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RESEARCH ARTICLE

Impact of leachate on soil microbial diversity and its treatment

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Abstract

This study analyzed the impact of leachate from a temporary landfill on soil microbial diversity in Tingo María, Huánuco region, Peru. Three treatments were used: untreated soil (S), addition of stream water (T0), leachate (T1), and leachate treated by coagulation and flocculation (T2), with 828.5 ml/week added in three weekly doses. Soil samples were collected from the Reserved Forest of the Universidad Nacional Agraria de la Selva. Twenty-one randomly distributed soil samples were taken and homogenized for analysis. Soil quality parameters measured included sand, clay, silt, texture, pH, organic matter, nitrogen, phosphorus and potassium. As for microorganisms, viable aerobes, lactobacilli, actinomycetes, fungi, nitrogen-fixing bacteria, and *Escherichia coli* were quantified using specific culture and counting methods for each of them. To evaluate the impact of the leachate on microbial diversity, equity indices (Shannon and inverse Simpson), dominance indices (complementary Simpson and Berger Parker) and the percentage composition of each microorganism per treatment were used. An ANOVA was performed to estimate differences in microbial diversity, with a Tukey test at a significance level of $\alpha = 0.05$. The study showed that leachates affect soil microbial diversity, reducing equity and increasing the dominance of certain species such as *E. coli*. They also alter physicochemical parameters, decreasing organic matter and nitrogen but increasing other elements such as phosphorus and potassium. This could have implications for soil health and functionality.

Keywords: Landfill leachate, temporary landfill; soil microbial diversity; soil quality parameters; coagulation - flocculation; water treatment.

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1. Introduction

The decomposition of solid organic waste in landfills and dumpsites involves intricate interactions between biological and physicochemical factors, resulting in the production of leachate (Salehi et al., 2020). Leachate, a liquid waste, has various detrimental environmental effects in the vicinity of waste disposal sites. For instance, it can lead to groundwater contamination with heavy metals and toxic organic compounds (Tchobanoglous et al., 1993) and can affect air quality by releasing gases like methane, a potent greenhouse gas (Gu et al., 2022).

In Tingo María, Peru, a temporary cell has been operational since 2021. This facility serves as a means for the safe disposal of municipal solid waste and is expected to remain in use for three years, providing a temporary solution until a long-term sanitary landfill can be established. The primary goal of this temporary cell is to mitigate the adverse impacts of improper domestic solid waste disposal. It includes a leachate collection pond, with an estimated monthly generation ranging from 5500 m^3 to 13000 m^3, in an area with annual precipitation of 1300 mm (López-Vega et al., 2021). In the Tingo María study area, where the annual precipitation reaches 3500 mm, the leachate amount could be even higher.

Leachate contaminants can harm various ecosys-tem components, including soil, groundwater, and biolo-gical communities (Dagwar & Dutta, 2024; Fida et al., 2024). Therefore, conducting studies that analyze key organisms and environ-mental quality indicators is crucial. Leachates contain toxic substances that can disrupt soil structure and function. Existing scientific literature suggests that assessing soil impact should include the examination of microbial diversity for a comprehensive understanding of soil quality. For example, studying the diversity and function of soil microorganisms can reveal how leachates influence ecological processes like decomposition and nutrient cycling (Wydro et al., 2022).

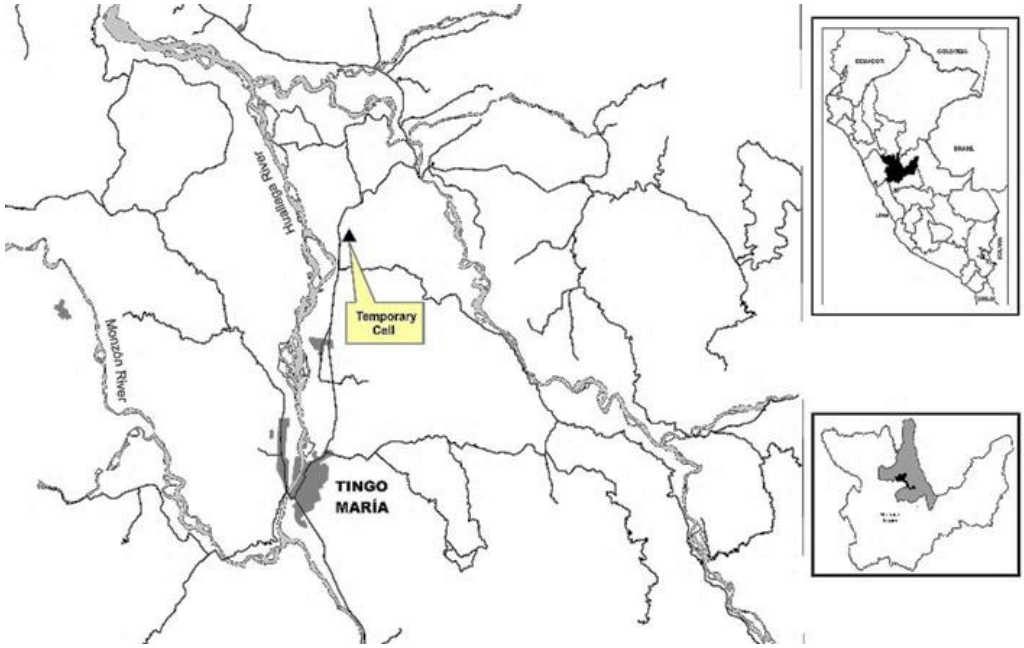

Figure 1. The geographic location of the temporary cell in Tingo María, Peru.

Despite implementing a coagulation-flocculation treatment for managing leachate in the temporary cell, its effectiveness remains uncertain. This treatment method requires a consistent contaminant load in the treated effluent. However, given Tingo María's location in a high-precipitation zone (Manrique De Lara, 2018), a more adaptable treatment approach capable of accommodating fluctuations in contaminant load and leachate volume becomes necessary. Studies indicate that in regions with a high rainfall regime, coagulation-flocculation treatment can achieve significant removal efficiencies for metals and organic load (Djeffal et al., 2019).

In this study, we formulated the following research questions:

1. What are the variations in microbial equity in soil exposed to landfill leachates?
2. What are the variations in microbial dominance in soil exposed to landfill leachates?
3. What are the variations in microbial composition in soil exposed to landfill leachates?

Therefore, this research has the following objectives:

1. Evaluate variations in microbial equity in soil exposed to landfill leachates.
2. Evaluate variations in microbial dominance in soil exposed to landfill leachates.
3. Evaluate variations in microbial composition in soil exposed to landfill leachates.

The results of this study will provide municipal authorities responsible for the temporary cell with the necessary foundation to make informed decisions aimed at improving the efficiency of leachate treatment. Additionally, this research will contribute to reducing the impact of the temporary cell on the surrounding natural resources.

2. Methodology

2.1. Study Area

The temporary cell is located on the outskirts of the city of Tingo María (9°12'38.93" S; 75°59'08.04" W), in the Luyando district, Leoncio Prado province, Huánuco region (Figure 1). The study area, situated in the ecological zone known as "Selva Alta" or highland rainforest, has reported average temperature between 24.34°C to 25.25°C and annual precipitation of 3,295.59 mm/year from 2007 to 2017 (Manrique De Lara, 2018). The cell covers an approximate area of 2.98 hectares.

2.2. Soil Sample Collection

We obtained soil samples from the Reserved Forest of the Universidad Nacional Agraria de la Selva (BRUNAS), a preserved area spanning 217.22 hectares predominantly populated by native forest species (Puerta y Cárdenas, 2009). BRUNAS ranges from 667 meters above sea level (masl) to 1092 masl. We conducted sampling within the altitude range of 667 to 850 masl, which reflected the typical conditions of the study area. We employed a simple random sampling method, resulting in 21 samples evenly distributed across the experimental altitude range, as shown in Figure 2. We considered three treatments with seven replicates each (Table 1). At each sampling location, we extracted soil cubes

measuring 20 cm x 24 cm in width and length, with a depth of 20 cm, while avoiding densely vegetated and rocky areas (Yeilagi et al., 2021). Once in the lab, soil samples were mixed and homogenized for subsequent analysis.

2.3. Formulation and application of treatments

We employed the Swiss method for leachate estimation to assess the amount of leachate that can infiltrate from a solid waste landfill into the soil (Gaudie Ley et al., 2021). The formula for this calculation is as follows:

$$Q = \left(\frac{1}{t}\right) \cdot P \cdot A \cdot K$$

Where Q represents the average percolated liquid flow rate (L/s); P stands for the annual average precipitation (mm); A denotes the landfill's surface area (m^2); t refers to the number of seconds in a year (31'536,000 s); and K is the coefficient depending on the degree of compaction of solid waste. For weakly compacted landfills with a specific weight ranging from 0.4 to 0.7 t/m^3, we estimated that leachate production is between 25% and 50% (K = 0.25 to 0.50) of the annual average precipitation corresponding to the landfill area (Pozo Bejarano et al., 2020; Zhou et al., 2024). This study considered K = 0.35 as the average value for weakly compacted landfills. The evaluation spanned three weeks and factored in a pot surface area of 20 cm by 24 cm, and an annual precipitation of 3,295.59 mm/year (Manrique De Lara, 2018).

Based on these parameters, we calculated the total volume of leachate liquid that needed to be added to the pots during that time. As a result, we determined that the volume required was 828.5 ml per week.

Table 1 summarizes the different treatments applied in the experiment, which spanned six weeks. We formulated the treatments to assess various soil conditions and manipulations:

- Treatment S (control): Represents the initial soil conditions in week 0, before applying any treatment.
- Treatment T0: Involved adding surface water from the Naranjal stream to simulate natural irrigation conditions.
- Treatment T1: Involved the addition of leachate, representing a contamination scenario.
- Treatment T2: Involved the addition of leachate treated with coagulation-flocculation, to study the effectiveness of this method.

For the leachate treatments (T1 and T2), we added a volume of 828.5 ml per week, inoculating it into the pots on three randomly selected days. We maintained soil moisture between 60% and 70% throughout the experiment (Wydro et al., 2022).

Table 1
Description of soil treatments

Treatment	Symbol	Quantity (ml/week)	Doses/week
Initial Soil (Control)	S	-	-
Stream water	T0	828.5	3
Leachate	T1	828.5	3
Treated leachate*	T2	828.5	3

*Alumina dosing until reaching a leachate pH of 6.9.

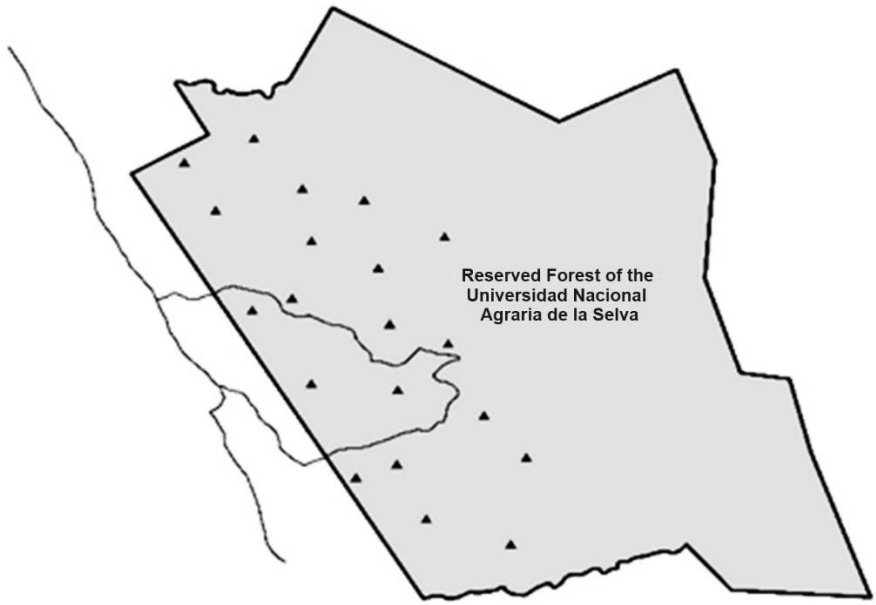

Figure 2. Location of Sampling Points within the Reserved Forest of the Universidad Nacional Agraria de la Selva.

2.4. Analysis of physicochemical soil parameters

Soil quality parameters are of significant importance when it comes to soil health and its capacity to support plant life. Factors such as sand, clay and silt, detailed in Table 2, are necessary to determine soil texture, which, in turn, affects permeability and the soil's ability to retain nutrients and water (Shukla et al., 2020). pH levels play a role in nutrient availability for both plants and microbial activity, while organic matter and essential nutrients like nitrogen, phosphorus, and potassium are fundamental for plant growth (Maurya et al., 2020). Additionally, changes in microbial activity can significantly impact soil quality by altering the decomposition of organic matter. Simultaneously, landfill leachates can introduce heavy metals and contaminants, potentially disrupting both soil structure and nutrient availability (Bünemann et al., 2018). Consequently, we measured a range of soil quality parameters, including Sand (%), Clay (%), Silt (%), Texture, pH, organic matter (OM) (%), N (%), P (ppm), and K (ppm) in the Soil Laboratory at the Universidad Nacional Agraria de la Selva.

2.5. Analysis of soil microbiological parameters

We employed specific protocols to quantify various soil microorganism groups, including viable aerobic microorganisms, lactobacilli, actinomycetes, fungi (both molds and yeasts), and nitrogen-fixing bacteria. These groups were subjected to a consistent methodology. We initiated the process by weighing precisely 10 grams of soil sample, which we then combined with 90 ml of 0.1% peptone water in a flask, resulting in a 10^{-1} dilution. Subsequently, we conducted serial dilutions to achieve the desired concentration for each microorganism group. Specific culture media, namely PCA Agar for viable aerobic microorganisms, MRS Agar for lactobacilli, Gauze Agar for actinomycetes, Sabouraud Agar for fungi, and Simms Agar for nitrogen-fixing bacteria, were employed for the seeding process. To seed each group effectively, we extracted 0.1 ml of inoculum from each dilution and applied the surface seeding method on the corresponding agar plates. Incubation conditions varied depending on the microorganism group: 30 °C for 48 hours for aerobic viable microorganisms, 37 °C for 72 hours for lactobacilli, 28 °C for seven days for actinomycetes, 25 °C for five days for fungi, and 28 °C for seven days for nitrogen-fixing bacteria. Following the incubation period, we enumerated the colonies that had developed on the plates using a counting device. The quantification of microorganisms per gram of sample was achieved through the following general formula:

$$UFC/g = Number\ of\ colonies \cdot Inoculum\ volume \cdot Dilution\ factor$$

The density of microorganisms per gram of soil was expressed in colony-forming units per gram (CFU/g). For the enumeration of *Escherichia coli*, our methodology began by selecting a sample for analysis and accurately weighing it, resulting in an initial weight of 10 grams. Subsequently, we transferred this sample to a sterile flask containing 90 ml of buffered peptone water solution, which led to a 1:10 dilution. We proceeded to vigorously agitate the sample in the flask on an orbital shaker for 1-2 minutes, ensuring thorough homogenization. Upon successful homogenization, we extracted 1 ml of this solution and transferred it to a sterile test tube containing 9 ml of the same buffered peptone water solution, achieving a 1:100 dilution. This procedure was repeated to generate other requisite decimal dilutions for the count. Moving forward, 1 ml was taken from each dilution and evenly spread onto 10 Petri dishes that contained MacConkey agar. This selective medium facilitated the proliferation of *E. coli* colonies while inhibiting the growth of other microorganisms. A sterile spreader was employed for uniform distribution of the liquid across the medium's surface. The Petri dishes were subsequently incubated at 37 °C for 24 hours. Following the incubation period, we scrutinized the Petri dishes for the presence of colonies exhibiting pink or red colors, indicative of *E. coli* growth on MacConkey agar. Finally, colony counting was conducted. To maintain precision, only plates bearing between 30 and 300 colonies were considered. The count was performed and subsequently multiplied by the corresponding dilution factor, yielding the Colony Forming Units per gram (CFU/g).

2.6. Analysis of equity

We evaluated the influence of landfill leachates on soil microbial equity using the Shannon-Wiener index, which was applied to the colony-forming units (CFUs) of each microorganism type within each treatment. In ecological studies, the Shannon-Wiener index typically yields values ranging from 1.5 to 3.5, with higher values suggesting greater richness and equity, although theoretically, the index can range from 0 to infinity. The formula employed for this index is as follows:

$$H' = -\sum p_i \cdot ln(p_i)$$

Where *H'* represents the Shannon-Wiener biodiversity index; $\Sigma(p_i)$ is the summation of the proportion of individuals belonging to each species; $ln(p_i)$ is the natural logarithm of the proportion of individuals belonging to each species. Furthermore, we utilized the inverse Simpson index, which emphasizes uni-

formity or evenness rather than richness. This index denotes the likelihood of selecting two different species when randomly picking individuals from a sample. The inverse Simpson index, represented as 1/D, spans from 1 (when the sample contains only one species) to S (total species within the sample). The formula for the inverse Simpson index is:

$$D = \frac{\sum n_i \cdot (n_i - 1)}{N \cdot (N - 1)}$$

Where *D* is the Simpson index; n_i is the total number of individuals in the i-th species; *N* is the total number of individuals in the community.

2.7. Dominance analysis

To assess dominance, we employed the Complementary Simpson Index, calculated as 1 - D, with D representing the original Simpson Index. The original Simpson Index gauges the probability of randomly selecting individuals belonging to the same species, while the Complementary Simpson Index reflects the likelihood of selecting individuals from different species. The index ranges from 0 (indicating that all individuals belong to the same species) to 1 (signifying that all individuals belong to different species).

In addition, we estimated the Berger-Parker Index, a measure of dominance that solely considers the most prevalent species in the ecosystem. This index is calculated as follows:

$$D = \frac{N_{MAX}}{N}$$

Where N_{MAX} is the number of individuals of the most abundant species; *N* is the total number of individuals.

The Berger-Parker Index varies between 1/S (where S is the total number of species, and all species are equally represented) and 1 (in scenarios where only one species is present).

2.8. Composition analysis

To evaluate the composition, we calculated the percentages that each species count represented in comparison to the total count of microorganisms for that specific measurement. These compositions varied among the different treatments. We conducted an analysis of variance (ANOVA) to assess the equity, dominance, and composition of the microbial species in the soil. I used Tukey's test to identify significant differences in these aspects at a significance level of 5%.

3. Results and discussion

3.1. Impact on diversity

Based on the results, it's evident that landfill leachates have a significant impact on soil microbial diversity. This impact is reflected in the changes observed in the Shannon and Inverse Simpson indices, as shown in Table 3 and Figure 4.

For the Shannon index, a p-value of 0.053 was obtained, indicating that there isn't a statistically significant difference in species diversity among the treatments. However, it's worth noting that the average Shannon index value is lower in treatments T1 and T2 compared to treatments S and T0. Although this difference doesn't reach statistical significance, it does suggest that the addition of leachates may be affecting the equity of microorganisms in the soil.

Regarding the Inverse Simpson index, the p-value is less than 0.05, signifying a significant difference between the treatments. The values of this index for T1 and T2 are lower than those for S and T0, indicating that the equity and species diversity are lower when leachates are added to the soil, either in normal doses or treated. This finding is relevant as it suggests that the addition of leachates can reduce the equity of microorganism species.

These changes might be associated with modifications in the physicochemical soil parameters. For instance, the addition of leachates significantly increases electrical conductivity (E.C.), phosphorus concentration, potassium, calcium, and magnesium, as well as the proportion of *E. coli* bacteria ($p < 0.01$ in all cases). This could favor certain microbial species over others, thus altering the community's equity.

These results are consistent with previous research showing that changes in soil physicochemical conditions, such as those caused by the addition of leachates, can impact the composition and structure of microbial communities (Jones et al., 2009; Semrau, 2011).

3.2. Impact on Dominance

The Complementary Simpson Index assesses the diversity of a community, where a higher value reflects greater diversity. In this study, we observed that this index decreased under treatments T1 and T2 compared to treatments S and T0. This suggests a reduction in the diversity of microorganisms with the addition of leachates.

The Berger-Parker Index represents the dominance of the most abundant species in a sample. In this case, we noticed an increase in the dominance of certain microorganisms under treatments T1 and T2, compared to treatments S and T0. This indicates that certain microorganisms may thrive in the presence of leachates, at the expense of others.

The decrease in diversity and the increase in the dominance of specific species may indicate an

alteration in the microbial balance of the soil. These alterations can have long-term impacts on soil health and functionality (Leff et al., 2015).

Significant changes in physicochemical parameters were observed under treatments T1 and T2. For instance, treatment T1 resulted in decreased levels of organic matter (OM), nitrogen, and carbon, whereas it led to an increase in elements like phosphorus, potassium, calcium, and magnesium.

These observed changes may be related to modifications in the diversity and dominance of microorganisms in the soil. Soil physicochemical changes can affect the composition of microorganisms, which, in turn, can influence diversity. Some microorganisms may thrive in environments rich in certain soil elements, such as phosphorus or potassium, explaining the increase in dominance. Additionally, the decrease in organic matter, nitrogen, and carbon could limit the growth of certain microorganisms, leading to a reduction in diversity.

It's crucial to note that nitrogen-fixing bacteria decreased under treatment T1. This group of bacteria plays a crucial role in nitrogen availability in the soil, and their decrease can have significant impacts on soil health and its ability to support plant growth (Zahran, 1999). Furthermore, a notable increase in *E. coli* is observed in the soil under treatment T1, which may indicate fecal contamination and pose a potential risk to human and animal health (Sinton et al., 2010).

3.3. Impact on composition

In the control sample (S), the proportion of viable aerobic microorganisms was 36.42% ± 5.07% (Table 3 and Figure 3). In the soil under treatment T1, this proportion significantly decreased to 9.79% ± 7.21%. In the soil with leachate treated by coagulation-flocculation (T2), it was slightly higher than in T1, at 10.84% ± 6.10%.

These reductions in the population of viable aerobic microorganisms may be directly related to the changes in physicochemical conditions induced by the leachates. Specifically, an increase in the concentration of heavy metals was observed in T1 and T2. Heavy metals can have toxic effects on soil microorganisms (Giller et al., 1998).

Furthermore, leachates can cause soil eutrophication, characterized by an excess of nutrients that can lead to uncontrolled growth of certain species and disrupt microbial composition (Smith, 2003). This could explain the decrease in viable aerobic microorganisms, which may be displaced by other species better adapted to these conditions.

The decrease observed in T2 suggests that the coagulation-flocculation treatment, although effectively removed solids, is not effective enough to eliminate the toxic components of the leachates, such as dissolved metals. This is consistent with Amokrane et al. (1997) who observed that this treatment method failed to remove all contaminants from the leachates.

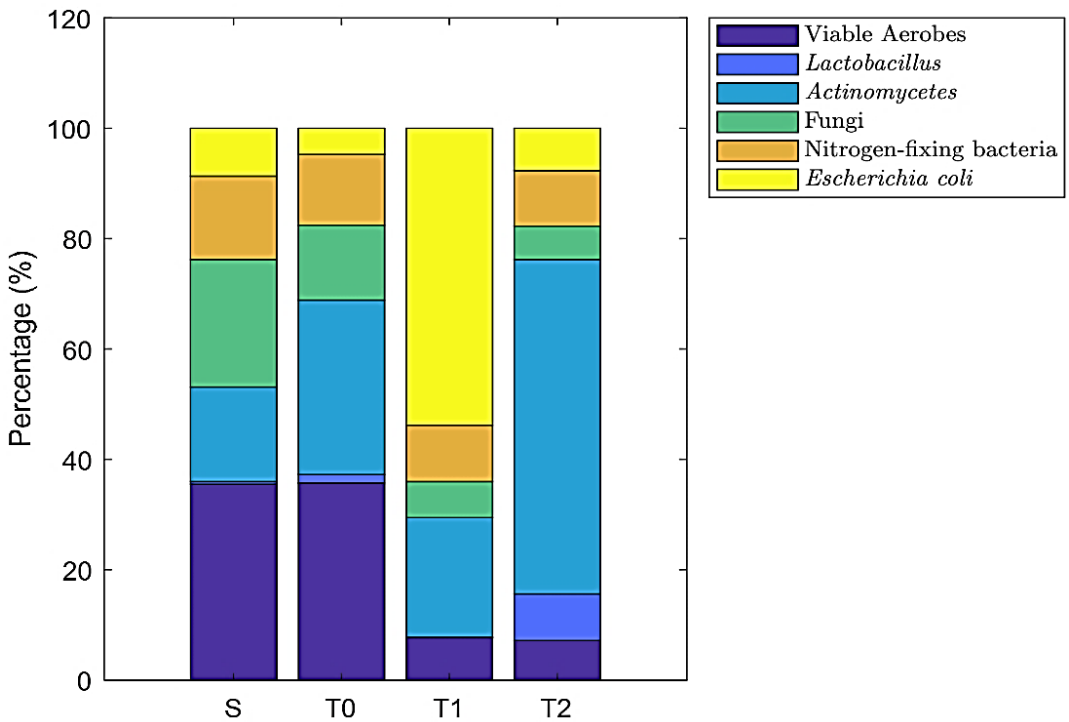

Figure 3. Change in the percentage composition of microorganisms in the treatments.

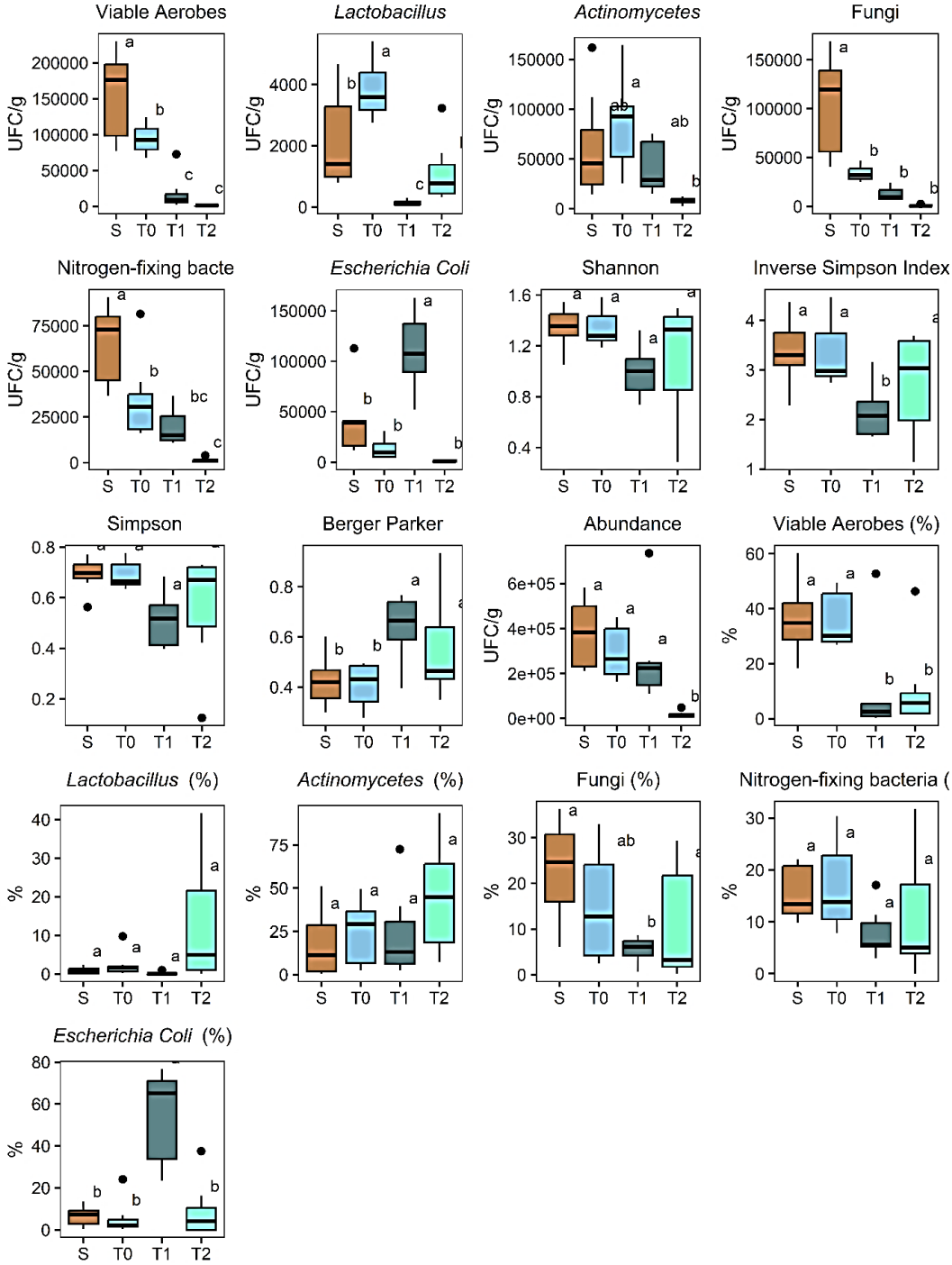

Figure 4. Box plot depicting the evaluated microorganisms (counted in CFU/g and percentage composition) and diversity indices, with Tukey grouping analysis.

Regarding *Lactobacillus*, the results show a statistically significant increase in the percentage of *Lactobacillus* in the soil with treated leachate (T2) compared to the other conditions. This increase may be linked to specific properties of the treated leachate. *Lactobacillus* are known to be facultative microorganisms, capable of surviving in both aerobic and anaerobic conditions, and they thrive in acidic environments. Although the pH levels increased in all treatments compared to the control sample (S), the soil treated with leachate (T2) maintained a slightly lower pH than the soil with surface water (T0) and the untreated leachate soil (T1). While the pH levels in T2 are not acidic, this slight decrease compared to the other treatments may have favored *Lactobacillus*.

Moreover, in treatment T2, there was an increase in organic matter compared to T0 and T1. *Lactobacillus* are heterotrophic microorganisms that require organic sources of carbon for their metabolism. The increase in available organic matter may have promoted their growth. Despite reductions in nitrogen levels compared to the control soil (S) in T2, phosphorus levels were considerably higher. Both nitrogen and phosphorus are essential nutrients for microbial growth. The higher availability of phosphorus may have further stimulated the growth of *Lactobacillus*, despite the decrease in nitrogen. These factors likely contributed to the higher proportion of *Lactobacillus* in the T2 treatment.

This finding aligns with previous research indicating that the introduction of treated leachates can significantly alter the microbial composition of the soil due to changes in environmental conditions (Song & Lee, 2010). While most studies tend to emphasize the negative aspects of these changes, this study demonstrates that there can also be positive effects, such as an increase in the proportion of Lactobacillus, a bacterial genus known to provide multiple benefits for soil and plant health (Wang et al., 2018). Concerning *E. coli*, the results indicate that the control soil sample (S) had an average concentration of 3.95×10^4 CFU/g, significantly lower than the average concentration of 1.11×10^4 CFU/g observed in sample T1, which received a normal dose of leachate. This increase in *E. coli* concentration suggests that leachate may create a favorable environment for the growth of these bacteria or introduce *E. coli* into the soil.

On the other hand, treatment T2, which involved treated leachate, exhibited a much lower concentration of *E. coli* (1.02×10^4 CFU/g), indicating that leachate treatment may effectively reduce the *E. coli* load.

Table 2

Summary of soil physicochemical parameters (mean ± standard error) per treatment (S, T0, T1, and T2), and Tukey's statistical grouping

Parameter	Units	S	T0	T1	T2	p-value
Sand	%	40.29 ± 3.42 (a)	24.00 ± 2.51 (b)	30.43 ± 2.90 (ab)	31.43 ± 4.17 (ab)	<0.05
Clay	%	38.29 ± 3.32 (a)	32.86 ± 2.02 (a)	33.71 ± 2.92 (a)	31.57 ± 4.87 (a)	0.549
Silt	%	21.43 ± 3.24 (b)	43.29 ± 3.22 (a)	35.71 ± 4.76 (ab)	36.86 ± 3.60 (a)	<0.01
pH		3.24 ± 0.07 (b)	5.35 ± 0.13 (a)	5.19 ± 0.46 (a)	4.96 ± 0.32 (a)	<0.01
CE	dS/m	0.32 ± 0.01 (b)	0.44 ± 0.04 (b)	1.10 ± 0.03 (a)	1.22 ± 0.05 (a)	<0.01
MO	%	3.04 ± 0.38 (a)	0.43 ± 0.11 (b)	0.98 ± 0.17 (b)	1.59 ± 0.44 (b)	<0.01
Nitrogen	%	0.18 ± 0.04 (a)	0.02 ± 0.00 (b)	0.07 ± 0.00 (b)	0.08 ± 0.02 (b)	<0.01
Carbon	%	0.60 ± 0.03 (b)	0.51 ± 0.05 (b)	0.59 ± 0.02 (b)	0.93 ± 0.03 (a)	<0.01
Phosphorous	ppm	9.10 ± 1.72 (ab)	3.67 ± 1.37 (b)	15.79 ± 4.26 (a)	16.21 ± 2.47 (a)	<0.01
Potassium	ppm	68.91 ± 3.17 (c)	74.77 ± 1.62 (c)	246.52 ± 1.36 (a)	210.10 ± 1.49 (b)	<0.01
Calcium	meq/100g	0.84 ± 0.01 (d)	1.88 ± 0.01 (c)	3.07 ± 0.00 (a)	2.39 ± 0.01 (b)	<0.01
Magnesium	meq/100g	0.11 ± 0.01 (c)	0.28 ± 0.02 (b)	0.42 ± 0.01 (a)	0.37 ± 0.02 (a)	<0.01
Exchangeable Potassium	meq/100g	0.35 ± 0.02 (b)	0.20 ± 0.05 (c)	0.51 ± 0.02 (a)	0.47 ± 0.03 (a)	<0.01
Sodium	meq/100g	0.17 ± 0.03 (a)	0.09 ± 0.02 (b)	0.21 ± 0.02 (a)	0.16 ± 0.02 (ab)	<0.01
Aluminum	meq/100g	4.39 ± 0.35 (a)	0.07 ± 0.04 (b)	0.09 ± 0.04 (b)	0.15 ± 0.07 (b)	<0.01
Hydrogen	meq/100g	0.43 ± 0.11 (a)	0.01 ± 0.01 (b)	0.06 ± 0.02 (b)	0.02 ± 0.01 (b)	<0.01
CEC		6.50 ± 0.87 (a)	2.92 ± 0.36 (b)	5.51 ± 0.47 (ab)	3.25 ± 0.83 (b)	<0.01
Exchangeable Bases	%	16.72 ± 2.07 (c)	84.96 ± 1.11 (b)	93.88 ± 1.02 (a)	89.55 ± 1.10 (ab)	<0.01
Exchangeable Acids	%	80.94 ± 2.49 (a)	13.40 ± 0.81 (b)	9.25 ± 2.84 (b)	12.32 ± 0.89 (b)	<0.01
Aluminum Saturation	%	63.86 ± 4.45 (a)	15.41 ± 5.14 (b)	4.87 ± 2.54 (b)	8.63 ± 2.12 (b)	<0.01

Table 3

Summary of soil microorganism counts. diversity indices. and composition (mean ± standard error) per treatment (S. T0. T1. and T2). and Tukey's statistical grouping

Parameter	Units	S	T0	T1	T2	p-value
Viable Aerobes	UFC/g	$1.54x10^5 \pm 2.32x10^4$ (a)	$9.40x10^4 \pm 7.76x10^3$ (b)	$1.83x10^4 \pm 9.44x10^3$ (c)	$9.40x10^2 \pm 2.09x10^2$ (c)	<0.01
Lactobacillus	UFC/g	$2.20x10^3 \pm 6.04x10^2$ (b)	$3.84x10^3 \pm 3.79x10^2$ (a)	$1.31x10^2 \pm 3.70x10$ (c)	$1.14x10^3 \pm 3.96x10^2$ (bc)	<0.01
Actinomycetes	UFC/g	$6.14x10^4 \pm 2.08x10^4$ (ab)	$8.46x10^4 \pm 1.79x10^4$ (a)	$4.27x10^4 \pm 9.97x10^3$ (ab)	$7.86x10^3 \pm 1.24x10^3$ (b)	<0.01
Fungi (molds/yeast)	UFC/g	$1.03x10^5 \pm 1.93x10^4$ (a)	$3.39x10^4 \pm 3.13x10^3$ (b)	$1.26x10^4 \pm 2.59x10^3$ (b)	$8.20x10^2 \pm 2.98x10^2$ (b)	<0.01
N-fixing bacteria	UFC/g	$6.44x10^4 \pm 8.18x10^3$ (a)	$3.43x10^4 \pm 8.71x10^3$ (b)	$1.97x10^4 \pm 3.74x10^3$ (bc)	$1.36x10^3 \pm 4.81x10^2$ (c)	<0.01
Escherichia coli	UFC/g	$3.95x10^4 \pm 1.31x10^4$ (b)	$1.29x10^4 \pm 3.88x10^3$ (b)	$1.11x10^5 \pm 1.49x10^4$ (a)	$1.02x10^3 \pm 2.53x10^2$ (b)	<0.01
Shannon	-	1.35 ± 0.06 (a)	1.34 ± 0.05 (a)	0.99 ± 0.08 (a)	1.10 ± 0.17 (a)	0.053
Inverse Simpson Index	-	3.38 ± 0.27 (a)	3.34 ± 0.24 (a)	2.15 ± 0.21 (b)	2.71 ± 0.39 (ab)	<0.05
Comp. Simpson Index	-	0.69 ± 0.03 (a)	0.69 ± 0.02 (a)	0.51 ± 0.04 (a)	0.56 ± 0.08 (a)	<0.05
Berger Parker	-	0.42 ± 0.04 (b)	0.41 ± 0.03 (b)	0.64 ± 0.05 (a)	0.56 ± 0.08 (ab)	<0.05
Abundance	UFC/g	$3.77x10^5 \pm 6.04x10^4$ (a)	$2.96x10^5 \pm 4.48x10^4$ (a)	$2.66x10^5 \pm 8.11x10^4$ (a)	$1.69x10^4 \pm 5.94x10^3$ (b)	<0.01
Aerobios Viables	%	36.42 ± 5.07 (a)	36.19 ± 3.84 (a)	9.79 ± 7.21 (b)	10.84 ± 6.10 (b)	<0.01
Lactobacillus	%	0.84 ± 0.32 (a)	2.41 ± 1.26 (a)	0.17 ± 0.14 (a)	13.11 ± 6.64 (a)	<0.05
Actinomicetos	%	17.66 ± 7.32 (a)	23.95 ± 7.00 (a)	23.08 ± 9.56 (a)	44.44 ± 12.04 (a)	0.207
Fungi (molds/yeast))	%	22.94 ± 4.14 (a)	14.96 ± 4.69 (ab)	5.52 ± 1.10 (b)	11.35 ± 4.72 (ab)	<0.05
N-fixing bacteria	%	15.70 ± 1.98 (a)	16.91 ± 3.25 (a)	7.94 ± 1.82 (a)	11.27 ± 4.40 (a)	0.168
Escherichia coli	%	6.44 ± 1.81 (b)	5.57 ± 3.19 (b)	53.49 ± 8.52 (a)	8.98 ± 5.23 (b)	<0.01

On the other hand, treatment T2, which involved treated leachate, exhibited a much lower concentration of *E. coli* (1.02 x 10^4 CFU/g), indicating that leachate treatment may effectively reduce the *E. coli* load.

Furthermore, the percentages of *E. coli* present in each treatment follow a similar pattern. While in samples S and T0, the percentages of *E. coli* fluctuate around 6.44% and 5.57%, respectively, the soil to which leachate was added (T1) shows a significant increase in this percentage, reaching 53.49%, confirming the previously mentioned trend. Treatment with treated leachate (T2) successfully reduces the presence of *E. coli* to 8.98%.

This increase in *E. coli* could have detrimental consequences for public health, as many *E. coli* strains are pathogenic and can cause diseases if ingested, for instance, through the consumption of food grown in contaminated soils or through direct contact. It may also disrupt the balance of the soil ecosystem by affecting other soil organisms.

These findings are in line with the work of Sinton et al. (2002), which identified an increase in the concentration of *E. coli* in soils irrigated with untreated wastewater, supporting the idea that leachate, often containing contaminants similar to those in wastewater, can have a similar impact on *E. coli* concentration in the soil.

4. Conclusions

The study's findings indicate a significant impact of landfill leachates on both the equity and diversity of soil microorganisms. This negative effect was evidenced by a reduction in the Shannon and Inverse Simpson indices. These changes were closely associated with alterations in the soil's physicochemical properties, including increased electrical conductivity, changes in nutrient concentrations, and shifts in bacterial populations. Given the crucial role of microorganisms in maintaining soil health, these observed changes may have long-lasting repercussions.

Furthermore, the introduction of leachates led to an increase in the dominance of specific microorganism species, potentially disrupting the overall microbial balance within the soil. These disturbances were particularly noticeable in shifts in physicochemical parameters, such as reduced organic matter, nitrogen, and carbon, as well as elevated levels of elements like phosphorus, potassium, calcium, and magnesium.

In addition to affecting equity, diversity, and dominance, the study revealed significant modifications in the composition of the soil's microbial community due to leachate exposure. This transformation was reflected in a decline in viable aerobes and an increased presence of specific bacteria such as *Lactobacillus* and *E. coli*. It is noteworthy that while leachate treatment offered some relief from these negative impacts, it did not entirely eliminate them. These findings emphasize the importance of considering the far-reaching consequences of leachate contamination on soil health and functionality.

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A. F. Cerna-Cueva: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **R. Puerta:** Data curation, Investigation, Methodology, Software, Visualization, Writing – review & editing. **C. Aguirre:** Investigation, Literature review, Methodology, Writing – review & editing. **J. Guerra:** Experimental design, Investigation, Sampling, Writing – review & editing. **M. Dueñas:** Investigation, Sampling. **W. Ríos:** Investigation, Sampling. **C. Paredes:** Writing – review & editing.

Conflict of interest statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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