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Soil Predisposing Factors to *Fusarium oxysporum* f.sp *Cubense* Tropical Race 4 on Banana Crops of La Guajira, Colombia

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Abstract: Fusarium wilt of banana caused by the soil-borne fungi *Fusarium oxysporum* f. sp. *cubense*, Tropical Race 4 (*Foc* TR4) (Syn. *Fusarium odoratissimum*), is a major threat to the global banana industry. Aiming to identify predisposing soil factors for Fusarium wilt of banana (FWB) TR4, the 23 physical and chemical soil properties were studied in three commercial banana farms in La Guajira, Colombia. Disturbed and undisturbed soil samples were collected from areas affected by the disease (affected plots) and disease-free areas (healthy plots). Five repetitions per farm were considered, with a total sample of $n = 30$. The data were analyzed using one-way analysis of variance (ANOVA). Subsequently, the debiased sparse partial correlation (DSPC) algorithm was applied. Organic matter (OM), pH, calcium (Ca), magnesium (Mg), zinc (Zn), and cation-exchange capacity on the exchange complex (ECEC), showed significant differences between the affected and healthy plots. In addition, the bulk density and saturated hydraulic conductivity (HC) were associated suggesting that physical attributes, such as soil compaction and poor drainage, create favorable conditions for FWB. According to the DSPC algorithm, the HC variable presented a grade of 5 and an intermediation of 14.67, which indicates that it has significant associations with BD, sand, porosity, ECEC, and OM and plays a critical role in the connection of other variables in the network and the differentiation of healthy and affected plots. These findings establish a baseline of information under field conditions in Colombia, which can be used to design soil management strategies to mitigate the detrimental effects of *Foc* TR4 by creating less favorable conditions for the pathogen.

Keywords: suppressive soil; calcium; organic matter; magnesium; pH; sand; saturated hydraulic conductivity

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1. Introduction

Fusarium wilt of banana (FWB) caused by the soil-borne fungi *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (*Foc* TR4) (Syn. *Fusarium odoratissimum*) is the most important and devastating banana disease affecting bananas worldwide. The pathogen causes severe damage to the plant's vascular system and blocks nutrient and water uptakes, which normally preclude fruit production. In addition, *Foc* TR4 can spread through planting material and any biotic or abiotic factor with the ability to carry water or soil particles. Furthermore, the pathogen survives in many alternative hosts and produces chlamydospores that persist in the soil for decades [1].

As *Foc* TR4 is a soil-borne pathogen it is anticipated that soil may play a crucial role in disease epidemiology and management. Different studies indicate that the chemical, physical, and biological factors of soil are associated with either suppressing or boosting FWB intensity [2,3]. Therefore, understanding which edaphic factors are involved in disease epidemics is critical to designing management strategies [4]. It is generally indicated that poorly drained soils facilitate the development of banana diseases, including FWB [5]. Additionally, soils with high bulk density and elevated penetration resistance values are frequently named conductive soils [6] and have been also associated with high FWB incidence [3]. For soil textural properties, contrasting results have been found, indicating that in some cases, suppressive soils are constituted by a high proportion of fine particles, while in other cases, the presence of soil aggregates (sandy loam or sandy soils) has a suppressive effect against FWB [7–9].

High FWB intensity is also frequently associated with low pH values [1,3,10]. It has also been reported that the use of ammoniacal fertilizers can reduce pH in the rhizosphere boosting the pathogen's virulence [8,11]. On the other hand, the use of nitrate-based fertilizers reduces FWB severity [10,12,13]. Low phosphorus content is frequently associated with higher FWB incidence [13,14], while high phosphorus levels are related to suppressive soils [15]. In addition, several studies have already indicated that the application of organic matter, calcium, and magnesium can increase soil pH with a consequent reduction of *Foc* populations [10,13,14,16]. It is generally assumed that soils with lower microbial diversity and/or abundance and lower biological activity are more disease-concentrated. In contrast, soils with a higher amount, diversity, and activity of microorganisms are considered suppressive against the disease [17–19].

In general, the following factors have been identified predisposing bananas to *Foc* TR4 epidemics: (a) waterlogged clayey soils with poor drainage, (b) acidic soils and calcium-poor areas, (c) soils with low organic matter and less microbiome richness, and (d) stressful situations leading to plant defenses reduction, such as poor-quality water, soil salinity, inadequate fertilization, and low temperatures, among others [17,20]. Therefore, integrated FWB management strategies should include the identification of these factors to promote soil suppressiveness together with other practices such as crop rotation, use of cover crops, and use of targeted fertilizer sources, among others [3,18,19,21–23].

The Guajira region has favorable edaphic and climatic conditions for FWB and has prompted an urgent need for research that explores the intricacies of soil-borne disease dynamics within this ecological setting. Soil properties and environmental conditions play a pivotal role in determining the success of pathogen establishment, survival, and subsequent disease development. This study aims to address this knowledge gap by investigating the physics and chemical soil predisposing factors that influence the prevalence and severity of *Foc* TR4 in banana crops across La Guajira, Colombia.

This study is novel in several ways, primarily due to the unique ecological conditions and geographical context of La Guajira, which has not been extensively explored regarding FWB. Through comprehensive soil analyses, we aim to identify the key drivers that underlie the susceptibility of this region to FWB, providing valuable insights for future disease management practices and contributing to the global effort to mitigate the impact of FWB on banana cultivation.

2. Materials and Methods

2.1. Field Location and Sampling

This study was conducted on three farms located in Dibulla county, La Guajira department, Colombia, represented by farms E44001-435, C44001-388ab, and A44001-288, identified as farms 1, 2, and 3, respectively. In the Dibulla municipality, the climate is classified as arid. The average temperature varies between 28 and 30 °C, the average annual precipitation is 1403 mm, the potential evapotranspiration is 1736 mm/year, the solar radiation is between 400 and 425 w/m^2, and the relative humidity is between 80 and 85% [24]. The predominant soils are of the inceptisols order, in the haplustepts group, and the alfisol order, in the natrustalfs group. The most representative soil series is aquic haplstepts, a fine loam family of kaolinitic soils that have recently evolved from medium and moderately fine alluvial sediments and that are moderately deep, with imperfect drainage, and are affected by flooding [25].

A detailed analysis was performed for each farm regarding the number of disease outbreaks, eradicated areas, and quantification of dispersion patterns into each farm (Appendix A, Table A1). Based on the geospatial and temporal analysis of disease outbreaks, five sites per farm were selected for sampling, considering affected areas that were localized and representative of disease outbreaks with similar aggregated patterns and characterized by the presence of symptomatic plants in different areas in each farm.

The Colombian Agricultural Institute (ICA) provided us with official information on the status of each field in terms of disease incidence and the location of TR4-affected areas. Under a collaboration framework with the agricultural sector, logistical, operational, and biosafety aspects were addressed for sampling and field analysis. Additionally, in coordination with the ICA, a chain of custody was implemented with strict biosafety protocols for soil sampling for the subsequent analysis of predisposing factors.

2.2. Sampling Criteria

For the study of soil predisposing factors, a stratified sampling methodology was applied, targeting conditions previously associated with the influence of the pathogen in eradicated areas and pathogen-free areas referred to as healthy zones. Based on the eradication protocol established by the ICA Resolution 00011912 dated 9 August 2019, soil samples were collected in Zone B, which is the area adjacent to the plant eradication zone (Zone A), and in the productive or healthy area (Zone C) for each outbreak (Figure 1). The sampling was carried out for 2019 and 2020.

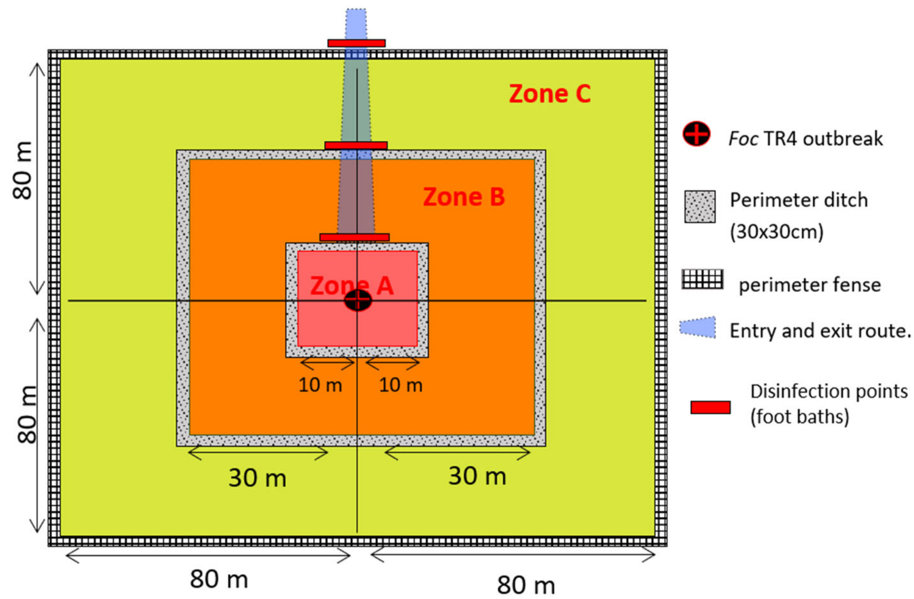


Figure 1. Diagram of intervention zones of Fusarium wilt of banana outbreaks. Zone A: eradication area. Zone B: delimitation (buffer) area. Zone C: productive or healthy (observation) area. Modified from Dita et al. [26].

The time from the moment of eradication to the moment of soil sampling was similar between farms, taking the samples with foci between 2 and 3 months after applying the eradication protocol. For each sampling site, Zone B and C, a central point was selected to excavate a trial pit to determine the soil horizons and effective depth for investigation. Additionally, soil sub-samples were taken at points near the central point for the evaluation of different soil properties. At least 5 sub-samples were collected at each zone, to ensure the representativeness of this study, at a depth from 0 to 20 cm. All samples were transported securely to the Agricultural Microbiology Laboratory at the Tibaitata Research Center of AGROSAVIA-Colombia for subsequent analysis. Finally, soil samples destinated for physical and chemical characterization were autoclaved two times at 120 °C, under 15 PSI of pressure for 20 min per cycle.

2.3. Soil Properties

2.3.1. Physical Properties

Undisturbed samples were collected for physical analysis in 5 × 5 cm volumetric rings using a Uhland-type auger. Three cylinders were collected within the first 0–20 cm soil layer, resulting in a total of six cylinders per site. In these soil samples, the bulk density (BD) (Mg/m^3), total porosity (Por) (%), and saturated hydraulic conductivity (HC) (cm/h) were determined. In the disturbed soil samples, particle size distribution analysis (% sand, silt, and clay) was conducted using 200 g of soil, following the methodologies described by González et al. [27].

2.3.2. Chemical Properties

Composite soil samples (5 composite samples per plot) of 500 g of soil were collected for a soil comprehensive fertility analysis like pH (1:2.5), electrical conductivity (EC) (dS/m), cation-exchange capacity on the exchange complex (ECEC) (cmol/kg), organic matter (OM) (%), total nitrogen (N) (%), total carbon (C) (%), phosphorus (P) (ppm), potassium (K) (cmol/kg), calcium (Ca) (cmol/kg), magnesium (Mg) (cmol/kg), sulfur (S) (ppm), iron (Fe) (ppm), zinc (Zn) (ppm), copper (Cu), manganese (Mn), boron (B)(ppm), and sodium (Na) (ppm). The methodologies used for chemical analyses were developed by the Agrosavia soil laboratory according to the NTC ISO/IEC 17025 standard.

2.4. Experimental Design

The design used was completely randomized, represented by three farms (F1, F2, F3), two conditions of the pathogen that were either present (affected plots, represented by the presence of symptomatic plants in eradication foci) or absent (healthy plots, represented by asymptomatic plants in areas without eradication foci), and 5 repetitions per farm (n = 30). The choice of a completely randomized design in this study offers several key advantages. Firstly, it ensures that each farm (F1, F2, and F3) is equally represented in the experimental design, mitigating the risk of bias associated with a more structured or systematic sampling approach. This random allocation of farms helps to account for the potential variability in soil conditions and the impact of the pathogen can be confidently assessed under various local conditions, improving the generalizability of its findings to a broader agricultural context.

Furthermore, the inclusion of two distinct conditions for the pathogen (present and absent) is a critical aspect of the experimental design. This binary distinction allows for a direct comparison of soil properties and disease outcomes in the presence and absence of the FWB. This approach not only enables the assessment of the disease's impact but also provides a means to identify soil factors that may predispose or deter *Foc* TR4 infection. The utilization of two conditions enhances the robustness of this study's results by offering a clear contrast in disease incidence and severity, which is vital for drawing meaningful conclusions regarding the relationship between soil properties and the prevalence of FWB in banana plantations. Additionally, the replication of each condition (affected and healthy) five times per farm further strengthens the statistical power of this study, enabling the researchers to detect subtle differences and relationships that may exist within the soil characteristics and disease dynamics in the region.

2.5. Analysis of the Results

2.5.1. Exploratory Analysis through Principal Component Analysis (PCA)

Before the analysis, data normalization was performed through log transformation and data scaling using Autoscaling in R software version 4.0.2 (R Core Team, Austria) [28].

The objective of performing principal component analysis (PCA) in this study is to reduce the dimensionality of the soil data and identify dominant patterns of variation among the variables. By transforming the original soil variables into principal components, PCA allows us to condense complex information while preserving the most significant variance in the dataset. This dimensionality reduction will help in simplifying the interpretation of the soil variables' relationships with *Foc* TR4. A first analysis with the 23 soil variables made it possible to eliminate those that added extraordinarily little and only contributed to distorting the analysis or making "noise". Subsequently, the data matrix was formed by n = 30 observations with p = 13 soil variables, using the statistical package in R and the "pca" function.

2.5.2. Analysis of Variance (ANOVA)

The physical and chemical properties of soil were analyzed using univariate statistical methods, descriptive statistics, and one-way analysis of variance (ANOVA). After testing the parametric assumptions ANOVA, the software Infostat Student Version 2016 [29] was used to perform Tukey's mean comparison test with a significance level of 5% (α = 0.005). The test distinguishes means into categories like 'a', ' b', and ' ab', it means that the groups being compared exhibit different levels of significance. 'a' indicates that the means are significantly different from each other, 'b' suggests significant differences, and 'ab' implies that there's no significant difference between those particular means.

2.5.3. Correlation Analysis

The purpose of using the Spearman rank correlation is to assess the non-linear relationships between soil variables and the incidence of the disease. By employing rank correlation, we can account for potential associations that may exist between soil factors and disease prevalence, providing a more robust and reliable analysis. The "cor. test" function in R was used for this analysis. This method allowed us to identify significant positive or negative monotonic relationships between soil variables and *Foc* TR4 occurrence, facilitating the detection of key predisposing factors in the soil ecosystem.

2.5.4. Debiased Sparse Partial Correlation (DSPC) Algorithm

To gain deeper insights into the complex interrelationships between soil variables and *Foc* TR4 in banana plantations, we employed the debiased sparse partial correlation (DSPC) algorithm with a network using MetaboAnalyst 5.0 [30]. This method aimed to identify the direct associations among soil variables while considering potential confounding factors, ultimately revealing the most influential variables in the disease predisposition network [31]. DSPC helped in removing indirect associations, reducing biases, and highlighting the key drivers in the soil ecosystem that contribute to the disease's prevalence.

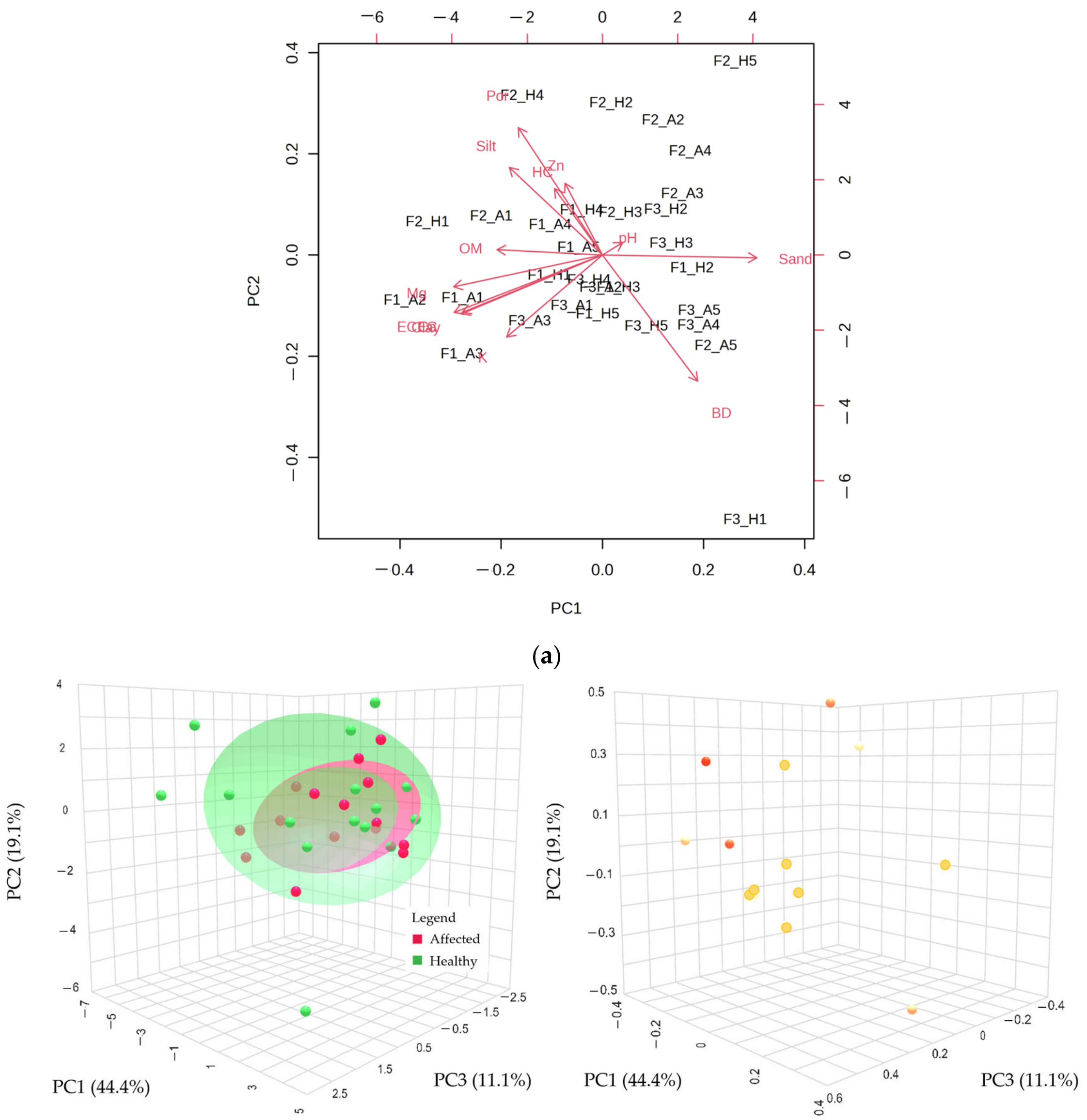
3. Results

3.1. Exploratory Analysis

In a PCA biplot (Figure 2a), the positions of all variables (Figure 2c) and observations (samples) (Figure 2b) are represented in a reduced-dimensional space. The first two principal components (PC1 and PC2) capture the most significant variance in the data (63.5%). PC1 explains the direction of the highest variance in the data. The loadings indicate the contribution of each variable to PC1 (44.4%) (Figure 2c). Variables with high positive loadings are positively correlated with PC1, while variables with high negative loadings are negatively correlated with PC1.

Sand has a positive correlation (0.38) with PC1. This suggests that higher sand content is associated with higher values of PC1. The variables like Mag (−0.37), CEC (−0.37), clay (−0.35), and Ca (−0.35) have negative correlations with PC1. This means that higher values of PC1 are associated with lower levels of these variables. PC1 seems to represent a gradient related to soil texture and cation-related properties. It separates samples with higher sand content from those with higher levels of Mg, CEC, clay, and Ca.

PC2 explains the direction of the second-highest variance in the data (19.1%). Silt content (0.34) has a positive correlation with PC2. An increase in silt content corresponds to higher PC2 scores. This suggests that the silt content plays a relatively important role in the variation captured by PC2. Bulk density (BD) (−0.49) shows a negative correlation with PC2. Higher bulk density is associated with lower PC2 scores. This indicates an inverse relationship between bulk density and the variation explained by PC2.



(**a**)

(**c**) (**b**)

Figure 2. (**a**) PCA biplot between the PC1 and PC2. (**b**) Synchronized 3D plot by score (samples) and (**c**) loading (soil variables). The colors of data points in the loading plot are based on their distances to the origin (0;0;0), with yellow for close and dark red for distant data points.

The porosity (por) (0.49) has a positive correlation with PC2. Higher porosity is linked to higher PC2 scores, implying a positive relationship between BD, and the variation represented by the PC2 potassium content (−0.32) is negatively correlated with PC2. Higher potassium content corresponds to lower PC2 scores. This suggests that potassium content is inversely related to the variation explained by PC2.

3.2. Comparative Analyses of Chemical and Physical Properties among Farms

Organic matter (OM), calcium (Ca), magnesium (Mg), zinc (Zn) content, and effective cation exchange capacity (ECEC) were the only soil chemical attributes showing differences among farms (Figure 3). The rest of the evaluated variables showed similar trends among farms. Farm 1 showed the highest values for OM, Ca, Mg, and ECEC (Figure 3). Farms 2 and 3 showed different patterns on these variables, except for the Zn content which was higher in farm 2.

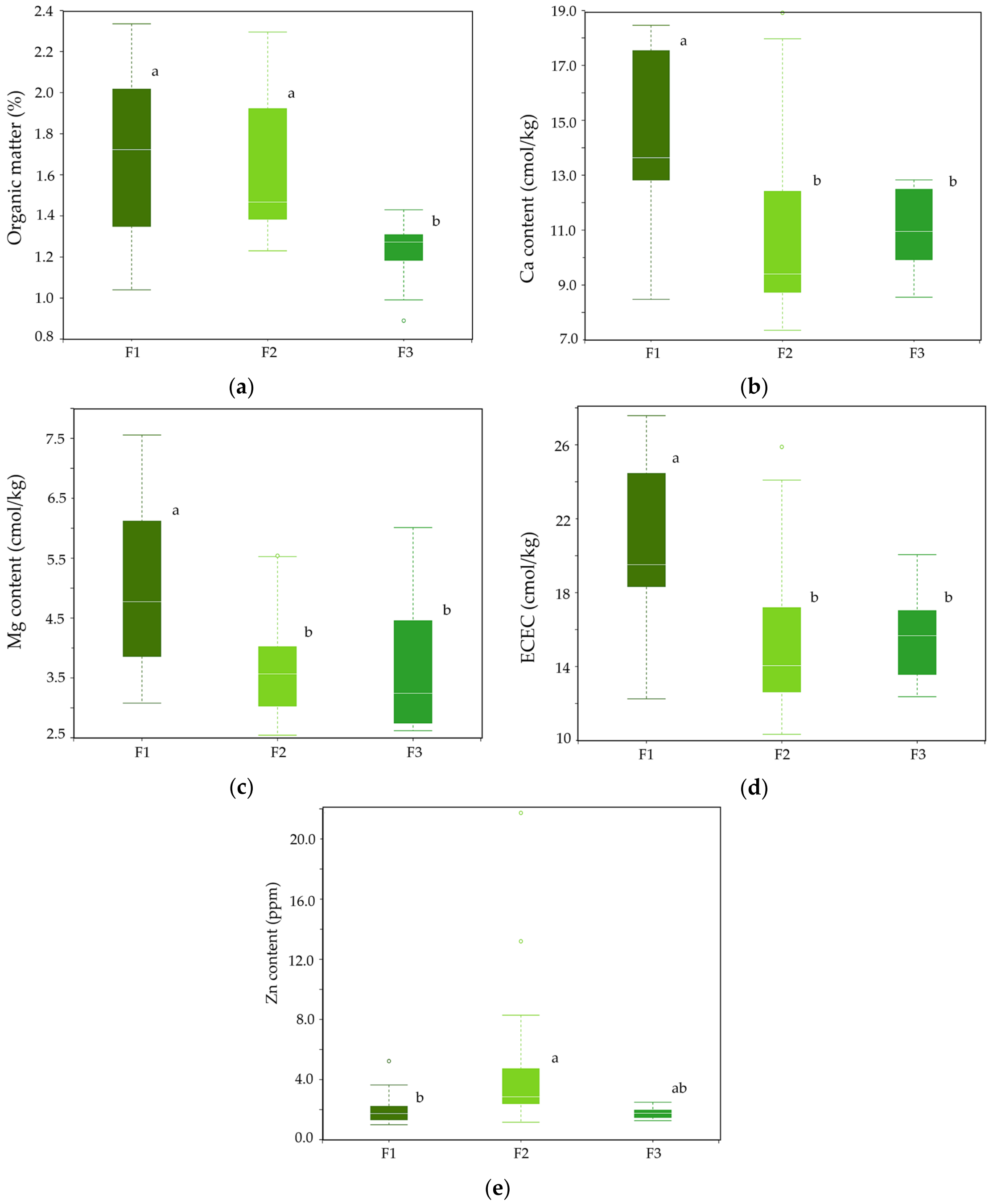
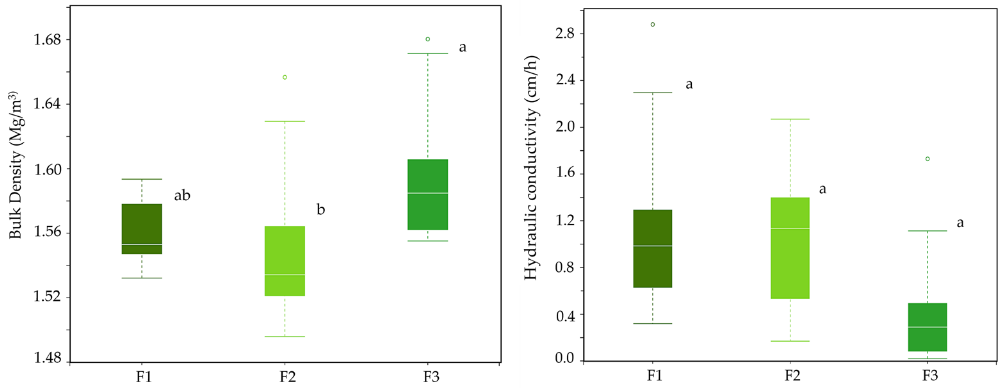


Figure 3. Soil chemical variables box plot for the three farms (F1, F2, and F3) with *Foc* TR4, in La Guajira, Colombia. (**a**) Organic matter, (**b**) calcium, (**c**) magnesium, (**d**) effective cation exchange capacity, and (**e**) zinc. The test distinguishes means into categories like 'a', 'b', and 'ab', it means that the groups being compared exhibit different levels of significance. 'a' indicates that the means are significantly different from each other, 'b' suggests significant differences, and 'ab' implies that there's no significant difference between those particular means.

Saturated hydraulic conductivity (HC), which is related to the water infiltration capacity in the soil, and bulk density (BD), which is associated with soil compaction, were the only physical properties showing differences among farms (Figure 4). Interestingly, farm 3 showed higher BD, but lower HC (Figure 4). During sampling, it was evident that farm 3 presented compacted soil with poor drainage, whereas the soil of the other two farms did not show any limitation.



(**a**) (**b**)

Figure 4. Physical soil properties box plot evaluated among the three farms with *Foc* TR4, in La Guajira, Colombia. (**a**) Bulk density, (**b**) saturated hydraulic conductivity. The test distinguishes means into categories like 'a', ' b', and ' ab', it means that the groups being compared exhibit different levels of significance. 'a' indicates that the means are significantly different from each other, 'b' suggests significant differences, and 'ab' implies that there's no significant difference between those particular means.

3.3. Comparative Analyses of Chemical and Physical Properties between Healthy and Affected Plots

Comparison at plot levels (affected versus healthy areas, corresponding to zones B and C, respectively) showed significant differences for both chemical and physical attributes (Table 1). From the 16 chemical variables evaluated, only four (pH, Ca, Mg, and ECEC) showed differences between healthy and affected plots (Table 1). Healthy plots presented the highest values of pH and contents of Ca, Mg, and UCEC. There were no significant differences in electrical conductivity, organic matter, total nitrogen, available phosphorus, available calcium, sulfur, sodium, iron, zinc, copper, manganese, and boron contents between affected and healthy plots.

Table 1. Soil chemical and physical properties in affected and healthy plots across three farms with different levels of incidence with the presence of *Foc* TR4 in La Guajira, Colombia. Values correspond to pooled data from all farms corresponding to the samples taken in the affected (n = 15) and healthy plots (n = 15). The test distinguishes means into categories like 'a', ' b', and ' ab', it means that the groups being compared exhibit different levels of significance. 'a' indicates that the means are significantly different from each other, 'b' suggests significant differences, and 'ab' implies that there's no significant difference between those particular means.

	Chemical Attributes				Physical Attributes	
Plot	**pH**	**Ca (cmol/kg)**	**Mg (cmol/kg)**	**ECEC (cmol/kg)**	**Sand (%)**	**HC (cm/h)**
Healthy	7.34 a	12.25 a	4.59 a	17.89 a	41.36 a	1.08 a
Affected	7.18 b	10.09 b	3.75 b	14.55 b	33.75 b	0.63 b
p-value	0.04	0.04	0.03	0.02	0.03	0.02
% CV	4.62	19.48	24.72	19.16	37.94	77.94

Sand content and HC were the only physical attributes showing differences between affected and healthy plots (Table 1). Healthy plots showed higher sand percentages and higher HC than affected ones. There were no significant differences in silt and clay percentages, bulk density, and total porosity between the affected and healthy plots.

3.4. Correlations

Correlation analyses between soil chemical and physical attributes in healthy and affected plots were performed (Figure 5). Each cell in the plot contains a correlation coefficient value, indicating the strength and direction of the correlation between two attributes.

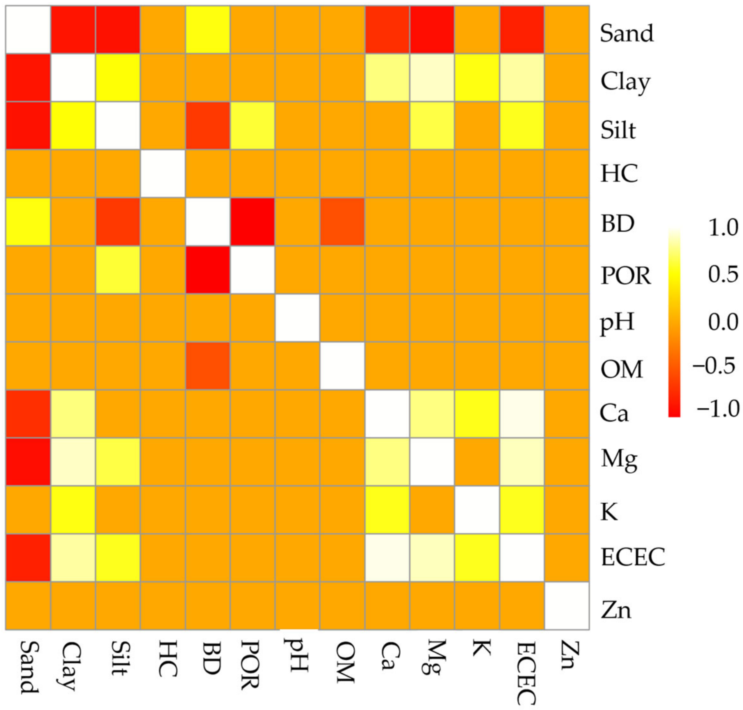


Figure 5. Heatmap of correlation coefficient among the chemical and physical soil attributes in banana healthy and affected plots by *Foc* TR4 in La Guajira, Colombia. HC: hydraulic conductivity; BD: bulk density; POR: porosity; OM: organic matter; Ca: calcium; Mg: magnesium; K: potassium, ECEC: effective cation exchange capacity; Zn: zinc.

The strongest negative correlation was observed between sand with Mg (−0.89), silt (−0.86), clay (−0.85), and CEC (−0.78). Bulk density (BD) and porosity also showed a moderately negative (−0.60) correlation. The strongest positive correlation was observed between Ca and CEC (0.95), Zn and OM (0.87), and Mg and CEC (0.76). Porosity and OM showed a moderately positive (0.51) correlation (Figure 5).

3.5. Debiased Sparse Partial Correlation (DSPC) Network

Figure 6 shows the DSPC network as a graph representation of the associations between different soil variables in areas affected by the disease *Foc* TR4 (affected plots) and disease-free areas (healthy plots). The nodes in the network represent soil variables, and the edges represent the association measures (likely partial correlations) between these variables after accounting for potential biases or confounding factors.

The variable HC is connected to five other variables in the network (Degree: 5), indicating that it has significant associations with multiple other soil variables. The high betweenness value (Betweenness: 14.67) suggests that this variable plays a critical role in connecting other variables in the network. Mg is connected to four other variables. It also has a high betweenness value (22.17), indicating that it serves as a critical bridge or intermediary between other variables.

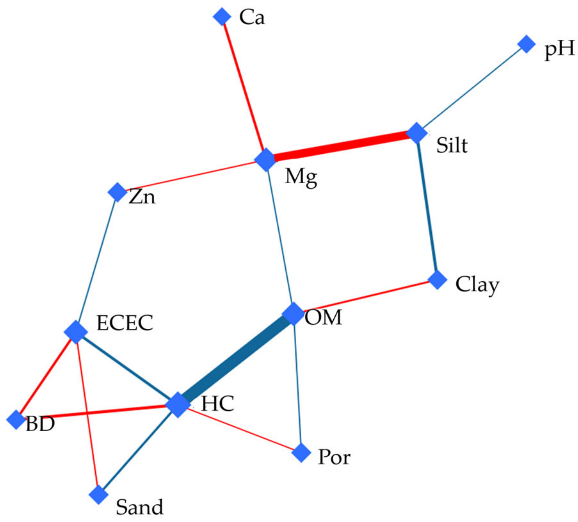


Figure 6. Partial correlation network of five evaluated variables. The size of the node indicates the direction of the change. The colored edges had a *p*-value <0.05 and the false discovery rate (FDR) adjusted *p*-value was <0.05. The red and blue borders show positive and negative correlations, respectively. HC: hydraulic conductivity; BD: bulk density; POR: porosity; OM: organic matter; Ca: calcium; Mg: magnesium; K: potassium, ECEC: effective cation exchange capacity; Zn: zinc.

The OM is connected to four other variables, and it also has a relatively high betweenness value (19.67), suggesting it plays an important role in the network's connectivity. The ECEC is connected to four other variables. Its betweenness value is lower than HC, Mg, and MO (7.33) but still higher than the other variables below.

The silt is connected to three other variables. It has a moderate betweenness value (11.5), indicating its role in connecting some other variables. Ultimately, the DSPC network provides valuable information on the associations between soil variables in areas affected by *Foc* TR4 and disease-free areas. Variables like HC, Mg, and MO play crucial roles in connecting different soil variables, while variables like Ca and pH have fewer associations and do not serve as key connectors.

4. Discussion

Soil attributes play an important role in the intensity of soil-borne diseases, such as FWB [2,4,10,13]. In this work, we showed that both chemical and physical attributes were associated with affected or healthy plots by *Foc* TR4 in banana commercial farms in La Guajira, Colombia. Key findings on these attributes and the implications for *Foc* TR4 management are discussed below making comparative analyses among farms and then between infected and healthy plots.

4.1. Comparative Analyses of Chemical and Physical Properties among Farms

Initially, we evaluated the chemical and physical properties of the three studied farms. In general, farm 1 presented better levels of fertility than the other two, showing the highest values for OM, Ca, Mg, Zn, and ECEC, especially in farm 3. When analyzing the levels of incidence and accumulated cases of *Foc* TR4 per farm, in the case of F1, there was the least number of foci, followed by F2 and finally F3, which presented the highest incidence and dispersion of the disease.

Organic matter is a regulator of different soil properties and a key factor in soil health and quality [9]. The higher soil organic matter content is generally associated with larger microbial populations with wide metabolic diversity [17,23]. The higher microbial diversity and microbial community structure represent an alternative for the mitigation of the negative effects of FWB. It has been already reported that in soils with high microbial activity, different processes such as competition and inhibition occur during antagonism against pathogens [20,32]. Metagenomic studies in the farms here are ongoing and should further complement our results.

Organic matter also releases nutrients in a plant-available form upon decomposition, improving root development, which might reinforce the first structural barriers against the pathogen. In addition, high OM contents improve the soil's physical structure, increasing infiltration and water retention [9,18,27,33]. However, we did not find such a correlation in this study.

Calcium and magnesium content have been previously associated with FWB suppressiveness [3,14,16]. It has been demonstrated that the application of Ca and Mg can increase soil pH and reduce FWB incidence [14,16,17]. On the other side, low contents of Ca, Mg, K, and P have also been associated with soils conducive to FWB [10,13,17,21,23].

Zinc deficiency has been already associated with higher FWB severity and its role in tylose formation as a defense response against *Foc* has been speculated [34–36]. Experiments conducted with *Foc* subtropical Race 4 in the Canary Islands, Spain, showed that Zn was not found to be associated with FWB intensity [12]. However, the authors indicated that Zn deficiencies affect the structure of chloroplasts and mitochondria at cellular levels and that, therefore, there may be a connection between zinc deficiency and *Foc* intensity in banana plants.

In terms of physical attributes, most of the evaluated sites presented textures between loam, silt loam, and clay loam. Farm 3 showed a bulk density value of 1.59 Mg/m^3, which has been reported as highly susceptible to compaction in soils with medium textures [37–41]. The higher content of sand found in this farm could be confusing when considering soil processes such as compaction or poor drainage. However, in this case, fine sands, which have smaller particle sizes and similar behavior to other finer particles such as silt might predominate. Sands were not divided in this work, but other studies reported similar compaction and slow infiltration responses of soils classified as loam [9,37,41]. Soil compaction has been already associated with higher FWB incidence [3,18,42].

Superficial and internal soil drainage (hydraulic conductivity) has been considered a predisposing factor to *Foc* TR4 since high humidity increases the FWB incidence and severity [3,4,18,19]. Farm 3 presented HC values of 0.41 cm/h, classified as poorly drained soils, while the other two farms showed HC values greater than 1.0 cm/h, considered as moderately well-drained soils [27,39]. When considering the number of total foci accumulated and spreading rate by the farm, the greatest number of foci occur on farms 2 and 3 (which have the lowest values associated with fertility). On the other hand, farm 1, with the lowest disease incidence and spreading rate, presented the least limiting or predisposing conditions to *Foc* TR4 in the chemical and physical properties of the soil (higher fertility and MO content, less compaction, and better drainage or water infiltration).

Overall, our results suggest that OM, Ca, Mg, and Zn levels must be considered in the fertilization plans carried out on farms in La Guajira to promote soil suppressiveness, boost plant defenses, and improve productivity [17]. In addition, physical attributes should be also evaluated and practices to reduce compaction and increase water infiltration, such as plowing and designing drainage networks to lower moisture saturation in the soil profile, must be considered [3,39,42,43].

4.2. Comparative Analysis through Affected and Healthy Plots

It was found that the affected plots showed lower pH and ECEC than the healthy plots. It is well documented that FWB is more severe in acid soils [3,4,42]. However, our results show that FWB can also be severe in soils considered slightly alkaline (>7.0). Interestingly, even under these alkaline conditions, the affected plots showed lower pH values (average 7.18) than the healthy ones (average value 7.34) suggesting more favorable conditions for FWB. Microbial diversity is restricted at low and high soil pH levels and is increased at moderate soil pH levels [43]. pH also affects enzyme activity, and microbial metabolism, thus influencing community composition [19].

Like the results found when farms were compared Ca, Mg, and ECEC also showed differences between affected and healthy plots, highlighting the role of these variables as predisposing factors to FWB [3,10,19,42]. These results indicated that TR4-affected plots present a lower pH value and fewer fertility conditions than the healthy plots, which is evidenced by the higher values of Ca, Mg, and ECEC in healthy plots.

Differences in sand content and hydraulic conductivity were also found between healthy and TR4-affected plots. In the affected plots, the soil is considered poorly drained (0.63 cm/h), while in the healthy plots, there is evidence of a notable improvement in the infiltration capacity (1.08 cm/h), above the critical value (1.0 cm/h), considered as moderately well-drained soils. These attributes have been previously reported as a predisposing factor to *Foc* TR4 [3,4,42].

Understanding the correlations in this study will help to prioritize and focus on the most critical soil factors when developing strategies for integrated FWB management. The PCA aided in identifying the main underlying factors that contribute to soil predisposition to the disease. According to [9] through the DSPC algorithm, it was determined that microbial activity actively participated in the decomposition of organic matter in banana soils in Venezuela. The application of DSPC in this study will offer a comprehensive view of the complex web of relationships between soil factors, enhancing our understanding of the underlying mechanisms driving *Foc* spread and impact on banana crops [44,45] The high intermediation degree of HC suggests that ECEC plays a central role in the DSPC network, acting as a key mediator of associations among various soil variables. The centrality of ECEC highlights its significance in understanding soil health and its potential role in the occurrence of FWB.

Mg is a crucial macronutrient for plants, involved in various physiological processes [46]. The high intermediation degree of Mg indicates its prominent role in the DSPC network, suggesting its strong association with other soil variables. Further investigation is needed to understand how magnesium levels might be linked to the prevalence of Fusarium wilt disease. Also, OM is an important component of soil, influencing soil structure, nutrient cycling, and water-holding capacity. The high intermediation degree of OM highlights its central role in information transfer within the DSPC network. This suggests that organic matter content could be a crucial determinant in distinguishing affected and healthy plots and understanding the dynamics of FWB. The network analysis allows us to gain insights into the complex relationships between soil variables and the disease's occurrence, which can help in understanding and managing FWB.

Recent studies on the influence of soil variables on pathogens like FWB have provided invaluable insights into disease management and prevention [47–49]. These investigations have shed light on the complex interplay between soil properties [50,51], microbial communities [52], and pathogen dynamics [53], offering a deeper understanding of the predisposing factors that contribute to disease outbreaks. Furthermore, the integration of machine learning techniques in agricultural research has revolutionized our ability to predict disease risks and optimize mitigation strategies [54,55]. These advancements enable Latin American countries, where Musaceae cultivation is economically significant, to harness data-driven approaches for more precise disease management [56]. By leveraging this knowledge and technology, these nations can bolster banana production, enhance food security, and safeguard their economies against the devastating impacts of *Foc* TR4 [57–59], ultimately ensuring the continued prosperity of this vital agricultural sector.

Recent findings in Venezuela [45] and Colombia [48] revealed that the incidence of *Foc* TR4 is closely related to environmental factors, with soil properties playing a prominent role in its prevalence. Unique environmental conditions in both countries, such as temperature, precipitation, and soil composition, were found to have a significant impact on the presence of the pathogen. These results highlight the undeniable importance of soil studies in unraveling the mysteries of this destructive pathogen, shedding light on its intricate relationship with the soil ecosystem [46,60]. The results allow the establishment of approximations in soil management in areas with a confirmed presence of *Foc* TR4 and preventive soil management in free areas. Our results highlight the need for preventive measures to avoid eventual *Foc* TR4 incursions in other banana-producing areas of Colombia, such as the Urabá region, which is characterized by acid soils and low natural fertility as well as soils with low infiltration and susceptibility to flooding [48].

5. Conclusions

This is the first work identifying soil attributes as predisposing to *Foc* TR4 in Latin America and the Caribbean. We found that both chemical and physical attributes were associated with *Foc* TR4. Lower values of pH, cation-exchange capacity on the exchange complex (ECEC), and saturated hydraulic conductivity (HC), as well as lower contents of organic matter (OM), Ca, Mg, Zn, and sand, were associated with the affected plots. Therefore, prevention strategies should not only consider biosecurity measures but also soil health-oriented practices to boost soil suppressiveness and improve the productivity of banana or plantain crops. The findings of this research underscore the importance of soil management practices, including pH adjustment, organic matter content, adequate fertilization with Ca, Mg, Zn, soil drainage improvements, and pathogen monitoring, in mitigating the risk of *Foc* TR4 infection. Furthermore, it highlights the need for tailored strategies and interventions to address the specific challenges faced by banana growers in Colombia, as well as for other producing regions in Latin America and the Caribbean.

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Appendix A

Table A1. Characteristics of each farm related to the presence and incidence of the disease *Foc* TR4 in La Guajira, Colombia.

Code	Farm	Accumulated Incidence (%) *	Estimated Eradicated Area (ha)	Total Area of the Farm (ha)	Affected Area (%)	Cultivated Clone
E44001-435	1	1.2	1.6	234.0	0.6	Williams (53%) Valery (47%)
C44001-388ab	2	2.5	8.4	393.0	2.1	Valery (88%) Williams (12%)
A44001-288	3	6.6	27.8	217.0	12.8	Valery (100%)

* Note: The information corresponds to two years (2019–2020) of data accumulated on each farm until the moment of soil sampling carried out in the research.

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