Original Research Article

EVALUATION OF PRESUMED DROUGHT IMPACTED SOILS THROUGH PHOSPHOLIPIDFATTY ACID (PLFA) BIOMARKERS

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: Correlational study design was used to analyze the PLFA biomarkers.examining the relationships between soil properties (e.g., organic carbon, nitrogen, pH) and PLFA biomarkers.

Place and Duration of Study: The study, conducted from May to October 2021, focused on the Sahel region of sub-Saharan Africa, encompassing Nigeria and the Niger Republic.

Methodology: Soil samples from 14 locations in Nigeria and the Niger Republic were collected, totaling 90. Phospholipids were extracted, analyzed using gas chromatography (GC), and quantified as biomarkers for different microbial groups. Principal component analysis was used to relate PLFA profiles to soil properties and environmental factors.

Results:.Drought can alter soil microbial communities, causing changes in PLFA biomarkers. It can decrease Gram-negative bacteria, which are associated with soil fertility and nutrient availability, and increase Gram-positive bacteria, which are involved in soil structure, organic matter turnover, and nutrient recycling. Low nutrient levels can also increase fungal dominance, favoring fungal PLFA biomarkers (e.g., 18:2ω6,9c). Drought-induced soils can increase the levels of PLFA biomarkers linked to stress tolerance and dormancy (cy17:0, 17:0, i17:0 and cy19:0). The imbalance between Gram-positive and Gram-negative populations and a shift towards fungal dominance can negatively affect soil health, causing changes in structure, fertility, nutrient cycling, and decomposition.

Conclusion: The study found fungal dominance in some locations, while bacteria were more abundant in others due to low nutrient levels. An increase in Gram-positive bacteria PLFA biomarkers (e.g., Actinobacteria and Firmicutes-15:0, i15:0, a15:0) in some areas may be due to low phosphorus and calcium levels.

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

1. INTRODUCTION

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. Quideau et al. [1] 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. MATERIAL AND METHODS

2.1 STUDY AREA

The study surveyed fields in the Sahel region, bordering Nigeria and the Niger Republic, including Dundaye, Makera, and Gidan Doki in Wamako, Daki Takwas in Birnin Tudu, Ribah in Danko, and Zodi in Zuru. Samples were collected from the Niger Republic, with Agadez being the driest region, and Diffa, Maradi, Tahoua, and Zinder in the neighboring Niger Republic.

2.2 Protocol

2.2.1precautionary measures

Quideau et al.'s [1] 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

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.

2.2.3Collection 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. 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 are processed simultaneously, with a set of 20 samples corresponding to a batch of 23 sample tubes. The extraction, separation, and methylation are conducted in batches of samples before preparing them for GC analysis. This helps identify errors and reduces the number of repeat extractions.

2.3 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.

2.3.1 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 is 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 N2 to avoid oxidation. Chloroform is evaporated off slowly, setting the N_2 flow to ruffle the liquid but not climb the sides of the vial. The samples are then stored in the freezer at -20 ºC wrapped in aluminium foil until ready to proceed with Step 2.

2.3.2 Lipid fractionation (Step 2)

The process involves solid phase extraction (SPE) using a column holder on a glass tank. New SPE columns are inserted, labeled, and conditioned by adding acetone and chloroform. The sample is re-dissolved and transferred to the column using a Pasteur pipette. Neutral lipids and glycolipids are eluted by adding chloroform and acetone, respectively. The solvent is then drained into the tank. Centrifuge tubes are inserted and labelled, and phospholipids are eluted by adding methanol. SPE columns are dried in a fume hood before disposal. Phospholipid fractions are dried under compressed N2 and purged. Samples are stored in the freezer at -20 ºC wrapped in aluminium foil until ready for Step 3. The process ensures accurate and consistent results.

2.3.3 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 dH2O. 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.

2.4 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) is prepared by adding MeC10:0 to hexane. The GC is turned on, and the gas supply is ensured. A good calibration is 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: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 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:

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

The study 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:

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) 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:

C19:0 sample = F X $(\frac{area\ C19:0}{area\ C10:0})$ 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:

C19:0 added = $(\frac{[19:0]std\ x\ V(19:0\ std\ added)}{M19:0})$ 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 is 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 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-1 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 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. 0 RESULTS AND DISCUSSION

Relationship between soil physiochemical properties and its impact on PLFA biomarkers were analysed using scatter plots (A–F) in (Figure 1).Plot A: Shows the relationship between pH and % OC (organic carbon). The p-value is 0.00, indicating a statistically significant relationship, and the correlation coefficient (r) is 0.34, showing a weak to moderate positive correlation. Plot B shows the relationship between pH and % OM (organic matter). With a p-value of 0.00 and a correlation coefficient of r=0.33, there is a weak to moderate positive and statistically significant correlation.Plot C represents the relationship between pH and nitrogen. The p-value here is 0.41, and r is 0.03, indicating a very weak, non-significant correlation between pH and nitrogen. Plot D represents the relationship between pH and phosphorus (P). The p-value is 0.23, and the correlation coefficient (r=0.06) indicates a very weak, non-significant positive correlation between pH and phosphorus. Plot E represents the relationship between pH and calcium (Ca). With a p-value of 0.01 and r=0.25, there is a weak positive and statistically significant correlation between pH and calcium. Plot F shows the relationship between pH and sodium (Na). The p-value is 0.05, which is marginally significant, and the correlation coefficient (r=0.15) indicates a weak positive correlation. Therefore significant positive correlations exist between pH , % OC (A), % OM (B), and Ca (E). Similarly, there is marginally significant positive correlation: pH and Na (F). While non-significant correlations exist between soil pH, nitrogen (C) and phosphorus (D). This confirms that soil pH may be associated with certain soil properties (like organic carbon, organic matter, and calcium), but has limited impact on nitrogen and phosphorus.

The Nature and Properties of Soils (14th ed.) by [2], 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 [3] 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 [4] 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.*[5].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.

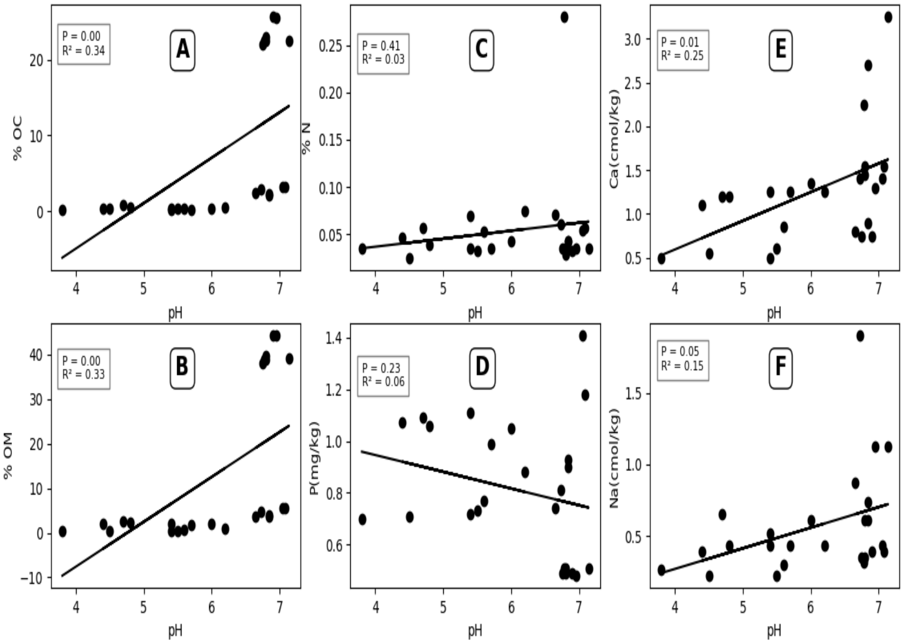

Figure 1: Correlations between soil pH and other soil physiochemical properties and its impact on PLFA biomarkers

3.1 Phospholipid Fatty Acid (PLFA) analysis

A GC-MS machine was used for the detection of PLFAs. Tables 1 and 2 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 1 and 2. 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 C16:1ɯ9 favoured higher soil conductivity and nitrate, and other PLFAs contributed more to the organic content. These are samples from Barkiawel, Nkonni, and Tahoua.

Table 1: 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
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, 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	C19:0
35.26	Anthracene, 9,10-dihydro-9,9,10-trimethyl-	C17:0

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

Based on the Table and Table 2, 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 [6] that fatty acids such as C16:0 and C17:0 are biomarkers for microbial communities in soil environments.

such as n-Hexadecanoic acid (C16:0) commonly found in microbial membranes and can indicate microbial activity around plant roots. Octadecanoic acid (C18:0) and Oleic acid (C18:1ω9) are ften associated with soil bacteria and fungi, indicating general microbial presence and activity. While Palmitoleic acid (C16:1ω9) is typically associated with gram-negative bacteria, which are common in nutrient-rich rhizosphere environments.Frostegård*et al.* [7]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.

The second group of volatiles from the table 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.* [8], 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 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[9] 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, [10] 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 [11] 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.*[12] 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 [13]. Schulz-Bohm *et al.* [14] 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 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.

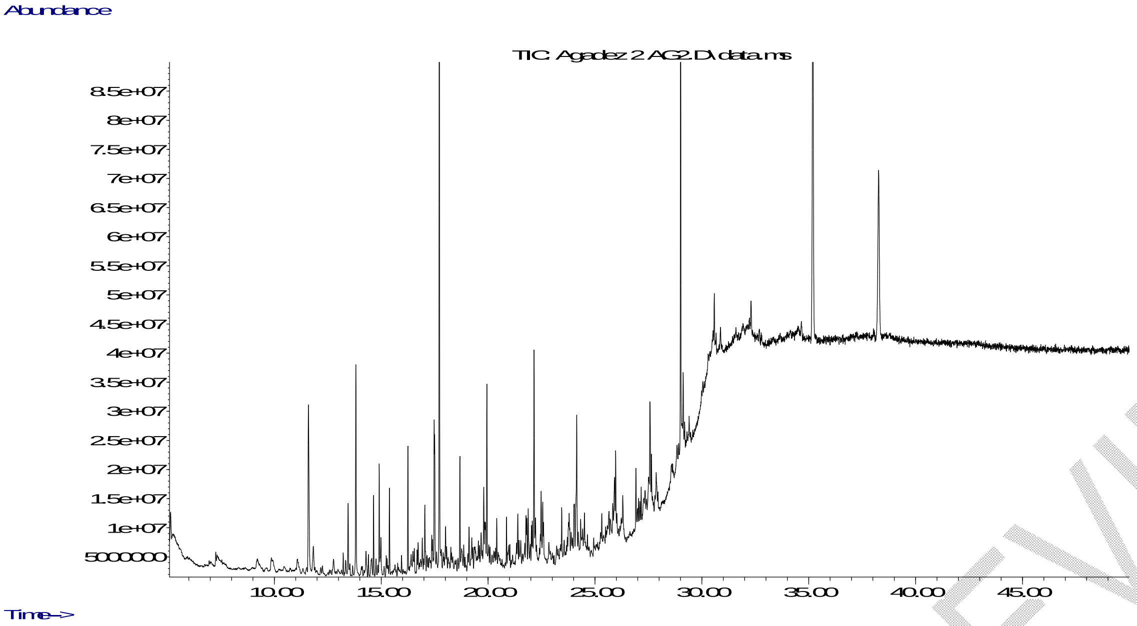

Figure 2: Representative GCMS chromatogram (Agadez 2) showing peak areas of microbial abundance eluted against time

Retention times and corresponding PLFAs and their peak area (pA) are indicated in Figure 2 for representative peaks. For clarity, not all peaks are indicated on the figure, although this particular sample yielded 74 identified PLFAs. Figure 2 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 19.8 min. The GC analysis separates the PLFAs based on their 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 addition, this analytical protocol can separate PLFAs based on their degree of unsaturation and the position of their double bond; for example, C18:0 elutes at 18.2 min, while C18:1ω9 (Table 1) and cyC18:1 (Table 2) elute at 24.09 min and 21.09 min, respectively.

Figure 3: 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= NdoungaNk= 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).

Figure 4: 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.

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 Daki Takwas, Dundaye, Gidan Doki, Makera, Ribah, and Zodi. Locations like Makera (Figure 4) 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.

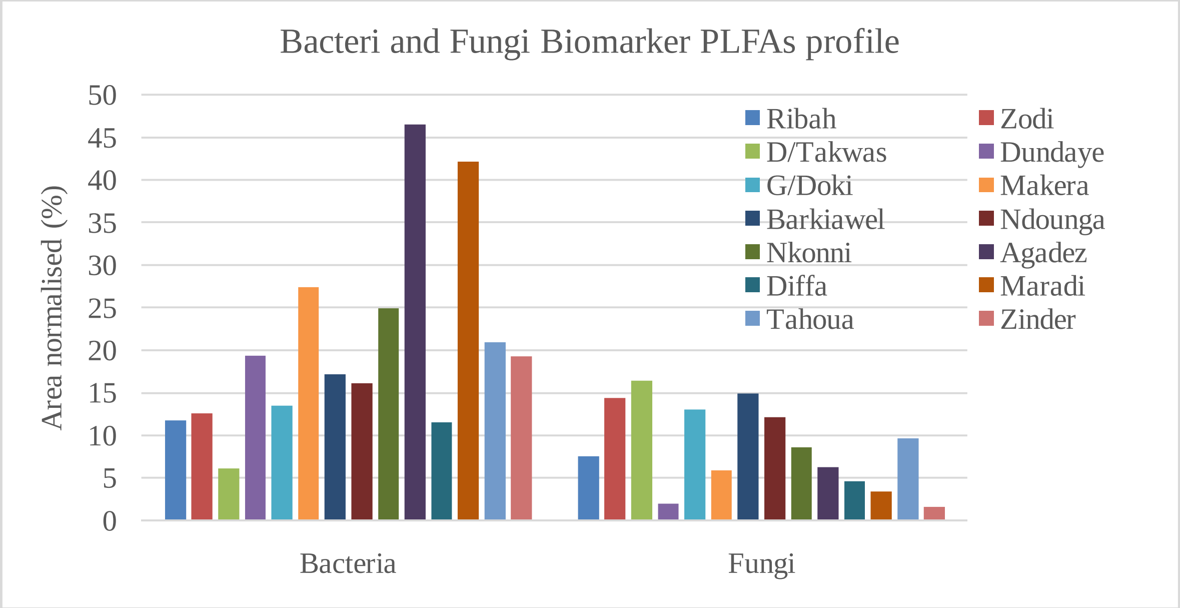

Figure 5: Bacteria and Fungi PLFA Biomarkers in nmol/g from soil samples across all locations.

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

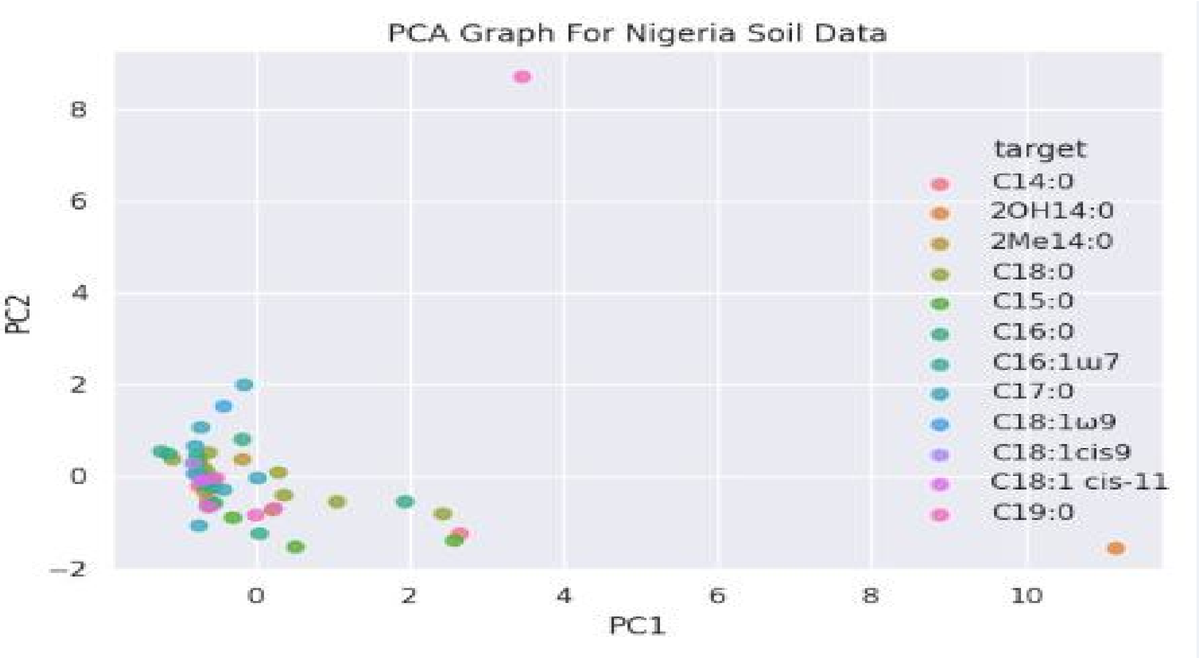

Figure 6: Principal Component Analysis (PCA) for Nigerian Soil PLFAs

PCA graph was used to represents a Principal Component Analysis (PCA) of soil data collected from Nigeria (Figure 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 points which are colored based on the "target" 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.

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

PCA plot derived from soil data collected in Niger (Figure 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 (Figure 7) 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.

4. CONCLUSION

Based on the analysis, the presence and types of volatile compounds detected in the agricultural soils of Niger and Nigeria, particularly in the sorghum rhizosphere, appear to reflect interactions between soil properties such as organic carbon, soil pH, and organic matter content. The soil pH is associated with the distribution of certain soil volatiles and nutrient availability, especially in influencing organic carbon and organic matter, which support microbial activity essential for soil health. While organic carbon and organic matter are likely enhanced by these microbial processes and contribute to the buildup of specific volatiles, soil pH shows limited impact on nitrogen and phosphorus availability. This suggests that pH is a critical regulator of the soil's chemical environment, affecting nutrient solubility and microbial activity, while organic matter serves as a source of carbon-based volatiles, playing a key role in the soil's biological interactions and potential resilience. Together, these properties highlight the importance of balanced pH and organic content for sustaining productive soils in sorghum cultivation systems.

REFERENCES

[1] Quideau SA, McIntosh AC, Norris CE, Lloret E, Swallow MJ, Hannam K. Extraction and analysis of microbial phospholipid fatty acids in soils. JoVE (Journal of Visualized Experiments). 2016 Aug 26(114):e54360.
[2] Brady, N.C. and Weil, R.R. (2008) The nature and properties of soil, 14 ed. Prentice-Hall, Upper Saddle River, New Jersey. 1082 p .
[3] Jenny, H. (1941) Factors of Soil Formation A System of Quantitative Pedology. Dover Publications, New York, 281 p.

[4]Marschner, H.(2012) Marschner's Mineral Nutrition of Higher Plants.Vol.89, Academic Press, London, 651 p.

[5] Zhao, Q., Zeng, D. H., & Fan, Z. P. (2010). Effects of vegetation and soil properties on soil pH buffering capacity in semiarid ecosystems of northeast China. *Environmental Earth Sciences*, 60(8), 1483–1490.

[6] Zelles, L. (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil. *Biology and Fertility of Soils*, 29(2), 111-129

[7] Frostegård, Å., Tunlid, A., &Bååth, E. (2011). Use and misuse of PLFA measurements in soils. *Soil Biology and Biochemistry*, 43(8), 1621-1625.

[8] Shi, S., Richardson, A. E., O'Callaghan, M., DeAngelis, K. M., Jones, E. E., Stewart, A., ... & Firestone, M. K. (2011). Effects of selected root exudate components on soil bacterial communities. *FEMS Microbiology Ecology*, 77(3), 600-610.

[9] Li, W., Ji, X., Zhang, W., Wang, F., & Liu, J. (2014). Hydrocarbon-degrading microbial communities in soil. *Frontiers in Microbiology*, 5, 59. This paper discusses hydrocarbons in the rhizosphere and their role in microbial metabolic processes.

[10] Saini, R., &Kookana, R. S. (2021). Microbial degradation and metabolization of hydrocarbons in the rhizosphere. *Microorganisms*, 9(4), 758.

[11] Bernards, M. A., & Lewis, N. G. (1992). Brominated alkanes in plant and soil systems: Ecological relevance. *Annual Review of Ecology, Evolution, and Systematics*, 23, 75-98.

[12] Wu, Y., Gu, X., Zhang, Y., Yu, X., & Xie, H. (2015). Biodegradation of dibutyl phthalate by a novel bacterium Acinetobacter sp. strain LMB-5 isolated from activated sludge. *PLOS One*, 10(12), e0144383

[13] Fu, Q., & Wang, Z. (2015). Microbial degradation of phthalate esters in the soil environment: A review. *Environmental Science and Pollution Research*, 22(4), 2465-2478. This review covers microbial degradation of phthalates and their presence in soil microbial communities.

[14] Schulz-Bohm, K., Geisen, S., & Wubs, E. R. J. (2018). The microbiome of root exudates: Mechanisms, complexity, and ecological functions. *Frontiers in Microbiology*, 9, 108.

[15] Friesen, M. L., Porter, S. S., Stark, S. C., von Wettberg, E. J., Sachs, J. L., & Martinez-Romero, E. (2011). Microbially mediated plant functional traits. *Annual Review of Ecology, Evolution, and Systematics*, 42, 23-46.

[16] Marschner, P., & Rengel, Z. (2012). Nutrient Cycling in Terrestrial Ecosystems. *Soil Biology*, 28, 1-15. This reference covers the role of sulfur compounds in nutrient cycling and their impact on rhizosphere microbial communities.

[17] Mukhopadhyay, B., &Stäbler, L. (2019). Microbial transformation of sulfur compounds in soil: Insights from molecular studies. *Current Opinion in Environmental Biotechnology*, 9, 22-27. This study discusses sulfur compounds in soil, including thiols, and their role in microbial ecology.

[18]Van Dongen, J. T., Schurr, U., & Rascher, U. (2013). Siloxanes in soil organic matter: Effects of roots and soil microbes on silicon-based compounds. *Plant and Soil*, 365(1), 1-12.