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CORRELATIONAL AND MULTIVARIATE ANALYSIS OF ELECTROCHEMICAL SIGNALS AND MICROBIAL COMMUNITIES IN TROPICAL PMFCS WITH CITRUS LIMON AND MANGIFERA INDICA

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ABSTRACT

Plant-microbial fuel cells (PMFCs) harness root exudates and electrogenic microbes to generate electricity while simultaneously monitoring soil status, yet their behaviour in tropical crops remains largely unexplored. For this study we assembled independent PMFC units with Citrus limon and Mangifera indica seedlings and ran them for 30 days under controlled tropical conditions (25 ± 2 °C, 60–70 % RH, 1.0–74.4 klux). Voltage, current, electrical conductivity, illuminance and time were logged every 12 h; data were analysed with Pearson correlation, principal-component analysis (PCA) and principal-coordinates analysis (PCoA) with PERMANOVA on 16S rRNA profiles. Both plant systems yielded consistently higher and more stable electrical outputs than unplanted controls. Voltage and current were almost perfectly correlated ($r \approx 1.00$), and the first two PCs explained 75.21 % of total variance, clearly separating planted from control units; illuminance and time acted as orthogonal, non-redundant drivers. Microbial ordination showed significant plant-dependent shifts (PERMANOVA $R^2 = 0.28$, $p = 0.001$) with enrichment of known electrogens at the anodes. These integrated electrochemical and microbiological responses confirm that lemon- and mango-based PMFCs function as robust, low-cost biosensors suited to tropical agroecosystems and provide a statistical baseline for their optimisation and field deployment across diverse tropical field conditions.

KEYWORDS: Plant Microbial Fuel Cell (PMFC), Bioelectrochemical System, Tropical Crops, Biosensor, Citrus Limon, Mangifera Indica, Multivariate Analysis, PERMANOVA.

1. INTRODUCTION

Growing global energy demand, together with the sustained dependence on fossil fuels, has markedly increased greenhouse gas emissions, thereby accelerating climate change. In this sense, the resulting environmental crisis has driven a global search for sustainable renewable energy alternatives, among which microbial fuel cells (MFCs) are noteworthy due to their capacity to generate electricity through the symbiotic interaction between electrogenic microorganisms and plant roots in the rhizosphere (Chong, Chuah, et al., 2025; Obileke et al., 2021). It has been shown that these systems can exhibit low environmental impact through the direct use of microbial activity and plant exudates, which provide a constant source of organic matter for bioelectric processes (Lepikash et al., 2024).

Recent research has evidenced that various plant species integrated into plant microbial fuel cell (PMFC) configurations are capable of sustaining bioelectricity generation, and that environmental and microbiological factors such as light availability, soil conditions, and community structure strongly influence their responses (Gan et al., 2024; Rusyn et al., 2022). However, despite the growing interest, PMFC studies remain limited in specific regional contexts, particularly in tropical environments such as those found in Ecuador. The current literature still lacks studies focusing on species commonly cultivated in Ecuador and in climatically similar regions of Latin America (Gupta et al., 2023; Lepikash et al., 2024), which restricts local optimization and the practical implementation of the technology.

In response to this gap, the present study evaluates two species of high agricultural importance in Ecuador Citrus limon (lemon) (Párraga et al., 2022) and Mangifera indica (Tommy Atkins mango) (Marcillo-Parra et al., 2021), employing PMFCs assembled with carbon electrodes under controlled environmental conditions in Daule, Guayas Province, Ecuador. The selection of these species is based on their economic relevance, adaptability to tropical conditions, and emerging evidence indicating that root-exuded organic compounds and rhizosphere interactions play a critical role in PMFC bioelectric performance (Gan et al., 2024; Gupta et al., 2023).

Rather than centering solely on maximum power output, the study focuses on characterizing statistical relationships among electrical variables (voltage, current), environmental drivers (illuminance, time), and microbiological attributes (community structure). It is hypothesized that: (i) voltage–current relationships exhibit robust and consistent patterns across treatments and species; and (ii) plant species influence detectable differences in rhizosphere microbial communities (Lepikash et al., 2024; Tongphanpharn et al., 2021).

Accordingly, the objective is to analyze PMFC responses with Mangifera indica and Citrus limon through correlation analysis (Pearson) and principal component analysis (PCA) to identify multivariate electrochemical patterns, as well as PCoA/PERMANOVA to compare microbial community composition, thereby assessing their potential as tropical platforms for bioelectric monitoring under controlled conditions. A detailed bioelectric setup was implemented, and electrical and environmental parameters were monitored over a 30-day period (Chong, Chuah, et al., 2025).

The results aim to contribute regional scientific evidence by providing initial insights into correlational and multivariate patterns, as well as community differences in PMFCs constructed with species adapted to the Ecuadorian climate. This information will serve as a baseline for advancing the effective implementation of sustainable bioelectrochemical technologies and environmental sensing in Ecuador and analogous tropical regions, reinforcing global strategies to mitigate environmental challenges through renewable and clean energy sources. In this context, recent literature underscores the importance of local, studies in Latin America to capitalize on bioelectric potential from regional species and to position PMFCs as tools for sustainable technologies and biosensing (Gupta et al., 2023; Rusyn et al., 2022).

2. MATERIALS AND METHOD

Citrus limon (lemon) and Mangifera indica cv. Tommy Atkins (mango) plants were obtained from the Universidad Estatal de Milagro (2°12′38,7″ S; 79°35′59,9″ W). Both species were systematically integrated into independent Plant Microbial Fuel Cell (PMFC) units for electrochemical and microbiological characterization under controlled laboratory conditions. Young seedlings of each species were cultivated in a clay-textured substrate with controlled moisture (20–30%). The experimental setup included carbon felt anodes, graphite cathodes, and cation exchange membranes (CEM) in selected units, together with individual test vessels. The instrumentation consisted of a digital multimeter (UNI-T UT61E, China), potentiostat (Gamry Interface 1010E, USA), conductivity meter (Hanna Instruments HI99300, Romania), and luxmeter (Extech LT300, USA). Ambient temperature was held at 25 ± 2 °C and relative humidity at 60–70 %, while illuminance varied from 1.0 klux at dawn to an occasional midday peak of 74.4 klux (median 4.2 klux), thanks to a west-facing translucent window; an HVAC system and reflective shading maintained thermal stability despite these light fluctuations.

2.1. Soil Characterization and Experimental Preparation

Prior to PMFC assembly, a single clay-textured soil was selected and subjected to comprehensive physicochemical and biological characterization to guarantee substrate uniformity across treatments; in this sense, any subsequent differences in system performance are attributable primarily to plant species rather than edaphic variability. The characterization encompassed pH, electrical conductivity (EC), total organic carbon (TOC), particle-size texture, macronutrients (total N, P, K), basal microbial activity, and redox potential.

The pH was determined in a 1:2.5 soil-to-distilled water suspension using a glass electrode potentiometer calibrated with standard buffer solutions at pH 4.0 and 7.0, following the ISO 10390:2021 standard. Electrical conductivity was measured in a 1:5 (w/v) soil-to-water suspension using a calibrated digital conductivity meter temperature-compensated to 25 °C. TOC was quantified via the modified Walkley–Black method in accordance with ISO 14235.

Microbial activity was estimated through soil respiration assays using airtight chambers with sodium hydroxide (NaOH) as a CO_2 trap, following the methodology described by Stotzky (Stotzky, 2016). Total phosphorus (P) was determined by acid digestion with a H_2SO_4–$HClO_4$ mixture and subsequent colorimetric detection at 880 nm using the ascorbic acid method. Total potassium (K) was quantified via wet digestion with HNO_3–$HClO_4$ and analyzed using flame atomic emission spectrometry, based on the AOAC 965.09 protocol.

This detailed characterization ensured that observed differences in the electrical performance of the PMFC systems could be primarily attributed to plant species variation rather than to edaphic heterogeneity. Moreover, the evaluation of redox conditions provided insights into whether the soil environment favored aerobic or anaerobic microbial metabolic pathways, thereby informing the interpretation of electrogenic activity.

2.2. Experimental Design

The present study was conducted following the principles of closed rhizosphere plant microbial fuel cell (C-R-PMFC) systems, to ensure consistent and reproducible conditions throughout the experimental period (Chong et al., 2025). In accordance with established standards in bioelectrochemical research and considering the low variability typically observed under controlled environments, plants were cultivated individually in separate containers for a duration of 30 days, with a sample size of n = 3.

Independent biological replication comprised n = 3 per treatment (lemon, mango, and unplanted control), each pot functioning as an independent PMFC unit. Electrical and environmental variables were recorded for every unit at 12-hour intervals across 30 consecutive days (≈ 60 repeated measures per unit). Accordingly, the pot is the experimental unit for between-treatment inference, while the 12-hourly readings constitute repeated measures within each unit and are used to stabilise within-unit estimates rather than to inflate the number of biological replicates. Resource and space constraints typical of proof-of-concept PMFC studies motivated this replication level; therefore, the study prioritises effect sizes and multivariate ordination over dichotomous hypothesis testing.

To maintain system stability, substrate moisture was continuously monitored using capacitive sensors, ensuring levels remained within the optimal range of 20–30%. This regulation was critical to preserve microbial stability within the rhizosphere, minimise fluctuations in ionic conductivity, and sustain the consistent activity of electrogenic bacteria. The irrigation system was meticulously managed to offset water losses resulting from evaporation and transpiration, thereby contributing to the maintenance of homogeneous environmental conditions throughout the experimental phase.

2.3. PMFC System Configuration

Each system functioned as an independent PMFC unit. Carbon electrodes were positioned in the root zone as anodes, while cathodes were placed at the soil–air interface to enhance oxygen availability. In selected units, a cation exchange membrane (CEM) was integrated between compartments to facilitate proton transport and prevent cross-contamination.

Electrodes were connected via insulated wires to an external circuit with adjustable resistors (100–1000 Ω). Voltage (V) and current (μA) were measured using a multimeter (UNI-T UT61E, China) and a potentiostat (Gamry Interface 1010E, USA). Electrical conductivity (EC; $\mu S\ cm^{-1}$) was recorded at a depth of 2 cm using a calibrated conductivity meter (Hanna Instruments HI99300, Romania), while illuminance was measured in situ with a luxmeter (Extech LT300, USA).

2.4. Data Collection of Electrical and Environmental Variables

Key electrical and environmental variables, namely voltage (V), current (I), electrical conductivity (EC; µS cm⁻¹), illuminance (lux), and time (days), were recorded every 12 h (morning/evening) across the 30-day period. Multiple readings per sample were obtained in each session to minimize geometric and sampling bias (Lepikash et al., 2024; Rusyn et al., 2022). Power was not retained as a response variable, since the analysis focuses on the correlational structure and multivariate patterns among fundamental electrical and environmental drivers.

2.5. Statistical Analysis

All data processing was performed using RStudio (version 4.3.0). A Pearson correlation matrix was computed and visualized using both the PerformanceAnalytics and corrplot packages (circular representation), facilitating the identification of significant inter-variable relationships (Gómez-Rubio, 2017).

In addition, Principal Component Analysis (PCA) was carried out to reduce data dimensionality and identify patterns in the electrochemical behavior of the PMFC systems. The PCA enabled a visual comparison of bioelectrochemical performance across plant species and experimental conditions. Prior to PCA, one-way ANOVA was conducted to assess statistical significance among treatments.

Given three independent biological replicates per treatment, nominal power to detect medium between-treatment effects at $\alpha = 0.05$ is limited. To address this, the analysis explicitly treats the pot as the independent experimental unit and uses the 12-hourly observations as repeated measures within each unit to improve precision of unit-level estimates rather than to increase the number of biological replicates. Consequently, emphasis is placed on estimation (e.g., standardised effect sizes with intervals where applicable) and on exploratory multivariate ordination (PCA for electrochemical variables; PCoA/PERMANOVA for 16S profiles) to characterise patterns without over-reliance on dichotomous significance testing.

2.6. Microbial Community Analysis

A Principal Coordinate Analysis (PCoA) based on Bray–Curtis dissimilarity indices was conducted to examine patterns of variation in microbial community structure across different PMFC treatments. Microbial samples were collected from anode biofilms, rhizosphere zones, and unplanted sediment controls after 30 days of PMFC operation. Total genomic DNA was extracted using the DNeasy PowerSoil Kit (Qiagen, Germany), and bacterial community profiles were generated through high-throughput sequencing of the V3–V4 regions of the 16S rRNA gene on the Illumina MiSeq platform (Mitter et al., 2017).

Raw sequencing data were processed in QIIME2 (version 2023.2) using the DADA2 plug-in to denoise sequences, remove chimeras, and generate amplicon sequence variants (ASVs). Taxonomic assignment was performed using the SILVA 138 reference database. The resulting ASV abundance table was normalized via rarefaction to account for variation in sequencing depth among samples (Callahan et al., 2016).

In addition to ordination, SILVA-based taxonomic assignments were inspected at higher ranks (family/genus) to aid interpretation of community structure across anodes, rhizospheres, and sediments. Given the small number of biological replicates (n = 3 per treatment), no formal differential-abundance testing was undertaken to avoid overinterpretation under compositional constraints.

A Bray–Curtis dissimilarity matrix was then computed to quantify differences in microbial community composition. This matrix served as input for the PCoA, implemented using the cmdscale() function from the vegan package (version 4.3.0) in R. The analysis projected the samples into a two-dimensional ordination space, with the first two principal coordinates selected based on the highest proportion of explained variance (Quintanilla et al., 2018).

3. RESULTS AND DISCUSSION

3.1. Physicochemical and Biological Characterization of the Soil used in PMFC Systems

To ensure the reliability and reproducibility of the experimental outcomes, a comprehensive physicochemical and biological characterization of the soil substrate employed in the plant microbial fuel cell (PMFC) systems was conducted. This assessment focused on parameters directly influencing electrogenic microbial activity and plant growth, with the objective of validating the suitability of the substrate to support microbial colonization, root development, and efficient ionic transport critical factors for the sustained functionality of PMFCs. The evaluated variables included pH, electrical conductivity (EC), total organic carbon (TOC), basal microbial respiration, and macronutrient concentrations of phosphorus (P) and potassium (K). A summary of these results is provided in Table 1 Physicochemical and biological properties of the soil used in PMFC systems (n = 3; values expressed as mean ± standard deviation).

Table 1: Physicochemical and Biological Properties of the Soil Used in PMFC Systems (n=3; Values Expressed as Mean ± Standard Deviation).

Parameter	Mean ± SD	Units
pH	7.65 ± 0.240	
Electrical conductivity	935.00 ± 4.53	μS cm^{-1}
Total Organic Carbon	4.30 ± 1.80	%
Microbial activity	0.003 ± 0.0011	g CO_2/g soil/day
Total Phosphorus	2230 ± 5.50	mg/kg
Total Potassium	4325.00 ± 1.06	mg/kg

The measured soil pH (7.65 ± 0.240) falls within the optimal range for electrogenic bacterial communities, which generally exhibit higher metabolic efficiency in neutral to slightly alkaline environments. Empirical evidence indicates that microbial fuel cells operating in near-neutral pH substrates exhibit enhanced voltage generation, primarily due to increased nutrient availability and reduced disruption of electron transfer mechanisms (Toczyłowska-Mamińska et al., 2025). The low variability among replicates, as reflected in the standard deviation, confirms the consistency and reliability of the pH measurements.

Similarly, the EC of the substrate (935.00 ± 4.53 μS cm-1) denotes a favorable ionic environment that facilitates proton mobility and minimizes internal resistance across the PMFC circuit. Previous studies have demonstrated that higher EC values correlate positively with improved current density and overall system stability (Aliyu & Dahiru, 2024). The minimal dispersion of EC values underscores the analytical robustness and electrochemical suitability of the medium.

The total organic carbon content (4.30 ± 1.80%) was notably high, consistent with tropical soil profiles rich in decomposed plant matter. This parameter is particularly relevant, as organic carbon serves as a primary energy source for electrogenic bacteria, which oxidize organic compounds to generate electrons for anode transfer (Liu et al., 2014). The moderate variability observed may be attributed to intrinsic heterogeneity in organic matter distribution across soil replicates. Elevated TOC levels have been linked to greater microbial diversity and enhanced bioelectrical output in bioelectrochemical systems (Saran et al., 2023).

Basal microbial activity, measured at 0.003 ± 0.0011 g CO_2/g soil/day, provides insight into the inherent metabolic potential of the microbial community under non-stimulated conditions. This level of respiration suggests an active microbial consortium capable of sustaining electron transfer processes in PMFCs. Comparable respiration rates have been associated with improved electron flow and energy yield in systems utilizing organically enriched soils (Amaral et al., 2012).

Regarding nutrient availability, the concentrations of total phosphorus (2230 ± 5.50 mg/kg) and potassium (4325 ± 1.06 mg/kg) were considerably elevated. These macronutrients play a vital role in promoting root elongation, cellular metabolism, and the exudation of rhizospheric compounds that serve as substrates for electrogenic bacteria (Sonu et al., 2025). Their abundance supports a favorable rhizosphere–microbe interaction, which has been identified as a determinant factor in optimizing PMFC energy generation (Yang et al., 2024). The low standard deviations associated with these measurements highlight the precision of the analytical methods and the uniformity of the experimental substrate.

Collectively, the physicochemical and biological attributes of the substrate indicate a highly conducive environment for PMFC operation. The synergistic contributions of both plant and microbial communities to energy generation are supported by substrate properties that favor microbial activity, ion transport, and nutrient cycling. Prior characterization of the growth medium is therefore essential to contextualize the electrochemical outputs and ensure the scientific rigor and reproducibility of PMFC performance assessments. The narrow dispersion of key parameters such as pH and microbial activity further reinforces the reliability of the findings.

3.2. Multivariate Visualization of Bioelectrical and Environmental Variables in PMFC Systems under Different Plant Treatments

To obtain an integrative perspective of the electrochemical behavior across PMFC treatments, a multivariate exploratory analysis was conducted. This approach enabled the examination of complex interdependencies among key experimental variables. Figure 1 illustrates the pairwise distributions and descriptive statistics of voltage, current, illuminance (lux), and exposure time across three experimental conditions: Citrus limon (blue), Mangifera indica (fuchsia), and unplanted soil controls (cyan). The presence of tightly clustered density ellipses in mango and lemon treatments suggests higher internal consistency and elevated electrochemical activity relative to the soil control.

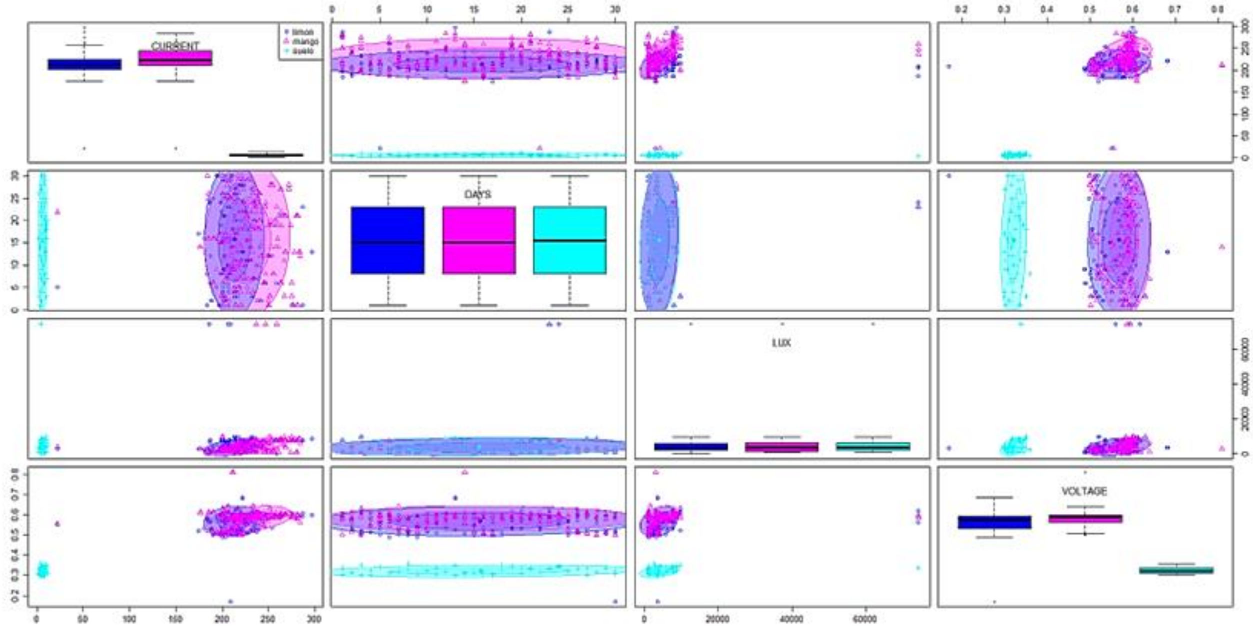


Figure 1: Pairwise Distributions and Density Ellipses of Electrochemical and Environmental Parameters (Voltage, Current, Illuminance, and Exposure Time) Recorded in PMFC Systems under Different Treatments. Color Codes Represent the Experimental Groups: Citrus Limon (Blue), Mangifera Indica (Fuchsia), and Unplanted Soil Control (Cyan). Density Ellipses Indicate the Multivariate Dispersion and Correlation Structure within Each Group.

Electrical output parameters specifically current and conductivity were significantly higher in both plant-based systems compared to the soil control. This enhancement is likely attributed to the greater ionic content and internal conductivity of plant tissues, as previously reported in studies on electrochemical responsiveness of fruit biomasses (Fadavi & Salari, 2019). These findings align with prior evidence positioning plant electrical conductivity (ECp) as a sensitive proxy for physiological processes modulated by environmental factors such as irradiance, ambient temperature, and relative humidity (Park et al., 2018). The observed variability in the plant groups may stem from differences in tissue maturity, cellular structure, and moisture retention capacity (Su et al., 2021), in contrast with the low EC values and reduced variability observed in the soil group. The latter's physicochemical stability underscores its role as a suitable inert control in PMFC studies (Jang et al., 2024).

Furthermore, it is well established that soil conductivity is influenced by moisture availability, granulometry, and salinity. Clayey textures and higher water retention typically result in increased ionic conductivity (Bertermann & Schwarz, 2018). Interestingly, plant root exudation has also been shown to locally modulate soil conductivity, as confirmed by geophysical imaging techniques (Filho et al., 2019), highlighting a complex interplay between plant activity and the surrounding substrate an area warranting further investigation.

Regarding voltage output, both plant treatments yielded consistently higher values than soil, with mango slightly outperforming lemon. This distinction may reflect compositional differences, such as organic acid concentration and tissue hydration, both of which can facilitate ionic mobility and enhance charge transfer (Bon et al., 2010; Srivastava et al., 2025). The low intra-group dispersion in voltage suggests stable electrochemical behavior over the monitoring period, a desirable feature for reliable bioelectric conversion systems (Huang et al., 2016). Notably, the scatterplots in Figure 1 reveal a robust positive correlation between voltage and current across plant treatments, evidencing electrochemical synergy a property that may be leveraged in the development of plant-based energy harvesting technologies.

Illuminance (lux), recorded as ambient light intensity over the canopy, emerged as a discriminant parameter. Soil samples recorded the lowest lux values, consistent with their low albedo and high opacity. In contrast, plant biomasses particularly lemon displayed greater variability, potentially due to differences in tissue translucency and epidermal morphology (Jang et al., 2024). While lux did not show strong linear correlations with electrochemical metrics, its physiological relevance remains significant, given its influence on stomatal conductance, transpiration, and photosynthetic activity. These factors are known to affect ionic gradients and, consequently, electrochemical outputs in plant-based systems (Yudina et al., 2022). The distinct lux profiles of each biomass group suggest its potential utility as a non-invasive classification parameter in precision phenotyping and agroenergy applications.

The temporal variable exposure time (in days) was uniformly distributed across all treatment groups, ensuring the elimination of temporal confounders and preserving the validity of intergroup comparisons. Given the transient nature of bioelectrochemical phenomena in plant-based systems (Huang et al., 2016), temporal control is critical for ensuring analytical robustness. This consistency is further substantiated by the shape and orientation of the density ellipses in the bivariate plots. While plant-based treatments exhibit elongated ellipses indicative of internal correlations between electrochemical parameters soil samples display more compact and circular ellipses, reflecting structural homogeneity and electrochemical passivity (Rodrigues et al., 2017; Valbuena Calderón et al., 2008).

Collectively, these multivariate patterns underscore the electrochemical complexity and biological heterogeneity inherent in plant-based PMFCs. The use of integrated statistical visualization techniques provides valuable insights into system dynamics, facilitating the identification of bioelectrical signatures that differentiate plant treatments from inert controls. Such findings reinforce the applicability of multivariate analysis as a robust tool for performance evaluation and optimization in bioelectrochemical research.

3.3. Evaluation of Correlations between Operational Time, Voltage, Current, and Illuminance in Plant Microbial Fuel Cells

Distributional asymmetries across the experimental variables indicate non-Gaussian behavior in the system. As illustrated in Figure 2, voltage and current display pronounced right skewness, while operational days follow an approximately uniform distribution. These patterns are characteristic of bioelectrochemical variability, where biological complexity often results in non-linear and asymmetric signal outputs (Šajn et al., 2024).

To account for such data structures and mitigate the influence of outliers and structured noise, robust estimators such as the Minimum Covariance Determinant (MCD) were applied(Mutalib et al., 2025; Tolner et al., 2022).

The uniformity observed in the distribution of "days" supports the validity of the experimental schedule, minimizing temporal confounding. Correlations between days and the remaining variables were negligible ($r = 0.11$ with lux; $r < 0.05$ with voltage and current), indicating that observed variance largely stems from intrinsic system dynamics.

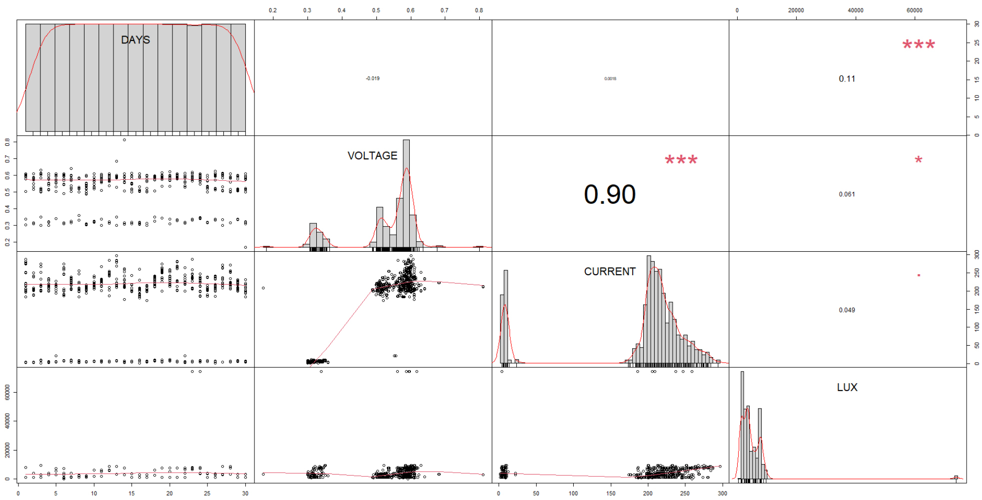


Figure 2: Correlation Matrix and Univariate Distributions of Experimental Variables. Significance Levels: ***p < 0.001; **p < 0.01; *p < 0.05.

As demonstrated in Figure 3, a strong positive correlation was observed between voltage and current (r ≈ +1), consistent with Ohmic behavior in plant-based bioelectrical systems. This relationship reflects key physiological traits such as internal resistance and electron transport efficiency, suggesting that both variables serve as reliable indicators of electrochemical performance. In contrast, correlations between lux and the electrical variables were weak, and days exhibited minimal association with voltage or current. This statistical independence is advantageous in multivariate modeling, as it reduces confounding and supports the role of "days" as a controlled covariate (Rao & Basak, 2023). The low collinearity also improves model stability and enhances the interpretability of regression outputs.

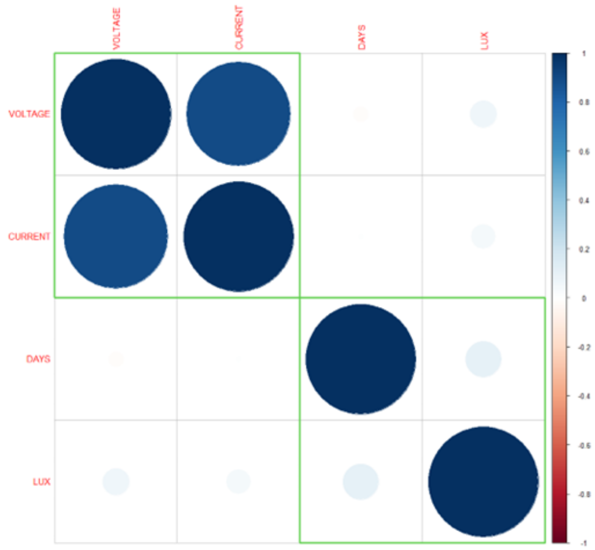


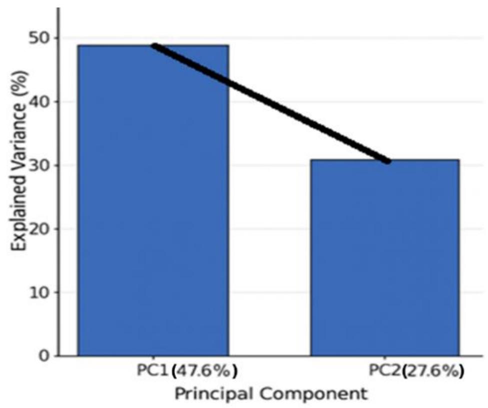
Figure 3: Pearson Correlogram among Bioelectrical and Environmental Variables. Circle Size and Color Intensity Indicate Correlation Magnitude and Direction; Darker Blue Signifies Stronger Positive Associations.

The observed correlation structure informs downstream dimensionality reduction strategies. Given the redundancy between voltage and current, Principal Component Analysis (PCA) becomes essential to transform these variables into orthogonal components for clearer data visualization and interpretation (Landwehr et al., 2021). In more complex settings, sparse PCA (sPCA) offers increased sensitivity to latent patterns and variable selection, particularly valuable in bioelectrical and environmental datasets.

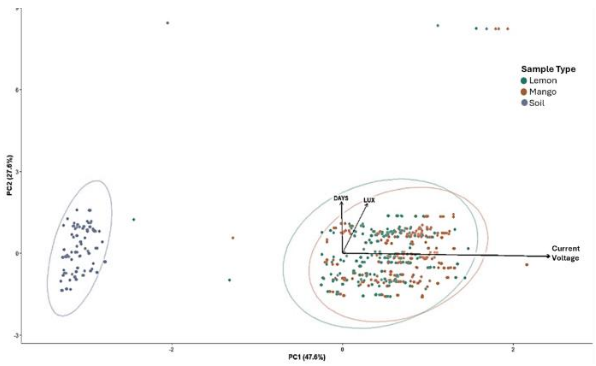
3.4. Principal Component Analysis (PCA) of Bioelectrochemical Variables

To uncover latent structure and reduce dimensionality, PCA was applied to normalized data for voltage, current, lux, and days. The first two components accounted for 75.21% of total variance (PC1: 47.63%; PC2: 27.59%), providing a compact yet informative representation of system variability (Fig. 4a).

Voltage and current contributed strongly to PC1, consistent with their high collinearity (r > 0.9), whereas lux and days aligned orthogonally in the biplot space (Fig. 4b), indicating statistical independence. The separation of lux from electrical variables suggests it captures optical inputs unrelated to bioelectrochemical output. Days acted as a temporally structured covariate with negligible multicollinearity. These findings validate the use of electrical parameters as core explanatory variables and support the incorporation of lux and days as nonredundant dimensions in multivariate modeling. Treatment-based clustering in PCA space (lemon, mango, soil) indicates differential system behavior potentially linked to substrate composition or microbial dynamics.



(a)



(b)

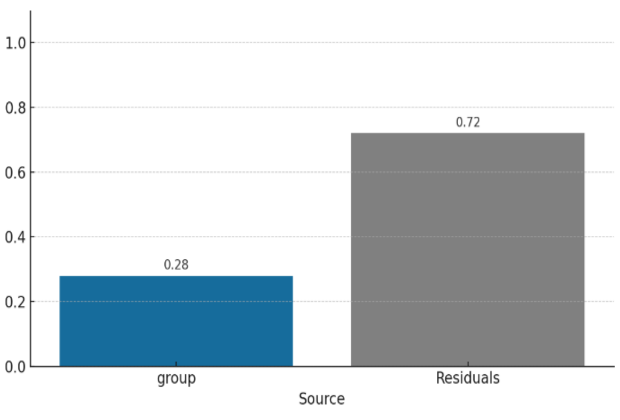
Figure 4: Principal Component Analysis of PMFC System Variables. (a) Variance Explained by PC1 and PC2. (b) Biplot Showing Sample Distribution by Treatment and Variable Loadings; Axes Are Labeled as PC1 (47.63%) and PC2 (27.59%).

These results offer insights into the electrical architecture of PMFC systems and their potential as biosensing platforms. While voltage–current dynamics remain central to system characterization, their loadings may also reflect deeper biochemical processes, such as membrane polarization or proton conduction across lignocellulosic networks (Zecchi & Heimburg, 2021). The combined PCA–correlation framework provides a scalable strategy for mapping bioelectrochemical complexity and supports the development of low-cost, plant-derived sensing technologies for environmental applications.

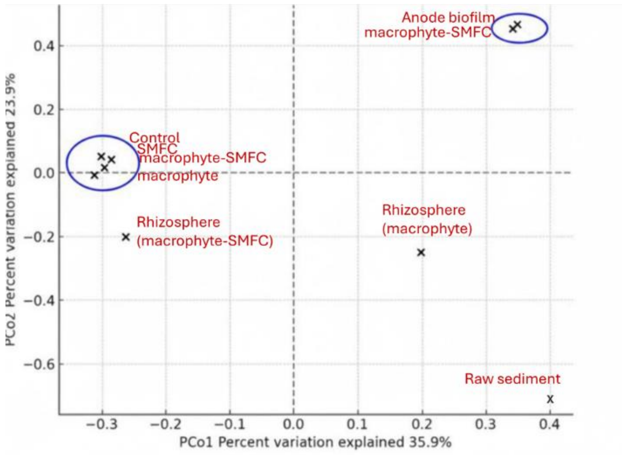
3.5. Microbial Community Structure Driven by Electrochemical Treatment: Ordination and Variance Partitioning

PCoA revealed distinct clustering of microbial communities by treatment, indicating divergence in community composition across experimental groups (Fig. 6a). Anode biofilms separated clearly from rhizosphere and sediment samples, suggesting selective enrichment of electroactive taxa such as Geobacter, Desulfuromonas, and Shewanella at the electrode surface (Logan et al., 2006; Park et al., 2018). In contrast, sediment samples grouped independently, reflecting a background microbial structure minimally shaped by electrochemical gradients. These spatial patterns support the hypothesis of redox-driven niche differentiation within PMFC systems (Guan et al., 2019).

To statistically validate these differences, a permutational analysis of variance (PERMANOVA) was conducted. Treatment explained 28% of total variance ($F = 2.59$, $R^2 = 0.28$, $p = 0.001$; 9,999 permutations), with the remaining variability attributed to residual environmental heterogeneity and stochastic effects (Fig. 6b). The R^2 value aligns with typical outcomes in soil microbiome studies, where fine-scale variability driven by moisture, pH, and microstructure accounts for a large proportion of unexplained variance (Su et al., 2021).



(a)



(b)

Figure 5: Microbial Community Structure in PMFC Systems. (a) PCoA Ordination of Microbial Profiles by Treatment. (b) Variance Partitioning by PERMANOVA.

These findings indicate that PMFC design exerts deterministic control over microbial assembly, potentially enhancing system-level functionality. The integration of ordination and robust statistical frameworks provides a scalable approach to quantify ecological responses to electrochemical conditioning and informs bioelectronic applications in environmental monitoring (Yang et al., 2024).

Despite the small number of biological replicates (n = 3 per treatment), the clear separation observed in the ordination spaces and the consistently large effect sizes support the biological relevance of the patterns reported here. Nevertheless, wide confidence intervals around some estimates reflect limited nominal power; thus, findings should be interpreted as a statistical baseline to be validated with increased replication in future studies (≥ 6 pots per treatment and field-relevant settings). By modelling the pot as the independent unit and using 12-hourly repeated measures to stabilise within-unit estimates, the study maximises information yield while maintaining appropriate inferential scope and reproducibility.

4. CONCLUSION

It is determined that PMFC systems integrating Mangifera indica and Citrus limon sustain stable bioelectrochemical performance under tropical, controlled conditions. Over the 30-day period, planted treatments yielded consistently higher outputs than unplanted controls, with M. indica exhibiting slightly higher median outputs than C. limon. In line with the study's approach, the emphasis remains on relative differences and temporal stability rather than absolute maxima.

Correlation analysis evidenced a near-perfect association between voltage and current ($r \approx +1$), confirming robust electrochemical coupling. PCA accounted for 75.21% of total variance (PC1: 47.63%; PC2: 27.59%) and separated treatments according to multivariate electrical patterns, while PCoA/PERMANOVA identified significant differences in microbial community composition ($R^2 = 0.28$; $p = 0.001$). In this sense, the results highlight the integrated nature of electrochemical and microbiological responses in planted PMFCs.

Accordingly, M. indica and C. limon emerge as promising platforms for biosensing and bioelectricity generation monitoring in tropical agroecosystems. Future work should standardize electrode-area and external-load protocols, explore modeling beyond PCA (e.g., partial least squares for prediction), and evaluate performance under field-relevant fluctuations (light, moisture) to generalize applicability. Likewise, data and code availability (e.g., raw 16S reads in a public repository; R scripts) will serve as a baseline to strengthen reproducibility and facilitate meta-analyses.

From a practical standpoint, forthcoming work will translate these PMFCs into deployable biosensing nodes for tropical agroecosystems by (i) calibrating voltage/current against soil electrical conductivity and moisture to deliver in situ diagnostics, (ii) testing early-warning capability for salinity and waterlogging stress, (iii) evaluating low-power telemetry integration for continuous field monitoring, and (iv) assessing cross-crop generalization (e.g., banana, cocoa) under routine irrigation and rainfall cycles.

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