

Original Research Article

**Evaluation Of Presumed Drought Impacted Soils Through Phospholipid Fatty Acid (Plfa) Biomarkers In The Sahel Region**

1 **ABSTRACT**

**Aims:** The aim of the study is to use biomarkers to indicate drought-induced changes in soil microbial communities on presumed drought-impacted soils in some selected Sahel region.

**Study design:** A correlational study design was used to examine the influence of soil pH in relation to other soil properties (e.g., organic carbon, nitrogen, pH), while PLFA biomarkers were analysed using principal component analysis and descriptive statistics.

**Place and Duration of Study:** The Sahel region of sub-Saharan Africa, which includes Nigeria and the Niger Republic, was the subject of the May–October 2021 study.

**Methodology:** A study using phospholipid fatty acid (PLFA) biomarkers investigated drought-induced changes in soil microbial communities in the Sahel region. Analysis of 90 soil samples revealed significant correlations between PLFA profiles and soil properties.

**Results:** The study emphasises the significance of site-specific soil management techniques, especially in regions with low levels of nutrients, nutrient availability, and soil organic matter (SOC). In regions with low SOC, SOM, and nutrients, targeted organic and inorganic additions can be required to improve soil fertility. While sandy soils in G/Doki (87.1%) would benefit from organic additions for better water retention and nutrient-holding capability, Nkonni's high clay concentration could necessitate better drainage management to avoid waterlogging. Certain PLFA indicators are linked to bacteria and fungus, including C18:1cis9 and C18:1ω9. PLFA biomarkers are impacted by drought's alteration of soil microbial populations. To promote soil health and fertility conservation, it is essential to monitor soil microbial dynamics and implement sustainable agricultural methods.

**Conclusion:**

Soil microbial populations are greatly impacted by drought, which alters PLFA indicators. While certain biomarkers, like C18:0 and C18:1, suggest broad microbial activity, others, including C18:1ω9 and C18:1cis9, are linked to fungus. The following markers are linked to Gram-positive bacteria: i15:0, a15:0, i16:0, i17:0, and cy17:0 for Gram-negative bacteria, which are useful tools for evaluating the effects of soil management techniques, environmental stressors, and climatic variability on agricultural soils. These changes demonstrate the potential of PLFA analysis to connect soil microbial dynamics with soil health and fertility. Drought affects soil microbial communities, affecting soil pH. Higher pH increases organic carbon, organic matter, and calcium levels, while nitrogen and phosphorus levels are minimally affected.

2 *Keywords:* [drought, soil microbial biomarkers, nutrient levels, Sahel region]

3  
4 **1. INTRODUCTION**

5  
6 Drought is a recurring event in arid regions. Improved land use and management practices are essential to mitigating  
7 its effects. Studies have shown that while climate change poses challenges, human land-use practices have a larger,  
8 more immediate impact on desertification [1]. Human and livestock activity has a more significant impact on arid  
9 environments than anticipated climatic changes [2]. Future climate change is likely to have significant feedback

10 effects on soil respiration through alterations in vegetation structure, particularly in heterogeneous ecosystems like  
 11 Mediterranean shrublands and deserts. According to [3], there is interconnectedness of precipitation, vegetation, and  
 12 soil carbon processes, with important implications for understanding dryland ecosystem responses to global climate  
 13 change.

14  
 15 An insight into how microbial communities have historically adapted to changing moisture regimes and informed  
 16 future predictions is best understood if the cumulative and lagged effects of drought, historical studies combining  
 17 sediment records, paleoecological methods, and PLFA biomarker analysis are extensively studied. Soil  
 18 physiochemical properties significantly influence microbial communities, which are reflected in phospholipid fatty  
 19 acid (PLFA) biomarkers. PLFA analysis is a sensitive tool for monitoring soil health and fertility. However, the  
 20 relationships between soil physiochemical properties and PLFA biomarkers remain poorly understood. Cruz-Paredes  
 21 *et al.* [4] discovered that drought decreases microbial biomass and alters community composition, with significant  
 22 changes in PLFA biomarkers under prolonged dry conditions. Similarly, Fierer *et al.*[5] proved that microbial  
 23 communities in arid soils respond rapidly to changes in water availability, highlighting the importance of long-term  
 24 studies on microbial adaptation. Drought is said to reduce microbial activity and functional diversity, with shifts  
 25 toward drought-tolerant organisms detectable through PLFA analysis [6].

26 Quideau *et al.* [7] used a statistical approach to identify patterns and correlations between PLFA biomarkers and soil  
 27 properties, such as organic carbon, nitrogen, and pH. This study investigates the relationships between soil  
 28 physiochemical parameters and PLFA biomarkers, providing insights into the complex interactions governing soil  
 29 ecosystem functioning.

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 31 **2. MATERIAL AND METHODS**  
 32 **2.1 STUDY AREA**

33 The study surveyed fields in the Sahel region, bordering Nigeria and the Niger Republic. These areas were  
 34 purposely and randomly selected due to the semi-arid nature of the locations. The Mobile Topographer application  
 35 was used to obtain the coordinates of both latitude and longitude of each study area (Table 1). A mobile topographer  
 36 application was used to capture the geographical details of each location, and the coordinates of latitude and  
 37 longitude points were recorded. These recorded points were later trained into *GeoPandas* and *Mapplotlib* to capture  
 38 the probable exact locations of points for sample collection on the map as reference points for further research  
 39 (Figure 1).

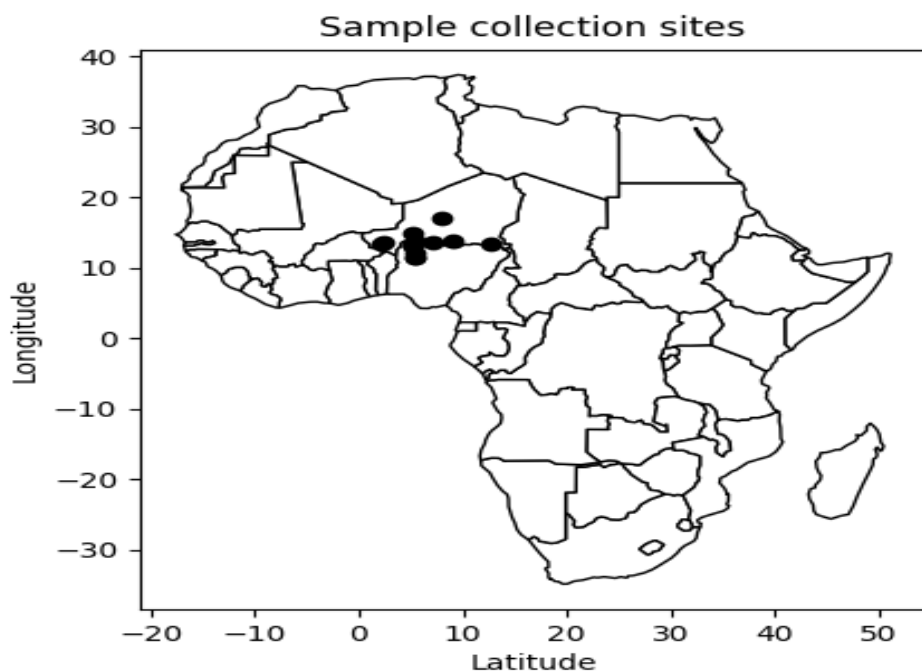
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42 Table 1: Geographic coordinates of the Study Areas

S/No.	Location point	Latitude (°)	Longitude (°)	Altitude (m)	Temperature (°)	Time collected (hours)
1.	Ndounga	13.371	2.252	193.72	39	12.42
2.	Ndounga	13.372	2.253	191.30	42	12.45
3	Ndounga	13.371	2.251	191.72	43	12.55
4.	Barkiawel	13.544	2.312	218	34	18.00
5.	Barkiawel	13.549	2.313	220.1	33	18.05
6.	Barkiawel	13.545	2.312	219.06	32	18.15
7.	Konni	13.796	5.250	272.94	43	12.55
8.	Konni	13.474	5.159	265.09	43	13.00
9.	Konni	13.79	5.256	262	42	13.05
10.	Dundaye	13.010	5.228	324.0	29	9.00

11.	Dundaye	13.134	5.194	259.31	29	9.05
12.	Dundaye	13.134	5.194	260.76	30	9.30
13.	Makera	13.137	5.189	271.11	30	9.38
14.	Makera	13.137	5.188	267.60	29	9.00
15.	Makera	13.137	5.188	271.16	32	9.30
16.	Gidandoki	13.138	5.191	271.21	29	8.55
17.	Gidandoki	13.138	5.191	272.93	30	9.35
18.	Gidandoki	13.138	5.190	271.18	31	9.45



**Figure 1:** Map of Africa showing sample collection sites across the borders of Nigeria-Niger Republic

## Collection of Soil Samples

Root samples (both rhizosphere and rhizoplane) of sorghum were collected under moist conditions during the rainy seasons and when rainfall began to decline. The soil samples were collected from fourteen (14) locations (Agadez 1 and Agadez 2, Barkiawel, DakiTakwas, Diffa, Dundaye, Makera, Maradi, Ndounga, Nkonni, Ribah, Tahoua, Zinder, and Zodi) within Nigeria and the Niger Republic located in the Sub-Saharan region. A total of 90 samples were collected. Barkiawel, Ndounga, and Nkonni samples were collected from May 24 to May 27, 2021; GidanDoki, Makera, and Dundaye (July 30 to August 1). While Agadez 1 and Agadez 2 (Adderbissonét), Diffa, Maradi, Tahoua, and Zinder were collected on September 28, 2021, Motsara and Roy's [8] method was adopted. Samples were pooled to prepare the composite soil sample. Next, these composites were air-dried and homogenised by sieving (fine earth, < 2 mm) which were used for comprehensive soil analysis.

## 2.2 Protocol for PLFA Analysis

### 2.2.1 Precautionary measures

Quideau et al.'s [7] method is a reliable soil ecological study method, but proper PPE is needed to prevent contamination. Nitrile gloves are recommended, and cleaned glassware should be rinsed with 70% alcohol.

### 2.2.2 Preparation of Glassware for Analysis

63 Disposable glassware, including centrifuge tubes, PTFE-lined caps, and reusable glassware, should be heated in a muffle furnace for 4  
64 and a half hours at 450 °C. PTFE-lined caps should be soaked in phosphate detergent, washed, and dried in an oven at 40 °C.

### 65 66 **2.2.3 Collection and Processing of Soil Samples Prior to PLFA Analysis**

67  
68 Soil samples were collected from 14 locations in Nigeria and the Niger Republic, resulting in 90 samples. After soil physiochemical  
69 analysis, the remaining samples were frozen until ready for freeze drying. The freeze-dried samples were transferred to new-labelled  
70 sterile bags and weighed into a pre-labelled muffled centrifuge tube for PLFA extraction. A general guideline is 0.5 g for organic  
71 materials and up to 3.0 g for mineral soil samples. For every 10 samples, an additional duplicate sample is weighed, and for every 20  
72 samples, a blank is included. Batches of sample tubes are processed simultaneously, with a set of 20 samples corresponding to a batch  
73 of 23 sample tubes. The extraction, separation, and methylation are conducted in batches of samples before preparing them for GC  
74 analysis. This helps identify errors and reduces the number of repeat extractions.

## 75 76 **2.3 PLFA Technique Steps**

77 The PLFA technique was conducted in a fume hood, using appropriate PPE and adhering to lab safety guidelines in each of the three  
78 steps.

### 79 **2.3.1 Extraction (Step 1):**

80 The process involves preparing a solution of KOH and citrate buffer, which are then adjusted to a pH of  $4.00 \pm 0.02$  by adding 5.0 M  
81 KOH. The citrate buffer is diluted to 1,000 ml and stored in the refrigerator. A PC(19:0/19:0) nonadecanoate surrogate standard is  
82 prepared daily by diluting 250 µl of the stock solution in 25 ml chloroform. The Bligh and Dyer extractant is added to the soil sample,  
83 followed by a second round of extractant and centrifugation at 226 x g for 15 minutes. The supernatant is transferred to a labelled 45-  
84 ml glass vial, and the remaining samples are added to the same vials. The samples are then placed under compressed N<sub>2</sub> to avoid  
85 oxidation. Chloroform is evaporated off slowly, setting the N<sub>2</sub> flow to ruffle the liquid but not climb the sides of the vial. The samples  
86 are then stored in the freezer at -20 °C wrapped in aluminium foil until ready to proceed with Step 2.

### 87 **2.3.2 Lipid fractionation (Step 2)**

88 The process involves solid-phase extraction (SPE) using a column holder on a glass tank. New SPE columns are inserted, labelled, and  
89 conditioned by adding acetone and chloroform. The sample is re-dissolved and transferred to the column using a Pasteur pipette.  
90 Neutral lipids and glycolipids are eluted by adding chloroform and acetone, respectively. The solvent is then drained into the tank.  
91 Centrifuge tubes are inserted and labelled, and phospholipids are eluted by adding methanol. SPE columns are dried in a fume hood  
92 before disposal. Phospholipid fractions are dried under compressed N<sub>2</sub> and purged. Samples are stored in the freezer at -20 °C  
93 wrapped in aluminium foil until ready for Step 3. The process ensures accurate and consistent results.

### 94 95 **2.3.3 Lipid methylation (Step 3).**

96 To prepare a sample for GC analysis, start by setting a hot water bath to 37 °C. Prepare 1M acetic acid by dissolving glacial acetic acid  
97 in 1,000 ml dH<sub>2</sub>O, which can be stored at room temperature for up to three months. Prepare a batch of methanolic KOH by dissolving  
98 0.45 g KOH in 40 ml methanol. Adjust the volume of KOH and methanol according to the anticipated batch size. Remove samples  
99 from the freezer and mix them with 0.5 ml chloroform and 0.5 ml methanol, followed by 1.0 ml methanolic KOH. Place sealed  
100 samples in a 37 °C bath for 30 minutes, ensuring the water level is 1-2 mm above the sample liquid. Label small glass vials with  
101 sample IDs, add 2.0 ml hexane, 0.2 ml of 1.0 M acetic acid, and 2.0 ml of dH<sub>2</sub>O. Vortex samples for 30 seconds, centrifuge samples at  
102 226 x g for 2 minutes, and transfer the top phase to clean, labelled vials. Evaporate solvent in a labelled 10-mm glass vial under N<sub>2</sub>  
103 and store samples in the freezer at -20 °C wrapped in aluminium foil until ready for GC analysis.

## 104 **2.4 Gas chromatograph (GC) analysis**

105 The identification and quantification of individual PLFAs can be achieved using a GC connected to an MS detector. The GC internal  
106 standard (ISTD) is prepared by adding MeC10:0 to hexane. The GC is turned on, and the gas supply is ensured. A good calibration is  
107 checked by running calibration standards containing a mix of fatty acids and a hexane blank. The identity of individual fatty acids can

be manually assigned based on retention times or automatically assigned using commercial software. A good calibration includes a flat baseline and no contamination in the hexane rinse. The sample is dissolved in 150 µl of the ISTD solution and transferred into the GC vial.

## 2.5 Representative Results

Fatty acids are identified using a X:Y<sub>ω</sub>Z designation, where X represents carbon atoms, Y represents double bonds, and Z indicates the first double bond position. The suffixes 'c' and 't' indicate geometric isomers, 'a' and 'i' indicate anteiso and iso branching, and Me and OH specify methyl and hydroxyl groups. After the GC run, samples were checked for adequate ISTD and internal standard responses. The areas of different peaks can be imported into a spreadsheet for further processing using:

$$\text{PLFA Content (nmol g}^{-1}\text{)} = \frac{F \times \left(\frac{\text{area PLFA}}{\text{area C10:0}}\right) \times \text{C10:0 std added} \times \left(\frac{\text{C19:0 std added}}{\text{C19:0 sample}}\right)}{\text{sample weight}}$$

The study uses a GC system to characterize soil PLFAs, adjusting for FID selectivity and molarity differences between fatty acids. The peak areas for each identified PLFA are expressed as peak areas, response, or %response. The areas can be assumed to be linearly proportional to the weights of fatty acids or small correction factors can be applied. Results are initially expressed on a weight percent basis, so they need to be normalized to yield molar amounts. Adjusting for molarity differences is achieved by taking into account molecular weights of individual fatty acids.

The amount of ISTD (nmol) added to each sample can be further calculated as:

$$\text{C10:0std added} = [\text{ISTD}] \times V (\text{STD added})$$

where [ISTD] is the concentration (nmol l<sup>-1</sup>) of the MeC10:0 (methyl decanoate) dissolved in hexane (Step 3) and V(STD added) is the volume (L) of prepared ISTD solution, added to each sample prior to the GC run (i.e., 150 µl according to Step 3).

The amount of C19:0 (nmol) present in each sample during GC analysis corresponds to:

$$\text{C19:0 sample} = F \times \left(\frac{\text{area C19:0}}{\text{area C10:0}}\right) \times \text{C10:0 std added}$$

where areaC19:0 is the peak area for C19:0, while the corresponding amount of C19:0 (nmol) added to each sample at the beginning of the PLFA extraction method (cf. Step 3.) is:

$$\text{C19:0 added} = \left(\frac{[19:0]\text{std} \times V(19:0 \text{ std added})}{M_{19:0}}\right) \times 2$$

where [19:0]Std (mg l<sup>-1</sup>) is the concentration of the C19:0 nonadecanoate surrogate standard dissolved in chloroform (Step 3), V(19:0 std added) is the volume of prepared surrogate standard added to each sample at the beginning of the PLFA extraction method (cf. Step 3), and M19:0 is the molecular weight of 1,2-dinonadecanoyl-sn-glycero-3-phosphocholine (PC(19:0/19:0)).

NOTE: One mole of C19:0nonadecanoate surrogate standard yields two moles of C19:0 following the methylation step, while the C10:0 standard is added after methylation. The following PLFAs are typically excluded from analysis of soil microbial communities: i) PLFAs that are <14 C and >20 C in length, and ii) PLFAs with less than 0.5% of total in peak area. Once these PLFAs have been excluded, the responses from all of the remaining PLFAs can be summed to obtain the total PLFA biomass (nmol g<sup>-1</sup> of dry soil).  
Univariate analysis of PLFA data

## 2.6 Data Analysis

The data generated from running the samples in the GC-MS machine were compared to library data and were further analyzed using Principal Component Analysis (PCA) and stacked bar charts to represent microbial composition across study areas. Statistical analysis and correlation matrices were also used to identify relationships between soil properties and PLFA biomarkers.

## 3. 0 RESULTS AND DISCUSSION

149 **3.1 Physicochemical Analysis of Selected Semi-Arid Soil in Nigeria and Niger Republic**

150 The findings from this study highlight significant spatial variation in soil physicochemical properties across the studied locations,  
151 revealing critical insights into soil fertility, nutrient dynamics, and potential agricultural productivity in these regions (Appendix I).

152 **3.2 Soil pH, Organic Carbon (SOC), and Organic Matter (SOM)**

153 The highest mean values for soil pH, SOC, and SOM were recorded in Agadez, suggesting that this location may have more favorable  
154 conditions for organic matter accumulation and microbial activity. In contrast, Zodi, DakiTakwas, and Makera had the lowest  
155 percentages of these parameters, indicating lower soil fertility and possibly reduced organic inputs or higher rates of organic matter  
156 decomposition. The distribution of SOC and SOM also highlights the variability in soil management practices and vegetation cover  
157 across the regions (Appendix I).

158 **3.3 Nitrogen (N) and Phosphorus (P)**

159 The study revealed minimal variation in soil nitrogen across locations, with the highest percentage recorded in Tahoua (0.28%) and  
160 the lowest in Diffa and G/Doki (0.028%). This consistency suggests that nitrogen availability is relatively constrained across the  
161 region, potentially limiting plant growth. However, soil phosphorus exhibited greater variability, with the highest levels observed in  
162 Ndounga (1.17 mg/kg) and the lowest in Agadez (0.485 mg/kg). The disparity in phosphorus levels may reflect differences in soil  
163 parent material, fertilizer application, or crop uptake (Appendix I).

164 **3.4 Cation Exchange Capacity (CEC) and Soil Nutrients**

165 Nkonni consistently showed the highest mean values for key nutrients, including potassium (K), sodium (Na), and calcium (Ca), as  
166 well as the highest cation exchange capacity (16.5 cmol/kg). This suggests that Nkonni soils have a greater ability to retain and  
167 exchange essential nutrients, supporting plant growth. In contrast, Makera and G/Doki exhibited the lowest levels of several key  
168 nutrients, including magnesium (Mg) and calcium (Ca), and a low CEC, indicating limited nutrient retention capacity and lower soil  
169 fertility (Appendix I).

170 **3.5 Soil Texture and Moisture**

171 Soil texture varied widely across the study locations. G/Doki had the highest percentage of sand (87.1%), which corresponds to lower  
172 water-holding capacity and reduced nutrient retention. On the other hand, Nkonni had the highest clay content (35.0%), which is  
173 typically associated with higher water retention and nutrient availability. Locations with higher clay content, such as Nkonni, are  
174 likely to support more stable soil ecosystems compared to sandy soils, which are more prone to leaching and erosion. Soil moisture  
175 content also varied significantly, with Ribah showing the highest levels (13.25%) and Barkiawel the lowest (0.5%). These variations  
176 suggest differences in rainfall, water infiltration, and soil management practices. The standard deviation analysis reveals the variability  
177 of soil properties across locations. Low standard deviation values suggest consistency in parameters such as nitrogen, while high  
178 standard deviations (e.g., for clay content) indicate significant spatial variability. This variability highlights the heterogeneity of soils  
179 within the region, which has implications for land management and agricultural planning(Appendix II).

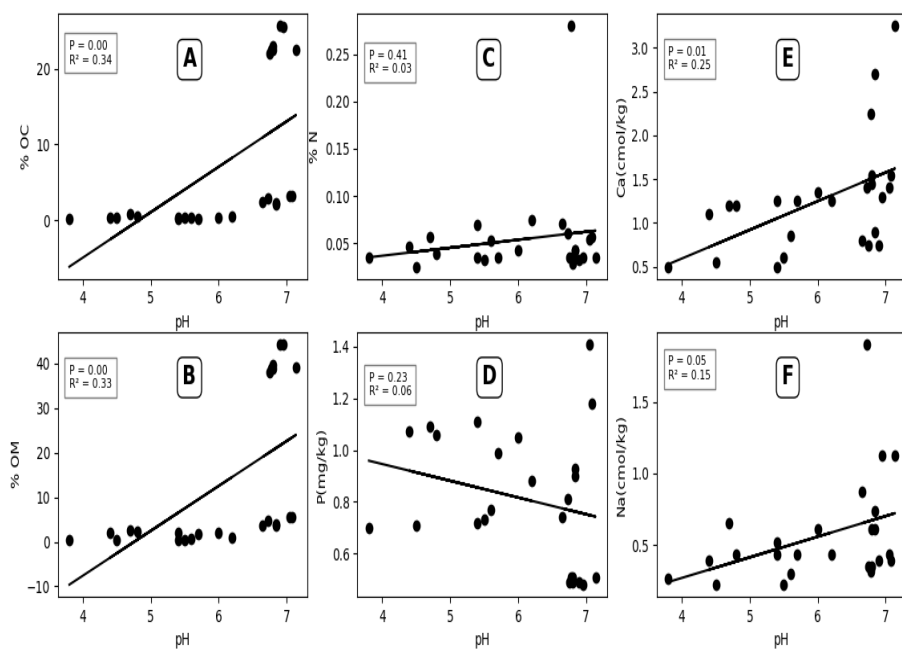
180 The study highlights the influence of soil physicochemical properties on soil fertility and agricultural potential in these regions.  
181 Locations like Nkonni with higher CEC, clay content, and nutrient levels show greater potential for sustained productivity, while areas  
182 with low SOM, SOC, and nutrient availability require significant management interventions. The findings provide valuable baseline  
183 data for informed agricultural decision-making, particularly in the context of climate variability and sustainable land management  
184 practices.

185 Relationship between soil physicochemical properties and soil pH and its impact on PLFA biomarkers were analysed using scatter plots  
186 (A–F) in Figure 2. soil pH may influence certain soil properties (like organic carbon, organic matter, and calcium), but has limited  
187 impact on nitrogen and phosphorus. These in turn determine microbial community abundance through PLFA biomarkers as indicators.  
188 Therefore, statistically significant trends ( $P < 0.05$ ) were observed for % OC, % OM, and Ca. However, weak or non-significant trends  
189 are observed for % N, P, and Na. In a nutshell, The  $R^2$  values across all charts are relatively low, indicating that pH alone is not a  
190 strong predictor of the soil properties, but it does play a meaningful role in some cases. While Organic content (OC, OM) and calcium  
191 are most strongly influenced by pH and could possibly be determinants of microbial diversity.  
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193 *The Nature and Properties of Soils* (14th ed.) by [9], highlights how soil pH can influence the availability of certain nutrients,  
194 particularly calcium, magnesium, and other base cations. It highlights that while soil pH affects organic carbon and organic matter  
195 decomposition rates, the availability of nitrogen and phosphorus is more complex and less directly influenced by pH. Nitrogen  
196 availability is more affected by microbial processes, while phosphorus availability is often limited by chemical reactions that form  
197 insoluble compounds at high and low pH. Jenny's classic work [10] on soil formation explains the relationships between soil pH and  
198 organic matter. Higher pH values often coincide with greater base saturation, which supports the accumulation of organic matter and  
199 organic carbon in soils. However, nitrogen availability is more influenced by organic matter content and microbial mineralization, and  
200 phosphorus availability is strongly controlled by reactions with iron and aluminum oxides, which are less directly affected by pH.  
201 Marschner [11] similarly explains that soil pH strongly influences calcium availability, as calcium is more soluble in neutral to slightly  
202 alkaline conditions. It also describes how pH indirectly affects organic matter decomposition rates and organic carbon content.  
203 However, nitrogen availability is controlled largely by microbial processes such as nitrification and ammonification, which are only  
204 indirectly influenced by pH. For phosphorus, pH can influence availability but typically requires very acidic or alkaline conditions to  
205 have a strong effect. In the same vein, study by Zhao *et al.*[12].indicates that soil pH is significantly correlated with organic carbon  
206 and calcium content in various soils, while its impact on nitrogen and phosphorus is minimal or inconsistent. The findings suggest that  
207 pH influences the microbial and chemical processes affecting organic carbon and calcium, but nitrogen and phosphorus availability  
208 depends more on specific microbial and chemical interactions that are less pH-dependent. These sources support the view that soil pH  
209 is closely related to properties such as organic carbon, organic matter, and calcium availability, but has limited influence on nitrogen  
210 and phosphorus due to the unique microbial and chemical processes governing these nutrients.

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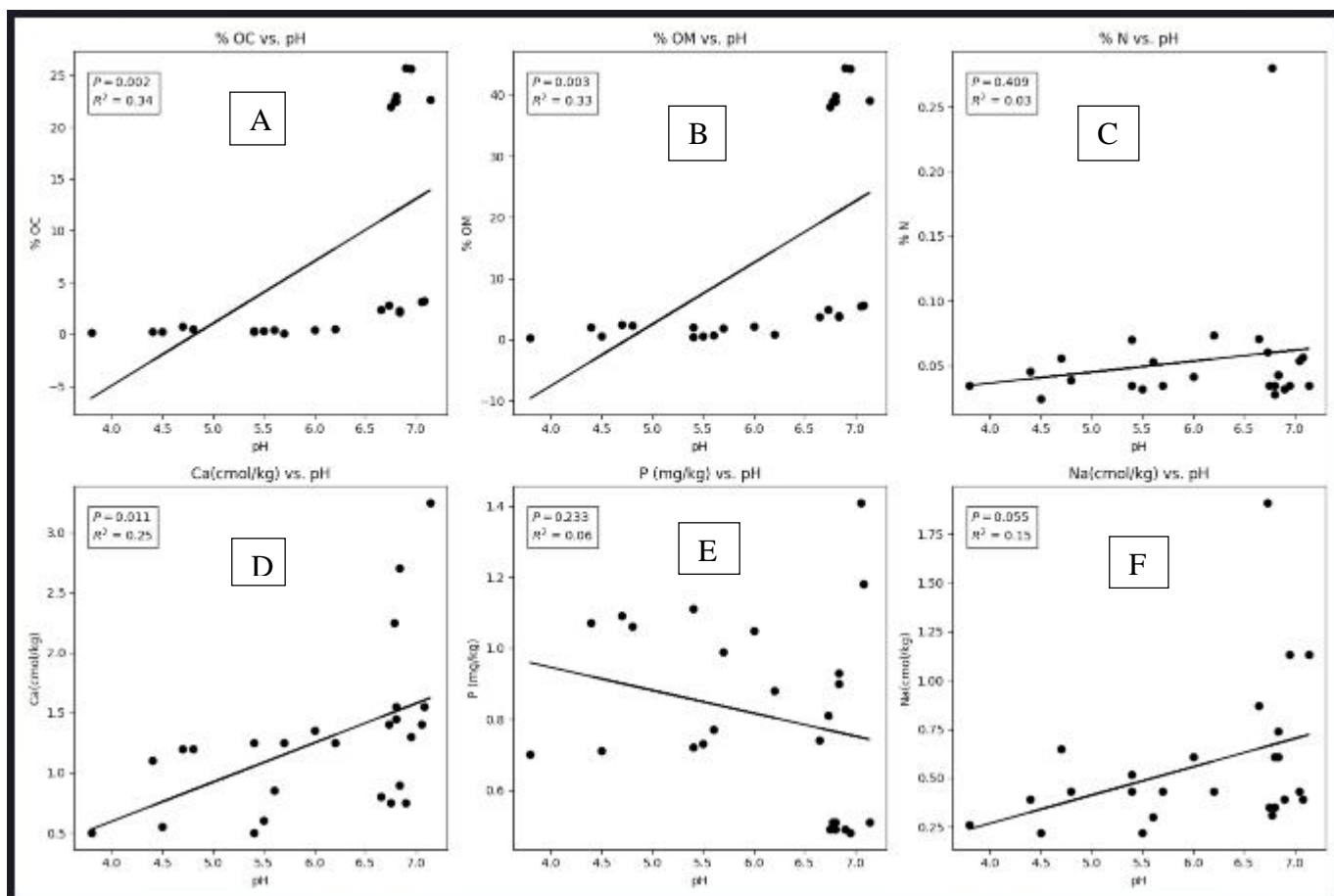
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**Figure 2:** Correlations between soil pH and other soil physiochemical properties and its impact on PLFA biomarkers

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### Key:

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- **Plot A (% Organic Carbon, OC vs. pH):** A significant positive correlation is observed ( $P = 0.002$ ,  $R^2 = 0.34$ : Around 34% of the variation in % OC can be explained by soil pH), indicating that higher soil pH is associated with increased organic carbon levels.
- **Plot B (% Organic Matter OM vs. pH):** Similarly, there is a significant positive correlation ( $P = 0.003$ ,  $R^2 = 0.33$ : 33% of the variation in % OM), suggesting that organic matter increases with soil pH.
- **Plot C (Nitrogen, N vs. pH):** No significant relationship ( $P = 0.409$ ,  $R^2 = 0.03$ : Only 3% of the variation is explained by pH), suggesting that nitrogen content is largely unaffected by changes in soil pH.
- **Plot D (Calcium, Ca vs. pH):** A significant positive correlation ( $P = 0.011$ ,  $R^2 = 0.25$ : 25% of the variation in Ca) shows that calcium availability increases with higher soil pH.
- **Plot E (Phosphorus, P vs. pH):** A weak and insignificant correlation/The relationship is not statistically significant. ( $P = 0.233$ ,  $R^2 = 0.06$ : Only 6% of the variation in P) indicates minimal impact of soil pH on phosphorus levels.
- **Plot F (Sodium, Na vs. pH):** A weak positive correlation/The relationship is borderline statistically significant. ( $P = 0.055$ ,  $R^2 = 0.15$ : 15% of the variation in Na) suggests a slight increase in sodium levels with pH.

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### 3.6 Phospholipid Fatty Acid (PLFA) analysis

A GC-MS machine was used for the detection of PLFAs. Tables 2 and 3 show the locations from which volatile compounds were detected. The retention times and corresponding PLFAs and their peak area (PA). For clarity, not all peaks are presented in the Tables 2 and 3. A total of 110 PLFAs were identified. cyC14:0, cyC16:0, and cyC18:1 was identified from locations like DakiTakwas, Gidandoki, Dundaye, Makera, Agadez, etc., and dihydroxymethyltetradecane (2OH14:0) was detected from Agadez 1 and 2, Barkiawel, Diffa, Maradi, Tahoua, and Zinder 1 and 2, respectively. Cis and 'trans' differentiate the isomeric configurations of the carbon chain at the double bond (C18:1cis9-oleic acid as observed in Makera and Ndounga). The dominance of PLFA hydroxylation was associated with microbial viability; bacteria rich in C16:0, C18:0, and C16:1 $\omega$ 9 favoured higher soil conductivity and nitrate, and other PLFAs contributed more to the organic content. These are samples from Barkiawel, Nkonni, and Tahoua.

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**Table 2:** Volatile Compounds detected from Methyl nonadecanoic Acid (i.e. extraction reagent) for Phospholipid Fatty Acid extraction from agricultural soils of Niger Republic.

RT	Volatile Compounds	FAN
10.552	l-(+)-Lactic acid, tert-butyldimethylsilyl ether	C17:0
14.546	1,3-Diphenyl-4H-1,2,4-triazoline-5-thione	C14:0
14.546	Carbonic acid, dodecyl vinyl ester	C15:0
14.546	2,6-Dihydroxyacetophenone, 2TMS derivative	C15:0

14.546	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C17:0
14.546	1,3-Propanediol, docosyl ethyl ether	C17:0
14.546	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C17:0
14.546	trans-13-Octadecenoic acid, methyl ester	C19:0
14.557	9H-carbazole-3,6-diamine, N3,N3,N6 ,N6-tetramethyl-	C16:0
14.643	Dodecane, 2,6,11-trimethyl-	C15:0
15.85	Fumaric acid, decyl 3-methylbut-3-enyl ester	C19:0
16.262	Tetradecane	C14:0
18.013	Tetradecane, 1-iodo-	C14:0
18.694	2,4-Di-tert-butylphenol	2OH14:0
19.244	Octadecane, 1-iodo	C18:0
19.781	Hexadecane (Benzaldehyde, 4-methoxy-3-(2,6-dimethylphenoxyethyl)-)	C16:0
19.804	Heptadecane	C17:0
20.434	Tetradecanoic acid	C14:0
20.771	Tetradecanoic acid, ethyl ester	C16:0
20.863	Octadecane	C18:0
21.395	Sulfurous acid, butyl tetradecyl ester	C18:0
21.469	Hexadecane, 1-iodo	C16:0
21.875	Nonadecane	C19:0
21.899	cis-Vaccenic acid	C18:1 cis-11
22.082	Hexadecanoic acid, methyl ester	C17:0
22.15	Octadecane	C18:0
22.156	Hexacosane, 1-iodo	C18:0
22.219	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C17:0
22.219	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C17:0
22.334	Palmitoleic acid	C16:1n9
22.471	n-Hexadecanoic acid	C16:0
22.482	n-Hexadecanoic acid	C16:0
22.545	1,2-Benzenedicarboxylic acid, butyl 1,2-methylpropyl ester	C16:0
22.545	1,2-Benzenedicarboxylic acid, butyl 1,2-methylpropyl ester	C16:0
22.591	Dibutyl phthalate	C16:0
22.74	Hexadecanoic acid, ethyl ester	C18:0
22.751	Hexadecanoic acid, ethyl ester	C18:0
23.054	Sulfurous acid, cyclohexylmethyltetradecyl ester	C19:0
23.398	Heptadecanoic acid	C17:0
24.09	Oleic Acid	C18:1n9
24.136	6-Octadecenoic acid	C18:0
24.285	Octadecanoic acid	C18:1cis9
24.33	Sulfurous acid, butyl tetradecyl ester	C18:0
24.359	Octadecanoic acid	C18:0
24.942	Oxalic acid, monomorpholide, undecyl ester	C17:0
25.252	Cyclooctasiloxane, hexadecamethyl	cyC16:0
26.001	Oxalic acid, cyclohexylmethylisohexyl ester	C15:0
26.425	Cyclononasiloxane, octadecamethyl	cyC18:0
26.916	1-benzylindole	C15:0

27.106	Carbonic acid, 2-ethylhexyl nonyl ester			C18:0
27.506	Phenanthro[1,2-b]furan-10,11-dione, 6,7,8,9-tetrahydro-6-(hydroxymethyl)-1,6-dimethyl-, (-)-			C16:0
29.011	Terephthalic acid, di(2-methoxyethyl) ester			2Me14:0
29.515	Oct-3-enoic acid, 2-methyloct-5-yn-4-yl ester			C17:0
34.447	Succinic acid, di(1-cyclopentylethyl) ester			C17:0
35.191	N-Benzyl-N-ethyl-p-isopropylbenzamide	Anthracene,	9,10-dihydro-9,9,10-	C19:0
35.26	trimethyl-			C17:0

**Key:** RT= Retention Time; FAN= Fatty Acid Notation

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**Table3:** Volatile Compounds detected from Methyl nonadecanoic Acid (i.e. extraction reagent) for Phospholipid Fatty Acid extraction from Nigerian agricultural soils.

RT	Volatile Compounds	FAN
5.74	Methyltris(trimethylsiloxy)silane	C14:0
12.6	3,4-Dihydroxybenzyl alcohol, tris(trimethylsilyl)-	C16:0
14.5	2-Thiopheneacetic acid, 3-tridecyl ester	C14:0

14.5	Octadecane, 1-iodo-	C18:0
14.5	Carbonic acid, 2-ethylhexyl nonyl ester	C19:0
17.42	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetra siloxane	C18:1 <sup>tu9</sup>
17.44	Cycloheptasiloxane, tetradecamethyl	cyC14:0
19.31	Tetradecane, 4-ethyl	C16:0
19.38	Cyclooctasiloxane, hexadecamethyl	cyC16:0
19.39	Cyclooctasiloxane, hexadecamethyl	cyC16:0
19.67	Sulfurous acid, cyclohexylmethylnonyl ester	C16:0
19.83	2-Bromotetradecane	C14:0
19.83	2-Bromotetradecane	C14:0
20.04	Methyl tetradecanoate	C15:0
20.77	Dodecanoic acid, ethyl ester	C14:0
21.09	Cyclononasiloxane, octadecamethyl	cyC18:1
21.47	Hexadecane, 1-iodo	C16:0
22.08	Pentadecanoic acid, 14-methyl-, methyl ester	C17:0
22.09	Hexadecanoic acid, methyl ester	C17:0
22.09	Heptadecane	C17:0
22.49	1,3-Propanediol, docosyl ethyl ether	C17:0
22.53	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	C16:0
22.54	Dibutyl phthalate	C16:0
22.74	Hexadecanoic acid, ethyl ester	C18:0
22.75	Hexadecanoic acid, ethyl ester	C18:0
23.01	Oleic Acid	C18:1 <sup>cis9</sup>
23.15	Isopropyl palmitate	C19:0 <sup>i</sup>
23.96	Methyl stearate	C19:0
24.56	Heptadecanoic acid, 15-methyl-, ethyl ester	C17:0
25.25	Hexadecane, 1-iodo-	C16:0
27.09	Carbonic acid, 2-ethylhexyl nonyl ester	C18:0
27.1	Carbonic acid, decyl 2-ethylhexyl ester	C18:0
27.11	Carbonic acid, 2-ethylhexyl nonyl ester	C18:0
27.66	tert-Hexadecanethiol	C16:0
27.67	Tetrahydrofuran-2-ylloxymethylene]-	C19:0
35.00	5-tert-Butyl-2,4-dihydroxybenzophenone, bis(tert-butyl dimethylsilyl) ether	C17:0

Key: RT= Retention Time, FAN = Fatty Acid Notation.

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Based on the Table 2 and Table 3, six (6) key volatile compounds that might be associated with the sorghum rhizosphere microbial community are fatty acids and their derivatives. For instance, fatty acids and esters (Methyl tetradecanoate, Pentadecanoic acid methyl ester and Hexadecanoic acid methyl ester,) i.e. Myristic acid, Pentadecylic acid and Palmitic acid respectively, denoted by C:14, C15:0 and C16:0 are common bio-markers of microbial communities, particularly bacteria in the rhizosphere. These fatty acids play a key role in microbial cell structure in the rhizosphere. This conforms to studies by Zelles [13] that fatty acids such as C16:0 and C17:0 are biomarkers for microbial communities in soil environments. While ~~such as~~ n-Hexadecanoic acid (C16:0) are commonly found in

340 microbial membranes and can indicate microbial activity around plant roots, Octadecanoic acid (C18:0) and Oleic acid (C18:1 $\omega$ 9) are  
341 ~~then~~ often associated with soil bacteria and fungi, indicating general microbial presence and activity. While Palmitoleic acid (C16:1 $\omega$ 9)  
342 however is typically associated with gram-negative bacteria, which are common in nutrient-rich rhizosphere  
343 environments. Frostegård *et al.* [14] supports these findings stating that these fatty acids are commonly found in microbial cell  
344 membranes and serve as biomarkers for soil microbial communities and are often reported in plant rhizospheres, reflecting microbial  
345 diversity and function.

346 The second group of volatiles from the table are the organic acids and esters like the L-(+)-Lactic acid, tert-butyl dimethylsilyl ether  
347 (suggestive of fermentation and microbial metabolism, possibly linked to lactic acid bacteria stimulated by root exudates. Fumaric  
348 acid, decyl 3-methylbut-3-enyl ester were similarly detected which could indicate microbial fermentation processes and interactions  
349 with root exudates. Shi *et al.* [15], paper highlights the role of organic acids, like lactic acid, in shaping the bacterial community  
350 structure in plant rhizospheres which is in line with the present study.

351 The third group of volatiles from the table are the Hydrocarbons and Alkanes such as Tetradecane (C14:0) and Hexadecane (C16:0)  
352 (commonly produced by soil microbes and can serve as energy reserves, often found in the rhizosphere. While Octadecane (C18:0) is  
353 a hydrocarbon indicative of microbial activity and adaptation in the soil environment such as stress tolerance. These hydrocarbons are  
354 associated with soil microbial communities, which produce them as secondary metabolites. Their presence in rhizospheres has been  
355 linked to microbial adaptation to the nutrient-rich environment provided by root exudates. These findings were supported by [16] in a  
356 paper on hydrocarbons reporting hydrocarbons like octadecane, tetradecane, and hexadecane are secondary metabolites produced by  
357 soil bacteria and fungi and how these compounds are often detected in the rhizosphere and can contribute to the soil's organic matter  
358 and their role in microbial metabolic processes. Adding to that, Saini and Kookana, [17] reports describes how hydrocarbons like  
359 tetradecane and hexadecane are found in plant rhizospheres and contribute to microbial metabolic processes. Moreover, Brominated  
360 alkanes such as 2-bromotetradecane can be produced by soil microbes or as degradation products of organic material. They are known  
361 to have antimicrobial properties, which can impact the microbial community structure in the rhizosphere. This conforms to the reports  
362 by Bernards and Lewis [18] that brominated alkanes in soil play important ecological roles in soils and influence microbial  
363 communities.

364 The fourth group are basically derivatives such as Phthalates and Aromatic Compounds like Dibutyl phthalate and 1,2-  
365 Benzenedicarboxylic acid derivatives. Though sometimes these derivatives are considered contaminants, these can also arise from  
366 microbial breakdown of complex organic compounds in the soil. Similarly, 2,4-Di-tert-butylphenol is known for its antimicrobial  
367 properties, which may play a role in microbial competition within the rhizosphere. Wu *et al.* [19] study discusses microbial  
368 degradation of phthalates, including dibutyl phthalate, in soil environments, adding that phthalates are sometimes reported as  
369 environmental contaminants; however, they can also be breakdown products of complex organic compounds in the rhizosphere.  
370 Similarly, certain soil bacteria are capable of degrading phthalates, making them a common feature in rhizosphere soil [20]. Schulz-  
371 Bohm *et al.* [21] review covers various antimicrobial compounds produced in the rhizosphere, including phenolic compounds, which  
372 influence microbial interactions and plant health reporting that 2,4-Di-tert-butylphenol as a compound known for its antimicrobial  
373 properties and has been observed in microbial communities that interact with plant roots. It can play a role in microbial competition  
374 within the rhizosphere.

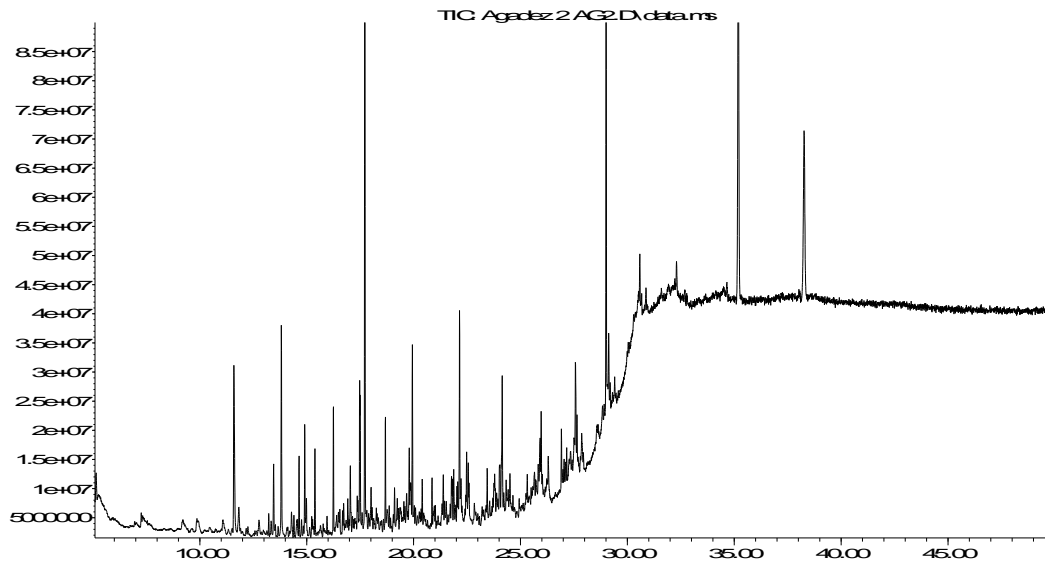
375 The fifth group are the Sulfur-containing esters such as Sulfurous acid, butyl tetradecyl ester (possibly linked to sulfur cycling  
376 microbes), which are important for nutrient availability in the rhizosphere. According to the findings of Friesen *et al.* [15], sulfur-  
377 containing compounds are linked to sulfur-cycling microbes, which are important for nutrient availability in the rhizosphere, adding  
378 that sulfur metabolism is a significant process in plant-microbe interactions including their impact on plant health. Sulfurous acid  
379 derivatives in the soil according to a report by Marschner and Rengel, [16], are associated with sulfur-cycling microbes, which  
380 contribute to nutrient availability for plants and are also known to influence microbial activity in the rhizosphere. Similarly, Niger  
381 agricultural soils which drought impacted soils harbor other Sulphur containing compounds such as thiols. Mukhopadhyay and  
382 Stähler [17] work highlights Thiols, like tert-hexadecanethiol, contain sulfur which are involved in microbial sulfur metabolism. They  
383 are often detected in soil and rhizosphere environments, where they play roles in microbial interactions and stress responses.

384 Siloxanes (Methyltris(trimethylsiloxy)silane, Cycloheptasiloxane, Cyclooctasiloxane, Cyclononasiloxane) are the last group of  
385 volatiles detected from Niger agricultural soils. Van-Dongen *et al.*, [18] study highlights the occurrence and potential functions of  
386 siloxanes in soil and plant rhizospheres adding that Siloxanes are organosilicon compounds commonly produced by soil microbes and  
387 can be found in rhizosphere environments. They are often associated with microbial adaptation to different environmental conditions  
388 and may indicate microbial diversity.

389 Therefore, the key volatiles in the sorghum rhizosphere include a mix of fatty acids, organic acids, hydrocarbons, phthalates, and  
390 sulfur-containing compounds. These volatiles suggest active microbial communities involved in nutrient cycling, fermentation, and

391 interactions with sorghum root exudates. This profile reflects a diverse microbial environment that supports soil health and plant  
392 growth in the rhizosphere.  
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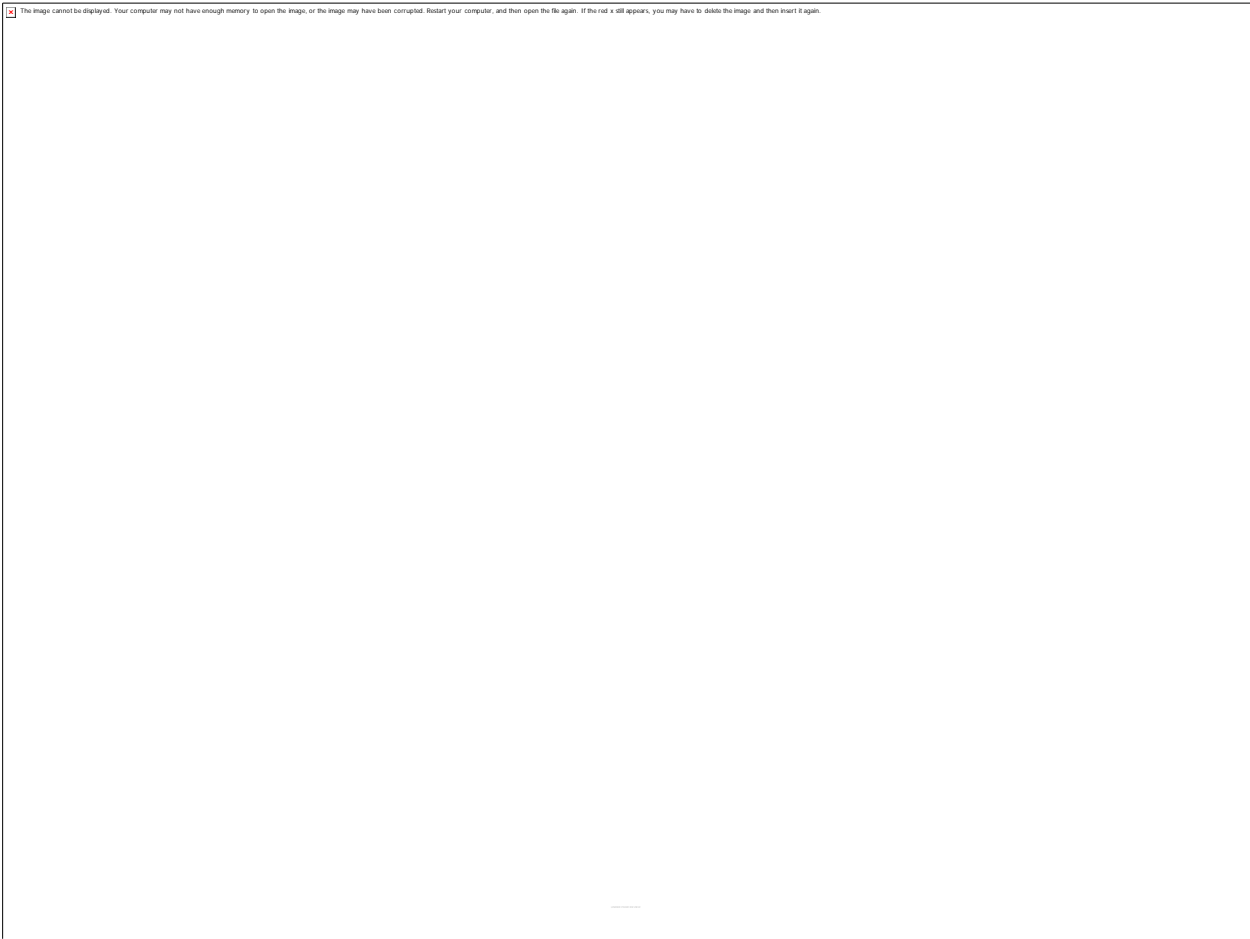


Time->

Figure 3: Representative GCMS chromatogram (Agadez 2) showing peak areas of microbial abundance eluted against time

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474 Retention times and corresponding PLFAs and their peak area (pA) are indicated in Figure 3 for representative peaks. For clarity, not  
475 all peaks are indicated on the figure, although this particular sample yielded 74 identified PLFAs. Figure 3 presents a representative  
476 sample run. The large hexane solvent peak characteristically appears at a retention time (RT) around 26.9 min. The ISTD standard  
477 peak (C10:0) appears at a RT of 5.1 min, while C19:0 has a RT of 19.8 min. The GC analysis separates the PLFAs based on their  
478 chain length, with longer chains eluting more slowly; for instance, C18:0 elutes at 18.2 min while C16:0 elutes at 11.8 min. In  
479 addition, this analytical protocol can separate PLFAs based on their degree of unsaturation and the position of their double bond; for  
480 example, C18:0 elutes at 18.2 min, while C18:1 $\omega$ 9 (Table 2) and cyC18:1 (Table 3) elute at 24.09 min and 21.09 min, respectively.  
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**Figure 4:** A Stacked barchart showing PLFA profiles across the different soils for Niger.

Key:

Ag1= Agadez 1 Ag2= Agadez 2 BK= Barkiawel DF= DiffaMr=Maradi

Nd= NdoungaNk= NkonniTh= Tahoua Zd1=Zinder 1 Zd2= Zinder 2

- C18: 1cis9 (oleic acid) is evidence of naturally occurring fatty acids- the phospholipids that make membranes bacteria/fungal PLFA.
- Cyclo-fatty acid (as in cyC17:0 and cyC19:0) show that G- bacteria are associated with such PLFAs and the content in the membrane of G- bacteria may allow them to withstand certain environmental conditions (drought stress).

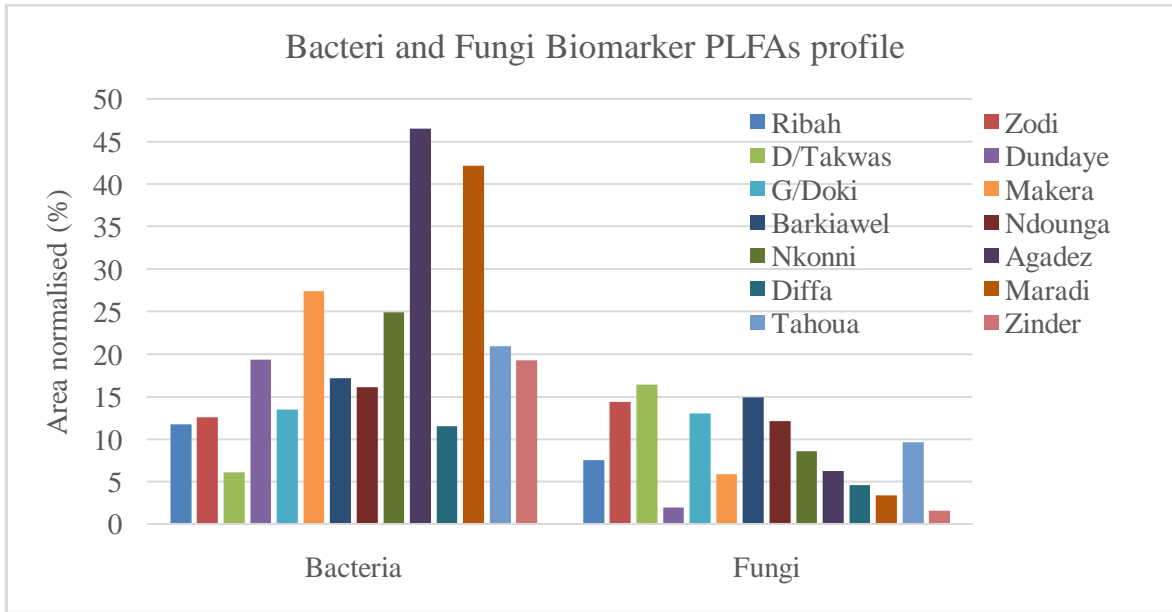


**Figure 5:** Stacked barchart showing PLFA profiles across the different soils for Nigeria.

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- Key:**
- DakiTakwas: The PLFA composition is mainly C18:1u9 (Purple) and C15:0 (Red), with a high diversity of around 10 different PLFAs.
  - Dundaye: Dominated by C18:1u9 (Purple) and C15:0 (Red), with a moderate diversity of around 8 different PLFAs.
  - Gidan Doki: More balanced distribution among various PLFAs, with a notable amount of C18:0 (Dark Blue) and C18:1u9 (Purple), and around 9 different PLFAs.
  - Makera: Highest in diversity with about 11 different PLFAs, and a significant presence of C18:1u9 (Purple) and C16:0 (Green).
  - Ribah: Lower in both total PLFAs and diversity, with prominent C18:1u9 (Purple).
  - Zodi: Characterized by a high presence of C18:1u9 (Purple) and C16:0 (Green), with a moderate diversity of around 6 different PLFAs.

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Y-Axis (left) in Figure 4 represents the percentage of total phospholipid fatty acids (PLFAs) for each category. While the Y-Axis (right) represents the number of distinct PLFAs in each location. The X-axis lists different locations where soil samples were collected. The locations are DakiTakwas, Dundaye, GidanDoki, Makera, Ribah, and Zodi. Locations like Makera (Figure 5 ) have a high diversity of PLFAs, indicating a richer microbial environment. Some locations have specific PLFAs that dominate the profile, such as C18:1u9 in Ribah. The combined bar and line graph provide a comprehensive view of both the composition and diversity of PLFAs across different locations. Therefore, Figure 3 and 4 effectively show both the distribution of specific PLFAs and the diversity of these PLFAs across various locations, providing insights into the microbial composition and potential environmental factors influencing these locations.



**Figure 6:** Bacteria and Fungi PLFA Biomarkers in nmol/g from soil samples across all locations.

Key:

- Dundaye soils have more bacteria PLFA's than fungi Zamfara (D/Tawas) soils harbour more Fungi PLFA'S compared to bacteria.
- Agadez and Maradi samples had the highest abundance of bacteria PLFA's

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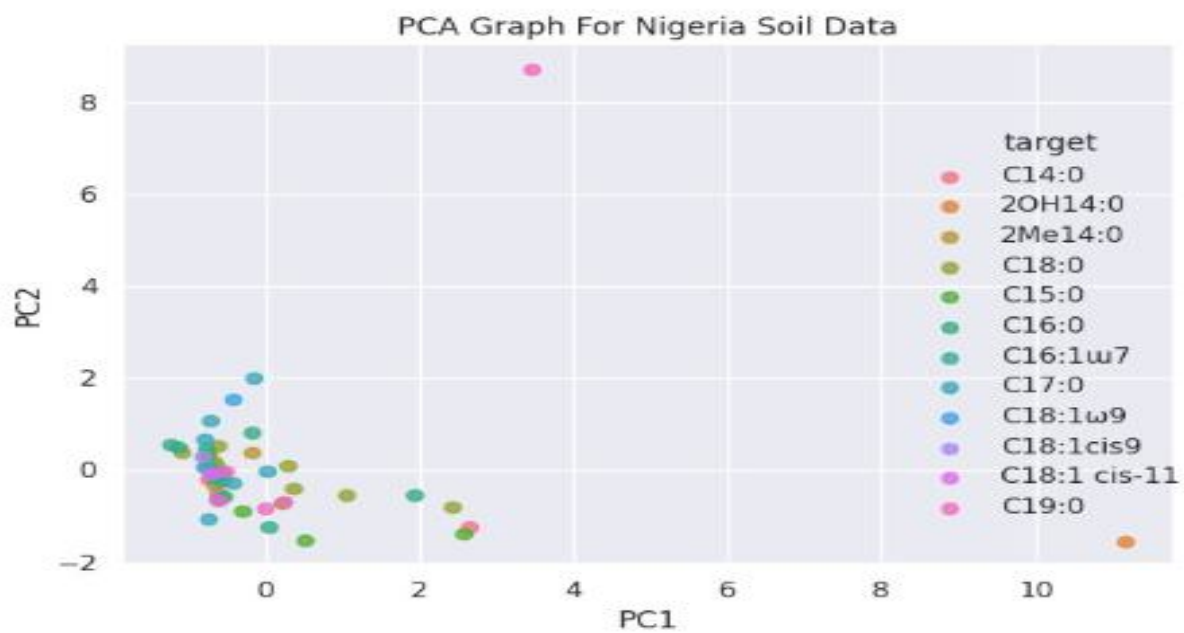


Figure 7: Principal Component Analysis (PCA) for Nigerian Soil PLFAs

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652 PCA graph was used to represents a Principal Component Analysis (PCA) of soil data collected from Nigeria (Figure 7). The PC2  
653 (Principal Component 2) i.e. the vertical axis (y-axis) was plotted against PC1 (Principal Component 1) - the horizontal axis (x-axis).  
654 The x-axis represents the first principal component. This component captures the maximum variance in the dataset. The vertical axis  
655 (y-axis) - PC2 (Principal Component 2) represents the second highest variance in the dataset, orthogonal to the first component. PC1  
656 explains the largest portion of the variance, while PC2 explains the second largest portion. By plotting PC1 against PC2, we can  
657 capture a significant amount of the data's variability in a two-dimensional plot, where each point on the graph represents a soil sample  
658 from the dataset. Similarly, the coordinates of each point are determined by its values on the first two principal components (PC1 and  
659 PC2). The legend indicates points which are colored based on the "target" variable, which represents different categories of PLFA  
660 biomarkers. For example, C14:0 is represented by pink, 2OH14:0 by orange, 2Me14:0 by green, and so on. Clusters points that are  
661 close to each other indicate that those samples have similar characteristics in terms of the variables used for PCA. The outliers' points  
662 that are far away from others, like the pink point (labeled C14:0) at (approximately) (4, 8), indicate a sample that is significantly  
663 different from the rest in terms of the principal components. Therefore, tight clustering of points in the lower left part of the graph  
664 suggests that many samples share similar properties. Similarly, the separation of points by color indicates how different categories of  
665 soil samples (based on fatty acid composition) relate to each other. The PCA therefore, is important as it reduces the complexity of the  
666 data by transforming it into a set of principal components, which are new variables that represent most of the variability in the data.  
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**Figure 8:**Principal Component Analysis (PCA) for Niger Soil PLFAs

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731 PCA plot derived from soil data collected in Niger (Figure 7) similarly indicate the first principal component (PC1), which captures  
732 the largest amount of variance in the dataset on the x-axis independent of the second largest amount of variance on the y-axis which  
733 represents the PC2 (Principal Component 2). Each point in the scatter plot (Figure 8) represents a soil sample from the dataset. The  
734 coordinates of each point are determined by its scores on the first two principal components (PC1 and PC2). For Niger samples, points  
735 are similarly color-coded which represents different categories of PLFA biomarkers. For example, cyC14:0: Pink, C14:0: Orange,  
736 C15:0: Yellow, C16:0: Green, cyC16:0: Cyan, C17:0: Dark Cyan, C18:0: Blue, C18:1u9: Purple, C18:1: Light Blue, C18:1cis9: Light  
737 Purple, C19:0: Pink (Different shade from cyC14:0 for distinction). Cluster points indicate that samples have similar characteristics in  
738 terms of the variables used for PCA. The outliers that are far away from others, such as the orange point at approximately (-3, 4),  
739 indicate a sample that is significantly different from the rest in terms of the principal components. Therefore, clustering of points by  
740 color indicates how different categories of soil samples (based on fatty acid composition) relate to each other. The orange outlier at (-  
741 3, 4) suggests that this particular sample, labeled "C14:0", is quite different from the others in the dataset in terms of the principal  
742 components. The general spread of points indicates the variance captured by the first two principal components, with most points  
743 centered around the origin and a few spread out.  
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#### 745 4. CONCLUSION

746 The presence of volatile compounds in agricultural soils in Niger and Nigeria, particularly in the sorghum rhizosphere, is influenced  
747 by soil properties such as organic carbon, soil pH, and organic matter content. Soil pH is crucial for nutrient availability and microbial  
748 activity, while organic matter is a source of carbon-based volatiles. Balancing pH and organic content is essential for sustaining  
749 productive soils in sorghum cultivation systems. Variations in soil properties across locations necessitate site-specific soil  
750 management strategies. A decrease in Gram-negative bacteria reflects stress from reduced water availability, while an increase in  
751 Gram-positive bacteria indicates resilience mechanisms. The imbalance between Gram-positive and Gram-negative populations and  
752 transition toward fungal dominance (such as C18:1ω9 and C18:1cis9) which can negatively impact soil health, leading to reduced  
753 fertility and altered decomposition rates. Additionally, drought stress promotes PLFA markers associated with microbial stress  
754 tolerance and dormancy (e.g., cy17:0, i17:0, cy19:0), reflecting a microbial strategy to endure harsh conditions. Therefore, Locations  
755 with low SOC, SOM, and nutrients, such as Zodi, DakiTakwas, and Makera, may require targeted organic and inorganic amendments  
756 to enhance soil fertility. High clay content in Nkonni (35.0%) may require improved drainage management to avoid waterlogging,  
757 while sandy soils in G/Doki (87.1%) would benefit from organic amendments to improve water retention and nutrient-holding  
758 capacity.

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767 Disclaimer (Artificial intelligence)

768 Option 2:

769 Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or  
770 editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well  
771 as all input prompts provided to the generative AI technology

772 Details of the AI usage are given below:

1. Author(s) hereby declare that generative AI technologies such as chatgpt -4o was used in interpretation of some charts such as Principal Component Analysis Charts and stacked bar charts. All the experiments for this research were performed on Intel Core i5-8250U CPU @ 1.60GHz × 8 processor with 8GB RAM. Ubuntu 20.04 64bit operating system was also used. The following are the software specifications and libraries used in the research;

1. Python 3.10 – a general purpose programming language usually used in machine learning problems.
2. Pandas – a python package for reading dataset.
3. Geopandas (<https://geopandas.org/en/stable>)
4. Matplotlib
5. Sk-learn – a python package for scientific calculation. It was used in this research for splitting dataset.
6. Pytorch - a python package for neural network.
7. Naive Bayes Classifier from Scratch in Python

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## APENDICES

### Appendix I: Standard Deviation and Data Distribution of Soil Nutrients Levels obtained from all Locations.

Location	Ph	% OC	% OM	% N	P (mg/k)	Ca (cmol/kg)	Mg (cmol/kg)	K (cmol/k)
Agadez	6.93 ±0.04	25.65±0.07	44.35±0.13	0.03 ±0.00	0.49±0.01	1.03 ±0.39	2.95±0.35	0.19
Barkiawel	6.87±0.30	2.84±0.60	4.67±1.37	0.06±0.01	0.96±0.31	1.18±0.53	0.93±0.32	0.45
D/Takwas	5.85±0.21	0.25±0.18	1.98±0.18	0.04±0.01	1.02±0.04	1.30±0.07	1.25±0.07	0.18
Diffa	6.80±0.00	22.5±0.00	38.9±0.00	0.03±0.00	0.51±0.00	1.45±0.00	3.00±0.00	0.15
Dundaye	5.90±0.42	0.43±0.07	0.75±0.12	0.06±0.02	0.83±0.08	1.05±0.28	0.63±0.32	0.53
G/Doki	5.00±0.71	0.30±0.03	0.52±0.05	0.03±0.01	0.72±0.01	0.58±0.04	0.70±0.07	0.21

Makera	4.60±1.13	0.21±0.04	0.36±0.07	0.04±0.00	0.71±0.01	0.50±0.00	0.70±0.07	0.27
Maradi	6.75± 0.00	22.0±0.00	38.04±0.0	0.04±0.00	0.49±0.00	0.75±0.00	2.40±0.00	0.1
Ndounga	6.95±0.15	2.71±0.63	4.68±1.09	0.05±0.01	1.17±0.34	1.15±0.35	0.78±0.18	0.44
Nkonni	6.79±0.08	2.48±0.48	4.29±0.83	0.05±0.01	0.86±0.06	2.05±0.92	2.35±0.57	0.78
Ribah	4.75±0.07	0.63±0.18	2.36±0.18	0.05±0.01	1.08±0.02	1.20±0.00	1.30±0.14	0.31
Tahoua	6.78±0.00	22.5±0.00	38.9±0.00	0.28±0.00	0.51±0.00	2.25±0.00	3.10±0.00	0.18
Zinder	6.97±0.24	22.8±0.28	39.43±0.49	0.04±0.00	0.50±0.01	2.40±1.20	2.95±1.06	0.35
Zodi	4.90±0.71	0.3±0.028	2.03±0.03	0.06±0.02	1.09±0.03	1.18±0.11	1.58±0.04	0.36

**Key:**

- **Standard deviation analysis reveals soil properties' variability across locations, with low values indicating consistency (e.g. in nitrogen).**
- **High standard deviation values indicate significant spatial variability.**

**Appendix II: Standard Deviation and Data Distribution of Soil Physiochemical Properties obtained from all Locations**

Location	Na (cmol/kg)	CEC (cmol/kg)	% Sand	% Silt	% Clay	% Moisture	
Agadez	0.76±0.52	8.70±3.54	76.50±2.12	19.50±2.12	4.00±0.00	2.25	±0.35
Barkiawel	0.63±0.34	3.10±0.42	59.50±8.49	25.5±1.41	15.0±7.07	0.50	±0.00
D/Takwas	0.52±0.13	9.50±0.42	72.35±1.34	17.45±1.34	10.20±0.0	4.50	±1.41
Diffa	0.35±0.00	6.60±0.00	71.00±0.00	22.0±0.00	7.00±0.0	2.50	±0.00
Dundaye	0.37±0.09	10.5±1.84	80.20±4.10	10.6±2.69	9.20±1.41	4.00	±0.72
G/Doki	0.22±0.00	6.70±0.42	87.10±0.00	4.70 ±0.00	8.20±0.00	2.50	±0.71

Makera	0.35±0.12	7.70±0.71	85.10±2.83	6.70 ±2.83	8.20±0.0	1.50	±0.00
Maradi	0.35±0.00	6.20±0.00	71.00±0.00	19.00±0.00	10.0±0.00	4.50	±0.00
Ndounga	0.52±0.13	3.00±0.57	61.55±9.97	21.9±19.16	16.5±9.19	1.25	±1.06
Nkonni	1.33±0.83	16.5±2.12	43.7±21.43	21.4±13.93	35±35.36	1.25	±1.06
Ribah	0.54±0.16	9.80±0.57	63.50±0.00	22.40±0.00	14.1±0.00	13.3	±0.35
Tahoua	0.31±0.00	9.80±0.00	75.00±0.00	21.00±0.00	4.00±0.00	2.4	±0.00
Zinder	0.87±0.37	7.00±1.41	78.00±0.00	18.00±0.00	4.00±0.00	7.3	±6.72
Zodi	0.46±0.09	11.1±2.12	65.5±2.828	24.3±2.828	10.20±0.00	7.0	±2.83

**Key:**

- **Standard deviation analysis reveals soil properties' variability across locations, with low values indicating consistency.**
- **High standard deviation values indicate significant spatial variability.**

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