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Evaluation of Metabolomic Profile and Growth of *Moringa oleifera* L. Cultivated with Vermicompost under Different Soil Types

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Abstract: *Moringa oleifera* is a highly versatile plant with potential use in the agro-food and biochemical industry. The goals of this study were to evaluate the effect of chemical fertilization and vermicompost on plant growth, and to analyze the metabolomic profile of *M. oleifera* crops cultivated over agricultural and native soils. The extracts were obtained from 90-day-old leaves via extraction with a hydroalcoholic mixture. Multivariate data analyses, such as principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA), were used to differentiate the distribution of leaf metabolites according to the soils or types of fertilizers used for the cultivation of *Moringa oleifera*. The results indicated that there was no significant effect on parameters such as plant height, root length and dry weight of leaves ($p < 0.05$). UPLC-ESI-MS/MS analysis of leaf extracts revealed a wide range of flavonoids, alkaloids and organic acids. The results of PCA and PLS-DA confirmed that the type of fertilizer had an effect on the metabolomic profile of *M. oleifera* leaves. The application of vermicompost induced changes in the metabolomic profile, but not in the morphometric variables of *Moringa oleifera*. These results are important for metabolite production via organic cultures and over different soil types in the industrialization of *Moringa*.

Keywords: secondary metabolite; *Moringa*; agriculture soil; leaves; UPLC-ESI-MS/MS

1. Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), one of the main challenges to be met in the future is the eradication of hunger worldwide [1]. For this reason, agriculture plays a very important role in combatting this problem. However, there is a worldwide increase in food demand, while primary resources in agriculture, such as soil and water, are rapidly lost. Therefore, agriculture should be an activity that safeguards organic matter in the soil and improves the conditions of those that have already been degraded to generate more arable land [2]. However, intensive agricultural practices negatively affect soil quality, as monocultures are sown for many seasons and agricultural inputs, such as chemical fertilizers, pesticides and herbicides, are used with

the aim of increasing yields [3]. Therefore, the use of organic inputs, such as vermicompost, can provide benefits for both plants and soil in order to improve soil fertility [4,5]. It has been found that organic supplies also provide growth-promoting hormones (auxins, gibberellins and cytokinins), which improve crop yield. The high content of organic acids in vermicompost, such as humic and fulvic acids, has proven to be effective in increasing yields in many crops, e.g., corn and oats, tobacco roots, soybeans, peanuts, among others [6–8].

On the other hand, the use of some plants with specific characteristics has been proposed, with the intention of solving problems due to food shortages in many parts of the world. Plants with high protein content that are capable of providing vitamins and minerals to complement a diet and to improve the nutrition of people are being put forward. *Moringa oleifera* is a native plant to Southern Asia and is the most widely cultivated species of the *Moringa* genus. Most parts can be used as food, as they are a high source of protein, essential amino acids, minerals and vitamins [9,10]. It is a multipurpose tree with important economic value around the world, as its leaves, flowers and seeds can be used by the agro-food and biochemical industries. It is used as a foodstuff, biopesticide, green manure, natural coagulant for turbid water, plant growth enhancer and is potentially a resource for biodiesel production [11]. It has also been reported that its leaves are rich in essential amino acids, antioxidants, minerals and vitamins [12,13].

Moringa growers have found that some of the plant's main properties include adapting to water scarcity and developing on dry soil [14]. Organic fertilizing of *Moringa oleifera* with supplies such as chicken and cow manure, grass clippings and cassava in different combinations have significantly promoted plant development [15]. Therefore, applying compost and organic fertilizers produces a direct impact on the growth and development of plants. This may be due to the interaction of the nutrients in the soil and growth-regulating substances (gibberellins, auxins and cytokinins).

Several studies [16,17] have reported the effect of organic fertilizers and biofertilization on the metabolomic profile in plants. However, few studies of this type have been conducted on *Moringa oleifera*. For example, the authors of [18] reported the nutritional quality of flour from *Moringa oleifera* leaves grown in South Africa. They also reported that the crude protein content (30% by weight) contains 19 amino acids, where alanine is the majority. The most prevalent macro-elements are calcium and potassium, whereas iron and selenium stand out as microelements. Furthermore, the use of biofertilizers, such as *Azospirillum silense* and *Herbaspirillum seropedicae*, induce microorganism–plant interactions, which exert a significant effect on the change of metabolites' profiles [19].

The employment and study of *Moringa oleifera* represents a worldwide alternative to feed the population due to its nutritional importance [20,21]. In Mexico, usage of this plant can bring direct positive benefits to consumers. However, it is first necessary to study its properties and to propose cultivating systems where the plant's exploitation does not have secondary effects on the environment. Therefore, the use of new agricultural techniques is proposed, attempting to decrease the environmental impact via application of organic supplies. Theoretically, the reduction of erosion and loss of nutrients in soil could be guaranteed. Thus, it is important to understand the impact that organic supplies have on the metabolomic profile of plants. Therefore, the aim of this work was to evaluate the effects of chemical fertilization and vermicompost on plant growth, and to analyze the metabolomic profile changes of *Moringa oleifera* crop cultivated in agricultural and native soils.

2. Materials and Methods

2.1. Site Description and Soil Sampling

Samples of native and agricultural soil and vermicompost were collected during the month of November 2014 from three ranches located in the state of Chiapas, Mexico. Native soil came from 'La Escondida' ranch (16°01'55.0" N and 92°50'53.8" W), and agricultural soil from 'La Majada' ranch (16°02'41" N and 92°22'32.1" W). Both of these are located in the municipality of La Concordia. Vermicompost came from the organic farm 'Luanda'

(16°45'36.7" N and 93°22'32.1" W), situated in the municipality of Ocozocoautla. Soil was sampled by randomly augering the top 15 cm layer of three plots, each with an approximate area of 0.5 ha. At each plot, 30 soil samples were taken and pooled, so that three soil samples could be obtained and characterized separately.

2.2. Characterization of Vermicompost and Soil

The characterization of soil samples and vermicompost was carried out as described in [22]. Sample pH was measured with a glass electrode in a 1:2.5 sample-H₂O suspension [23]. The WHC was measured in soil and in water-saturated vermicompost in a funnel and left to stand overnight. The electrolytic conductivity (EC) was determined in a saturated solution extract as described by [24]. The organic C in vermicompost and soil was measured with a total organic carbon analyzer TOC-VCSN (Shimadzu, USA). Total N was measured by the Kjeldahl method [25]. Soil particle size distribution was determined by the hydrometer method, as described by [26].

2.3. Cultivation of *Moringa* in the Greenhouse

The experimental design was completely randomized and performed in triplicates. *Moringa* was cultivated in agricultural soil, for which three treatments were applied: (1) uncultivated and unfertilized soil, (2) plants fertilized with 0.6 g of urea applied per soil column or an equivalent of 150 kg N ha⁻¹, and (3) soil mixed with vermicompost (39.17 g of vermicompost per column). In this way, plants were fertilized with a final concentration of 150 kg N ha⁻¹, taking into consideration that all mineral N in the vermicompost and that 40% of the organic N in the vermicompost was mineralized during the experimental period.

Seventy-two sub-samples of soil, each with 9 kg, were added to PVC tubes (length 50 cm and diameter 16 cm). At the bottom, each tube was filled with 7 cm of gravel and topped up with 3 cm of sand. *Moringa* seeds were provided by 'La Escondida' farm. Three *Moringa* seeds were planted at a depth of 1 cm in each of the 72 PVC tubes. The PVC tubes were placed at random spots inside the greenhouse. Throughout the 90-day experiment (beginning on 2 December), a volume of 2000 mL of water was added to each column every four days. The amount of water applied was limited in order to avoid leaching. After 30 and 90 days, the entire soil column was removed from the PVC tube and plant roots and shoots were characterized.

Each treatment was performed in triplicate, whereby a total of 72 experimental units were obtained. Evaluation of variables included plant height, weight of dry leaves and metabolomic profile of leaves. This evaluation was performed at a time of 45 and 90 days after germination.

2.4. Sample Preparation for Metabolomics Analysis

Leaves were air-dried at room temperature for 72 h and ground to a fine powder before being stored. Afterwards, extraction was performed as described in [27]. Briefly, 25 g of *Moringa oleifera* leaves were soaked for 7 days in a mixture of methanol:water (80:30) at room temperature. The extract was then filtered and concentrated by using an R-210 Buchi rotary evaporator (BÜCHI Labortechnik AG, Switzerland), and then lyophilized to preserve the sample until analysis.

2.5. Analysis of Metabolites by UPLC-ESI-MS/MS

In order to improve the extraction of metabolites, 20 mg of lyophilized residue was reconstituted in 500 µL of methanol for 10 min in an ultrasound bath at 20 °C, and then centrifuged (11,356 × g, 10 min). Supernatants were collected and filtered through a 0.22 µm polypropylene membrane filter (ANOTOP10 plus; Whatman, Maidstone, UK). Each sample (10 µL) was analyzed in a UPLC-ESI-MS/MS system (LCQ Fleet, Thermo Finnigan, San Jose, CA, USA). This equipment used a C18 Hypersil gold column (50 × 2.1 × 1.9 mm) [28]. Operating conditions were as follows: oven temperature of 38 °C, flow rate of 350 L/min, where gradient mobile phase A was water in 0.1% formic acid and mobile phase B was

methanol in 0.1% formic acid. The gradients of solvent B were 35% (0–1.5 min), 35–86% (1.5–3 min), 86–100% (3–25 min), 100% B (25–27 min) and 35% B (27–27.5 min). Subsequently, individual sample data were downloaded into “mzXML” digital files and analyzed with programming software and bioinformatics. Results were analyzed with Mzmine3 [29], MetaboAnalyst [30] and R Software [31]. In addition, the results were evaluated in order to select the best treatment that could allow samples to be arranged as Gaussian behavior [32]. The chemometric analysis, with the help of PCA, was used for determining optimal linear transformation to allow generated data to be screened according to variability, thus making comparisons less complicated. Subsequently, PLS-DA was used as a multivariate regression technique [32]. The putative identification of metabolites was cross-referenced against information related to monoisotopic mass, mass spectra and most probable molecular mass using PlantCyc [33], MetaCyc [34], PubChem [35] and Massbank [36].

2.6. Statistical Analysis

A multivariate analysis of variance (ANOVA) was carried out with the Tukey test with the Statgraphics Centurion XVI software analysis, for which the $p < 0.05$ threshold was applied for statistically significant differences.

3. Results

3.1. Characteristics of Vermicompost and Soil

The physicochemical composition of soils and vermicompost is provided in Table 1. Native and agricultural soils showed significant differences ($p < 0.05$) in all related parameters. The vermicompost had a higher pH value (7.41) compared to the other samples. Likewise, WHC, humidity, EC, organic C, total N and C/N ratio were higher for vermicompost than for the SN and SA samples.

Table 1. Physicochemical characterization of soils and vermicompost.

Treatment	pH	WHC (g g ⁻¹ Soil)	Humidity%	EC (dS m ⁻¹)	Organic C (g kg ⁻¹ Soil)	Total N (g kg ⁻¹ Soil)	C/N	Textural Classification
SN	6.77 ^b	0.41 ^b	5.99 ^b	4.31 ^b	9.65 ^c	1.77 ^b	5.5 ^c	Sandy Clay
SA	5.45 ^c	0.42 ^b	1.18 ^c	3.69 ^b	14.12 ^b	1.49 ^c	9.5 ^b	Sandy Clay
* V	7.41 ^a	0.92 ^a	49.25 ^a	8.00 ^a	233 ^a	11.8 ^a	19.7 ^a	-
LSD ($p < 0.05$)	0.024	0.019	0.021	1.153	1.154	0.116	0.199	

* SN: native soil, SA: agricultural soil and V: vermicompost. Significant differences within treatments ($p < 0.05$) are indicated in columns by use of different lowercase letters.

3.2. Evaluation of Growth

The growth parameters were evaluated at days 45 and 90. Based on the results, it was observed that no significant difference between *Moringa oleifera* fertilization treatments occurred, as seen in Table 2. However, differences were found between soil types after 45 days (Table 3).

Table 2. Effect of the application of different types of fertilization on the growth parameters of *Moringa oleifera*.

Treatment	Plant Height (cm)		Root Length (cm)		Dry Weight Leaves (g)	
	Day		Day		Day	
	45	90	45	90	45	90
Vermicompost	45.83 ^a	66.33 ^a	12.33 ^a	31.16 ^a	1.44 ^a	2.11 ^a
Urea	43.83 ^a	66.50 ^a	13.00 ^a	28.50 ^a	0.83 ^a	2.52 ^a
Soil + Plant	43.33 ^a	52.83 ^a	16.33 ^a	26.50 ^a	1.39 ^a	1.96 ^a
LSD ($p < 0.05$)	6.30	12.05	6.17	10.51	0.66	0.66

Significant differences within treatments ($p < 0.05$) are indicated in columns by use of different lowercase letters.

Table 3. Effect of two soil types on growth parameters of *Moringa oleifera*.

Type of Soil	Plant Height (cm/Plant)		Root Length (cm/Plant)		Dry Weight Leaves (g/Plant)	
	Day		Day		Day	
	45	90	45	90	45	90
Native	54.33 ^a	66.00 ^a	17.77 ^a	29.22 ^a	1.93 ^a	2.47 ^a
Agricultural	34.33 ^b	57.77 ^a	10.00 ^b	28.22 ^a	0.51 ^b	1.93 ^a
LSD ($p < 0.05$)	5.14	9.84	5.04	8.58	0.54	0.54

Significant differences ($p < 0.05$) are indicated in columns by use of different lowercase letters within treatments.

3.3. Metabolomic Profile Changes in *Moringa* Leaves

Informatic analysis and statistical visualization were performed with MetaboAnalyst software. The first step of the chemometric analysis was a Data Integrity Check, with the objective of evaluating peaks via the m/z and tr relationship. A total of 186 peaks (m/z , tr) were detected, 12 of which were discarded.

Upon completion of proofreading of obtained data integrity, filtering of the data was performed in order to eliminate noise or non-informative variables in the dataset. As part of data filtering, a normalization curve was generated. Global data coherence was thereby improved, so that significant biological comparisons could be evaluated statistically. Data were transformed into a matrix, where samples were inserted into rows and variables (compounds and peaks) into columns. The normalizing of columns of variables was performed in order to make values in each row comparable to one another. Presently, there is no common accord as to which normalization methods work better for the different types of metabolomic data. However, results must be evaluated in order to select the best treatment that will allow samples to be arranged as Gaussian behavior.

Subsequently, the effect of the organic amendment (vermicompost) was evaluated by exploratory multivariate statistical data analysis. This allowed to find variations in the effect on the metabolomic profile of *Moringa oleifera* leaves. For this purpose, a two-sample t -test was used to test a coefficient significance $\alpha = 0.05$. The results obtained with the test indicated that an effect exists on the m/z relationship (Figure 1), as the 19 pink circles correspond to peaks (m/z , tr) presenting statistical differences in vermicompost-supplemented plants when compared with the average of the control (soil without vermicompost).

In principal component analysis (PCA), obtained data were projected over a two-dimensional coordinate system. As a result of this analysis, five PCs were obtained, which represented total data variability (100%) (Figure 2). However, in order to observe the effect of vermicompost application on a graph, only two dimensions were taken into consideration (PC1 and PC2). With the addition of its components (48.5% and 20%), the effect of vermicompost application on plants could be described, as these two PCs account for over 60% of the data variability.

With the help of PCA with PC1 and PC2 (Figure 3), it was demonstrated that differences exist between vermicompost-treated samples (green dots) and control data (red dots). Two out of three samples are on the same side of PC1 (right to left), representing 48.5% of the total data variability.

With the PCA test, it was possible to describe the behavior of the data in the metabolomic profile. However, it was necessary to perform a partial least squares regression with discriminant analysis (PLS-DA), which is a method of grouping and sorting monitored data. Unlike PCA, PLS-DA uses multivariate regression techniques. As part of the chemometric analysis (R system), the "PLSR" package was used to perform the PLS-DA regression. This tool was used in order to maximize the separation between different groups for comparison, which allowed to confirm the existence of statistical differences in the metabolomic profile of vermicompost-supplemented *Moringa oleifera* leaf extracts, as compared to control samples. Therefore, five components relating to the PLS-DA were generated (Figure 4). These results indicated that 100% of the variance of the sample data was generated.

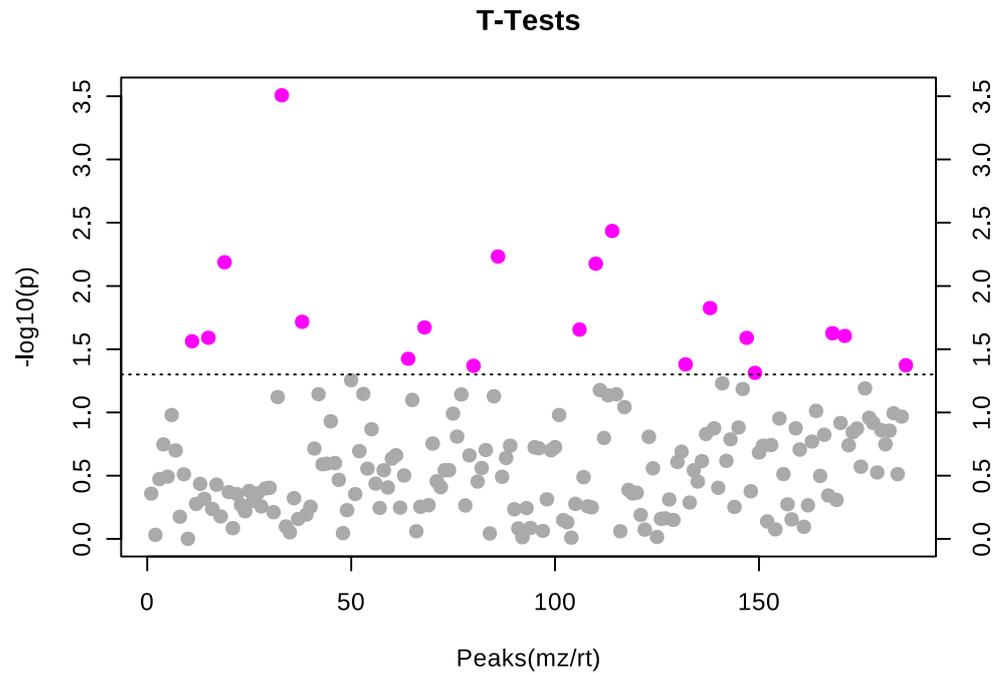


Figure 1. T-test of the peaks (mz/rt) identified in samples of *Moringa oleifera* extracts. Pink dots represent the m/z ratios that were statistically different when the plants were amended with vermicompost as compared to an unamended control group.

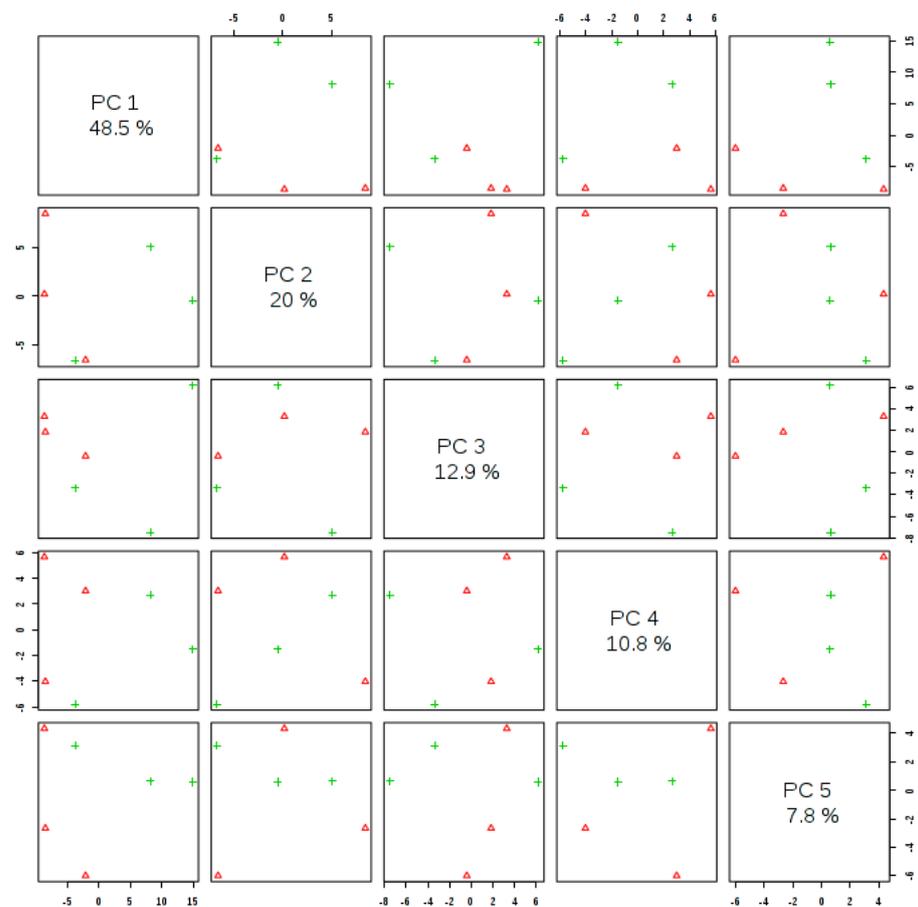


Figure 2. PCs generated in the PCA, which represent 100% of the variability of the data of extracts from *Moringa oleifera* leaves. Green triangles represent the samples treated with vermicompost and red cross represents data from the control treatment.

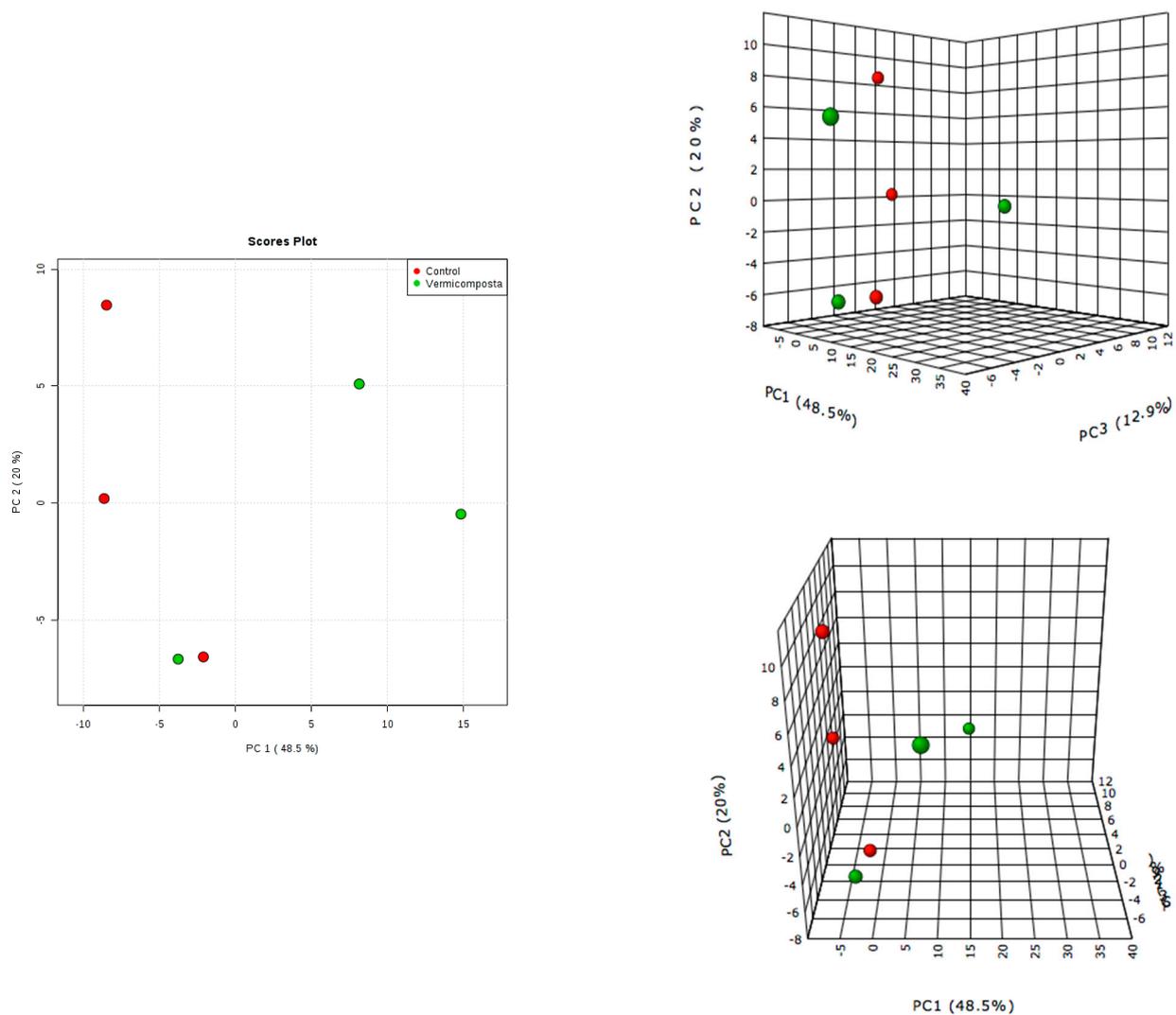


Figure 3. PCA samples amended with vermicompost group vs. control samples.

For analysis and interpretation, PC1 and PC3 were used to plot two-dimensional data, as the amount of both components accounted for 61.1% of the variance of the data. The results of PLS-DA analysis (Figure 5) allowed to conclude that the type of fertilizer factor has an effect on the metabolomic profile of *Moringa oleifera* leaf extract. This was observed due to data grouping. Samples supplemented with vermicompost were grouped on the right side of the first component of the PLS, which are positively related, while the control groups remained on the left side of the first component. This means that they are negatively related to samples supplemented with vermicompost.

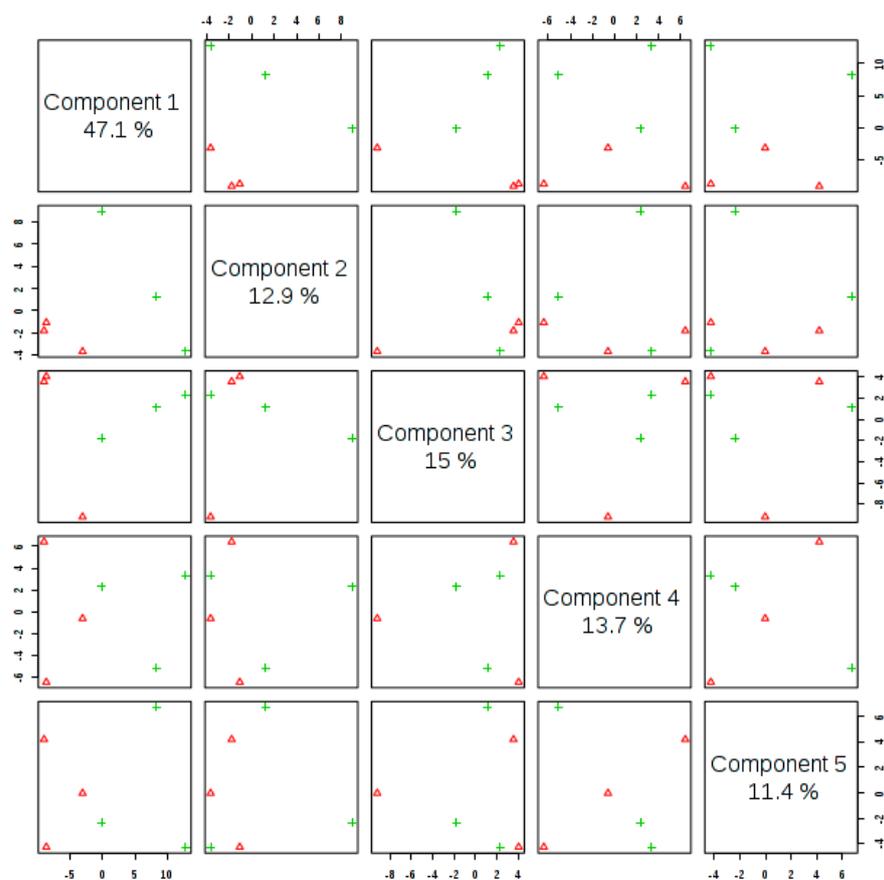


Figure 4. Principal components of the PLS-DA were used for the generation of the two-dimensional graph, which explained 62.1% of the variability of all the data in the metabolic profile of *Moringa oleifera* leaves extracts. Green triangles represent the samples treated with vermicompost and red cross represents data from the control treatment.

The next step of the chemometric analysis was the heat map metabolomic profiling of *Moringa oleifera* leaf extracts. This analysis was performed by using the m/z and tr relations of the 186 peaks mentioned previously (Figure 6). Using a heat map, the behavior of the metabolomic profile can be viewed based on the m/z ratio, which pertains to the metabolites present in the samples of *Moringa oleifera* leaf extracts when they are compared for diversity, abundance and type of supplement used.

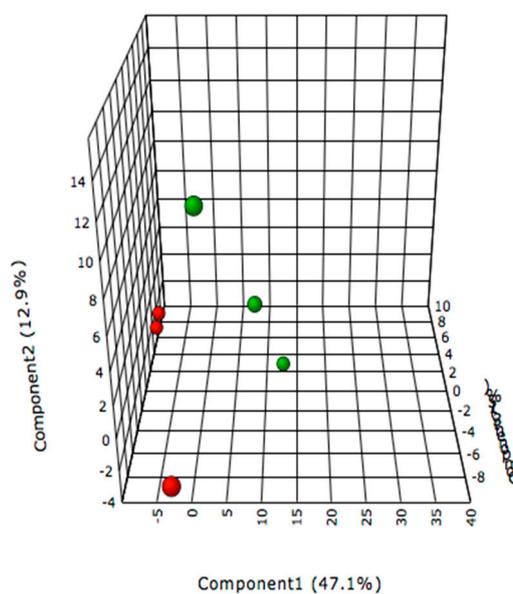
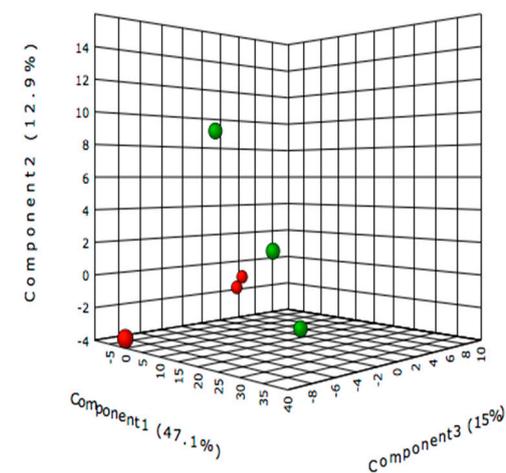
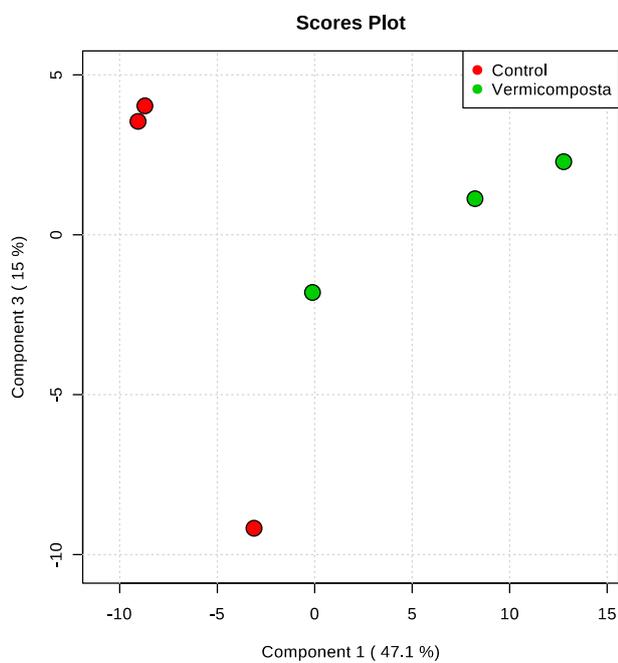


Figure 5. PLS-DA of samples amended with vermicompost vs. samples from the control group.

3.4. Metabolite Identification

In this study, metabolites were identified in *Moringa oleifera* leaf extracts, based on their m/z and tr ratio. These results indicated that the identified putative metabolite family groups belong to alkaloids, flavonols/flavonoids, phospholipids, glycerophospholipids, fatty acids and organic acids (Table 4). The metabolome database of different soil types is shown in Supplementary Table S1.

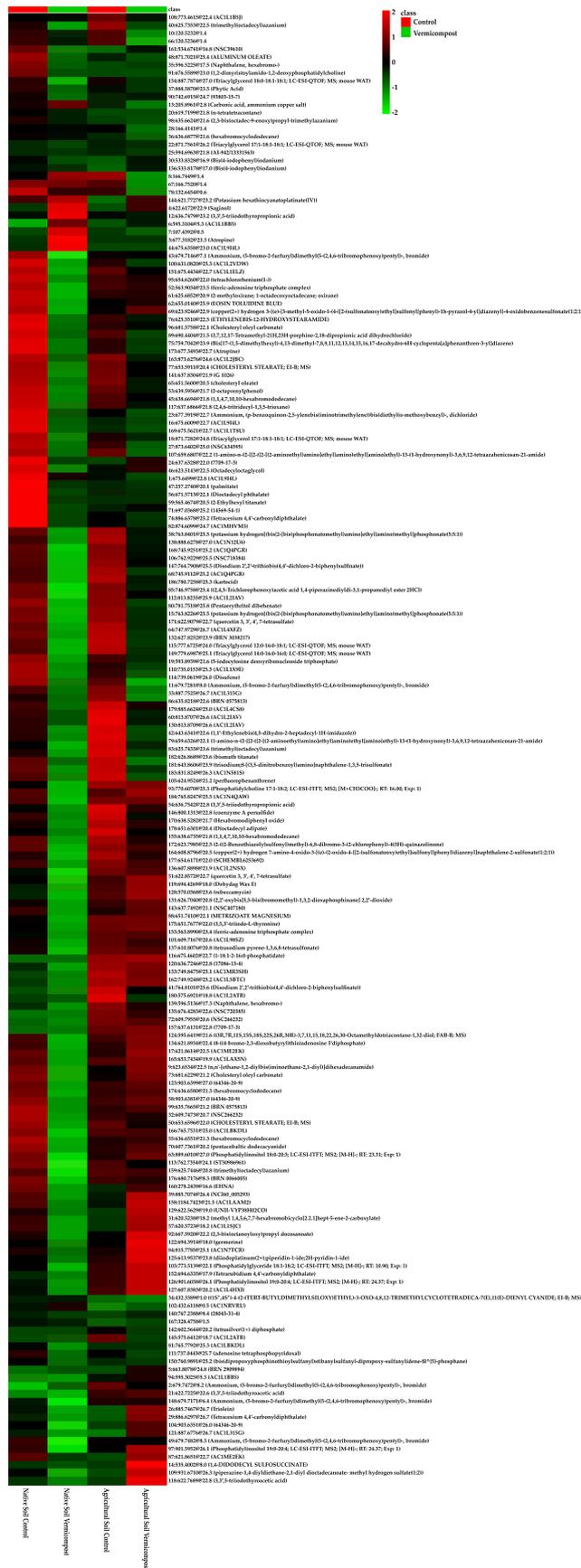


Figure 6. Heat maps corresponding to *Moringa oleifera* leaves' extracts cultivated with vermicompost over agricultural and native soils.

Table 4. Identification of putative metabolites from *Moringa oleifera* with biological potential.

Treatment	Metabolites	<i>m/z</i>	<i>rt</i>	Activity	Reference
Agricultural soil vermicompost	Germerine	694.3914	18	Hypotensive	[37]
	piperazine-1,4-diyl diethane-2	931.671	26.3	Anticancer	[38]
	hexanedioic acid	651.6301	20.4	Antibacterial	[39]
	Quercetine 3,3',4',7-tetrasulfate	622.857	22.7	Antioxidant	[40]
	Triolein	885.7467	26.7	Antioxidant	[41]
Agricultural soil control	Phosphatidylcholine	770.607	22.5	Drought tolerance	[42]
	Hexabromodiphenyl oxide	638.528	21.7	Anticoagulant, antiplatelet and profibrinolytic activities	[43]
	Phosphatidylinositol	889.601	27	Ion homeostasis	[44]
	ferric-adenosine triphosphate complex	563.9034	23.5	Intracellular iron transport	[45]
Native soil vermicompost	Atropine	677.3182	23.3	Anticholinergic activity	[46]
	palmitate	257.274	20.1	Anticancer	[47]
	3,3',5-triiodothyroacetic acid	622.7689	22.8	Treatment of hyperthyroidism	[48]
Native soil control	palmitate	257.274	20.1	Anticancer	[47]
	n-tetratetracontan	619.7199	21.8	Antibacterial	[49]
	3,3',5-triiodothyroacetic acid	622.7689	22.8	Treatment of hyperthyroidism	[48]
	Germerine	694.3914	18	Hypotensive activity	[37]
	Atropine	677.3182	23.3	Anticholinergic activity	[46]

4. Discussion

Native and agricultural soils have pH values of 6.77 and 5.45, respectively. It has been reported that the presence of chemical fertilizers, which increase crop productivity, can be one of the factors involved in decreasing pH in soils [50]. However, these phenomena relate to a dynamic process in which various natural (soil, climate and biological) and anthropogenic factors are included, where the latter have been the factor that has worsened and accelerated the process of soil acidification [51]. Regarding moisture percentage, native and agricultural soils have a value of 5.99% and 1.18%, respectively. For its part, electrical conductivity for native and agricultural soil is at an estimated 4.31 and 3.69 ds m⁻¹, respectively. The total nitrogen content (1.77 g kg⁻¹ soil) in the native soil was higher compared to the agricultural soil. These values are consistent with those reported by the authors of [22], who reported similar values (1.9 g kg⁻¹ soil). The C/N ratio is an indicator of fertility and soil productivity. This ratio was higher for soils that have not been cultivated or plowed. Changes in the C/N ratio are attributed to climate change [52]. However, a relationship with values below 10 for arable soils is associated with a high amount of nitrogen, mainly due to the excessive use of fertilizers [52]. This result indicates that biomass in soil organic matter (SOM) is not sufficient for nitrogen mineralization, which generates leachates that contaminate the soil [53]. This ratio was higher (19.7) for vermicompost, as compared with native and agricultural soil (5.5 and 9.5, respectively). In products obtained by biological fermentation of organic matter, such as vermicompost, there are many microorganisms capable of improving soil fertility [54]. Lastly, a C/N ratio greater than 12 indicates that the composting is ripe and suitable for use.

4.1. Evaluation of Growth

The growth parameters were evaluated at days 45 and 90. Based on the results, it was observed that no significant difference was found between *Moringa oleifera* fertilization treatments (Table 2). However, differences are first observed between soil types after 45 days (Table 3). The obtained results suggest that the fertilization rate (150 kg N ha⁻¹) of vermicompost used in this work does not have an influence on increasing the growth

parameters such as plant height, root length and dry weight of leaves. In a perennial crop such as *Moringa*, removal of soil nutrients due to the growth and development of the plant eventually leads to a reduction in biomass yields [55]. Moreover, the pH of the soil has a direct impact on the parameters of plant growth, as there is a pH range which allows the absorption or availability of nutrients.

4.2. Metabolite Identification

The metabolomic analysis in *Moringa* leaves revealed a clear discrimination between the different treatments, indicating that organic amendment had an impact on the metabolic profile (Figure 6). Biologically important metabolites were identified in the *Moringa oleifera* leaf extracts, based on their m/z and rt relationship. These results indicated that the identified putative metabolite family groups belong to alkaloids, flavonols/flavonoids, phospholipids, glicerophospholipids, fatty acids and organic acids (Table 4). These groups pertain to the peaks identified on the total ion chromatogram (TIC), as they are the most well-defined. In order to identify the m/z ratios, the following databases were used: PlantCyc [33], MetaCyc [34], PubChem [35] and Massbank [36]. Furthermore, our results show that the application of vermicompost in *Moringa* crops on agricultural soils induces a higher level of various alkaloids. Among the alkaloid-related groups, the following are suggested: germerine (m/z 694.3914, rt 18 min), which is a metabolite reported in poisonous perennial herbs of the Melanthiaceae family with hypotensive activity [37], piperazine (m/z ratio of 931.6710 and 746; rt of 26.3 and 15.4 min) and piperidine (m/z 613.9537, rt 23.8 min). To our knowledge, the latter groups have not been reported for *Moringa oleifera* leaves. However, it has been reported that these alkaloids have antibacterial, antifungal and antiparasitic activity [56], and have been found in plants of the *Prosopis* genus (Leguminosae, Mimosaceae) and in *Piper retrofractum* Vahl [57]. In addition, the presence of alkaloid groups known as moringine and moringinine has been reported in *Moringa* leaves [58]. Other identified alkaloids are N, a-L-rhamnopyranosyl vincosamide [59], trigonelline [60], pyrrolemarumine 42-O- α -L-rhamnopyranoside and 4'-hydroxyphenylethanamide [61]. These are known for possessing cardiogenic activity and anti-hypertensive effects [62].

Among the flavonoid groups found, the presence of quercetin 3,7,3',4'-tetrasulphated is suggested. The existence of this metabolite has been reported in *Moringa oleifera* leaves and flowers [41,58]. In the results reported herein, the presence of palmitic acid is suggested (m/z 257, rt 20 min). Palmitic acid is one of the most frequently found fatty acids in plants, including *Moringa oleifera* [18]. Finally, triolein or glycerine trioleate (m/z 885, rt 26.7), which is a compound belonging to the triglyceride group, has been reported previously in *Moringa oleifera* seed oil [63].

5. Conclusions

To our knowledge, there are few studies analyzing the relationship between metabolomic profiling of *Moringa oleifera* leaves and their fertilization with organic fertilizers (such as vermicompost) using different types of soil. The overall results obtained in the present study revealed that the application of vermicompost induces significant differences in metabolomic profiles as compared to the control. However, this was not observed in the morphometric variables of *Moringa oleifera*. For future studies, carrying out in vitro and in vivo testing could contribute more information to the medicinal and food industries.

Supplementary Materials: The following is available online at <https://www.mdpi.com/article/10.3390/agronomy11102061/s1>. Table S1. The metabolome Database of *Moringa oleifera* L. Cultivated with Vermicompost under Different Soil Types.

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draft preparation, L.A.M.-G. and V.M.R.-V.; writing—review and editing, L.A.M.-G. and V.M.R.-V.; visualization, L.A.M.-G., N.R.-L. and F.A.R.-M.; supervision, R.R.-R., F.A.G.-M., R.W. and V.M.R.-V.; project administration, V.M.R.-V.; funding acquisition, V.M.R.-V. All authors have read and agreed to the published version of the manuscript.

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