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Effects of Vermicompost Leachate versus Inorganic Fertilizer on Morphology and Microbial Traits in the Early Development Growth Stage in Mint (*Mentha spicata* L.) And Rosemary (*Rosmarinus officinalis* L.) Plants under Closed Hydroponic System

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Abstract: The objective of this study was to compare the morphology of *M. spicata* and *R. officinalis* plants, and the relative abundance quantification, colony-forming units, ribotypes, and biofilm former bacteria under an inorganic fertilizer and the use of vermicompost leachate in the rhizosphere under a closed hydroponic system. In mint (*Mentha spicata*) plants treated with the vermicompost leachate, growth increase was determined mainly in root length from an average of 38 cm in plants under inorganic fertilizer to 74 cm under vermicompost leachate. In rosemary (*Rosmarinus officinalis*), no changes were determined between the two treatments. There were differences in the compositions of microbial communities: For *R. officinalis*, eight ribotypes were identified, seven for inorganic fertilizer and four for vermicompost leachate. For *M. spicata*, eight ribotypes were identified, three of them exclusive to vermicompost leachate. However, no changes were observed in microbial communities between the two treatments. Otherwise, some changes were observed in the compositions of these communities over time. In both cases, the main found phylum was Firmicutes, with 60% for *R. officinalis* and 80% for *M. spicata* represented by the *Bacillus* genus. In conclusion, the use of vermicompost leachate under the hydroponic system is a viable alternative to achieve an increase in the production of *M. spicata*, and for both plants (mint and rosemary), the quality of the product and the microbial communities that inhabited them remained unaltered.

Keywords: organic fertilizer; hydroponic; ribotypes; vermicompost leachate

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1. Introduction

At present, the growing global population has put pressure on agriculture in different ways: the increase in demand for food and the need to meet this demand in an environmentally friendly manner. Although the use of chemical fertilizers has led to an enhancement in crop production, several major health- and environment-related concerns are associated with their use [1,2]. Pollution and the increase in global temperature are predicted to have negative consequences for agriculture in the coming decades [3]. Likewise, future climate-change scenarios predict a more frequent occurrence of extreme conditions [4]. In this sense, hydroponic systems have emerged as an alternative to improve yield, product quality, water management, land saving, nutrient recycling, and environmental and pathogen control. Hydroponic systems are cultivation technologies that use nutrient solutions rather

than soil substrates, and can use natural or artificial media to provide physical support to plants [5–7]. However, there is an intense debate about which hydroponic practices align or do not align with the Organic Foods Production Act (OFPA) and USDA organic regulations [8]. Furthermore, hydroponic systems are a form of soilless food production, and one of the points of conflictive in points in organic agriculture is the use of inorganic nutrition in water solutions, which many people strongly believe should not be allowed [8]. Hydroponic production has increased in recent years due to its multiple benefits. Thus, it is convenient to understand the role of microorganisms and natural sources of nutrients to improve hydroponic systems for the production of healthy food beyond reaching certification in organic agriculture. At present, the use of vermicompost leachate coupled with hydroponic systems seems to be a viable alternative. Vermicompost is the resulting product from the processing of organic waste in the digestive tract of earthworms [9,10]. This process involves the bio-oxidation and stabilization of organic compounds by the joint action of earthworms and microorganisms [11]. Consequently, the obtained vermicompost is a fertilizer with available nutrients for plants and a strong charge of beneficial bacteria [12,13]. Likewise, vermicompost is an effective technique to reduce the toxicity of waste material [14]. Vermicompost leachate is a subproduct of the vermicompost process with nutrients, microorganisms, and biologically active substances, such as fulvic acids and humic acids, and the released water during the decomposition of the organic material [15,16]. One of the positive effects of the use of vermicompost leachate is an increase in the population of plant-growth-promoting bacteria (PGPB) [17]. PGPB can promote plant growth by both direct and indirect mechanisms. Direct mechanisms include the production of auxin, ACC deaminase activity, cytokinin, gibberellin, the nitrogen fixation process, phosphorus solubilization, and the sequestration of iron by bacterial siderophores. Indirect mechanisms refer to the bacterial capability to inhibit the proliferation of plant pathogenic organisms, such as fungi and bacteria [2,18]. Most studies on hydroponic systems reported the role of indigenous bacteria and the effects of bacterial addition, and indirect bacterial mechanisms for biological pathogen control, but scarce data are available about the existence of differences between the bacteria content and plant growth when applying vermicompost leachate to a hydroponic system [13,19]. The influence of agricultural management practices on plant microbial communities is not completely clear [20]. Opportune microorganism identification in hydroponic systems which uses vermicompost leachate as a low-cost organic fertilizer is essential to select the most adequate microorganisms for an efficient pathogen biocontrol program, also to define a fertilization protocol for this system environmentally friendly and accessible to any producer [21]. Mint (*Mentha spicata* L.) and rosemary (*Rosmarinus officinalis* L.) are two plants of agronomic importance belonging to the *Lamiaceae* family [19,22], a family with many wild and cultivated officinal species, rich in essential oils and antioxidant compounds that are useful to humans [23,24]. The leaves of *M. spicata* are dried and used for tea infusions, and cultivated for the production of essential oils that are widely used in the pharmaceutical and cosmetic industries [19]. *R. officinalis*, besides its culinary uses due to its characteristic aroma, is also widely employed by indigenous populations in areas where it spontaneously grows. Rosemary extracts are used as a natural antioxidant, improving the shelf life of perishable foods [22,25]. This study assessed the effect of two types of fertilizer (inorganic versus organic fertilizer) on the growth of mint (*M. spicata*) and rosemary (*R. officinalis*) plants under a hydroponic production system, as an alternative agronomic method contributing to a reduction in pollution, water use, and fertilizer consumption, and low-cost production.

2. Materials and Methods

2.1. Study Area

The experiment was conducted in a shade-enclosure environment that served as a greenhouse facility in La Paz, located in a Bw (h') hw (e) climate, which is considered to be semiarid and sustains the xerophytic vegetation of Baja California Sur, northwest Mexico, at 7 m above sea level. Mean, maximal, and minimal temperature in the shade-enclosure

facility were 21.4, 31.8, and 8.9 °C, respectively, with a mean of 70% relative humidity. Meteorological records were obtained during the study from an automated weather station located inside the shade-enclosure facility.

2.2. Plant Cultivation Conditions and Hydroponic System

The experiment was carried out from September to November. *M. spicata* and *R. officinalis* cuttings were obtained from mother plants within their regional cultivars and were placed in pots with vermiculite until they developed enough roots to be able to absorb nutrients from fertilizers after applying the treatments. The pots were placed in 30 propylene containers of 20 L (24.5 × 16 × 10 cm (length × width × height)) filled with water. Oxygen supplementation in containers was provided with a Blogger Sweetwater pump (model SST20, 50 Hz). The water volume was maintained constant to build a closed hydroponic system; there was no recirculating water because the study was on the early vegetative stage (September to November).

2.3. Treatments and Experimental Design

The experimental design consisted of two treatments: one applying vermicompost leachate (L) and the other applying inorganic fertilizer (SS; control group) [26]. Vermicompost leachate (L) was produced at the CIBNOR experimental field according to recommendations by Gunadi et al. [27]. The vermicomposting process was carried out in 200 L containers cut in half, to which 5 holes were made in its base. Subsequently, a 5 cm thick layer of gravel and an antiaphid mesh were placed to separate the gravel from the bed where the earthworms developed. Kitchen waste and manure were used as food for the earthworms in a ratio of 1:1 volume:volume. Both kitchen waste and manure were precomposted for 21 days before being used as food for the earthworms. The feeding process was carried out using 5 cm thick layers of precomposted food every week for 12 weeks. The vermicomposting process was considered to have ended when a homogeneous material was observed without the presence of remnants of the original material. The vermicompost was separated to be laid and sheltered in a dry place and away from light for 90 days for its mineralization. Vermicompost leachate was obtained according to the methodology described by García-Galindo et al. [28], where 5 kg of vermicompost was placed in a container. Three liters of distilled water was poured into the container, and the leachate was collected. Information of the nutrient content of both inorganic fertilizer and vermicompost leachate is shown in Table 1. The experiment was established under a completely randomized design with 15 replicates for each treatment (vermicompost leachate and inorganic fertilizer). Each replicate consisted in a container before descripted with 12 pots, each pot with one plan. Treatments were applied once at five days after sowing (DAS), for inorganic fertilizer a commercial fertilizer of 17% NPK was used to prepared 10 mL that contained 0.0079, 0.000087, 0.070 (parts per million of N, P K, respectively) diluted in 40 L of top water (the capacity of pot container). For the vermicompost-leachate treatment, 140 mL that contained 0.00709, 0.000259, and 0.074 (parts per million of N, P K, respectively) was diluted in 40 L of tap water. The nutrient doses of N–P–K corresponded to the minimum established for these crops in the region to examine if any differences could be detected in microbial and morphological traits in the use of an organic versus inorganic fertilizer. Plants were analyzed in early-stage growth at 35 days after fertilizer application.

2.4. Morphological Traits and Relative-Growth Analysis

Stem length (SL, cm), fresh stem weight (FSW), dry stem weight (DSW), foliar area (FA), fresh foliar weight (FFW), dry foliar weight (DFW), root length (RL), fresh root weight (FRW), and dry root weight (DRW) were evaluated in five *M. spicata* plants and five *R. officinalis* rosemary plants before treatment application and at the end of the experiment (35 DAS). Stem and root weights (g) were obtained using an analytical scale (Mettler Toledo, AG204); for dry weights, an oven was used with forced air circulation at 70 °C (Shel-Lab®, FX-5, series 1000203) until constant weight. Data of initial and final dry weights were used

to calculate total relative growth rate (TGR), foliar growth rate (FGR), root growth rate (RGR), and stem growth rate (SGR) in grams per day, according to Hunt [29], following Formula (1):

$$TGR = ((lnDW2) \times (lnDW1))/(t2 - t1), \quad (1)$$

where DW2 and DW1 are the total plant (TGR), foliar (FGR), root (RGR) and stem (SGR) dry weight (g), recorded at times t2 (time of sampling) and t1 (beginning of the experiment), respectively. The difference (t2 − t1) is expressed in days. TGR, FGR, RGR, and SGR are expressed in g^{-1} day^{-1}.

Table 1. Solution-component analyses of nutritional source for *M. spicata* and *R. officinalis* in hydroponic system.

SS		L	
pH	5.5–6.5	pH	5.5–6
Electric conductivity (dS/m)	1.84	Electric conductivity (dS/m)	1.36
	mg L^{-1}		mg L^{-1}
Potassium nitrate	53,330	Potassium nitrate	5490.6
Ammonium nitrate	10,200	Ammonium nitrate	0.021
Monoammonium phosphate	14,800	Nitrite	0.012
Calcium nitrate	60,200	Nitrate	1.500
Magnesium sulphate	42,200	Potassium total	0.074
Ferrous sulfate	2000	Nitrogen total	1.5
Manganese Sulfate	50	Manganese Sulfate	6.38

SS: inorganic fertilizer, L: vermicompost leachate.

2.5. Photosynthetic Pigments

For *M. spicata* and *R. officinalis* plants under organic and inorganic treatments, we determined chlorophyll with seven plants (one leaf per plant) per treatment. *M. spicata* SPAD values [30,31] were recorded for 20 consecutive days after the beginning of both organic and inorganic treatments application. In *R. officinalis* plants, chlorophyll was evaluated two times: before any treatment application, and 20 days after both treatment applications. For *R. officinalis*, the chlorophyll was extracted following the acetone extraction methodology from leaf tissue, and the absorbance measure was carried out with a UV/visible spectrophotometer (model HELIOS OMEGA, Thermo Scientific, Vantaa, Finland). Chlorophyll a and b concentrations were estimated by applying the following functions [32]:

$$\text{Chlorophyll a (mg mL}^{-1}) = 11.64\ (A663) - 2.16\ (A645) \quad (2)$$

$$\text{Chlorophyll b (mg mL}^{-1}) = 20.97\ (A645) - 3.94\ (A663), \quad (3)$$

where A663 and A645 correspond for the absorbance values at wavelengths (λ) of 663 and 645 nm, respectively.

2.6. Sampling for Bacterial-Community Characterization

To determine the influence of organic and inorganic fertilizers on rhizobial microbial communities from the plant rhizosphere, samples of the root rhizosphere were taken in the hydroponic system as follows: a water sample of 50 mL with the roots (0–0.5 cm) from three different reservoirs at three times (1, 7, and 35 DAS). The collected samples were processed immediately for: (i) total DNA isolation from water (rhizosphere) samples, and (ii) bacterial isolation from *R. officinalis* and *M. spicata* root samples with the methodology that follows below. Vermicompost was free of pathogens.

2.7. Colony-Forming Units (CFU) Quantification and Isolation of Bacteria from M. spicata and R. officinalis Cultivated by Hydroponic System

The water and root samples were vorticed for 30 s. Then, 25 mL of the sample was transferred to a new tube for DNA isolation. The remaining 25 mL was used to determine

the colony-forming units (CFU). One milliliter of the remaining sample was used to perform serial dilutions in saline solution 0.85% (*w*/*v*) (from 10-2 to 10-7). Lastly, 100 µL for each dilution (from 10-2 to 10-7) was plated on nutrient agar (NA) and incubated for 24 h at 30 °C. After 24 h, the CFU count was performed.

After the CFU count, bacterial colonies were isolated on the basis of their morphology. A representative colony of the five most abundant colonial morphologies was reseeded by streak dilution in a new plate of NA and incubated at 30 °C overnight. This step was repeated until a pure isolate in each case (a single bacterial morphology per isolate) was obtained. The obtained pure isolates were stored in glycerol 30% (*v*/*v*) at −80 °C until their use.

2.8. DNA Isolation

The total DNA isolation of the water samples and bacterial isolates was carried out according to the protocol with slight modifications [33]. For water samples, 25 mL was centrifuged at 5000× *g* for 10 min, and the supernatant was discarded. For bacterial isolates, 3 mL of liquid culture was placed in nutrient broth (NB) at 30 °C overnight and centrifuged at 5000× *g* for 5 min, and the supernatant was discarded. Both the pellet from water samples and the bacterial isolate pellets were processed in the same way. The resulting pellet was resuspended in 1 mL of a lysis buffer (15% sucrose, 0.3 mg/mL lysozyme, 0.05 M EDTA and 1 M Tris, pH 8) and incubated for 30 min at 37 °C. Then, 100 µL of 10% SDS (*w*/*v*), 100 µL of 5 M NaCl, and 5 µL of proteinase K (0.4 mg/mL) were added and incubated under agitation for 1 h at 50 °C. After incubation, 200 µL of phenol–chloroform–isoamyl alcohol (25:24:1) was added to 500 µL of the solution, briefly vorticed, and then centrifuged at 12,000× *g* for 5 min. The aqueous phase was recovered, and 200 µL of ammonium acetate (7.5 M) and 500 µL (1 volume) of absolute ethanol were added to be mixed by inversion and precipitate at 4 °C overnight to centrifuge at 4 °C at 12,000× *g* for 15 min. The supernatant was discarded, and the pellet was washed twice with 100 µL of ethanol 70% (*v*/*v*). The DNA was dried at room temperature, resuspended in molecular-biology-grade water, and stored at −20 °C until use.

2.9. Relative-Abundance Quantification by qPCR

The relative abundance of the bacterial population was assessed through qPCR to determine the effect of treatments. The qPCR was performed on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) according to the instructions of the iTaq™ Universal SYBR® Green Supermix (Bio-Rad, Hercules, CA, USA). The relative abundance of the total bacteria in the rhizosphere samples for each treatment was assessed according to the methodology described by López-Gutiérrez et al. [33] with slight modifications.

2.10. Characterization of Bacterial Communities by Ribotype Assay Analysis (16S rRNA Gene)

Ribotype assay analysis was conducted according to the Bogino et al. [34] methodology. A total DNA of 36 water samples (3 samples × 3 times × 2 treatments × 2 species of plants = 36 samples in total) and 60 bacterial isolate strains (30 isolate strains for each plant for both organic and inorganic fertilization treatments) were characterized by amplified ribosomal DNA restriction analysis (ARDRA). Bacterial genomic DNA was extracted from each isolate as mentioned previously. For 16S rRNA gene amplification, we used primers fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and rD1 (5′- AAGGAGGTGATCCAGCC-3′). PCR amplification products (~1500 bp) were processed by a restriction endonuclease assay with HaeIII (Thermo Fisher Scientific), and the resulting fragments were electrophoretically separated on a 2% (*w*/*v*) agarose gel, stained with ethidium bromide to visualize them with UV radiation, and the corresponding image was photographed. Ribotype identification is directly associated with a specific restriction fragment fingerprint. The community structure dendrogram was constructed on the basis of ribotypes of the bacterial isolates with GelCompar II software. Bacterial isolate strains belonging to either unique majority ribotypes or common ribotypes were selected for further identification through 16S rRNA

gene nucleotide sequence analysis with primers COM 1 (5′-CAGCAGCCGCGGTAATAC-3′) and COM 2 (5′-CCGTCAATTCCTTTGAGTTT-3′) with the methodology described by Stach et al. [35]. The 16S rRNA gene sequences were analyzed using the BLAST (blastn) search program (National Center for Biotechnology Information (NCBI)).

2.11. Biofilm-Formation Assay

Biofilms are microbial communities that adhere to surfaces and are enclosed in a protective matrix; this is also the primary structure from which bacteria interact with plants and other eukaryotes. Thus, to characterize the bacterial capability of the rhizosphere (water samples) isolate strains from *M. spicata* and *R. officinalis* to form biofilms, we carried out the crystal violet (CV) staining quantitative assay of Labrie et al. [36] with slight modifications. CV staining absorbance was measured at 590 nm using a spectrophotometer (Multiskan Spectrum, Thermo Scientific, Wilmington, DE, USA).

2.12. Statistical Analysis

Data were analyzed using univariate and multivariate analysis of variance (ANOVA and MANOVA) for one-way classification, and the nutrition source was the study factor. For chlorophyll content, multiple analysis of variance (MANOVA) and significant differences between means for each recorded date were determined by two-way analysis of variance (ANOVA). Least significant differences (LSD) in Tukey's HSD test ($p = 0.05$) were estimated for one-way ANOVA. For all cases, significant differences between means were considered to be significant at $p < 0.05$. All statistical analyses were performed with Statistica software program v10.0 and GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Plant Morphology and Photosynthetic Pigments

3.1.1. *M. spicata*

Stem height (SL), dry foliar weight (DFW), fresh foliar weight (FFW), foliar area (FA), and root length (RL) showed a significant increase in the vermicompost leachate treatment compared with the inorganic treatment for *M. spicata* (Table 2). There was no difference between the vermicompost leachate treatment and the inorganic treatment for relative growth rates of leaves (FGR), stems (RGS), total growth rate (TGR), and roots (RGR), which was lower for vermicompost leachate than inorganic fertilizer was (Table 3). Chlorophyll a and b, and total content did not show any differences between plants with vermicompost leachate or inorganic treatment (Table 4 and Figure 1).

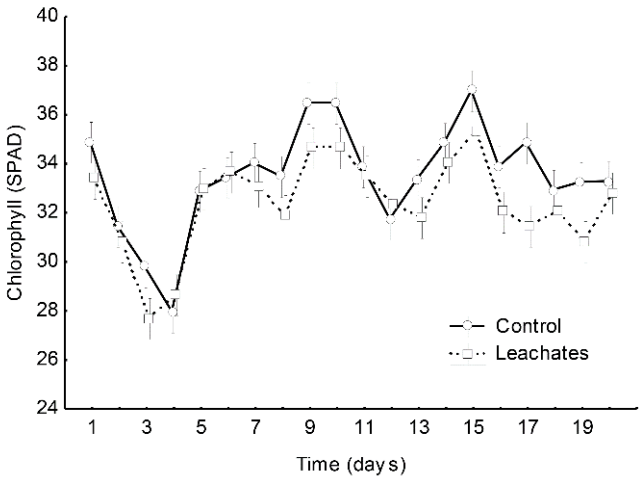

Figure 1. Chlorophyll SPAD readings in *M. spicata* plants under leachates of inorganic and vermicompost leachate fertilizers. Vertical bars represented mean ± standard error.

Table 2. Morphometric parameters in *M. spicata* and *R. officinalis* plants under fertilization treatments.

		SL (cm)	FSW (g)	DSW (g)	FA (cm^2)	FFW (g)	DFW (g)	RL (cm)	FRW (g)	DRW (g)
M. spicata	SS	11.8 ± 0.5 b	1.6 ± 0.5 a	0.4 ± 0.1 a	123 ± 51 b	4 ± 0.3 b	1 ± 0.1 b	38 ± 5 b	10 ± 2 a	1.5 ± 0.3 a
	L	14.7 ± 0.7 a	3 ± 0.5 a	0.6 ± 0.1 a	246 ± 21 a	7 ± 0.5 a	2 ± 0.2 a	54 ± 7 a	12 ± 2 a	1.8 ± 0.3 a
R. officinalis	SS	4.5 ± 0.5 a	4.5 ± 1 a	3.5 ± 0.6 a	32.7 ± 7 a	7.9 ± 1.4 a	4.6 ± 1 a	8.2 ± 0.6 a	10 ± 1 a	4 ± 0.6 a
	L	5.2 ± 0.4 a	4.9 ± 0.2 a	3.4 ± 0.1 a	33.5 ± 2 a	8 ± 0.8 a	4.5 ± 0.1 a	8.8 ± 0.5 a	11 ± 0.7 a	4.3 ± 0.2 a

SS: inorganic fertilizer, L: vermicompost leachate, SL: stem length, FSW: fresh stem weight, DSW: dry stem weight, FA: foliar area, FFW: fresh foliar weight, DFW: dry foliar weight, RL: root length, FRW: fresh root weight, DRW: dry root weight. Data represent means ± standard error (n = 3). *M. spicata* and *R. officinalis* data were treated as independent ANOVA analyses. Different letters for each column denote statistical difference.

Table 3. Total growth rate (TGR), foliar growth rate (FGR), root growth rate (RGR), and stem growth rate (SGR) expressed in grams per day of *M. spicata* and *R. officinalis* plants.

		TGR	FGR	RGR	RGS
M. spicata	SS	0.03 ± 0.00 a	0.023 ± 0.01 a	0.057 ± 0.02 a	0.013 ± 0.0 a
	L	0.02 ± 0.00 a	0.021 ± 0.01 a	0.048 ± 0.01 a	0.015 ± 0.0 a
R. officinalis	SS	0.0339 ± 0.008 a	0.0218 ± 0.01 a	0.0424 ± 0.001 a	0.034 ± 0.01 a
	L	0.0239 ± 0.007 a	0.0206 ± 0.01 a	0.0282 ± 0.001 b	0.022 ± 0.00 a

SS: inorganic fertilizer, L: organic fertilizer (vermicompost leachate). Data represent means ± standard deviation (n = 5). Different letters denote statistical differences.

Table 4. Chlorophyll (Chl) a and b, and total ($mg \cdot mL^{-1}$) content in *M. spicata* and *R. officinalis* plants under different nutrient sources in two times before (BT) and after (AT) application of vermicompost leachate and inorganic treatments.

	Treatment	Date *	Chl a	Chl b	Chl Tot
M. spicata	SS	BT b	60 ± 11 a	5 ± 0.4 a	86 ± 16 a
	L	AT a	67 ± 10 a	5.4 ± 0.6 a	97 ± 17 a
R. officinalis	SS	BT b	63 ± 9 a	4.9 ± 0.4 a	87 ± 13 a
	L	AT a	74 ± 10 a	5.5 ± 0.4 a	105 ± 15 a

SS: inorganic fertilizer, L: organic fertilizer (vermicompost leachate). Data represent means ± standard deviation (n = 5). Different letters denote statistical differences. * Denote statistical differences between sampling dates.

3.1.2. *R. officinalis*

For all morphological traits, there were no differences between the vermicompost leachate and inorganic treatments (Tables 2 and 3) except for rosemary under treatment with leachate in RGR, which showed lower growth (Table 3). Organic treatment did not affect chlorophyll a and b, and total content did not undergo alterations in either organic or inorganic treatment, and the only variable that exerted an effect was the time (date) of chlorophyll sampling (Table 4).

3.2. CFU Quantification and Relative Abundance of Bacterial Communities

The relative abundance of total bacterial communities due to the effect of treatments was assessed by CFU estimation and by a qPCR-based assay. For both *M. spicata* and *R. officinalis*, no differences were determined between the vermicompost leachate and inorganic treatments regarding the abundance of bacterial populations; however, an increase in relative abundance in time was more evident for the vermicompost leachate (Figure 2).

Bacterial community structure kinetics between both vermicompost leachate and inorganic treatments was analyzed. Thirty-six total DNA water samples were analyzed by amplified ribosomal DNA restriction analysis (ARDRA). As this test showed for *M. spicata* and *R. officinalis*, bacterial community structures underwent changes through time without a significant effect between treatments (Figure 3a,b). Thus, these results highlight the feasibility of replacing inorganic fertilizer with the vermicompost leachate without significant impact on the bacterial abundance or bacterial community structures of *M. spicata* and *R. officinalis* in hydroponic systems.

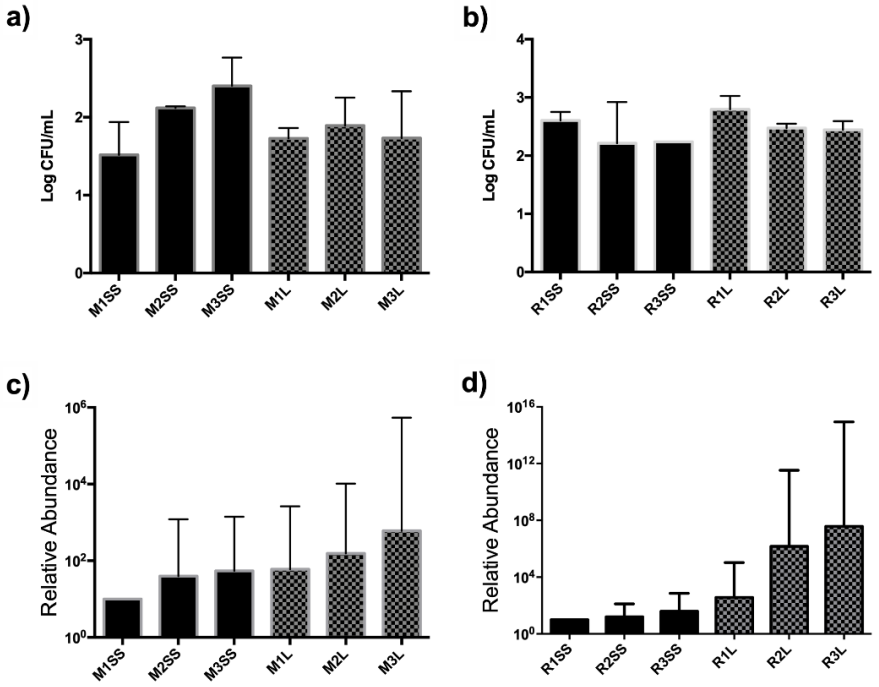

Figure 2. Colony-forming unit (CFU) quantification and relative abundance (qPCR) of bacterial communities in *M. spicata* and *R. officinalis*. CFU quantification in (**a**) *M. spicata* and (**b**) *R. officinalis*; relative abundance (qPCR) of bacterial communities in (**c**) *M. spicata* and (**d**) *R. officinalis* (M1SS: mint composed sample, time 1, inorganic fertilizer; M2SS: *M. spicata* composed sample, time 2, inorganic fertilizer; M3SS: *M. spicata* composed sample, time 3, inorganic fertilizer; M1L: *M. spicata* composed sample, time 1, vermicompost leachate; M2L: *M. spicata* composed sample, time 2, vermicompost leachate; M3L: *M. spicata* composed sample, time 3, vermicompost leachate; R1SS: *R. officinalis* composed sample, time 1, inorganic fertilizer; R2SS: *R. officinalis* composed sample, time 2, inorganic fertilizer; R3SS: *R. officinalis* composed sample, time 3, inorganic fertilizer; R1L: *R. officinalis* composed sample, time 1, vermicompost leachate; R2L: *R. officinalis* composed sample, time 2, vermicompost leachate; R3L: *R. officinalis* composed sample, time 3, vermicompost leachate.

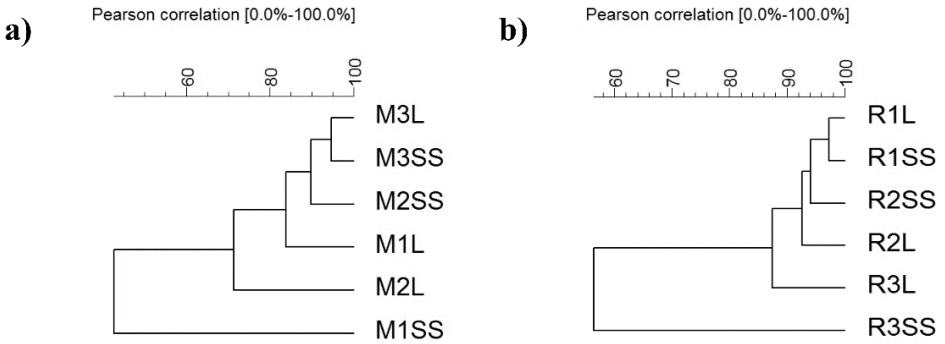

Figure 3. Dendrogram of general distribution of bacterial composition of communities between treatments in (**a**) *M. spicata* and (**b**) *R. officinalis* (M1SS: *M. spicata* composed sample, time 1, inorganic fertilizer; M2SS: *M. spicata* composed sample, time 2, inorganic fertilizer; M3SS: *M. spicata* composed sample, time 3, inorganic fertilizer; M1L: *M. spicata* composed sample, time 1, vermicompost leachate; M2L: *M. spicata* composed sample, time 2, vermicompost leachate; M3L: *M. spicata* composed sample, time 3, vermicompost leachate; R1SS: *R. officinalis* composed sample, time 1, inorganic fertilizer; R2SS: *R. officinalis* composed sample, time 2, inorganic fertilizer; R3SS: *R. officinalis* composed sample, time 3, inorganic fertilizer; R1L: *R. officinalis* composed sample, time 1, vermicompost leachate; R2L: *R. officinalis* composed sample, time 2, vermicompost leachate; R3L: *R. officinalis* composed sample, time 3, vermicompost leachate).

3.3. Composition and Diversity of Bacterial Communities

A total of 60 bacterial isolate strains (30 isolate strains for each plant for both vermicompost leachate and inorganic fertilization treatments) were characterized by ARDRA. From ARDRA, 15 ribotypes were identified in *M. spicata* and *R. officinalis* according to the yielded fingerprint after the restriction assay with the HaeIII restriction enzyme (Table 5). In the case of *R. officinalis*, eight different ribotypes were identified (Figure 4). Of these eight ribotypes, seven were present in inorganic treatment, and four in the vermicompost leachate. Of the ribotypes present in the inorganic treatment, four were exclusively present in this treatment, while only one ribotype was exclusive of the vermicompost leachate. In the case of *M. spicata*, there were also eight different ribotypes for both the vermicompost leachate and the inorganic treatment. For the inorganic treatment, there were five ribotypes, and none was exclusive to this treatment. For the vermicompost leachate treatment, eight ribotypes were present, and three ribotypes were exclusive of this treatment. However, it was not possible to characterize the ribotype to which three bacterial isolates from *M. spicata* belonged (two from inorganic treatment and one from organic treatment).

Representative bacterial strains were identified by 16S rRNA gene sequencing. Bacterial isolate strains were selected according to ribotype ARDRA profiles (Table 6). Most bacterial isolate strains belonged to the Firmicutes phylum, which was mainly composed of the Bacilli class, the Bacillaceae family, and the *Bacillus* genus. Bacterial isolate strains belonging to Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria classes from the Proteobacteria phylum were found (Table 6).

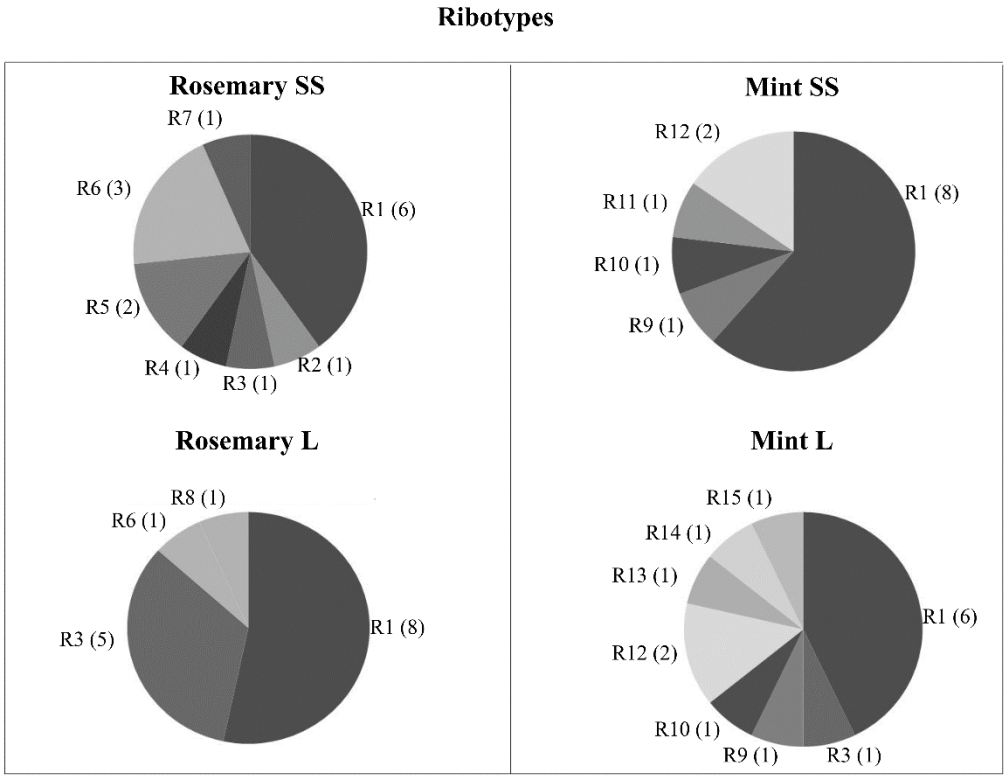

Figure 4. Ribotypes present in *M. spicata* and *R. officinalis* obtained by amplified ribosomal DNA restriction analysis (ARDRA; R: ribotype, number: number of ribotypes, and number in parentheses: number of isolates corresponding to each ribotype).

Table 5. Ribotypes of bacteria isolated from hydroponic system in *M. spicata* and *R. officinalis* plants.

Ribotype	Isolates from *M. spicata*		Isolates from *R. officinalis*	
	SS	L	SS	L
1	MSS1, MSS5, MSS10, MSSR1, MSSR4, MSS2, MSS6, MSSR5	ML6, ML7, ML8, ML10, MLR3, MLR5	RSS1, RSS5, RSS7, RSS8, RSS9, RSSR1	RL4, RL5, RL6, RL7, RL8, RL9, RL10, RLR2
2			RSS2	
3		MLR4	RSS3	RL2, RLR1, RLR3, RLR4, RLR5
4			RSS4	
5			RSS6, RSS10	
6			RSSR2, RSSR3, RSSR4	RL3
7			RSSR5	
8				RL1
9	MSS3	ML1		
10	MSS4	MLR2		
11	MSS7			
12	MSS8, MSS9	ML2, ML4		
13		ML5		
14		ML9		
15		MLR1		

SS: inorganic fertilizer, L: vermicompost leachate, MSS-number or MSSR-number: isolates from *M. spicata* inorganic fertilizer, ML-number or MLR-number: isolates from *M. spicata* vermicompost leachate, RSS-number or RSSR-number: isolates from *R. officinalis* inorganic fertilizer, and RL-number or RLR-number: isolates from *R. officinalis* vermicompost leachate. Note: MSSR2, MSSR3, and ML3 are missing from the table because they were unclassified.

Table 6. Identities of bacterial strains isolated from hydroponic system in *M. spicata* and *R. officinalis* plants.

Isolated	Rt	Most Closely Related Sequence (Accession Number) (Id %)	Phylogenetic Affiliation
RSS-1	1	*Bacillus koreensis* (NR_043084.1) (98)	*Firmicutes*
RSS-5	1	*Bacillus aryabhattai* (NR_118442.1) (99)	*Firmicutes*
MSS-2	1	*Bacillus aryabhattai* (NR_118442.1) (99)	*Firmicutes*
ML-6	1	*Bacillus vietnamensis* (NR_113995.1) (98)	*Firmicutes*
RSS-2	2	*Enterobacter cloacae* (NR_118568.1) (99)	*Gammaproteobacteria*
RSS-3	3	*Herbaspirillum chlorophenolicum* (NR_114143.1) (99)	*Betaproteobacteria*
RSS-4	4	*Bacillus pseudomycoides* (NR_114422.1) (99)	*Firmicutes*
RSS-6	5	*Bacillus subtilis* (NR_102783.1) (99)	*Firmicutes*
RSSR-2	6	*Novosphingobium pokkalii* (NR_149820.1) (94)	*Alphaproteobacteria*
RSSR-5	7	*Lysinibacillus tabacifolii* (NR_132691.1) (99)	*Firmicutes*
RL-1	8	*Novosphingobium capsulatum* (NR_113.591.1) (99)	*Alphaproteobacteria*
ML-1	9	*Bacillus paralicheniformis* (NR_137421.1) (99)	*Firmicutes*
MSS-4	10	*Pseudomonas entomophila* (NR_102854.1) (99)	*Gammaproteobacteria*
MLR-2	10	*Pseudomonas entomophila* (NR_102854.1) (99)	*Gammaproteobacteria*
MSS-7	11	*Brevibacterium frigoritolerans* (NR_117474.1) (99)	*Firmicutes*
MSS-8	12	*Staphylococcus petrasii* (NR_136463.1) (99)	*Firmicutes*
MSS-9	12	*Staphylococcus petrasii* (NR_136463.1) (99)	*Firmicutes*
ML-5	13	*Bacillus oceanisediminis* (NR_118440.1) (98)	*Firmicutes*
ML-9	14	*Bacillus flexus* (NR_118382.1) (99)	*Firmicutes*
MLR-1	15	*Bacillus toyonensis* (NR_121761.1) (98)	*Firmicutes*

Rt: ribotype.

Ribotypes found in rosemary bacterial isolate strains belonged to Firmicutes (60%), mainly composed of the *Bacillus* genus. Comparing the vermicompost leachate and inorganic treatments, we determined that the Firmicutes phylum was the most abundant between treatments, and the Alphaproteobacteria and Betaproteobacteria classes, and Gammaproteobacteria showed greater abundance in inorganic treatment than in the vermicompost leachate treatment (Figure 4, Table 6). The ribotypes found in *M. spicata* bacterial isolate strains belonged to Firmicutes (80% and were mainly composed of the *Bacillus* genus. Interestingly, 10% of the bacterial isolate strains were unclassified. Comparing the vermicompost leachate and inorganic treatments, the most abundant phylum was Firmicutes, followed by the Gammaproteobacteria class (Tables 5 and 6). For the vermicompost leachate, the Betaproteobacteria class showed greater abundance in the vermicompost

leachate treatment than in inorganic treatment (Tables 5 and 6). Therefore, the Firmicutes phylum was the most abundant in both *R. officinalis* and *M. spicata* plants, and in both the vermicompost leachate and the inorganic treatment.

3.4. Biofilm-Forming Ability of Bacterial Communities

All bacterial isolate strains from *R. officinalis* (30 isolates) and *M. spicata* (30 isolates) were assessed for adhesion and biofilm-establishment capability with a CV assay. The CV assay showed that all bacterial isolates were able to adhere to the surface and establish biofilms (Figure 5). Differences were found in biofilm formation that were categorized according to the capability to retain CV measured by the OD at 595 nm (CV-OD595) [28], for all bacterial isolate strains as follows: weak (<0.6), moderate (0.6–1.2), and strong (>1.2). *R. officinalis* bacterial isolate strains with the vermicompost leachate treatment showed that 3 bacterial isolates formed a moderate biofilm, 2 a strong biofilm, and the remaining 10 a weak biofilm. For the bacterial isolate strains from the inorganic treatment, 4 bacterial isolates formed a moderate biofilm, 1 a strong biofilm, and the remaining 10 a weak biofilm. The *M. spicata* bacterial isolate strains with the vermicompost leachate treatment showed that 1 bacterial isolate formed a strong biofilm, 2 a moderate biofilm, and the remaining 12 formed a weak biofilm. For the inorganic treatment, 2 bacterial isolates were able to form a strong biofilm, 1 a moderate biofilm, and the remaining 12 a weak biofilm. Altogether, for the *R. officinalis* and *M. spicata* plants and both the vermicompost leachate and the inorganic treatment, most bacterial isolates were able to form weak biofilms in the conditions assessed in this study.

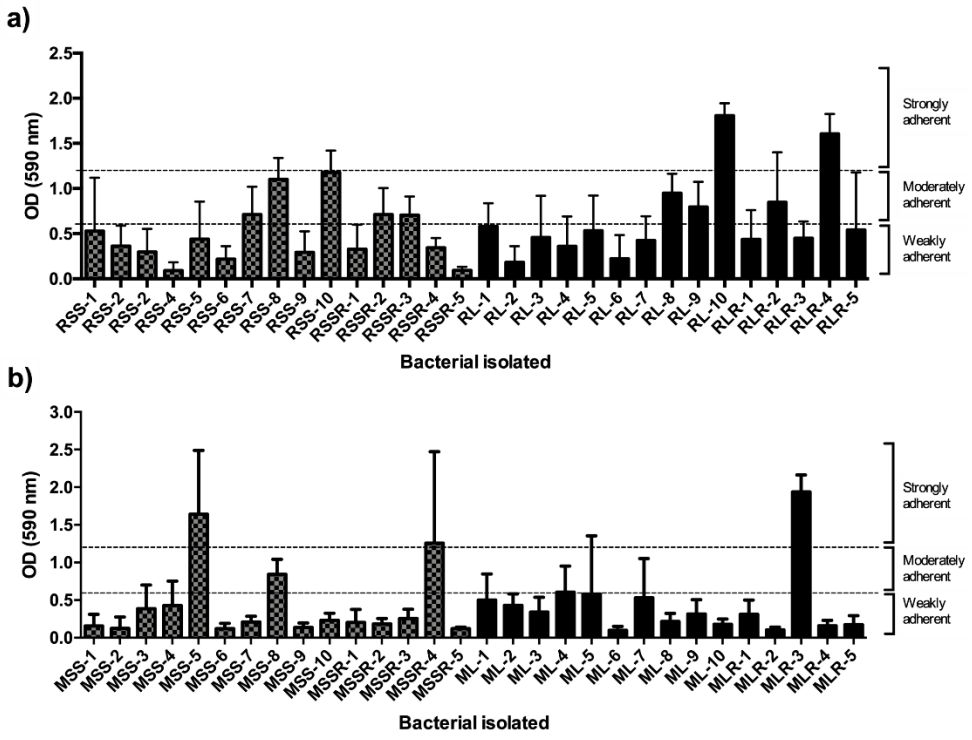

Figure 5. Biofilm formation quantified by staining with crystal violet of isolates from (**a**) *R. officinalis* and (**b**) *M. spicata* (RSS-number or RSSR-number: isolates from *R. officinalis* inorganic fertilizer, RL-number or RLR-number: isolates from *R. officinalis* vermicompost leachate, MSS-number or MSSR-number: isolates from *M. spicata* inorganic fertilizer, and ML-number or MLR-number: isolates from *M. spicata* vermicompost leachate.

4. Discussion

The vermicompost leachate treatment for both *M. spicata* (mint) and *R. officinalis* (rosemary) plants did not affect their growth; even for *M. spicata* plants, we were able

to determine a growth increase for several morphometric parameters. Moreover, for *R. officinalis* plant growth, for all morphometric parameters, there were only differences for root growth, which was lower for vermicompost than for inorganic leachate; similar results were found by Peng et al. [37]. This is important since the aim of healthy food production is avoiding the application of inorganic fertilizer [25,38–41]. Furthermore, vermicompost leachate contains a high amount of plant hormones, such as auxins, gibberellins, and cytokinins from microbial origin, giving rise to plant-growth enhancement, and acting as a liquid fertilizer [15,42–45]. Emperor and Kumar [45] determined that organic matter processed in the earthworm gut and then excreted as vermicast undergoes an increased level of microbial population, microbial respiration, microbial enzyme activity, and N, P, and K enrichment, bacterial exopolysaccharide production, lignocellulolytic activity establishment, nitrifying, and nitrogen-fixing microorganism proliferation. The above allow for us to conclude that the use of vermicompost to replace inorganic fertilizers is a viable option under the use of hydroponic systems [43,46–49].

The bacterial communities' relative abundance showed no differences between the vermicompost leachate and inorganic treatments for both *R. officinalis* and *M. spicata* plants, showing time-related differences, as expected, in accordance with previous works, where the analyzed bacterial communities underwent the same behavior [50,51]. The bacterial-community structure for the *R. officinalis* and *M. spicata* plants and for both treatment types were mainly composed by the Firmicutes phylum, followed by the Proteobacteria phylum, which was represented by the Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria classes; we were also able to determine the presence of beneficial bacteria from the *Bacillus* (Firmicutes phylum) and *Pseudomonas* (Proteobacteria phylum) genera. Those bacteria are designated as beneficial or plant-growth-promoting (PGPB), and the characterization of the bacterial-community structures of the rhizosphere for other plant members (*Thymus vulgaris*, *T. citriodorus*, *T. zygis*, *Santolina chamaecyparissus*, *Lavandula dentata*, and *Salvia miltiorrhiza*) of the Lamiaceae family showed that Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Acidobacteria, and Gemmatimonadetes were among the most abundant bacterial phyla [5,52–56].

Lastly, the capability to establish biofilms was assessed for all 60 bacterial isolate strains from the *M. spicata* and *R. officinalis* plants and both treatments, with no differences highlighting the essential role of biofilm development in bacterial survival and physiology [36]. We determined that most of the isolates (66.67% in *R. officinalis* and 80% in *M. spicata*) had weak capacity (CV-OD595) to form a biofilm; a smaller proportion were able to produce a strong biofilm for both *M* plants and both treatments. In an aqueous environment, such as a hydroponic system, biofilm establishment follows other mechanisms that are not yet characterized. Authors should discuss the results and how they can be interpreted from the perspective of previous studies and working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

5. Conclusions

In this study, we showed that the substitution of inorganic fertilizer by vermicompost leachate in a hydroponic system allows for us to maintain or increase the production of two crop plants with agricultural importance (*M. spicata* (mint), and *R. officinalis* (rosemary)). Furthermore, we determined that this fertilizer substitution modifies neither the bacterial communities for both plants nor their ability to form biofilms. Through time, the vermicompost leachate tendency showed an increase in relative abundance, which is important to consider for future studies. Therefore, we propose the use of vermicompost leachate fertilizer as a feasible replacement for inorganic fertilizer in hydroponic systems to achieve sustainable and ecofriendly agricultural production, in agreement with our results and recent research conducted on open-field cultures, to face the challenge of a growing population and pollution derived from the use of inorganic fertilizers.

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