EFFECT OF *ATTA MEXICANA* WASTES ON THE NUTRITIONAL AND MICROBIOLOGICAL CHARACTERISTICS OF SOIL

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Abstract. Ants are considered as ecosystem engineers due to their ability to modify nutrient availability for other organisms, promote microbial activity, and enhance energy flow. In this study, we conducted a chemical characterization and estimated the microbial activity of the fungal garden residues of *Atta mexicana*, as well as the soils directly in contact with the waste and reference soils that do not interact with the residues. The objective was to evaluate the effect generated by the residues on the soils where the waste dumps are located. The chemical variables evaluated included pH, electrical conductivity (EC), concentrations of nitrogen (N), phosphorus (P), potassium (K), and organic carbon (OC), and microbial activity was estimated based on CO_2 emissions. Our results demonstrated that fungal garden residues increased the nutrient content of the underlying soils in the waste dumps, particularly the organic carbon content (291.86 ± 58.6) and the concentrations of N (8.27 ± 6.92), P (5.21 ± 5.4), and K (15.19 ± 5.5). Additionally, microbial respiration in the interacting soils increased by 7.03 ± 2.35 times. The evidence gathered suggests that the waste dumps of *Atta mexicana* fungal garden residues provide ecosystem services that enhance nutrient availability and microbial activity in the soils.
Keywords: *Attini, ant compost, soil fertility, Leucoagaricus gongylophorus, ecosystem engineers*

Introduction

Fungus-growing ants are widely distributed in the Neotropical region of the American continent. *Atta mexicana* Smith (Formicidae: Myrmicinae: Attini) is the species with the greatest distribution in Mexico and is considered one of the main agricultural pests due to its defoliating capacity. However, it also provides various ecosystem services such as organic matter recycling and soil aeration in Neotropical soils (Abril, 2011; Vázquez-Bolaños, 2011).

On the other hand, fungus-growing ants have been of great scientific interest due to the mutualistic interactions they establish with various microorganisms, among which the main microbial symbiont is the cultivated fungus species *Leucoagaricus gongylophorus* (Basidiomycota: Agaricales). This fungus represents the sole source of food for the ants in their larval stage (Abril, 2011; Schultz, 2020, 2022).

The production system of the fungus *L. gongylophorus* emerged approximately 60 million years ago. Ant fungiculture is complex and shares many similarities with human agriculture (Schultz, 2020, 2022; Jesovnik and Schultz, 2022). In general, *A. mexicana* ants defoliate plant material to construct a specific substrate for the inoculation and maintenance of their crop. Subsequently, the symbiotic fungus generates globular structures called “gongylidia,” which are rich in fatty acids, proteins, and carbohydrates. These gonglids are harvested by the ants (Aylward et al., 2015; Khadempour et al., 2021; Schultz, 2020, 2022).

The symbiotic fungus lacks the ability to assimilate complex biomolecules; therefore, communities of organic matter decomposer microorganisms transform the complex compounds into simpler ones that are easier to assimilate. When the substrate has lost the desired characteristics to promote the optimal growth of the cultivated fungus, the ants discard it and replace it with a freshly prepared substrate using fresh materials (Schultz, 2020, 2022).

The discarded residues from fungal gardens are accumulated in specialized mounds located outside the nests. However, due to their nutritional and microbiological characteristics, they can cause changes in the chemical and microbiological properties of the soils where they are deposited, similar to what happens with other ant species (Boots et al., 2012; Farji-Brener and Werenkraut, 2017; Majeed et al., 2018; Delgado-Baquerizo et al., 2019; Swanson et al., 2019; Parker and Kronauer, 2021).

Several studies concur that the habits of ants lead to the accumulation of organic matter, increased nutrient concentrations, and enhanced enzymatic activities of soil microorganisms. As a result, ants modify nutrient availability and promote energy flow through trophic networks in their ecosystems (Fernández et al., 2014; Wu et al., 2015; Farji-Brener and Tadey, 2016; Farji-Brener and Werenkraut, 2017; Delgado-Baquerizo et al., 2019). Based on the above, several authors agree that ants are ecosystem engineers (Wu et al., 2015; Swanson et al., 2019; Parker and Kronauer, 2021). Based on the above, the following question was set up: “What effect do waste dumps of *Atta mexicana* fungal garden residues have on the chemical and microbiological characteristics of the soil?”

In this study, the chemical characterization and microbial activity estimation of the fungal garden residues of *Atta mexicana*, the soils in direct contact with the waste, and reference soils that do not interact with the residues were performed. The objective was to evaluate the effect generated by the residues on the soils where the waste dumps are located.

Materials and methods

Sampling

Five waste dumps from fungal gardens of *Atta mexicana* Smith ants were located in the municipality of Tezontepec de Aldama, Hidalgo State, Mexico (*Fig. 1a*). *Figure 1b* schematizes the three types of samples; to obtain samples from each waste dump (Waste), three points along each dump were identified, and organic material was collected from the surface of the mound down to its base. For each mound, three composite samples were obtained, each consisting of five subsamples that were blended in disinfected bags, resulting in homogeneous samples weighing approximately 500 to 1000 g. Each bag was appropriately labeled and stored in the shade until processing. To collect samples from the soils underlying, and in direct contact with, each waste dump (SW), the organic waste atop the dump was removed until reaching the soil surface. Subsequently, three points were selected within the soil, and soil was collected to a depth of 10 cm. Each SW sample comprised roughly 250 g of soil. Reference soils

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(Soil) were sampled at a distance of 20 m from each waste dump, ensuring the absence of other nearby mounds. Three reference soil samples weighing 500 g were taken.

A total of five Waste samples, four SW samples, and four Soil samples were obtained. The fifth waste dump was located inside a building, thus there was no underlying or reference soil. The samples were labeled and dried in the shade to minimize microbial activity. Upon arrival at the laboratory, the samples were homogenized using a 2 mm sieve and stored in the shade until processing.

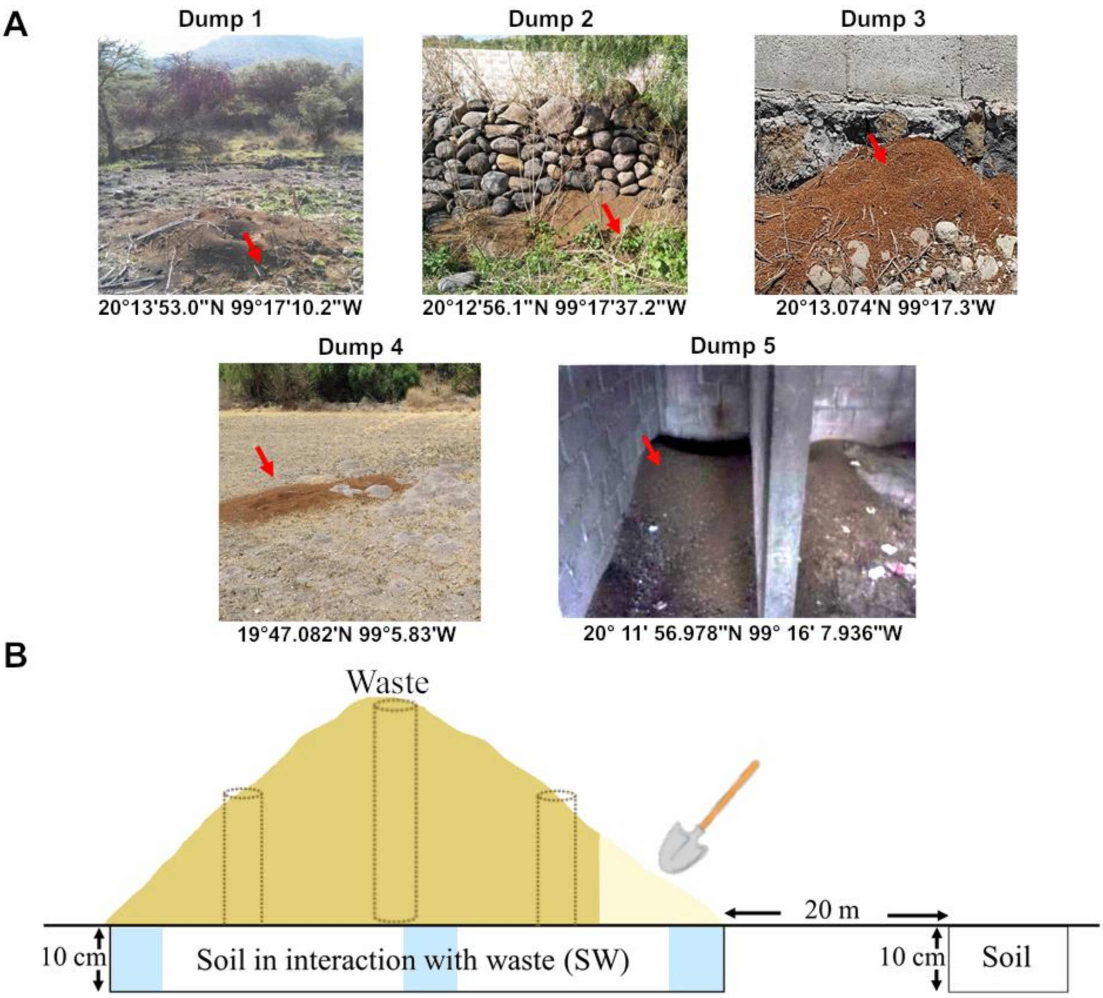

Figure 1. *Waste dumps of Atta mexicana fungal gardens (a); sampling design scheme (b). The red arrows indicate the mounds of organic waste from fungal gardens of Atta mexicana; the cylinders positioned on the mounds depict how the "Waste" samples were collected; in SW, the blue boxes represent the sampled points, for which it was necessary to previously remove the organic waste*

Description of the sampling sites

Waste dump 1 and waste dump 2 were in minimally or undisturbed areas, with a nearby shallow rainwater stream. According to the locals, they were considered "old" ant nests (over 5 years old). The reference soil was sampled in an undisturbed area. Waste dump 3 was located next to the wall of a building, and based on its size, it was

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considered a new dump. The reference soil was collected from a rainfed cornfield. Waste dump 4 was found within an agricultural cultivation plot where rainfed corn has been grown every year for the past 10 years. According to the owner of the plot, the mound was new (less than 1 year old), and the reference soil was sampled from the same plot. Waste dump 5 was located inside an abandoned building and was the largest dump (approximately 50 cm in height).

It is important to highlight that, even though site where Waste 5 dump was located lacked SW and (reference) Soil, it was considered as an additional biological replicate within the study for the assessments of microbial activity in the dumps. The inclusion of this replicate provided insights into microbial activity values when the waste is protected from ambient conditions.

Chemical characterization

Due to the nature of the fungal garden residues of *A. mexicana*, the chemical analyses were conducted based on the chemical characterization methodology for composts by Sadzawka et al. (2005). The variables evaluated were pH and electrical conductivity (EC) using a 1:5 w/v aqueous extract; organic carbon percentage (OC) estimated from the organic matter, determined by calcination at 550°C; total nitrogen (N), obtained by the micro-Kjeldahl method; available phosphorus, using the vanadomolybdic acid method and measured by spectrophotometry at a wavelength of 660 nm; potassium (K) concentration was quantified by atomic absorption of the samples previously digested with HNO_3/$HClO_4$. Atomic absorption spectrophotometry was developed with a model 932 Plus, GBC®. Each determination was performed in triplicate.

Microbial activity

It was estimated through the measurement of generated CO_2 as an indicator of microbial respiration. The evaluations were carried out using the closed incubation method with 50 g of moistened sample at 80% field capacity. 5.0 mL of NaOH 1N were used as a CO_2 absorbent to generate $Na_2CO_3 + H_2O$. Carbonates were precipitated with 2.0% $BaCl_2$, and the amount of residual NaOH was calculated through back titration with H_2SO_4 0.1N using 2.0% phenolphthalein as an indicator. Respiration was estimated in mg of C-CO_2 g-1 day-1, following the methodology described in Guerrero-Ortiz et al. (2010) and Valerio-Luna et al. (2016). Quantification was carried out every five days during a twenty-day incubation period. Each treatment was replicated four times.

Statistical analysis

Waste, SW, and Soil were considered as treatments. For the chemical characterization, four biological replicates of Waste, four of SW, and four of Soil were taken into account. For the evaluation of microbial activity, Waste consisted of five replicates. The treatments were considered as independent groups; therefore, a one-way ANOVA was selected as the statistical test to determine the significance among the treatments.

Additionally, an ANOVA analysis was conducted using a factorial experimental design, wherein the collection sites were considered as “factor one” and the treatments as “factor two”, aiming to account for interactions between these two factors. Means were grouped using a Tukey test ($\alpha \leq 0.05$). Means were grouped using a Tukey’s test ($\alpha \leq 0.05$).

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The incubation experiment used to estimate microbial activity was carried on under a completely randomized design. An ANOVA and a hypothesis test of linear regression were used to determine if there was a significant difference between the slopes of CO2 accumulations in SW and their respective reference soils (Soil). The ANOVA, hypothesis tests, and mean comparisons were conducted using the R language in RStudio version 4.0.3.

Multivariate analyses

A Pearson correlation analysis was conducted to analyze the interaction between all variables. Besides, a Multiple Correspondence Analysis (MCA) was used to group the treatments based on their similarities. The FitoPac2 version 2.1 software was utilized for these analyses.

Results

Chemical characterization

The reference soils (Soil) and SW obtained slightly acidic pH values, while the residues were practically neutral. The electrical conductivity (EC) was higher in the reference soils, while SW and the residues had similar mean values. However, the differences in pH ($p = 0.506$) and EC ($p = 0.412$) among the treatments were not significant. *Tables 1* and *2* show the comparison of the chemical characteristics among the three treatments (Waste, SW, and Soil).

Significant differences were observed in the concentrations of macronutrients. Waste showed significantly higher concentrations compared to the reference soils ($p = 0.0001$), but not compared to SW, suggesting that the residues from *A. mexicana* fungal gardens serve as reservoirs of this macronutrient. Additionally, although the difference in total N concentrations between WS and Soil was not significant, there was an increase in the macronutrient content in WS. *Table 3* shows that the total N concentrations of the SW replicates were significantly higher than their respective reference soils ($p = 0.0001$). This suggests that the underlying soils of the waste dumps were enriched with the remaining nitrogen from the residues of *A. mexicana* fungal gardens.

The concentration of phosphorus was significantly lower in Waste compared to the SW and reference soils ($p = 0.000883$). *Table 1* does not show significant differences between SW and Soil; however, *Table 3* clearly shows an increase in phosphorus concentrations in the SW compared to Soil1 and Soil2 ($p = 0.0001$). This was not the case for Soil3 and Soil4, as their phosphorus concentrations were already high, which could be attributed to them being agricultural plots treated with mineral fertilizers. This suggests that phosphorus from the waste dumps has been translocated to the SW, although there might be a bias caused by reference soils Soil3 and Soil4. Future studies should use undisturbed soils as reference and agricultural soils as an independent group.

The potassium concentrations in SW were significantly higher than in Soil and Waste ($p = 1.22e^{-10}$), with concentrations more than double compared to the other treatments (*Table 2*), indicating the enrichment effect of this macronutrient in SW soils. It is interesting to note that Soil3 and Soil4 did not exhibit elevated potassium concentrations as observed with phosphorus. Based on *Table 3*, all SW samples were statistically higher than their respective reference soils ($p = 0.0001$).

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The increases in potassium in transient soils (SW) could be related to the type of material used by ants to construct their fungal gardens; multiple studies concur that *Atta mexicana* employs flowers, seeds, and young leaves as raw materials for building their cultivation substrate (Farji-Brener and Tadey, 2012; Fernández et al., 2014; Meriño-Cabrera et al., 2014). These plant organs are rich in nutrients, especially N, K, and Mg; therefore, the accumulation of organic matter in the waste dumps promotes the buildup of micronutrients. This phenomenon has also been observed in various species of ants that collect plant-derived organic materials or even insect cadavers (Farji-Brener and Tadey, 2012; Fernández et al., 2014; Farji-Brener and Werenkraut, 2017; Leroy et al., 2017; Pirk et al., 2020). Nevertheless, it remains an open question what could lead to a doubled potassium concentration in the SW layer. Future studies should delve into the translocation pathways that macro- and micro-nutrients follow within the ant-waste dump-soil systems.

With respect to the organic carbon concentration, Waste showed significantly higher values, followed by SW, while Soil had the lowest organic carbon concentration ($p = 2e^{-16}$) (*Table 2*). This suggests that SW has been enriched through its interaction with Waste. In general, the fungal garden residues of *Atta mexicana* are composed of organic carbon sources and macronutrients that can be translocated to the underlying soils of the waste dumps.

The C/N ratio is an indicator related to the mineralization processes of OC and N due to the effect of soil microbial activity (Cayuela et al., 2009; Pergola et al., 2018). The highest C/N ratio was found in Waste, suggesting that it is composed of abundant carbon sources that have not yet been mineralized by its microbiota. Organic materials with C/N ratios > 30 are considered to be poorly stabilized, and therefore cannot be used as organic amendments or organic fertilizers. However, by observing the chemical characterization in *Table 3*, it can be observed that Waste1 and Waste2 have C/N ratios < 20, indicating that they are stabilized organic materials. On the other hand, Waste3 and Waste4 represent less stabilized landfills. This suggests that not all landfills of fungal garden waste from *A. mexicana* will exhibit the same level of mineralization of their organic carbon sources. The C/N ratios of SW and Soil were not significantly different; however, their ratios close to 20 can be considered ideal for promoting microbial activity, as they maintain sufficient organic carbon as a useful energy source for soil microbiota (Cruz et al., 2010).

Table 1. *Summary of ANOVA for the chemical characteristics of Atta mexicana residues, residues-associated soils (SW), and non-residue-associated soils (Soil) (α = 0.05)*

Parameter	Site (factor one)			Treatment (factor two)			Site*treatment		
	Df	MS	Pr > F	Df	MS	Pr > F	Df	MS	Pr > F
pH	3	1.791	< 0.0001	2	0.94	< 0.0001	6	5.465	< 0.0001
EC	3	1.541	< 0.0001	2	3.837	< 0.0001	6	15.767	< 0.0001
OM	3	23302	< 0.0001	2	712858	< 0.0001	6	10611	< 0.0001
N	3	107.22	< 0.0001	2	161.42	< 0.0001	6	52.61	< 0.0001
P	3	4161	< 0.0001	2	20884	< 0.0001	6	35535	< 0.0001
K	3	39	< 0.0001	2	5166	< 0.0001	6	263	< 0.0001
OC	3	7697	< 0.0001	2	235453	< 0.0001	6	3505	< 0.0001
C:N	3	16954	< 0.0001	2	29583	< 0.0001	6	8865	< 0.0001

Df: degrees of freedom; MS: mean square

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Table 2. *Means of the chemical and nutritional characteristics of Atta mexicana residues. (mean ± SE); means followed by the same letter were not statistically different (Tukey; p ≤ 0.05)*

	pH	CE (ds m^{-1})	N (g kg^{-1})	P (ppm)	K (g kg^{-1})	OC (g kg^{-1})	C/N ratio
Soil	6.5±0.96a	2.3±2.4a	1.1±0.69b	53.26±50.66a	12.3±12.57b	29.57±23.43c	23.46±11.09b
SW	6.6±1.01a	1.5±1.02a	5.3±3.39ab	59.99±29.57a	47.99±8.9a	83±30.47b	20.59±12.69b
Waste	7.01±1.24a	1.4±1.3a	8.27±6.92a	5.21±5.45b	15.19±5.53b	291.86±58.6a	108.12±96.49a

Table 3. *Chemical characteristics of Atta mexicana waste, soils directly associated with the waste (SW), and soils not associated with the waste (Soil) (mean ± SE); means followed by the same letter were not statistically different (Tukey; α ≤ 0.05)*

	pH	CE (ds m^{-1})	N (g kg^{-1})	P (ppm)	K (g kg^{-1})	OC (g kg^{-1})	C/N ratio
Soil1	7.35±0.07c	0.04±0.001f	0.59±0.07de	6.76±1.28ef	4.71±0.14gh	7.02±0.22f	11.95±1.6d
Soil2	7.38±0.04c	0.03±0.0005f	0.52±0.04e	3.82±0.58ef	4.24±0.2h	6.76±0.22f	13.01±0.6d
Soil3	5.3±0g	5.04±0.3a	1.7±0.01de	109.79±0.47a	21.51±0.19e	53.67±1.97ef	31.42±1.16cd
Soil4	5.8±0f	4.65±0a	1.01±0.01de	100.72±4.73ab	7.22±0.11gh	37.66±0.9ef	36.99±0.7c
SW1	6.57±0.04d	1.46±0.27d	10.19±1.32b	97.74±10.94b	60.09±0.47a	110.94±17.21d	10.89±1.07d
SW2	5.17±0.03h	3.06±0.12b	5.06±0.39c	68.46±6.83c	48.76±1.05b	59.06±15.57def	11.54±2.13d
SW3	7.6±0b	0.69±0e	2.33±0.01de	35.2±0.47d	38.15±0.34d	66.74±1.44de	28.54±0.82cd
SW4	7.4±0c	0.75±0e	2.76±0.01d	28.22±1.72d	44.56±3.44c	90.6±41.05de	32.79±15.02cd
Waste1	5.7±0f	2.07±0.15c	15.3±2.08a	0.17±0.01f	8.03±0.2g	270.11±5.74b	18.02±3.03cd
Waste2	5.97±0.05e	3.17±0.25b	14.3±0.57a	0.15±0.01f	12.33±1.19f	214.55±21.75c	15.15±2.18cd
Waste3	8.2±0a	0.34±0ef	1.84±0.01de	12.08±0.72e	20.92±0.26e	346.39±1.52a	187.56±2.41b
Waste4	8.2±0a	0.17±0f	1.58±0.01de	8.43±0.31ef	19.47±0.92e	336.37±35.73a	211.74±21.83a

Microbial activity

Throughout the experiment, the waste (Waste) showed significantly higher microbial activity compared to SW and Soil (*Fig. 2a*). Waste4 and Waste3 exhibited the highest CO_2 emissions as a result of microbial activity. As mentioned earlier, Waste3 and Waste4 were the least stabilized materials (based on their high C/N ratios), indicating that they had a greater amount of easily assimilable organic C sources for the microorganisms responsible for mineralization (Guerrero-Ortiz et al., 2012). Waste4 accumulated a total of 628.25 ± 7.07 mg CO_2 g^{-1} over 20 days of incubation, which was significantly higher than the other treatments ($p = 2e^{-16}$). Waste3 (577.43 ± 11.45) and Waste5 (429.84 ± 11.21) ranked second and third, respectively. The evidence suggests that the waste materials maintain a high microbial activity and may potentially enhance the microbial activity of the soils they interact with. *Figure 2a* illustrates the kinetic production of C-CO_2 from the waste (Waste), SW, and reference soils (Soil) during the 20-day incubation period.

Based on the hypothesis tests of the accumulation slopes, there was evidence that SW exhibited significantly higher microbial activity compared to their respective reference soils. SW1 (203.06 ± 12.7 mg C-CO_2 g^{-1} day^{-1}) was significantly higher than Soil1 (24.22 ± 6.5 mg C-CO_2 g^{-1} day^{-1}) ($p = 6.03e^{-5}$); in second place, SW4 (182.16 ± 16.85 mg C-CO_2 g^{-1} day^{-1}) was significantly higher than Soil4

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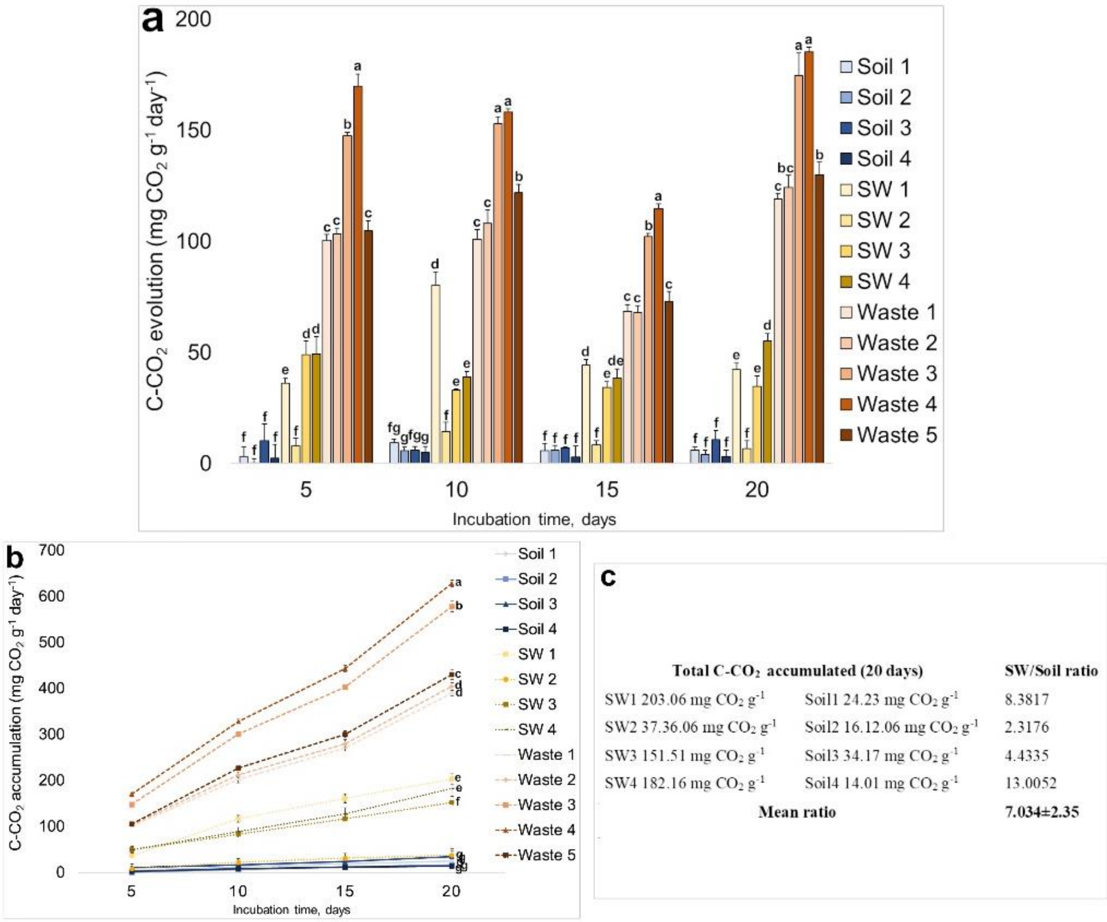
(14.006 ± 4.8 mg C-CO_2 g^{-1} day^{-1}) (p = 0.0057); in third place, SW3 (151.506 ± 2.1 mg C-CO_2 g^{-1} day^{-1}) was significantly higher than Soil3 (34.17 ± 11.5 mg C-CO_2 g^{-1} day^{-1}) (p = 9.3e^{-6}); finally, SW2 (37.36 ± 13.16 mg C-CO_2 g^{-1} day^{-1}) was significantly higher than Soil2 (16.12 ± 6.5 mg C-CO_2 g^{-1} day^{-1}) (p = 0.005). This overall demonstrated that the interaction of the waste from fungal gardens increased, on average, 7.03 ± 2.35 times the microbial respiration of the SW compared to the reference soils (*Fig. 2c*). This suggests that the waste from *Atta mexicana* fungal gardens promotes microbial activity in the soils they interact with.



Total C-CO_2 accumulated (20 days)		SW/Soil ratio
SW1 203.06 mg CO_2 g^{-1}	Soil1 24.23 mg CO_2 g^{-1}	8.3817
SW2 37.36.06 mg CO_2 g^{-1}	Soil2 16.12.06 mg CO_2 g^{-1}	2.3176
SW3 151.51 mg CO_2 g^{-1}	Soil3 34.17 mg CO_2 g^{-1}	4.4335
SW4 182.16 mg CO_2 g^{-1}	Soil4 14.01 mg CO_2 g^{-1}	13.0052
Mean ratio		**7.034±2.35**

Figure 2. *Microbial respiration kinetics during 20 days of incubation (a); accumulation of C-CO_2 produced by microbial activity during 20 days of incubation (b); comparison of accumulated CO_2 between residue-associated soils (SW) and reference soils (Soil) (c). Measurements with the same letter were not statistically different (Tukey; α ≤ 0.05). The error bars represent the statistical error*

Interaction between microbial activity and chemical characterization

Soil microbial activity (MA) depends on various micro-environmental soil factors. *Figure 3* demonstrates that the chemical characteristics with the greatest influence on MA were organic C concentration (R^2 = 0.97) and the C/N ratio (R^2 = 0.77), underscoring the significance of organic C as an energy source for soil microbial biological processes (Guerrero-Ortiz et al., 2012; Fernández et al., 2014).

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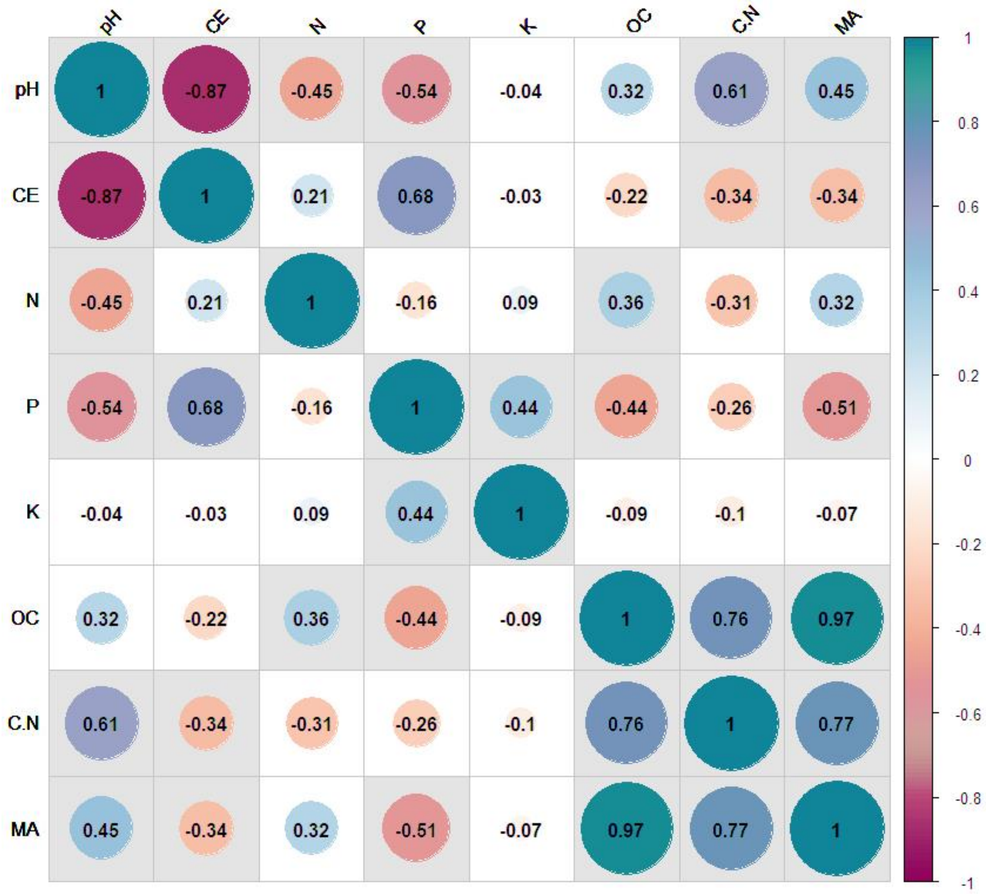

	pH	CE	N	P	K	OC	C.N	MA
pH	1	-0.87	-0.45	-0.54	-0.04	0.32	0.61	0.45
CE	-0.87	1	0.21	0.68	-0.03	-0.22	-0.34	-0.34
N	-0.45	0.21	1	-0.16	0.09	0.36	-0.31	0.32
P	-0.54	0.68	-0.16	1	0.44	-0.44	-0.26	-0.51
K	-0.04	-0.03	0.09	0.44	1	-0.09	-0.1	-0.07
OC	0.32	-0.22	0.36	-0.44	-0.09	1	0.76	0.97
C.N	0.61	-0.34	-0.31	-0.26	-0.1	0.76	1	0.77
MA	0.45	-0.34	0.32	-0.51	-0.07	0.97	0.77	1

Figure 3. *Pearson correlation matrix of chemical variables and microbial activity. The shaded boxes indicate correlations that were statistically significant ($p \leq 0.05$)*

Other less significant positive correlations were found between total N concentration ($R^2 = 0.32$) and pH ($R^2 = 0.45$). Additionally, it was observed that the correlation between total N concentration and organic C concentration was positive ($R^2 = 0.36$). Despite its minimal strength, the relevance lies in the fact that microorganisms obtain N and energy (organic C sources) from the organic matter of *A. mexicana* fungal garden residues (Scott et al., 2010; Lewin et al., 2016).

Besides, a positive correlation of $R^2 = 0.68$ was observed between phosphorus concentration and electrical conductivity (EC), suggesting that phosphate ions may influence EC. However, further evaluations are needed to determine the concentrations of other soluble ions (Fernández et al., 2014; Quevedo-Martínez et al., 2020).

The multiple correspondence analysis (*Fig. 4*) reveals three groups. Group 1 consisted of three subgroups. The Wastes were grouped into two separate subgroups within Group 1, while the third subgroup consisted solely of Soil1 and Soil2. Group 2 exclusively comprised SW. Group 3 consisted exclusively of Soil 3 and Soil 4.

The overlap observed between Group 1 and Group 2 suggests that, due to the changes that SW have undergone in terms of their chemical and microbial characteristics, they can no longer be grouped together with Soil or Waste. However, their differentiation is not such that the groups are completely separated. Additionally, the multiple correspondence analysis reveals that the most influential factors in the organization of Groups 1 and 2 were the concentration of N, OC, pH, C/N ratio, and MA.

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On the other hand, Group 3, consisting solely of Soil3 and Soil4, was positioned at a greater distance from the previous two groups, indicating a greater difference between these treatments and the rest. These differences may be influenced by the agricultural use that these reference soils have received. Future studies should evaluate undisturbed reference soils and agricultural soils as distinct independent groups.

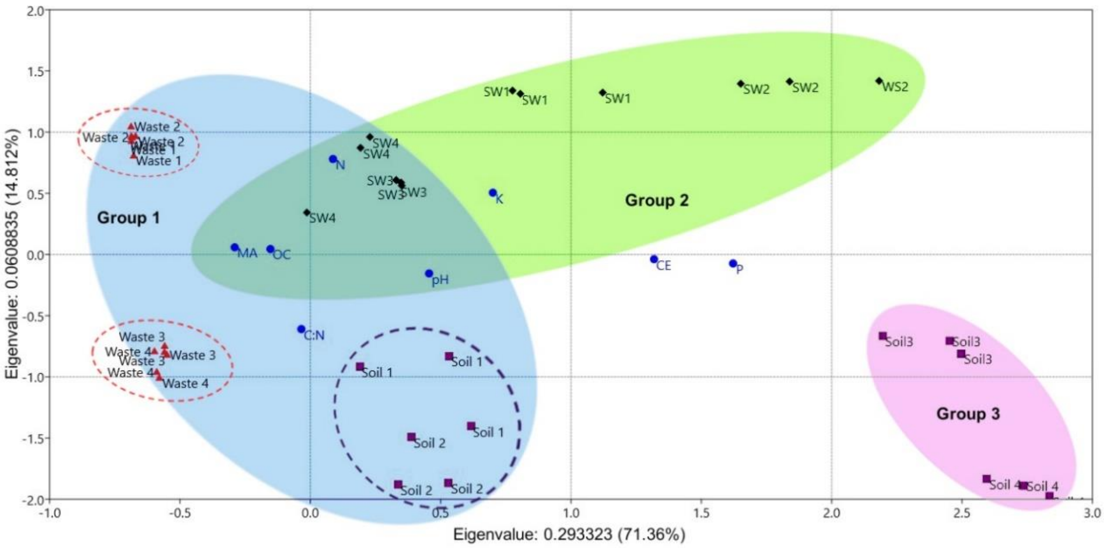

***Figure 4.** Multiple correspondence analysis of waste from Atta mexicana fungal gardens (Waste), soils associated with the waste (SW), and reference soils (Soil)*

Discussion

Chemical characteristics

Ants are often considered as ecosystem engineers due to their ability to modify nutrient availability and enhance energy flow through trophic networks of the organisms they interact with (plants, microorganisms, or other arthropods) through their diverse feeding habits (Wu et al., 2015; Swanson et al., 2019; Parker and Kronauer, 2021).

Leaf-cutting ants have the habit of discarding old fungal gardens in specific dumpsites; these accumulations of organic matter significantly modify the chemical and microbiological characteristics of soils in different ecosystems (Farji-Brener and Tadey, 2009; Fernández et al. 2014; Sousa-Souto et al., 2012). The evidence suggests that the main changes in soil chemical characteristics are caused by the accumulation of nutrient-rich organic matter; Farji-Brener and Silva (1995) and Farji-Brener and Tadey (2016) agree with the results obtained in this study. Their studies have confirmed that accumulations of leaf-cutting ant residues mainly increase the concentrations of N, P, and K, as well as other soluble ions such as Ca and Mg.

It is interesting that, due to the nutrient concentrations in fungal garden residues, some studies have evaluated their application as organic fertilizers in agricultural production (Fortanelli and Servín, 2002; Quevedo-Martínez et al., 2020).

The results obtained suggest that fungal garden residues of *A. mexicana* represent a reservoir of nutrients, primarily N, P, and K. Additionally, these nutrients are translocated to the soils near the waste dumps. P is a less mobile element in the soil, and its main form of mobilization is through leaching. Therefore, the movement of this

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nutrient to the underlying soil largely depends on precipitation. On the other hand, N is more mobile, and the enrichment of the underlying soils also depends on leaching. However, microbial activity is a factor that promotes the flow of nutrients through biogeochemical cycles (Zamudio-González et al., 2011; Majeed et al., 2018). Boots et al. (2012) discovered the presence of microorganisms actively involved in nitrogen redox processes in the majority of microbial niches associated with several ants species.

Pinto-Tomás et al. (2009) and Sapountzis et al. (2015) reported nitrogen-fixing microorganisms in different microniches associated with fungus-growing ants, including the residues of fungal gardens. Therefore, the nutrient enrichment of these materials, as well as the soils they interact with, could be influenced by various microbial metabolic processes (Boots et al., 2012; Delgado-Baquerizo et al., 2019).

On the other hand, other studies have shown that the accumulation of organic matter can promote physical changes in soils, such as increased moisture retention and aggregate formation, which enhances soil fertility (Zamudio-González et al., 2011); these results suggest that the residues from leaf-cutting ant fungal gardens could serve as soil restorers; however, the Mexican standard NMXFF-109-SCFI-2008 establishes that organic materials used as fertilizers or organic amendments must have C/N ratios lower than 20/1. Therefore, not all residues can be used without prior evaluation of their level of stabilization (Cruz et al., 2010). For example, based on the C/N ratios obtained in this study, Waste1 and Waste2 could be used as fertilizers or organic amendments because their C/N ratios are lower than 20. On the contrary, Waste3 and Waste4 obtained C/N ratios higher than the established limit, and therefore, they need to be processed to promote their stabilization and reduce their C/N ratios. One way they could be processed is through composting, as this process promotes the mineralization of organic C and N sources by the microorganisms responsible for biodegradation. During the composting process, organic C sources are oxidized into CO_2, while N is mineralized or immobilized in microbial biomass and eventually released in forms assimilable by plants (Cruz et al., 2010; Fernández et al., 2014).

On the other hand, the waste from the fungal gardens of *Atta mexicana* has gained interest as a natural resource for horticulture, as they possess similar characteristics to other organic fertilizers such as compost (Fortanelli and Servín, 2002). Composting is defined as a process of biodegradation of organic matter resulting from biotic factors, such as the activity of saprophytic microorganisms under aerobic conditions, favored by abiotic factors such as moisture and temperature (Pergola et al., 2018; Sagdeeva et al., 2018). Therefore, the by-product of ant fungiculture (the fungal garden waste) could be considered as compost. Quevedo-Martínez et al. (2020) have already coined the term “ant compost” and have demonstrated that its effects in horticulture can be comparable to those produced by crops fertilized with other composts.

Microbial activity

Microorganisms utilize organic C as an energy source to increase their populations and carry out enzymatic processes. However, not all sources of organic C are equally assimilable for microorganisms. The most labile sources correspond to simple biomolecules such as carbohydrates, proteins, and lipids, while another fraction that is more difficult to assimilate consists of complex biomolecules such as cellulose, hemicellulose, and pectins. Additionally, there is a smaller fraction considered to be recalcitrant, which primarily includes lignins (Cayuela et al., 2009; Khadempour et al., 2020).

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When the fungal garden is optimal, it remains inside the ant nest. During this time, it serves as a substrate rich in labile sources of organic C that are easily assimilated by microorganisms, especially the fungus cultivated by the ants. However, over time, the labile fractions of organic C become depleted, leaving behind sources that are more difficult to assimilate. Previous studies have shown that the cultivated fungus does not produce exoenzymes capable of degrading these complex biomolecules. However, the substrate is abundant in microorganisms capable of degrading cellulose and other complex sources of organic C (Abril and Bucher, 2002; Abril, 2011; Moreira-Soto et al., 2017; Khadempour et al., 2020).

When the substrate becomes old, it has depleted the more labile sources of organic C, but it remains abundant in recalcitrant C sources. At this stage, the ants discard it into waste dumps, but the microbial activity does not cease. Scott et al. (2010), Lewin et al. (2016), and Moreira-Soto et al. (2017) discovered that the most abundant microbial populations in the waste dumps are capable of degrading sources of C that are difficult to break down. Therefore, the residues of *A. mexicana* serve as a reservoir of microorganisms responsible for the biodegradation of organic matter, which can enhance microbial activity in the soil (Somera et al., 2015). The findings of Fernández et al. (2014) concur with our results, indicating that the residues of fungal gardens from fungus-cultivating ants promote microbial activity involved in the nutrient recycling of soil organic matter. Thus, they represent an alternative for recycling nutrients present in the organic matter of the soil. Moreover, the accumulation of organic matter provides benefits in ecosystems where organic matter is scarce.

Potential practical aspects

The waste from fungal gardens of fungus-growing ant species has been sparsely evaluated from a practical perspective for its application in agricultural production. The works of Fortanelli and Servín (2002) and Quevedo-Martínez et al. (2020) provide significant evidence that the byproduct of waste from *Atta mexicana* fungal gardens holds potential as organic fertilizer in small-scale horticulture for resource-limited agricultural producers in Mexico. However, the primary limitation of this resource lies in the restricted volume attainable in the field. Hence, strategies to optimize its practical potential need to be assessed. For instance, based on their microbial characteristics, the waste constitutes a source of microbial resources that can facilitate the decomposition of organic matter, a decisive factor in the composting process (Pinto-Tomás et al., 2009; Scott et al., 2010; Aylward et al., 2012; Lewin et al., 2016; Khadempour et al., 2020).

Composting is a technique aimed at expediting the biotransformation process of the nutrients composing organic matter into forms assimilable by plants, such as ammonium, ammonia, nitrites, or nitrates (mineral forms of N), and orthophosphates (mineral form of P). Aylward et al. (2012) and Khadempour et al. (2020) reported that fungal gardens maintain an exceptionally diverse bacteriome, abundant in bacterial groups tasked with biodegrading complex organic compounds to transform them into simpler forms for assimilation by the symbiotic fungus. However, the works of Scott et al. (2010) and Lewin et al. (2016) demonstrated that, within fungal garden waste, the most abundant microbial communities functioned in degrading recalcitrant plant material, such as lignin, cellulose, or hemicellulose. Based on the above and on our findings, fungal garden waste represents materials with potential for bioaugmentation in the composting process. Future research should assess the bioaugmentative effect of fungal gardens in composting.

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Additionally, bacterial genera of interest for agricultural biotechnology due to their ability to promote plant growth, such as *Pantoea*, *Klebsiella*, or *Acinetobacter*, have been reported (Pinto-Tomás et al., 2009; Aylward et al., 2012; Khadempour et al., 2020); hence, fungal garden waste represents an underexplored source of microbial resources with biotechnological potential. Fungal garden waste constitutes a significant area of opportunity for future research focused on obtaining microorganisms with potential for plant growth promotion and nutrient recycling.

Conclusion

In general, the residues of fungal gardens increased the nutrient content of the waste dumps, particularly the organic carbon content and the concentrations of N, P and K. This also contributed to an increase in the microbial activity of the directly affected soils. Therefore, the accumulation of fungal garden residues from *Atta mexicana* provides ecosystem services that enhance nutrient availability in the underlying soils.

Additionally, these residues represent an alternative for improving soils in semi-arid areas such as those found in Tezontepec de Aldama. However, further studies are necessary to evaluate the effect of these organic matter accumulations in other, more humid ecosystems, where microbial activity is higher, and organic matter is more abundant.

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