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Evaluation of Presumed Drought Impacted Soils through Phospholipid Fatty Acid (PLFA) Biomarkers in the Sahel Region

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study is to utilize biomarkers to detect drought induced changes in soil microbial communities within presumed drought impacted soils in selected regions of the Sahel.
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 and Phosphorus), while PLFA biomarkers were analysed using principal component analysis and descriptive statistics.
Place and Duration of Study: The study, conducted from May to October 2021, focused on the Sahel region of sub-Saharan Africa, specifically within the territorial boundaries of Nigeria and the Niger Republic.

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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 highlights the significance of site-specific soil management techniques, particularly in regions characterized by low nutrient levels and depleted soil organic matter (SOC) and carbon (SOM). In such regions, targeted applications of organic and inorganic amendments can be necessary 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 fungi, 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 fungi. 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.

Keywords: Drought; PLFA biomarkers; nutrient levels; Sahel region.

1. INTRODUCTION

Drought is a recurring event in arid regions. Improved land use and management practices are essential to mitigating its effects. Studies have shown that while climate change poses challenges, human land-use practices have a larger, more immediate impact on desertification [1]. Human and livestock activity has a more significant impact on arid environments than anticipated climatic changes [2]. Future climate change is likely to have significant feedback effects on soil respiration through alterations in vegetation structure, particularly in heterogeneous ecosystems like Mediterranean shrublands and deserts. According to Talmon et al. [3], there is interconnectedness of precipitation, vegetation, and soil carbon processes, with important implications for understanding dryland ecosystem responses to global climate change.

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

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

2. MATERIALS AND METHODS

2.1 Study Area

The study surveyed fields in the Sahel region, bordering Nigeria and the Niger Republic. These

areas were purposely and randomly selected due to the semi-arid nature of the locations. A mobile topographer application was used to capture the geographical details of each location, and the coordinates of latitude and longitude points were recorded (Table 1). These recorded points were later trained into *GeoPandas* and *Mapplotlib* to capture the probable exact locations of points for sample collection on the map as reference points for further research (Fig. 1).

Table 1. Geographic coordinates of the study areas

S/No.	Location point	Latitute (^{O})	Longitude (^{O})	Altitude (m)	Temperature (^{O})	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.	Gidan doki	13.138	5.191	271.21	29	8.55
17.	Gidan doki	13.138	5.191	272.93	30	9.35
18.	Gidan doki	13.138	5.190	271.18	31	9.45

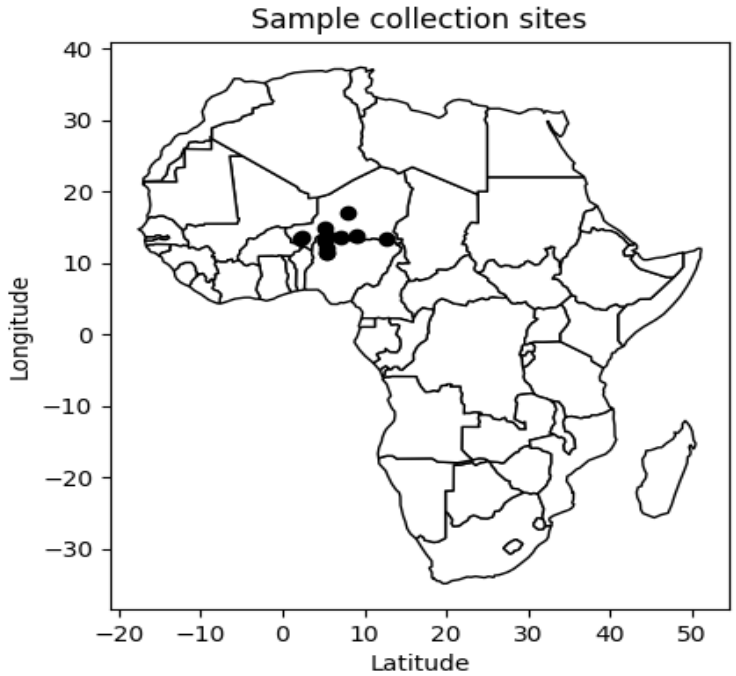

Fig. 1. Map of Africa showing sample collection sites across the borders of Nigeria-Niger Republic

2.2 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, Daki Takwas, 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: Gidan Doki, Makera, and Dundaye (July 30 to August 1). While Agadez 1 and Agadez 2 (Adderbisonnét), 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.

2.3 Protocol for PLFA Analysis

2.3.1 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.

2.3.2 Preparation of glassware for analysis

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

2.3.3 Collection and processing of soil samples prior to PLFA analysis

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

2.4 PLFA Technique Steps

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

2.4.1 Extraction (Step 1)

The process involves preparing a solution of KOH and citrate buffer, which are then adjusted to a pH of 4.00 ± 0.02 by adding 5.0 M 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 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, followed by a second round of extractant and centrifugation at 226 x g for 15 minutes. The supernatant was transferred to a labelled 45-ml glass vial, and the remaining samples are added to the same vials. The samples are then placed under compressed N_2 to avoid oxidation. Chloroform was evaporated off slowly, setting the N_2 flow to ruffle the liquid but not climb the sides of the vial. The samples were then stored in the freezer at -20ºC wrapped in aluminium foil until ready to proceed with Step 2.

2.4.2 Lipid fractionation (Step 2)

The process involves solid-phase extraction (SPE) using a column holder on a glass tank. New SPE columns are inserted, labelled, and conditioned by adding acetone and chloroform. The sample was re-dissolved and transferred to the column using a Pasteur pipette. Neutral lipids and glycolipids were eluted by adding chloroform and acetone, respectively. The solvent was then drained into the tank. Centrifuge tubes were inserted and labelled, and phospholipids were eluted by adding methanol. SPE columns were dried in a fume hood before disposal. Phospholipid fractions were dried under compressed N_2 and purged. Samples were stored in the freezer at -20 ºC wrapped in aluminium foil until ready for Step 3. The process ensured accurate and consistent results.

2.4.3 Lipid methylation (Step 3)

To prepare a sample for GC analysis, start by setting a hot water bath to 37 ºC. Prepare 1M acetic acid by dissolving glacial acetic acid in 1,000 ml dH2O, which can be stored at room temperature for up to three months. Prepare a batch of methanolic KOH by dissolving 0.45 g KOH in 40 ml methanol. Adjust the volume of KOH and methanol according to the anticipated batch size. Remove samples 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 samples in a 37 ºC bath for 30 minutes, ensuring the water level is 1-2 mm above the sample liquid. Label small glass vials with sample IDs, add 2.0 ml hexane, 0.2 ml of 1.0 M acetic acid, and 2.0 ml of dH_2O. Vortex samples for 30 seconds, centrifuge samples at 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_2 and store samples in the freezer at -20 ºC wrapped in aluminium foil until ready for GC analysis.

2.5 Gas Chromatograph (GC) Analysis

The identification and quantification of individual PLFAs can be achieved using a GC connected to an MS detector. The GC internal standard (ISTD) was prepared by adding MeC10:0 to hexane. The GC was turned on, and the gas supply was ensured. A good calibration was 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 determined using commercial software. A good calibration includes a flat baseline and no contamination in the hexane rinse. The sample was dissolved in 150 µl of the ISTD solution and transferred into the GC vial.

2.6 Representative Results

Fatty acids were identified using a X:YωZ 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:

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

The study used a GC system to characterize soil PLFAs, adjusting for FID selectivity and molarity differences between fatty acids. The peak areas for each identified PLFA were 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 were 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:

C10:0std added = [ISTD] × V (STD added)

where [ISTD] is the concentration (nmol l^{-1}) of the MeC10:0 (methyl decanoate) dissolved in hexane (Step 3) and V(STD added) was 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:

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

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

$$\text{C19:0 added} = \left(\frac{[19{:}0]std\ x\ V(19{:}0\ std\ added)}{M19{:}0}\right) \text{ x 2}$$

where [19:0]Std (mg L^{-1}) 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 was the molecular weight of 1,2-dinonadecanoyl-sn-glycero-3-phosphocholine (PC(19:0/19:0)).

NOTE: One mole of C19:0 nonadecanoate surrogate standard yields two moles of C19:0 following the methylation step, while the C10:0 standard was added after methylation. The

following PLFAs were 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-1 of dry soil).

2.7 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 a 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. RESULTS AND DISCUSSION

3.1 Physicochemical Analysis of Selected Semi-arid Soil in Nigeria and Niger Republic

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

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

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

3.3 Nitrogen (N) and Phosphorus (P)

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

3.4 Cation Exchange Capacity (CEC) and Soil Nutrients

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

3.5 Soil Texture and Moisture

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

The study highlights the influence of soil physiochemical properties on soil fertility and agricultural potential in these regions. Locations like Nkonni with higher CEC, clay content, and

nutrient levels show greater potential for sustained productivity, while areas with low SOM, SOC, and nutrient availability require significant management interventions. The findings provide valuable baseline data for informed agricultural decision-making, particularly in the context of climate variability and sustainable land management practices.

Relationship between soil physiochemical properties and soil pH and its impact on PLFA biomarkers were analysed using scatter plots (A–F) in Fig. 2. soil pH may influence certain soil properties (like organic carbon, organic matter, and calcium), but has limited impact on nitrogen and phosphorus. These in turn determine microbial community abundance through PLFA biomarkers as indicators. Therefore, statistically significant trends ($P<0.05$) were observed for % OC, % OM, and Ca. However, weak or non-significant trends 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 strong predictor of the soil properties, but it does play a meaningful role in some cases. While Organic content (OC, OM) and calcium are most strongly influenced by pH and could possibly be determinants of microbial diversity.

The Nature and Properties of Soils (14th ed.) by Brady and Weil [9], highlights how soil pH can influence the availability of certain nutrients, particularly calcium, magnesium, and other base cations. It highlights that while soil pH affects organic carbon and organic matter decomposition rates, the availability of nitrogen and phosphorus is more complex and less directly influenced by pH. Nitrogen availability is more affected by microbial processes, while phosphorus availability is often limited by chemical reactions that form insoluble compounds at high and low pH. Jenny's classic work [10] on soil formation explains the relationships between soil pH and organic matter. Higher pH values often coincide with greater base saturation, which supports the accumulation of organic matter and organic carbon in soils. However, nitrogen availability is more influenced by organic matter content and microbial mineralization, and phosphorus availability is strongly controlled by reactions with iron and aluminum oxides, which are less directly affected by pH. Marschner [11] similarly explains that soil pH strongly influences calcium availability, as calcium is more soluble in neutral to slightly alkaline conditions. It also describes how pH indirectly affects organic matter decomposition rates and organic carbon content. However, nitrogen availability is controlled largely by microbial processes such as nitrification and ammonification, which are only indirectly influenced by pH. For phosphorus, pH can influence availability but typically requires very acidic or alkaline conditions to have a strong effect. In the same vein, study by Zhao et al. [12]. indicates that soil pH is significantly correlated with organic carbon and calcium content in various soils, while its impact on nitrogen and phosphorus is minimal or inconsistent. The findings suggest that pH influences the microbial and chemical processes affecting organic carbon and calcium, but nitrogen and phosphorus availability depends more on specific microbial and chemical interactions that are less pH-dependent. These sources support the view that soil pH is closely related to properties such as organic carbon, organic matter, and calcium availability, but has limited influence on nitrogen and phosphorus due to the unique microbial and chemical processes governing these nutrients.

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 Daki Takwas, Gidan doki, Dundaye, Makera, Agadez, etc., and dihydroxymethyl tetradecane (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 C16:1ω9 favoured higher soil conductivity and nitrate, and other PLFAs contributed more to the organic content. These are samples from Barkiawel, Nkonni, and Tahoua.

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 PLFAs such as n-Hexadecanoic acid (C16:0) are commonly found in microbial membranes and can indicate microbial activity around plant roots, Octadecanoic acid (C18:0) and Oleic acid (C18:1ω9) are ften often associated with soil bacteria and fungi, indicating general microbial presence and activity. While Palmitoleic acid (C16:1ω9) however, is typically associated with gram-negative bacteria, which are common in nutrient-rich rhizosphere environments. Frostegård et al. [14] supports these findings stating that these fatty acids are commonly found in microbial cell membranes and serve as biomarkers for soil microbial communities and are often reported in plant rhizospheres, reflecting microbial diversity and function.

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-dimethylphenoxymethyl)-)	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)d eca-6,9-diene-2,8-dione	C17:0
22.334	Palmitoleic acid	C16:1ɯ9
22.471	n-Hexadecanoic acid	C16:0
22.482	n-Hexadecanoic acid	C16:0
22.545	1,2-Benzenedicarboxylic acid, buty l 2-methylpropyl ester	C16:0
22.545	1,2-Benzenedicarboxylic acid, buty l 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, cyclohexylmethyl tetradecyl ester	C19:0
23.398	Heptadecanoic acid	C17:0

RT	Volatile Compounds	FAN
24.09	Oleic Acid	C18:1ω9
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, cyclohexylmethyl isohexyl 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

Table 3. 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	C18:1ω9
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, cyclohexylmethyl nonyl 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*cis*9
23.15	Isopropyl palmitate	C19:0i
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

RT	Volatile Compounds	FAN
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-yloxymethylene]-	C19:0
35.00	5-tert-Butyl-2,4-dihydroxybenzophenone, bis(tert-butyldimethylsilyl) ether	C17:0

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

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

The third group of volatiles from the Table 3 are the Hydrocarbons and Alkanes such as Tetradecane (C14:0) and Hexadecane (C16:0) (commonly produced by soil microbes and can serve as energy reserves, often found in the rhizosphere. While Octadecane (C18:0) is a hydrocarbon indicative of microbial activity and adaptation in the soil environment such as stress tolerance. These hydrocarbons are associated with soil microbial communities, which produce them as secondary metabolites. Their presence in rhizospheres has been linked to microbial adaptation to the nutrient-rich environment provided by root exudates. These findings were supported by Li et al. [16] in a paper on hydrocarbons reporting hydrocarbons like octadecane, tetradecane, and hexadecane are secondary metabolites produced by soil bacteria and fungi and how these compounds are often detected in the rhizosphere and can contribute to the soil's organic matter and their role in microbial metabolic processes. Adding to that, Saini and Kookana, [17] reports describes how hydrocarbons like tetradecane and hexadecane are found in plant rhizospheres and contribute to microbial metabolic processes. Moreover, Brominated alkanes such as 2-bromotetradecane can be produced by soil microbes or as degradation products of organic material. They are known to have antimicrobial properties, which can impact the microbial community structure in the rhizosphere. This conforms to the reports by Bernards and Lewis [18] that brominated alkanes in soil play important ecological roles in soils and influence microbial communities.

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

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

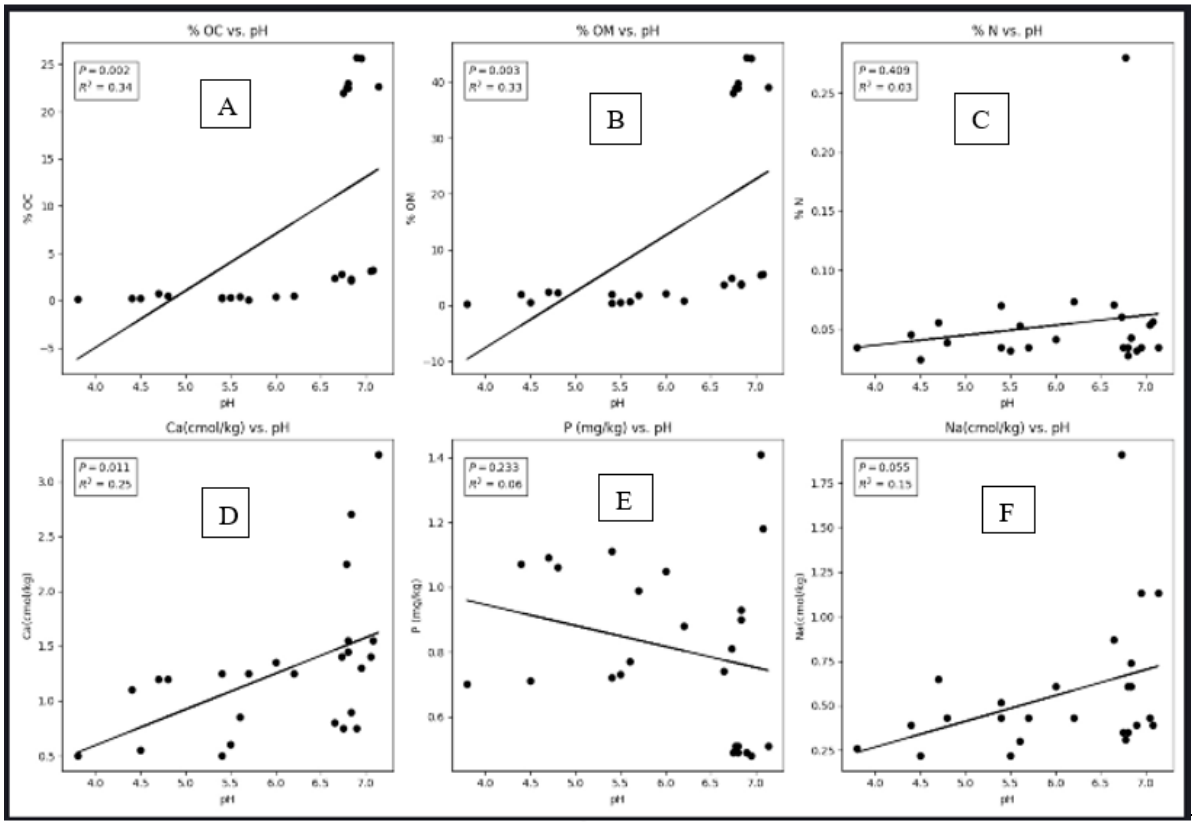

Fig. 2. Correlations between soil pH and other soil physiochemical properties and its impact on PLFA biomarkers

Key:

- ***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, C 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.*

involved in microbial sulfur metabolism. They are often detected in soil and rhizosphere environments, where they play roles in microbial interactions and stress responses.

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

Therefore, the key volatiles in the sorghum rhizosphere include a mix of fatty acids, organic acids, hydrocarbons, phthalates, and sulfur-

containing compounds. These volatiles suggest active microbial communities involved in nutrient cycling, fermentation, and interactions with sorghum root exudates. This profile reflects a diverse microbial environment that supports soil health and plant growth in the rhizosphere [22,23].

Retention times and corresponding PLFAs and their peak area (pA) are indicated in Fig. 3 for representative peaks. For clarity, not all peaks are indicated on the figure, although this particular sample yielded 74 identified PLFAs. Fig. 3 presents a representative sample run. The large hexane solvent peak characteristically appears at a retention time (RT) around 26.9 min. The ISTD standard peak (C10:0) appears at a RT of 5.1 min, while C19:0 has a RT of 14.5 min. The GC analysis separates the PLFAs based on their chain length, with longer chains eluting more slowly; for instance, C18:0 elutes at 14.5 min while C16:0 elutes at 12.6 min. In addition, this analytical protocol can separate PLFAs based on their degree of unsaturation and the position of their double bond; for example, C18:1ω9 (Table 2) and cyC18:1 (Table 3) elute at 24.09 min and 21.09 min, respectively [24,25].

Y-Axis (left) in Fig. 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 Daki Takwas, Dundaye, Gidan Doki, Makera, Ribah, and Zodi. Locations like Makera (Fig. 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, Figs. 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.

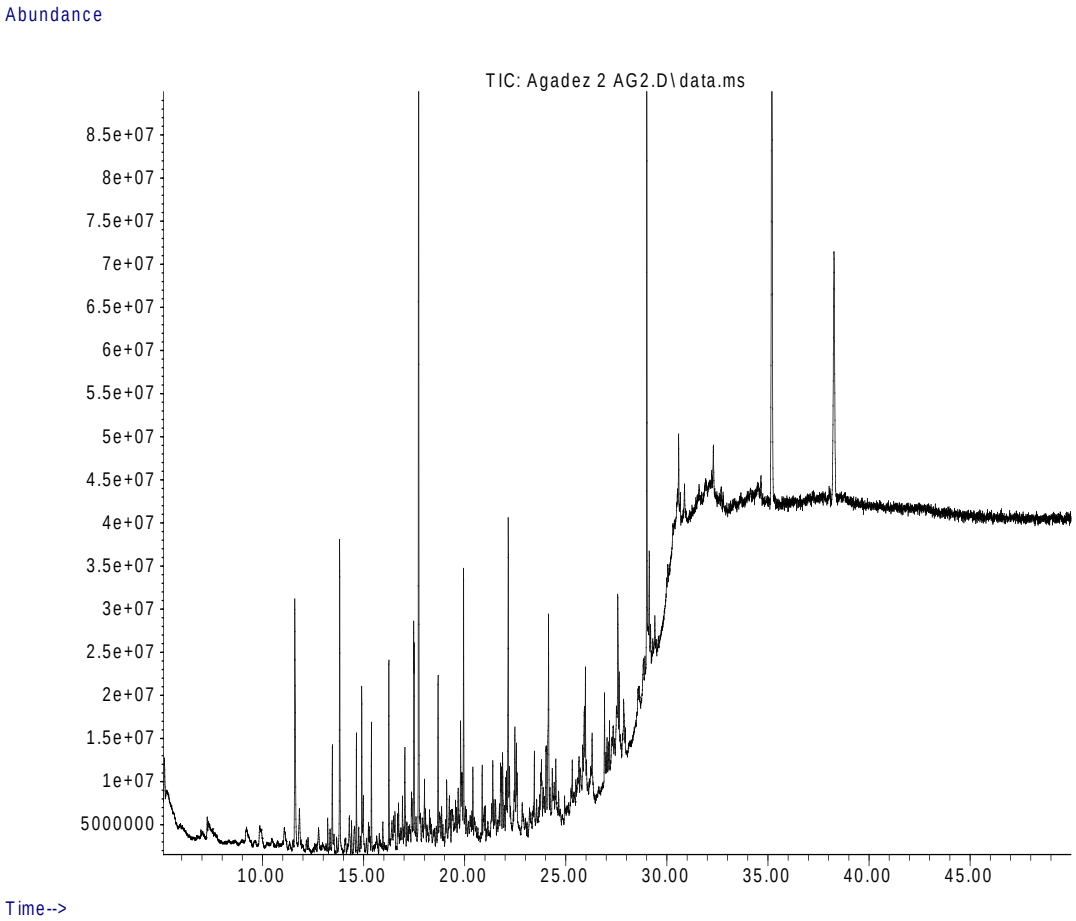

Fig. 3. Representative GCMS chromatogram (Agadez 2) showing peak areas of microbial abundance eluted against time

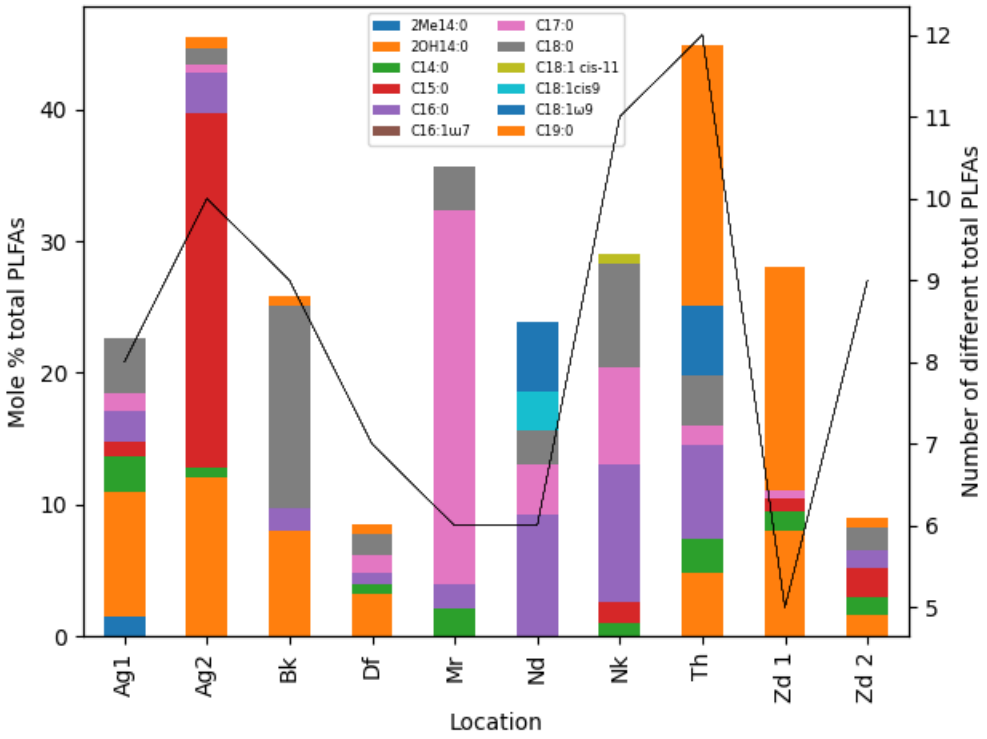

Fig. 4. A Stacked barchart showing PLFA profiles across the different soils for Niger

Key:

Ag1= Agadez 1 Ag2= Agadez 2 BK= Barkiawel DF= Diffa Mr=Maradi

Nd= Ndounga Nk= Nkonni Th= 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).*

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

PCA plot derived from soil data collected in Niger (Fig. 7) similarly indicate the first principal component (PC1), which captures 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 represents the PC2 (Principal Component 2). Each point in the scatter plot (Fig. 8) represents a soil sample from the dataset. The coordinates of each point are determined by its scores on the first two principal components (PC1 and PC2). For Niger samples, points are similarly color-coded which represents different categories of PLFA biomarkers. For example, cyC14:0: Pink, C14:0: Orange, 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 Purple, C19:0: Pink (Different shade from cyC14:0 for distinction). Cluster points indicate that samples have similar characteristics in terms of the variables used for PCA. The outliers that are far away from others, such as the orange point at approximately (-3, 4), indicate a sample that is significantly different from the rest in terms of the principal components. Therefore, clustering of points by color indicates how different categories of soil samples (based on fatty acid composition) relate to each other. The orange outlier at (-3, 4) suggests that this particular sample, labeled "C14:0", is quite different from the others in the dataset in terms of the principal components. The general spread of points indicates the variance captured by the first two principal components, with most points centered around the origin and a few spread out (Fig. 8).

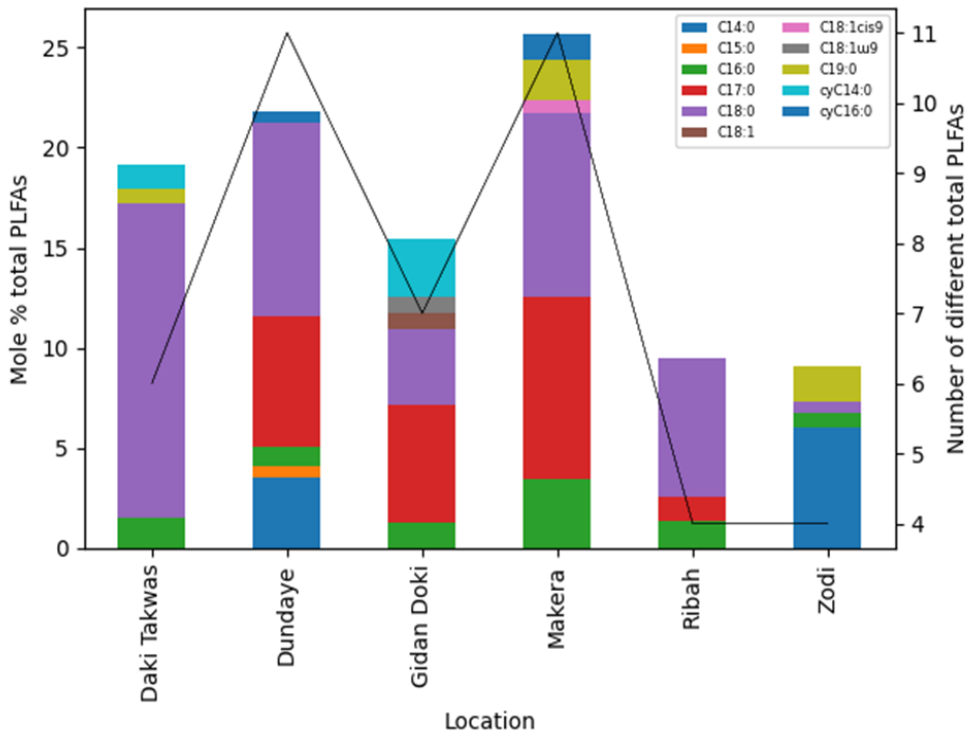

Fig. 5. Stacked barchart showing PLFA profiles across the different soils for Nigeria

Key:

- *Daki Takwas: 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.*

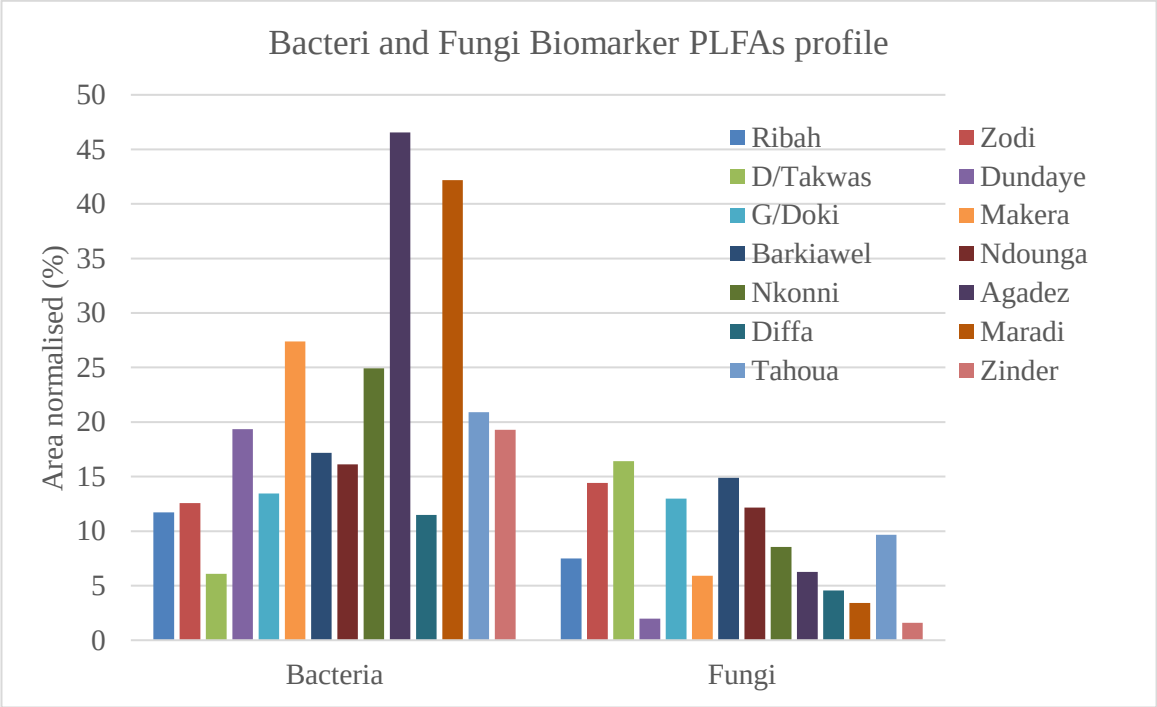

Fig. 6. Bacteria and Fungi PLFA Biomarkers in nmol/g from soil samples across all locations

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*

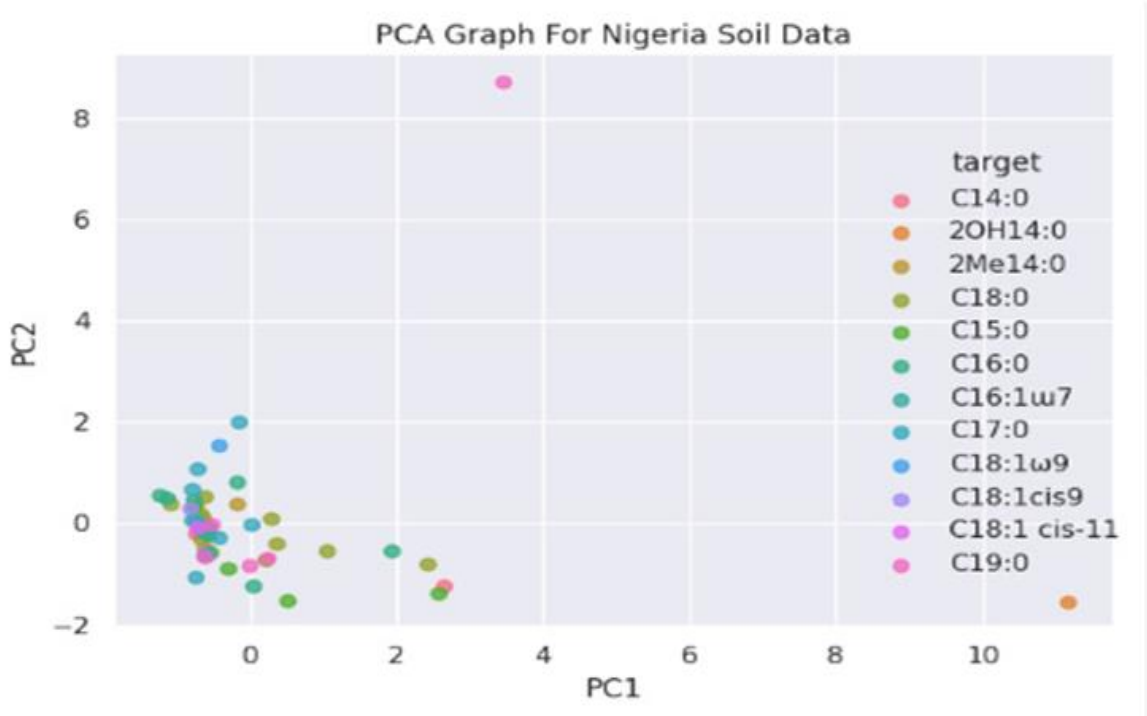

Fig. 7. Principal Component Analysis (PCA) for Nigerian Soil PLFAs

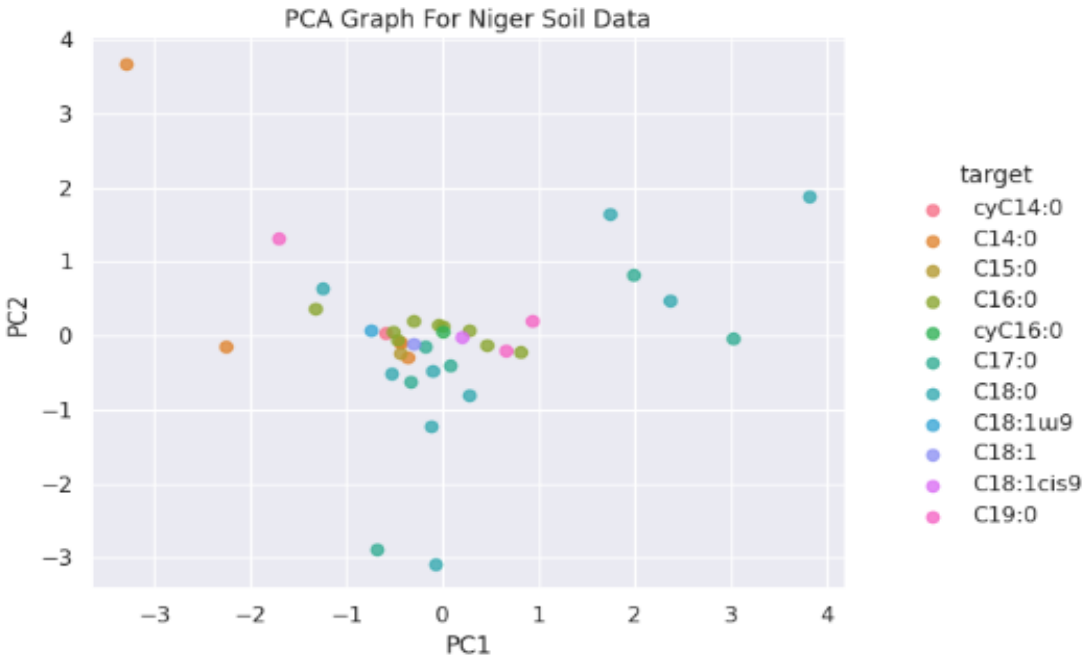

Fig. 8. Principal Component Analysis (PCA) for Niger Soil PLFAs

4. CONCLUSION

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

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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

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 starked barcharts. 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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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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