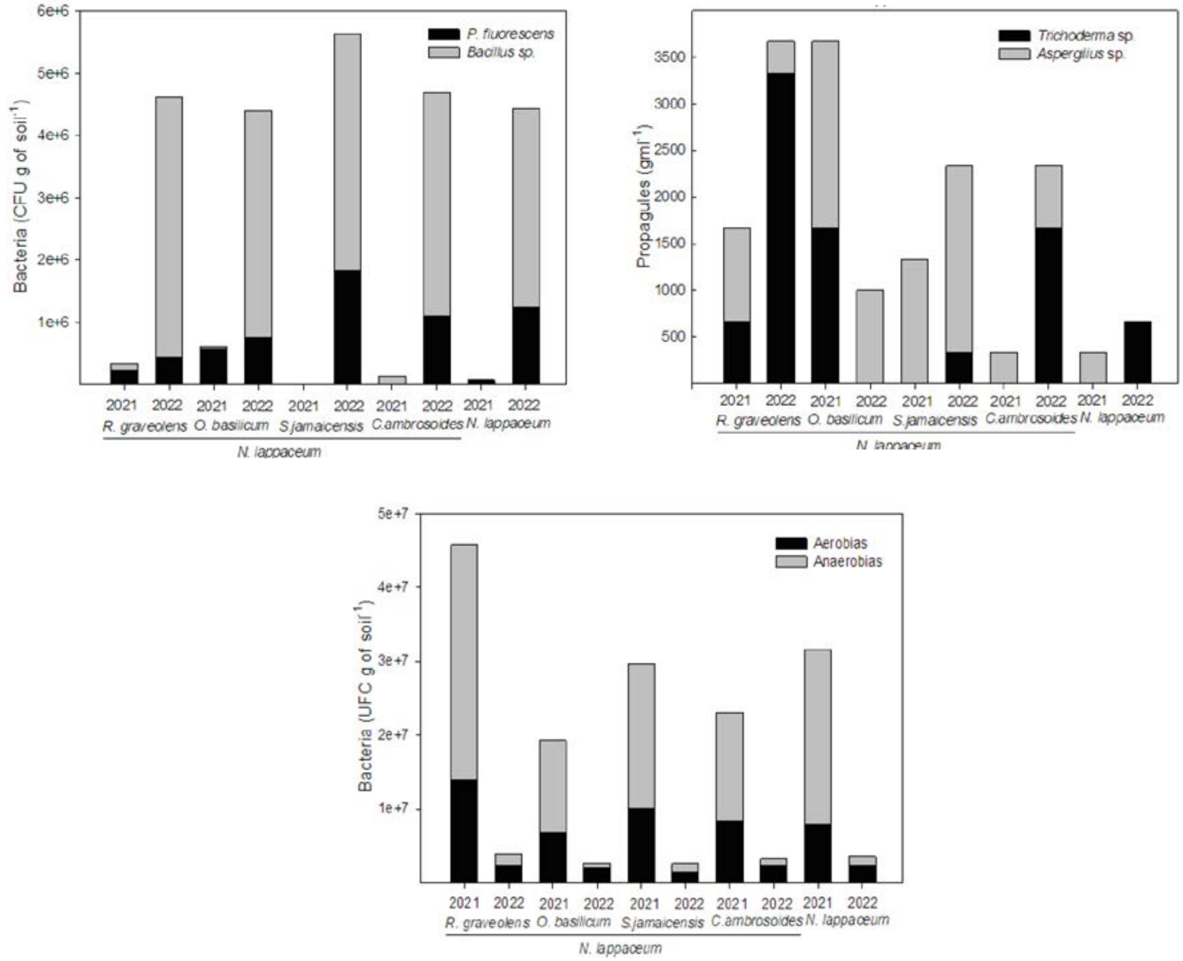

Figure 3. Bacteria and fungi (colony forming units per g of soil) present in a soil from Soconusco Chiapas established with *N. lappaceum* L. and associated with medicinal plants.

There were no significant differences in the number of worm castings among the treatments. However, visual observations indicated a greater presence of castings in *S. jamaicensis* and *R. graveolens*, with lower visibility in *C. ambrosoides* and *N. lappaceum*.

The higher nutrient content in the soil during the first sampling is considered to be associated with the phenological stage of rambutan, as this sampling coincided with the vegetative stage. The second sampling was conducted during the fruiting stage and was characterized by a decrease in soil nutrients. In this regard, Sosa-Rodríguez and García-Vivas (2020) reported that *N. lappaceum* extracts high concentrations of nutrients throughout the year, with a greater demand for N (41%), Ca (20%), Mg (5%), and S (4%) for the formation of leaves and branches. In contrast, P (6%) is primarily required for seed development, while K is mainly concentrated in the rambutan peel (24%). However, the nutrient content in the different structures of the trees may vary depending on agroclimatic conditions and tree age. Reyes-Moreno *et al.* (2020) further note that nutrient deficiencies in the soil may occur after fruit production.

The high nitrogen (N) demand during fruiting is associated with its role in the photosynthesis process (Combatt-Caballero *et al.*, 2020) and as a component of various organic acids (Reyes-Moreno *et al.*, 2020).

In the case of medicinal plants, limited information is available regarding their nutrient requirements. However, it has been reported that *O. basilicum* requires high amounts of nitrogen (ranging from 100 to 190 kg ha^{-1}) and potassium, between 125 and 235 kg ha^{-1} (Combatt-Caballero *et al.*, 2020), while *C. ambrosoides* demands higher amounts of nitrogen compared to other nutrients (Aguilar-Carpio *et al.*, 2021).

Regarding beneficial microorganisms, it has been demonstrated that they exert both direct and indirect effects on plants. They release compounds, minerals, and phytohormones, and can modify soil structure by participating in the mineralization of certain nutrients and acting as biological control agents against pathogenic microorganisms (Pedraza *et al.*, 2010).

Regarding microbial populations, their interactions may have influenced the development of other microbial communities. It has been documented that *Trichoderma* sp. is capable of controlling *Phytophthora nicotianae*, *P. aphanidermatum*, *P. parasitica*, *P. capsici*, *Rhizoctonia solani*, and *Pythium* spp. (Companioni-González *et al.*, 2019), and the population of this microorganism increased in the presence of *R. graveolens. Aspergillus* sp. was also present in the soil of medicinal plants associated with rambutan. Its importance lies in its role in the decomposition of organic matter and in host defense against pathogenic microorganisms (Sacheri-Viteri *et al.*, 2022), such as *Fusarium*, which causes wilting or stem rot (Villa-Martínez *et al.*, 2015).

Nitrifying bacteria such as *Pseudomonas* sp. and *Bacillus* spp. are important in nitrogen mineralization (Másmela-Mendoza *et al.*, 2019) and can also control various pathogens (Pedraza *et al.*, 2020). The abundance of *Bacillus* has also been reported in soils cultivated with medicinal plants such as *Matricaria chamomilla* L., *Calendula officinalis* L., and *Solanum distichum* Schumach. & Thonn., with positive effects on promoting plant growth and increasing flavonoid content (Solaiman and Anawar, 2015). There is a close relationship between vegetation and the rhizospheric microbiota, as rhizosphere bacteria can act as signaling molecules and produce chemical substances that may trigger physiological and morphological changes in plants (Montaño-Arias *et al.*, 2006).

In our case, the highest populations of *S. jamaicensis* favored the abundance of *P. fluorescens* and *Aspergillus* sp. Likewise, the greatest amount of earthworm excreta was found in this soil. This may be related to the presence of arbuscular mycorrhizal fungi. It has been established that bacterial populations increase in the presence of arbuscular mycorrhizal fungi, and this plant is known to be colonized by mycorrhizae of the genus *Glomus* spp. (Aggangan *et al.*, 2015). Mycorrhizae, a symbiotic association between higher plants and microorganisms, are commonly found in nature. In this symbiotic relationship, the host plant provides carbohydrates to the mycorrhizal fungi through photosynthesis, while the fungi provide nutrients in return. It has been demonstrated that a large number of bacteria inhabit the zone around mycorrhizae (Frey-Klett and Garbaye, 2005). Arbuscular mycorrhizal fungi and bacteria known as mycorrhizal helper bacteria (MHB) are interdependent; the mycorrhizal fungi provide nutrients for bacterial growth through the release of secretions, and the bacteria supply low molecular weight nutrients (Frey *et al.*, 1997; Rangel-Castro *et al.*, 2002). The pathogenic microorganisms were not uniformly found in the soil, but their presence in certain treatments may indicate competition among the increased populations of microorganisms associated with the exudates of medicinal plants. In the case of aerobic bacteria, their high presence indicates that the soil is not saturated and has good aeration, meaning the soil's porosity allows for proper development of the plant root system.

CONCLUSIONS

The association of bioactive plants results in changes in the chemical properties of the soil, with fluctuations in nutrient contents identified over the two years of evaluation, both with and without the association of medicinal plants. The association of medicinal plants and rambután interferes with the population growth of both beneficial and harmful microorganisms. The diversity of beneficial microorganisms increases, and the presence of pathogens in low populations did not result in damage to the plants. The contrasting presence of beneficial microorganisms in the soil during the evaluation years suggests dependency on exudates from the medicinal plants, such as *P. fluorescens* and *Bacillus* spp. with *S. jamaicensis* and *Trichoderma* and *Aspergillus* with *R. graveolens* and *O. basilicum*.

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