Electrical response of a soil microbial fuel cell under $CoCl_2$-induced chemical perturbations

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Soil microbial fuel cells (SMFCs) are bioelectrochemical systems capable of generating electrical signals from microbially mediated oxidation processes occurring within the soil matrix. This study evaluates the electrical response of a soil microbial fuel cell (SMFC) subjected to controlled chemical perturbations induced by cobalt chloride ($CoCl_2$). SMFC microcosms were established under four treatments: control, low (50 mg), medium (100 mg), and high (200 mg) $CoCl_2$, with four replicates per treatment. Soil physicochemical parameters, including pH, conductivity, moisture, and nutrient content, were monitored together with voltage and current outputs. $CoCl_2$ addition produced transient soil acidification and increased ionic strength. Current exhibited strong sensitivity to these changes, whereas voltage remained comparatively stable. Spearman correlation analysis revealed a consistent negative relationship between current and soil pH, identifying pH as the dominant controlling variable. A theoretical electrochemical framework confirmed that $CoCl_2$ acts as a chemical modulator rather than an electron source. These results highlight the potential of SMFCs as responsive bioelectrochemical indicators of soil chemical dynamics.

Keywords: Soil microbial fuel cells; electrochemical perturbation; cobalt chloride; soil pH dynamics; bioelectrochemical response.

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1. Introduction

Soil microbial fuel cells (SMFCs) are bioelectrochemical systems capable of converting part of the chemical energy stored in soil organic matter into electrical energy through the metabolic activity of soil microorganisms. In these systems, organic compounds naturally present in the soil matrix, including the partially decomposed organic matter, are oxidized by electroactive microorganisms in the anode zone. This process releases electrons that are transferred through an external circuit toward a cathodic electron acceptor, typically oxygen, enabling continuous electricity generation driven by microbial metabolism within the soil environment. Although early developments of microbial fuel cells were largely focused on plant-associated configurations, the fundamental electrochemical principles described in these studies are directly applicable to soil-based SMFC systems [1–3].

Unlike conventional microbial fuel cells operated with liquid electrolytes, SMFCs function within heterogeneous and dynamic soil environments, where physicochemical conditions vary temporally and spatially. As a consequence, the electrical output of SMFCs is strongly influenced by soil properties such as pH, moisture, temperature, ionic conductivity, and nutrient availability. These variables affect microbial activity, proton transport, internal resistance, and extracellular electron transfer pathways, all of which are strongly governed by the structure and functional dynamics of the microbial community. Comprehensive reviews on plant microbial fuel cells have highlighted the central role of electroactive microorganisms and their interaction with environmental conditions in regulating system performance [4, 5]. Although PMFC configurations differ from soil-only systems, the underlying microbial electrogenic mechanisms are shared across bioelectrochemical platforms.

Among these factors, pH has been identified as a key parameter governing the performance of bioelectrochemical systems. Variations in pH influence microbial metabolism, proton accumulation near the anode, and electrochemical losses associated with polarization phenomena. Similarly, soil conductivity controls ionic transport between electrodes

and contributes to the overall ohmic resistance of the system. Consequently, changes in soil chemical conditions often lead to pronounced fluctuations in current, whereas voltage typically exhibits a more buffered response due to thermodynamic constraints imposed by electrode potentials [3].

Although the power density achieved by SMFCs remains low compared with conventional renewable technologies such as photovoltaic systems, their relevance lies in applications requiring ultra-low power levels. Recent studies have demonstrated the feasibility of coupling SMFCs with energy harvesting and power management circuits to operate autonomous sensors, positioning these systems as promising platforms for long-term, self-powered environmental monitoring [6, 7].

Beyond energy harvesting, SMFCs have been proposed as sensitive bioelectrochemical indicators of environmental disturbances. Chemical inputs, nutrient amendments, or ionic stressors can modify the structure of the microbial community and metabolic pathways, resulting in detectable changes in electrical signals. In particular, dissolved ions and metal species have been reported to influence extracellular electron transfer mechanisms and biofilm behavior in microbial electrochemical systems, even when these species do not participate directly in redox reactions [8, 9].

Cobalt chloride ($CoCl_2$) is a highly soluble salt that readily dissociates in aqueous environments into Co^{2+} and Cl^{1-} ions, producing transient changes in ionic strength and soil acidity. Although $CoCl_2$ is not an electron donor for microbial fuel cells, its addition can indirectly alter soil chemical conditions by modifying pH, ionic strength, and microbial stress responses. Such characteristics make $CoCl_2$ a useful agent for inducing controlled chemical perturbations in soil systems.

Despite growing interest in SMFCs, experimental studies quantifying the relationship between soil physicochemical parameters and electrical output under chemically perturbed conditions remain limited. In particular, the identification of the variables that most strongly govern current and voltage behavior under different levels of ionic stress is still poorly understood.

Therefore, the objective of this study is to evaluate the electrical response of a soil-based SMFC subjected to increasing concentrations of $CoCl_2$, with particular emphasis on the temporal evolution of soil pH and its relationship with current and voltage generation. By combining continuous electrical measurements with physicochemical monitoring and correlation analysis, this work seeks to identify the main variables associated with SMFC performance under controlled chemical perturbation and to examine the differential sensitivity of current and voltage as indicators of bioelectrochemical activity.

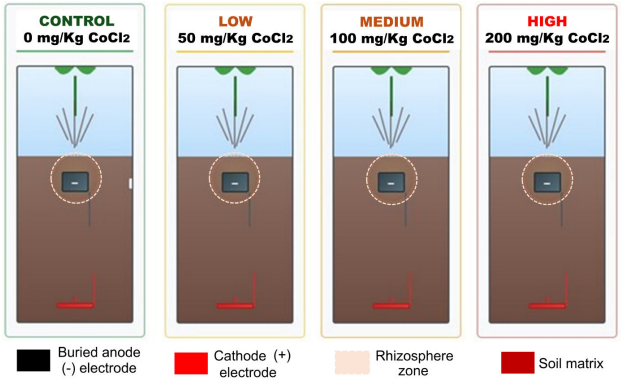

FIGURE 1. Schematic representation of the SMFC configuration used in this study, showing the soil matrix, buried anode, and cathode. $CoCl_2$ treatments (control, low, medium and high concentrations) were applied to induce controlled chemical perturbations of the soil environment.

2. Materials and methods

2.1. Experimental design and SMFC configuration

The experiment was conducted using SMFC established in individual pots under controlled laboratory conditions. Each SMFC is equipped with a pair of metallic electrodes acting as the anode and cathode. The anode was fabricated from a 3 m long copper wire arranged in a spiral configuration, covering an effective circular area of approximately $78.5\ \mathrm{cm}^2$. The copper wire is inserted into a flexible plastic tube. The cathode consisted of a zinc electrode with an exposed surface area of $100\ \mathrm{cm}^2$. Both electrodes were embedded within the soil matrix and positioned at a fixed separation distance of 5 cm. Both electrodes were connected to allow periodic electrical measurements under open-circuit and short-circuit conditions. The SMFC configuration was identical for all treatments, ensuring that observed differences in electrical response were exclusively attributable to chemical perturbations rather than to geometric or material variability (Fig. 1).

2.2. $CoCl_2$ treatments and experimental conditions

Cobalt chloride ($CoCl_2{\cdot}6H_2O$, 98% purity; Fagalab) was selected as a highly soluble inorganic salt capable of inducing controlled, transient chemical perturbations in soil via ionic dissociation. Four experimental treatments were established: a control without $CoCl_2$ addition, a low-concentration treatment (50 mg $CoCl_2$), a medium-concentration treatment (100 mg $CoCl_2$), and a high-concentration treatment (200 mg $CoCl_2$) (as seen in Fig. 1). Reagent quantities were accurately weighed using an analytical balance (PA214C, OHAUS, Parsippany, NJ, USA). The external working humidity and temperature were 79% and $T = 24$–$26\,^{\circ}\mathrm{C}$, respectively. The sixteen SMFCs were the total result of each treatment being replicated four times.

Prior to application, $CoCl_2$ was dissolved in water and uniformly distributed within the soil of each pot to mini-

mize spatial heterogeneity. Upon dissolution, $CoCl_2$ dissociates into Co^{2+} and Cl^{1-} ions, increasing soil ionic strength and promoting transient acidification. All soil matrices were maintained under identical environmental conditions throughout the experiment. Soil was irrigated with $250\ \mathrm{mL}$ of water per pot on a weekly basis, for 4 months.

2.3. Physicochemical measurements

Soil physicochemical conditions were monitored continuously throughout the experimental period using *in situ* measurements. Data acquisition was performed with a portable multiparametric soil probe integrating simultaneous sensors for temperature, volumetric moisture content, electrical conductivity (EC), pH, and macronutrient availability (potassium (K), nitrogen (N), phosphorus (P)). The probe *portable 8-in-1 soil parameter detector (USB-C type, commercial device)* was interfaced with a mobile device, and measurements were recorded and visualized in real time via a dedicated smartphone application (*Soil Parameter Detector*), which stored the acquired data in comma-separated values (CSV) format for subsequent processing and analysis.

Soil pH was measured directly in the soil solution, as it constitutes a key variable influencing microbial metabolism and electrochemical behavior. Electrical conductivity was monitored to track changes in ionic strength associated with $CoCl_2$ dissociation. Nutrient concentrations were used to evaluate potential links between microbial activity and electrical output.

2.4. Electrical measurements

Voltage and current generated by each SMFC were measured once per day over a period of 106 consecutive days (approximately three months) using a calibrated digital multimeter (Fluke Corporation, USA). Open-circuit voltage (OCV) was measured directly across the electrodes. Short-circuit current (I_{SC}) was determined by directly connecting the electrodes through the ammeter function of the multimeter, effectively imposing negligible external resistance. Electrical measurements were systematically recorded and subsequently analyzed as time series to capture transient responses associated with chemically induced perturbations.

2.5. Data preprocessing and statistical analysis

Raw experimental data were organized by treatment, replicate, and time. To avoid pseudoreplication arising from high-frequency measurements, daily mean values were calculated for each replicate prior to statistical analysis.

Descriptive statistics, including mean and standard deviation, were computed to summarize temporal trends and treatment-level variability. Heatmaps were constructed to visualize heterogeneity among replicates and to identify periods of enhanced electrical activity.

Correlation analyses were performed using Spearman's rank correlation coefficient (ρ), selected due to the non-normal distribution of several variables and the monotonic relationships expected between physicochemical parameters and electrical outputs. Correlations were evaluated independently for each treatment, and statistical significance was assessed using a threshold of $p < 0.05$. All data processing, statistical analyses, and graphical representations were conducted using MATLAB (MathWorks, USA), ensuring full reproducibility of the analytical workflow.

2.6. Theoretical electrochemical framework associated with $CoCl_2$ addition

To contextualize the magnitude of the electrical response observed in the SMFC system, a theoretical electrochemical framework was considered based on the amount of $CoCl_2$ added to the soil. This analysis does not imply that cobalt chloride acts as an electron donor within the SMFC; instead, it provides an upper theoretical limit for the charge that could be associated with the applied compound under hypothetical electrochemical conditions [10].

Cobalt chloride readily dissociates in aqueous environments according to:

$$\mathrm{CoCl_2} \rightarrow \mathrm{Co^{2+}} + 2\mathrm{Cl^{1-}}. \tag{1}$$

Under soil conditions, neither Co^{2+} nor Cl^{1-} are expected to undergo spontaneous redox reactions that directly release electrons. However, assuming a hypothetical complete electrochemical reduction of cobalt ions,

$$\mathrm{Co^{2+}} + 2\mathrm{e^-} \rightarrow \mathrm{Co^0}, \tag{2}$$

each mole of $CoCl_2$ would correspond to a maximum of two moles of electrons. The total theoretical charge Q can thus be estimated as:

$$Q = n_e F, \tag{3}$$

where n_e is the number of moles of electrons and F is Faraday's constant ($96485\ \mathrm{C\ mol^{-1}}$).

This framework provides an upper bound that contextualizes the order of magnitude of the measured currents and reinforces the interpretation of $CoCl_2$ as a chemical modulator rather than an electrochemical fuel.

3. Results

3.1. Chemical perturbation induced by $CoCl_2$ addition

The addition of cobalt chloride generated clear and reproducible chemical perturbations in the soil system. Temporal monitoring of soil pH and electrical conductivity revealed an immediate response following $CoCl_2$ application, characterized by transient acidification and increased ionic strength (Fig. 2).

All treatments receiving $CoCl_2$ exhibited a marked decrease in pH during the initial experimental phase, whereas the control treatment remained comparatively stable. This

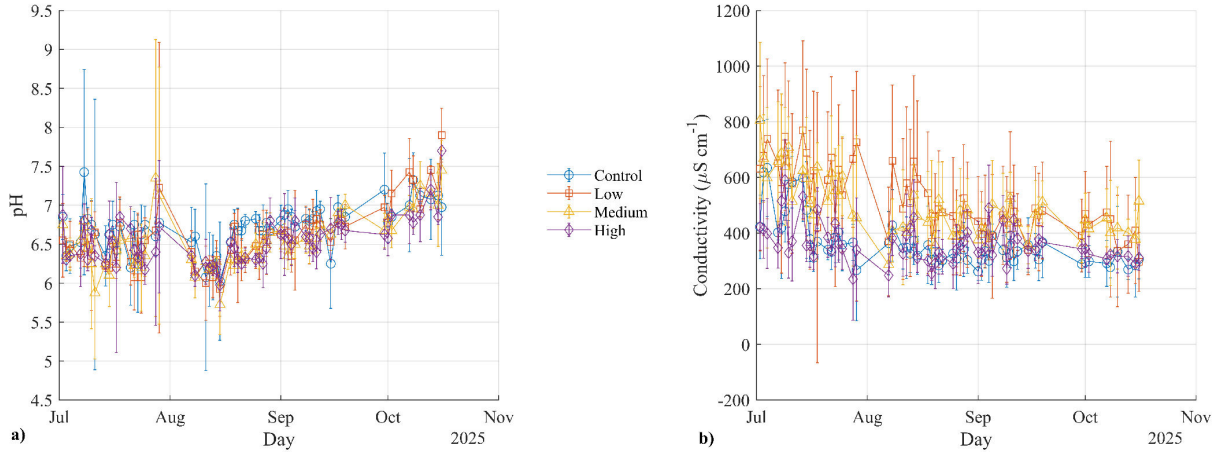

FIGURE 2. Temporal evolution of soil a) pH and b) electrical conductivity for the different $CoCl_2$ treatments.

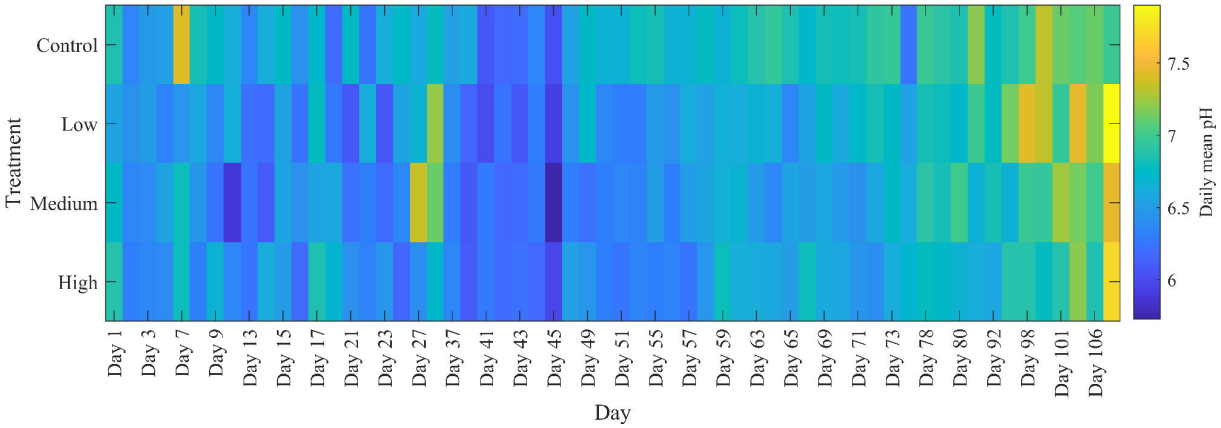

FIGURE 3. Heatmap showing temporal variability across replicates for soil pH under different $CoCl_2$ treatments. Color intensity reflects normalized values.

acidification was transient, with pH values progressively recovering toward near-neutral conditions within several days. The temporal behavior was consistent among replicates, as evidenced by the pH heatmap (Fig. 3), indicating that the chemical response was primarily driven by the applied salt rather than stochastic variability.

Soil electrical conductivity increased proportionally with $CoCl_2$ concentration, with the highest values observed under the high-dose treatment. Conductivity fluctuations were most pronounced during the early stage of the experiment and subsequently stabilized, suggesting the establishment of a new ionic equilibrium within the soil matrix. Together, these results confirm that $CoCl_2$ addition induces a short-term but measurable modification of soil chemical conditions, providing the initial perturbation to which the SMFC system responds.

3.2. Electrical response of the SMFC system to chemical perturbation

The chemical perturbations described above were translated into a measurable electrical response of the SMFC system. Temporal profiles of current and voltage are shown in Fig. 4.

I_{SC} exhibited pronounced variability during the early experimental phase, particularly in treatments containing low and medium $CoCl_2$ concentrations. Periods of elevated current coincided temporally with the phase of reduced pH and increased conductivity. In contrast, the control treatment showed comparatively stable current levels throughout the monitoring period.

Voltage displayed a distinct behavior. Although slight temporal fluctuations were observed, voltage values remained within a narrower range compared with current, indicating a more buffered electrical response. The heterogeneity of current generation across time and replicates is further illustrated in the current heatmap (Fig. 5), where localized periods of enhanced electrical activity were observed, particularly under chemically perturbed conditions. These patterns confirm that the SMFC system responds dynamically to changes in soil chemistry rather than maintaining a constant electrical output.

3.3. Distribution of electrical outputs among treatments

The distribution of daily mean current and voltage values across treatments is summarized in Fig. 6. Current distribu-

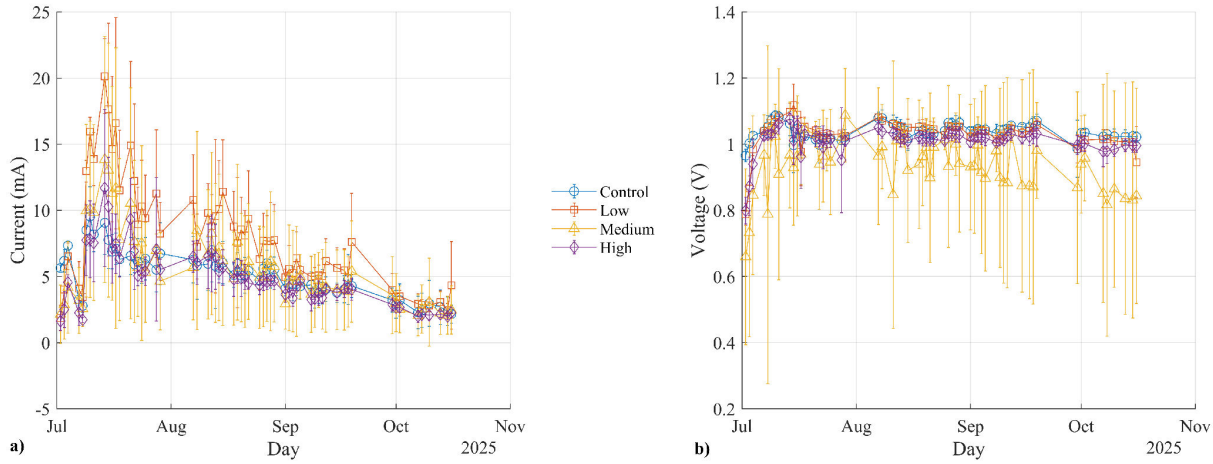

FIGURE 4. Temporal evolution of a) current, and b) voltage for the different $CoCl_2$ treatments. Shaded areas indicate the initial perturbation phase following salt addition.

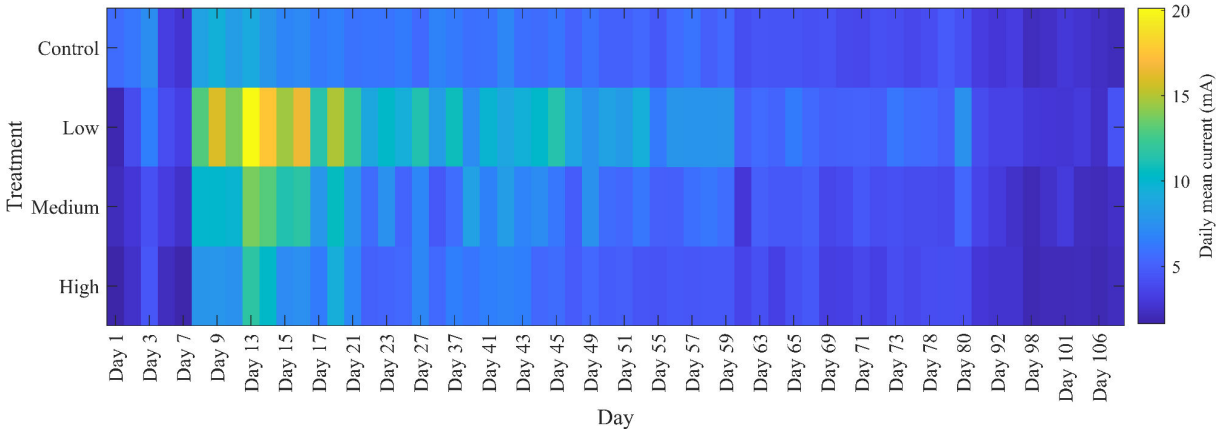

FIGURE 5. Heatmap showing temporal variability across replicates for current generation under different $CoCl_2$ treatments. Color intensity reflects normalized values.

tions differed among treatments, with broader interquartile ranges observed under low and medium $CoCl_2$ concentrations. These distributions indicate higher variability in current generation when the system was exposed to moderate chemical perturbation.

In contrast, the high-concentration treatment exhibited a narrower current distribution, suggesting a constrained electrical response at elevated $CoCl_2$ levels. Voltage distributions were comparatively more uniform across treatments, with substantial overlap observed among all conditions. This reinforces the observation that voltage is less sensitive to changes in soil chemical conditions than current.

Overall, these results indicate that current constitutes the primary responsive electrical variable in the SMFC system, whereas voltage behaves as a more stable parameter under chemical perturbation.

3.4. Differential sensitivity of current and voltage

A direct comparison of current and voltage responses reveals a clear difference in environmental sensitivity. While current responded strongly to temporal changes in soil chemistry, voltage exhibited weaker associations and reduced variability.

This differential sensitivity is reflected in the correlation analyses, where current displayed significant relationships with several soil physicochemical parameters, whereas voltage correlations were generally weaker or non-significant. These contrasting behaviors indicate that current more directly reflects variations in bioelectrochemical activity, while voltage is constrained by system-level electrochemical potentials.

Accordingly, current was selected as the principal indicator for identifying the variables controlling SMFC electrical performance.

3.5. Identification of controlling variables for current generation

The relationships between current and soil physicochemical parameters are presented in Fig. 7. Spearman rank correlations calculated from daily mean values revealed a strong and

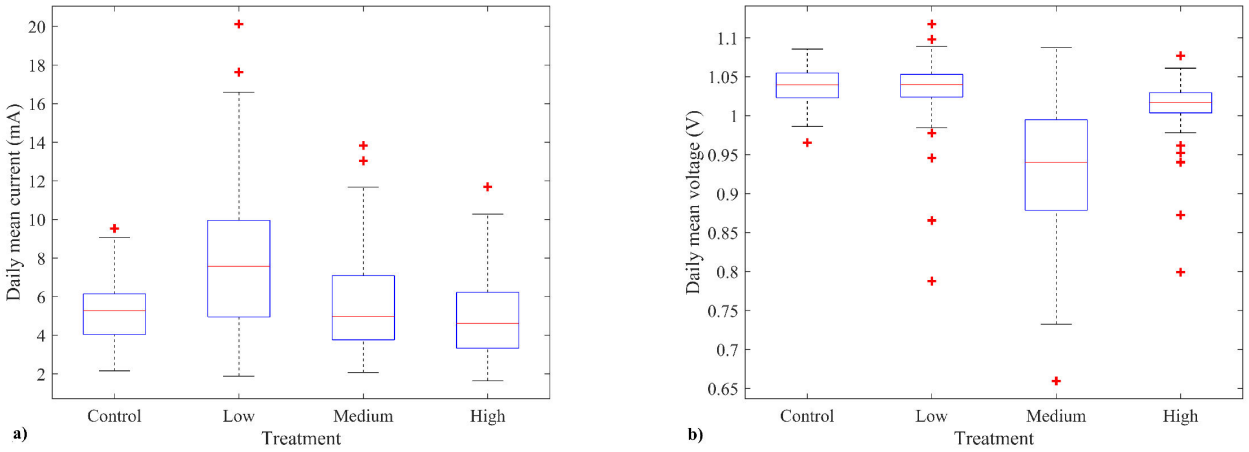

FIGURE 6. Distribution of daily mean a) current and b) voltage values across experimental treatments. Boxes represent interquartile ranges, horizontal lines denote medians, and whiskers indicate data dispersion.

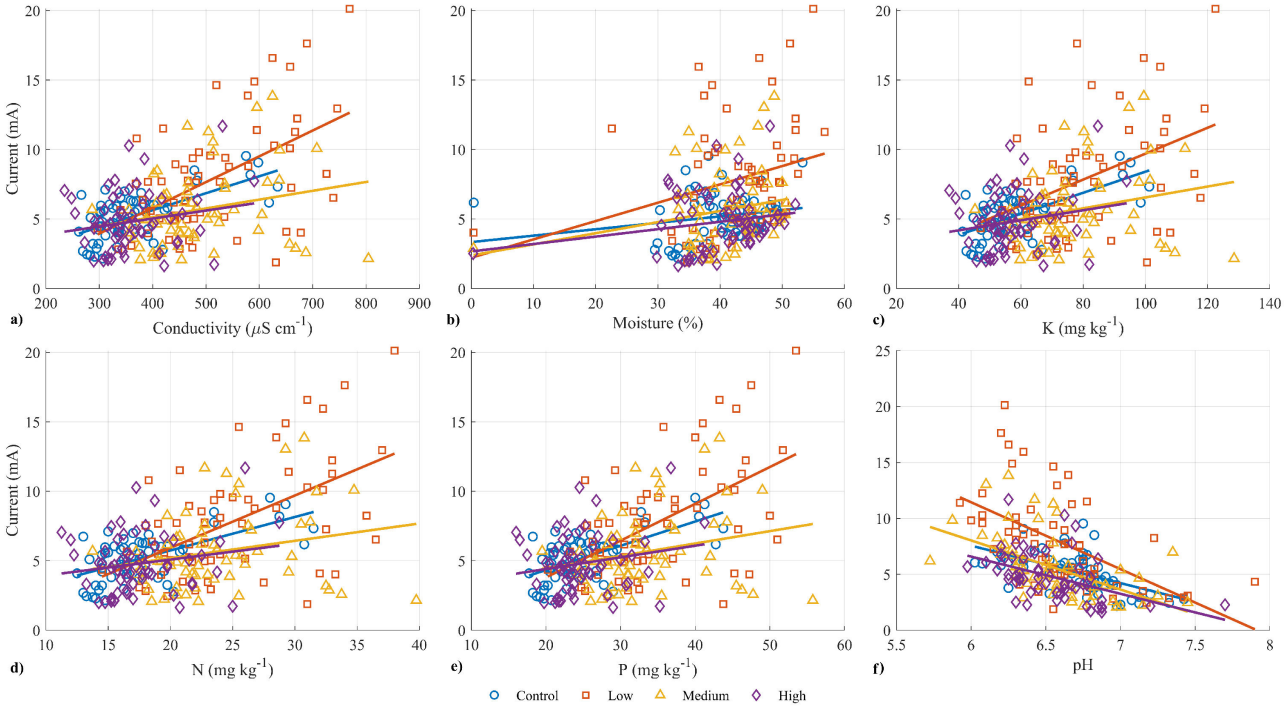

FIGURE 7. Relationships between current generation and soil physicochemical parameters under different $CoCl_2$ treatments: a) electrical conductivity, b) soil moisture, c) potassium concentration, d) nitrogen concentration, e) phosphorus concentration, and f) soil pH. Symbols represent individual daily mean values for each treatment (control, low, medium, and high $CoCl_2$), and solid lines correspond to linear fits shown for visual guidance.

consistent negative relationship between current and soil pH across all treatments [Fig. 7a)].

Lower pH values were systematically associated with higher current generation, with statistically significant correlation coefficients observed regardless of $CoCl_2$ concentration. This relationship emerged as the dominant control on current variability.

In addition to pH, current exhibited moderate positive correlations with nitrogen and phosphorus concentrations [Fig. 7c)–7d)], particularly under control and low $CoCl_2$ treatments. These associations weakened at higher salt concentrations, suggesting that nutrient-driven enhancement of current becomes less effective under stronger chemical perturbation.

Correlations between current and potassium content, moisture, and conductivity were generally weaker and more treatment-dependent [Fig. 7b), 7e)–7f)], indicating secondary or indirect influences. Overall. Within the experimental conditions evaluated in this study, pH emerged as the primary controlling variable for current generation, while nutrients

TABLE I. Spearman rank correlation coefficients (ρ) between daily mean current and soil physicochemical parameters under different $CoCl_2$ treatments ($n = 60$ per treatment). Statistically significant correlations ($p < 0.05$) are indicated with an asterisk (*).

Variable	Control	Low	Medium	High
Conductivity (μS cm^{-1})	0.534*	0.477*	0.256*	0.113
Moisture (%)	0.246	0.329*	0.245	0.118
K (mg kg^{-1})	0.533*	0.399*	0.252	0.119
N (mg kg^{-1})	0.523*	0.475*	0.244	0.108
P (mg kg^{-1})	0.538*	0.472*	0.242	0.109
pH	-0.677*	-0.626*	-0.678*	-0.539*

modulated the magnitude of the response under less chemically stressed conditions. The quantitative results of the Spearman correlation analysis are summarized in Table **??**. Current exhibited statistically significant positive correlations with soil conductivity, potassium, nitrogen, and phosphorus under control and low $CoCl_2$ treatments. These associations weakened under medium concentrations and became non-significant under high $CoCl_2$ conditions.

In contrast, soil pH showed a strong and statistically significant negative correlation with current across all treatments, with the highest magnitude observed under control and medium conditions ($\rho \approx -0.68$). This pattern indicates a consistent inverse relationship between soil acidity and current generation, independent of cobalt concentration.

3.6. Modulation of system sensitivity by $CoCl_2$ concentration

The magnitude and structure of current–soil relationships varied with $CoCl_2$ concentration. Under low and medium treatments, correlations between current and multiple soil variables were stronger and more consistent. In contrast, the high-concentration treatment exhibited reduced correlation strength and lower variability.

This pattern indicates that increasing chemical perturbation alters the sensitivity of the SMFC system, potentially shifting it from a metabolically responsive regime toward a more constrained state. These results highlight the importance of considering concentration-dependent effects when evaluating chemical inputs in SMFC systems.

4. Discussion

The present study demonstrates that transient chemical perturbations induced by $CoCl_2$ addition produce measurable and reproducible modifications in the electrical response of the SMFC system. Although conceived as a preliminary assessment, the experimental results reveal consistent relationships between soil physicochemical variables and electrical output that can be interpreted within a general electrochemical framework governing bioelectrochemical systems [5, 11].

The most robust outcome of this work is the strong and persistent negative correlation between current and soil pH across all treatments. Independent of cobalt concentration, lower pH values were systematically associated with higher current generation. From an electrochemical standpoint, variations in pH modify proton activity within the soil electrolyte and can influence proton-coupled redox processes occurring at the anode. Because current under short-circuit conditions reflects the combined influence of reaction kinetics and internal transport limitations, changes in proton availability are expected to affect measurable current more strongly than equilibrium voltage.

Although interfacial resistances and polarization components were not independently quantified in this study, the observed correlations are consistent with pH-dependent modulation of electrochemical kinetics. Similar pH-related effects have been reported in other microbial fuel cell configurations, where enhanced proton transport is associated with increased current densities [11, 12]. While those studies were conducted in liquid-phase systems, the underlying principles of proton-coupled electron transfer are applicable to soil-based SMFCs [2, 3]. Nevertheless, the present results establish correlation rather than direct mechanistic quantification.

In contrast to current, voltage exhibited comparatively limited variability and weaker correlations with soil parameters. This divergence reflects fundamental electrochemical considerations: cell voltage is largely determined by the difference between anodic and cathodic equilibrium potentials, whereas current depends on kinetic and transport factors. Consequently, moderate physicochemical perturbations may significantly alter current without proportionally modifying voltage. The relative stability of voltage observed here is consistent with previous SMFC studies in which current density variations exceed voltage fluctuations [5].

The transient increase in soil electrical conductivity following $CoCl_2$ addition indicates enhanced ionic strength in the soil matrix. Increased ionic concentration may influence ionic transport properties and potentially reduce the ohmic component of internal resistance. However, because resistance contributions were not directly resolved, this interpretation remains qualitative. The moderate positive correlations

between current and conductivity under control and low treatments are consistent with improved charge transport under increased ionic availability, but these associations weakened at higher $CoCl_2$ concentrations.

Positive correlations between current and nutrient concentrations (N and P) were observed primarily under control and low treatments and diminished as cobalt concentration increased. Rather than attributing this attenuation exclusively to microbial stress, the data may indicate a shift in dominant limiting factors controlling current generation. Under mild perturbation, nutrient availability may indirectly influence electron production rates, whereas under stronger ionic modification, electrochemical constraints appear to play a comparatively larger role in determining system behavior.

The concentration-dependent reduction in correlation strength under high $CoCl_2$ treatment suggests that excessive ionic perturbation modifies the sensitivity of the SMFC system. Although cobalt ions do not function as electron donors, their addition alters soil acidity and ionic composition, which in turn modulates electrochemical conditions at the electrode-soil interface. Similar concentration-dependent effects of metal ions on bioelectrochemical performance have been described in related systems [8]. Within the experimental range evaluated here, the persistence of the pH-current relationship across all treatments indicates that proton dynamics constitute the most stable predictor of current variability.

Overall, the results indicate that current acts as the primary responsive electrical variable under chemically perturbed conditions, while voltage behaves as a comparatively buffered parameter. Within the limits of the present experimental design, soil pH emerged as the dominant controlling factor for current generation. Although microbial community composition and internal resistance components were not independently resolved, the observed patterns are consistent with established electrochemical principles governing low-power bioelectrochemical systems.

Future investigations incorporating electrochemical impedance spectroscopy, polarization analysis, and microbial community characterization would enable quantitative separation of ohmic, activation, and mass transport contributions and provide deeper mechanistic resolution of SMFC operation under chemical perturbation [11].

5. Conclusions

This study demonstrates that the electrical behavior of a soil microbial fuel cell (SMFC) is strongly influenced by transient chemical perturbations of the soil environment induced by cobalt chloride ($CoCl_2$) addition. Among the monitored physicochemical variables, soil pH emerged as the dominant factor controlling current variability, with lower pH values consistently associated with enhanced current generation across all treatments.

In contrast, voltage exhibited comparatively limited variation in response to changes in soil chemical conditions, highlighting a clear differential sensitivity between the two electrical outputs. Moderate positive associations between current and soil nutrient availability, particularly nitrogen and phosphorus, were observed under control and low $CoCl_2$ concentrations, whereas increasing ionic stress at higher $CoCl_2$ levels reduced the strength and consistency of these relationships.

The theoretical electrochemical framework confirms that $CoCl_2$ does not function as an electron source within the SMFC system but instead acts as a chemical modulator that alters soil conditions governing microbial metabolism and extracellular electron transfer. The concentration-dependent response observed in this work suggests the existence of a threshold beyond which chemical perturbation constrains, rather than enhances, bioelectrochemical activity.

Overall, the results indicate that SMFC electrical signals-particularly current-respond reproducibly to short-term chemical perturbations and can serve as sensitive indicators of soil bioelectrochemical dynamics. These findings support the potential application of SMFCs not only as ultra-low-power energy harvesting devices but also as self-powered sensors for investigating soil chemical and microbial processes under controlled environmental modifications.

Acknowledgements

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