Sensor-Based Assessment of Plant–Microbe Energy Interactions

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Abstract*—The BIONOTE-L voltametric sensor was used in this work to exploit cyclic voltammetry as an operating principle to interact with the plant/soil system. Indeed, in bacteria, the metabolic process oxidizes organic compounds from the soil organic matter with extracellular electron transfer. Plants can also release electrons into the rhizosphere—in what are known as plant microbial fuel cells (PMFCs). The electrons obtained, when appropriately conveyed, generate an electric current. On this basis, the innovation of a sensor system "interrogating" the plant/soil system via cyclic voltammetry is proposed. This work tries to place a new paradigm: Rather than exploiting the energy autonomously produced, the plant-microorganism system and the interaction that occurs between them can be analyzed in energetic terms, which is novel with respect to the standard approach measuring other parameters related to plant growth. An external input is provided, and the response of the system to this solicitation is observed. The energy extracted does not have the purpose of powering other devices or being stored. Instead, it is treated as data on the state of health of the plant-microorganism system. This test has been structured as a step-by-step experimental investigation, with a gradual increase in complexity. The BIONOTE-L was tested to analyze the behavior of four different genera of bacteria in solution: *Rhizobium*, *Bacillus*, *Enterobacter*, and *Kosakonia*. The behavior of *Bacillus* was then analyzed in interaction with the soil and with a corn plant (*Zea mays* L.). The gradual growth of the bacteria in the solution was assessed using a spectrophotometer (optical density: O.D.) and predicting O.D. with BIONOTE-L with root mean square errors in cross validation from 0.004 to 0.2 (depending on the different bacteria). Specific fingerprints were detected for each hour of bacterial growth (therefore specific for the concentration of bacteria in solution) and for each different genus of bacteria. Finally, the electrical response of *Bacillus* in interaction with the soil and plant was characterized, thus providing a specific electrical signature to be monitored in a real-world scenario.

***Index Terms*—Chemical sensors, cyclic voltammetry, energy harvesting, microbial fuel cells (MFCs), plant microorganisms.**

I. Introduction

THIS study presents a series of tests conducted using the BIONOTE-L voltammetric sensor [1], [2] to analyze the bacteria–soil–plant system and monitor its capacity to generate electricity (ability previously demonstrated in the literature and summarized in this introduction). Soil bacteria can generate electricity, and microbial fuel cells (MFCs) use microbial catabolic activity to convert biomass into electricity [3], [4]. MFCs exploit organic matter oxidation to generate power, offering diverse energy sources [5]. Some bacteria can access energy sources unavailable to other organisms. While animals rely on oxygen as the terminal electron acceptor, bacteria use alternatives such as iron, sulfate, and nitrate. MFCs have been successfully applied to wastewater and organic waste treatment [6], [7], functioning in sludge, soils, marine and riverbeds [8], [9], [10], and rice paddies.

Beyond MFCs, plant microbial fuel cells (PMFCs) generate electricity by oxidizing plant-produced organic compounds, expanding conventional technology. PMFCs rely on photosynthesis and do not require external substrates or nutrients, making them a long-term, sustainable source of clean bioenergy [11], [12], [13], [14], [15].

PMFCs use electrochemically active microorganisms in mixed or pure cultures, advancing self-powered systems for indoor and outdoor use. Strik et al. (2008) [16] first demonstrated PMFCs' energy potential, estimating a theoretical maximum of 21 GJ/ha/year (5800 kWh/ha/year) in Western Europe. Deng et al. (2012) [17] reported annual outputs of 1266–1638 kWh/ha, depending on plant type. Power density is crucial for PMFCs as a renewable source. Strik et al. (2011) [18] estimated a maximum power density of 1.6–3.2 W/m^2 projected geometric anode area (PGA), comparable to conventional bioenergy sources, which reach up to 220 mW/m^2 PGA.

PMFC power density has increased up to more than 240 mW/m^2, showing promise as a future energy source [19].

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Long-term adoption requires maintaining plant vitality and stable energy production while preventing electrode deterioration. Selecting durable, cost-effective, and fouling-resistant plants and electrodes is crucial [20].

In this work, the scientific question is not about the amount of energy produced or its exploitation, but about its meaning in terms of plant conditions. The energy produced by MFCs and PMFCs can serve as an indicator of plant health. Rather than exploiting this energy for power generation, as in conventional MFC and PMFC approaches, this work focuses on evaluating its informational content. The innovation introduced through the use of the BIONOTE-L voltammetric sensor [1], [2], [21] lies in a new paradigm in which the extracted energy is treated not as a power source or storage medium, but as data describing the state of the plant–microorganism system, together with standard environmental monitoring parameters. A bottom-up approach was used, focusing on the microorganisms and their metabolic activity as drivers of the electrical activity of the system. The experiment began by studying microbial electrical activity in solution, then progressed to their interaction with soil and a plant, analyzing different levels of organization. Detecting the produced energy is like "listening" to the system's energy production, while stimulating with a voltage input is like "speaking" to it. Thus, the resulting current output is then "listening" to the system's response. Indeed, to understand plant growth and physiology, a holistic approach is necessary, considering all levels of organization from molecules to ecosystems. Plants have an intimate relationship with microorganisms, and the plant-microbiome should be seen as a holobiont [22]. Information and energy flow back and forth between plants and their microbiota through their roots in the rhizosphere. In-situ sensing of soil and environment by means of a distributed network of heterogeneous sensors is revealing the most viable solution for precision agriculture, enabling the identification of harmful factors for crops and environment, thus transforming information into knowledge-enabling decisions [23].

The study was divided into three stages with increasing complexity. First, the operation of the BIONOTE-L sensor was evaluated using six solutions: Three with different concentrations of ascorbic acid ($C_6H_8O_6$) and three with different concentrations of citric acid ($C_6H_8O_7$). As these acids are standard components involved in microorganism-mediated soil transformations [24], the aim was to verify whether their presence generated an electrical response and whether the sensor could produce specific fingerprints for each acid and concentration. Second, the behavior of different bacterial genera in solution was analyzed to assess the sensor's ability to detect microbial electrical activity and to evaluate variations in sensor output with bacterial concentration and genus. The gradual growth of the bacteria in the solution was assessed using a spectrophotometer (optical density: O.D.) and predicting O.D. with BIONOTE-L with root mean square errors in cross validation (RMSECV) from 0.004 to 0.2 (depending on the different bacteria).

Finally, the behavior of a genus of bacteria in the interaction first only with the soil, then both with the soil and a specific plant, was analyzed. The aim was to choose the best measurement procedure for measuring soil samples and to evaluate whether the electrical activity expressed by the bacteria that showed the best behavior in solution was modified (in terms of pattern (signature) detected by the BIONOTE-L) in the interaction with the soil and in the interaction with the soil and the plant. The electrical response of *Bacillus* in interaction with the soil and plant was characterized, thus providing a specific electrical signature to be monitored in a real-world scenario.

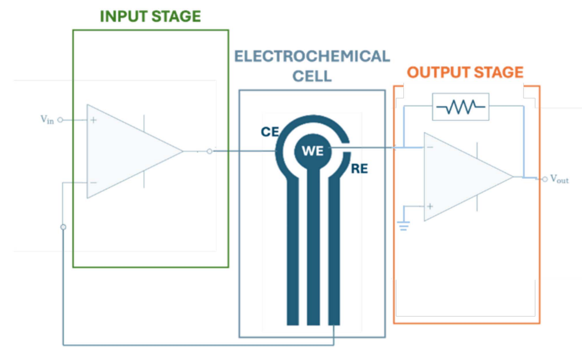


Fig. 1. Scheme of the BIONOTE-L composed of three main stages: the input stage, the electrochemical cell, and the output stage.

II. Methods

A. Sensor

The sensor employed in this work, known as BIONOTE for Liquids (BIONOTE-L), was developed at the Unit of Electronics for Sensor Systems of the University Campus Bio-Medico of Rome [1], [2], [21]. As shown in Fig. 1, the sensor's structure includes an electronic interface that provides the input signal consisting of a potentiostat (indicated in Fig. 1 as "Input Stage"), a transimpedance circuit to record the output signal (indicated in Fig. 1 as "Output Stage"), and an electrochemical cell. As a detection terminal, a screen-printed carbon membrane electrode is used, where the working electrode, diameter 0.40 cm, is made of gold, the counter electrode (CE) of platinum, and the reference electrode (RE) of silver [SPE; DRP-250BT; all from Metrohm (Herisau, Switzerland)].

The input signal applied to the electrochemical cell is a triangular wave with an amplitude in the interval [-1 V; $+1$ V] and a frequency of 0.01 Hz. The output signal is recorded every 200 ms, generating 500 samples per measurement cycle. The amplifiers are powered by a dual voltage between $+6$ and -6 V. After the transimpedance stage, there is a first-order low-pass filter with a cutoff frequency of 10 Hz. The stability of the sensor response (granting measurements reproducibility) is granted by the opamp feedback circuit, which is able to adjust CE voltage in response to RE voltage variations.

B. Calibration to Standard Solution

Six different solutions were examined: three solutions with three different concentrations (30 mM, 20 mM, 10 mM) of ascorbic acid ($C_6H_8O_6$) and three solutions with three different concentrations (30 mM, 20 mM, 10 mM) of citric acid ($C_6H_8O_7$). The volume of water used for each solution was 100 ml.

C. Selection of Plant Growth-Promoting Bacteria (PGPB)

PGPB exhibit several characteristics that positively contribute to plant growth: the ability to change the redox state of metals

TABLE I
INFORMATION RECORDED FOR EACH SELECTED BACTERIAL STRAIN

Bacteria's genus	Accession number	Host plant	Isolation place (Portugal)	Main characteristic	Strain
Rhizobium	KY491644.1	*Pisum sativum* L.	Elvas	Acidification of the growth medium, N_2 fixation	E20-8
Bacillus	MK713663.1	*Phaseolus vulgaris* L.	Vagos	Production of siderophores	J25
Enterobacter	MK318777.1	*Phaseolus vulgaris* L.	Vagos	Phosphorus solubilization	C11
Kosakonia	MG517427.1	*Phaseolus vulgaris* L.	Vagos	Potassium solubilization	O21

(such as iron), to fix atmospheric nitrogen, to produce phytohormones, antimicrobial compounds, and hydrolytic enzymes, to solubilize phosphorus, potassium, and other components essential for plant development. These bacteria can exert an indirect influence on pathogen control through induced systemic resistance or the production of antibiotics and lytic enzymes and can increase plant tolerance to abiotic stresses [25]. To select a bacterial genus for the inoculation of soil samples, some of which also contained plant seeds, four PGPB strains (Rhizobium spp., Bacillus spp., Enterobacter spp., and Kosakonia spp.) were used. Each strain can fulfill one or more of the characteristics (cited above) that contribute to promoting and improving plant growth and health.

The genera of the selected bacteria, accession number, host plant from which they were isolated, isolation location, primary plant growth-promoting characteristics, and strains are detailed in Table I.

D. Motivations for Rhizobium Selection

Bacteria of the genus Rhizobium enable symbiotic nitrogen fixation, reducing atmospheric nitrogen into ammonium (NH4+) in legume root nodules [26]. To fix nitrogen, plants supply organic compounds from photosynthesis, which bacteria use metabolically. This process involves redox reactions, releasing electrons and protons detectable via an electrochemical cell. Similar reactions occur when bacteria degrade organic substrates in soil or solution, a trait common to many bacterial genera. Rhizobium also releases organic acids such as citric and succinic acid, acidifying the surrounding medium by releasing protons. These protons may contribute to electric current production, as they migrate to the cathodic compartment, aiding oxygen or electron acceptor reduction.

E. Motivations for Bacillus Selection

Bacillus is one of the predominant bacterial genera in soil (particularly in the rhizosphere) [27]. Since the Bacillus genus has a high capacity to degrade different substrates, including recalcitrant compounds, this genus of bacteria can be used to generate electric current and at the same time degrade contaminants. Indeed, they are known to enact a series of direct and indirect mechanisms to promote plant growth and health, solubilization and mineralization of phosphorus and other nutrients, production of phytohormones, siderophores, antimicrobial compounds, hydrolytic enzymes, induced systemic resistance, and protection from abiotic and biotic stresses [28]. Another important characteristic associated with this genus of bacteria is their ability to degrade organic matter present in the soil, which has a significant impact on plant growth and agriculture [29].

F. Motivations for Enterobacter Selection

Some soil microorganisms can solubilize phosphorus and convert it into forms that can be used by plants. Bacteria belonging to the soil microbiome that have this ability are known as phosphate-solubilizing bacteria (PSB) [30], [31]. Enterobacter is one of these PSB, therefore, bacteria of the genus Enterobacter can contribute to the production of electric current through two processes: oxidation of organic compounds that occurs during the metabolic process common to all bacterial genera and the release of acids into the surrounding environment in the solubilization of phosphorus. In the process of phosphorus solubilization, bacteria of the genus *Enterobacter* produce organic and inorganic acids that lower the soil pH. The production of acids leads to the release of protons (H^+ ions). The protons generated during this process can be considered an additional reason why this bacterium is believed to contribute to the generation of electric current, beyond the redox metabolic processes common to all bacterial genera. In fact, the protons produced in the anode compartment can migrate to the cathode compartment, where they participate in the reduction of oxygen (O_2) or another electron acceptor.

G. Motivations for Kosakonia Selection

Potassium is an essential macronutrient and major component of all living cells. Along with nitrogen and phosphorus, it plays a key role in plant growth, metabolism, and development [32]. Plant resistance to diseases, parasites, and abiotic stress, is obtained activating various enzymes responsible for plant and animal processes such as energy metabolism, starch synthesis, photosynthesis, and sugar degradation [33]. Bacteria responsible for converting the soil's mineral/insoluble potassium into forms that can be assimilated by plants are known as potassium-solubilizing bacteria (KSB), including Kosakonia is one of them. Most of the evidence of the relationship between Kosakonia bacteria and plants comes from the isolation or detection of different Kosakonia species from the rhizosphere of some plants such as corn or wheat [34]. When growing in a K free medium it is expected that these bacteria will begin to produce acids, lowering the pH of the solution and contributing significantly to the generation of electric current.

H. Monitoring of Bacteria Growth

The measurements were carried out at the University of Aveiro (Portugal), in the Center for Mechanical Technology and Automation (Department of Mechanical Engineering), in the Center for Environmental and Marine Studies, and at the Department of Biology; and at the Campus Bio-Medico University of Rome, in the labs of the Electronics Unit for Sensor Systems.

1) Monitoring of Bacteria Growth in Tubes: For bacterial growth the yeast mannitol broth (YMB) at 6.8 pH was used. Tubes with 5 ml YMB were inoculated with 100 μL of a preinoculation grown during the previous night. Bacteria were grown at 26 °C, 150 rpm, during a 24 hours period. During the 24 hours growth period, bacterial growth was monitored measuring O.D. at 600 nm and electric current at nine intervals, with a rate of 1 hour and after 24 hours (H1, H2, H3, H4, H5, H6, H7, H8, and H24). Different samples of the same bacterium

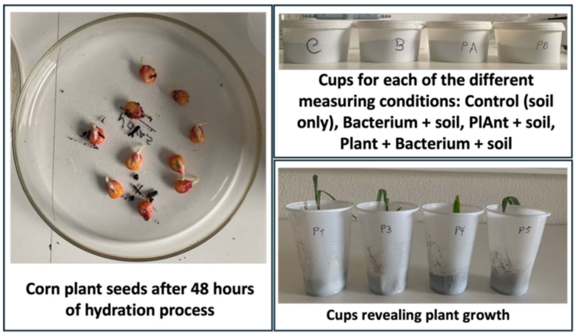
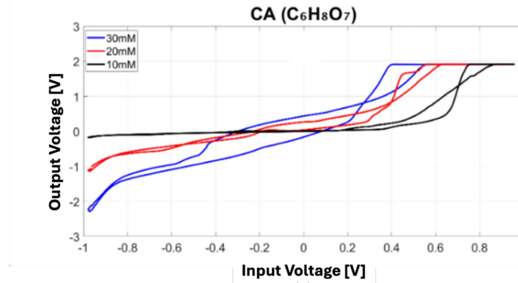
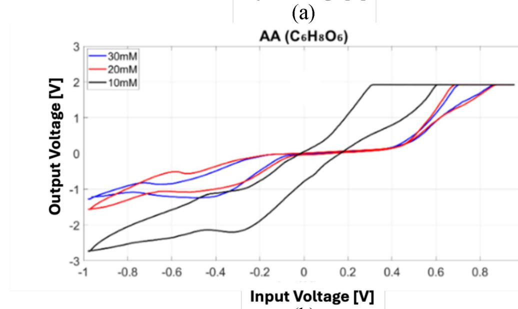


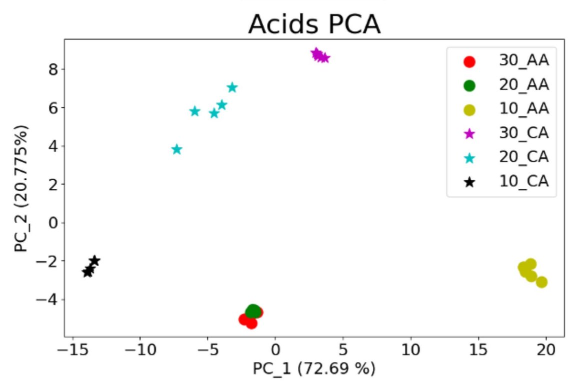
Fig. 2. Preparation of samples for the third experiment with soil and plants. Corn seeds after hydration; the four types of cups of soils tested; the plants evidently grew in the prepared cups.



(a)



(b)



(c)

Fig. 3. (a) Graph of the three average voltammograms from the last five measurement cycles (blue, red, and black) corresponding to the measurements of three different citric acid concentrations (30, 20, and 10 mM). (b) Graph showing the three average voltammograms from the last five measurement cycles (blue, red, and black) corresponding to the measurements of the three different concentrations of ascorbic acid (30, 20, 10 mM). The different shapes of the voltammograms accounts for different samples: the direction of the modification also follows the increasing concentration levels (black, red, blue). (c) PCA scoreplot of the voltammograms represented in panels a and b.

in different time-condition (time: Hi) have been measured for 15 consecutive cycles.

2) Monitoring of Bacteria Growth in Soil: The selection of the plant Zea mays L. (corn) for the following experiments is motivated by its fast-growing rate, and because it is the most produced cereal worldwide, and bacteria inoculation in soil can promote maize growth and production [35]. Since Bacillus was the bacterial strain generating higher voltage (in the experiment described above) it was chosen for the subsequent growth. Zea mays seeds (certified trial seeds, Pioneer Optimum AQUAmax hybrids, variety P9911) were kindly offered by Cooperativa Agrícola de Esposende and Pioneer. Zea mays seeds were hydrated for 2 days in running water. Containers (150 ml) were filled with 100 g of artificial soil (OECD 208). Four conditions were used: artificial soil with 1 ml of nonincubated medium (Ci), artificial soil with 1 ml of nonincubated medium and one hydrated corn seed (Pi), artificial soil inoculated with 1ml (108 cells/ml) Bacillus strain inoculant (Bi), and artificial soil inoculated with 1 ml (108 cells/ml) Bacillus strain inoculant and with one hydrated corn seed (PB). A total of 5 replicates $\times$ 3 were performed, totalizing 15 containers. Containers were placed 14 days under artificial light (1450 μmol/m^2/s) and a photoperiod of 12 hours and 26 $\pm$ 1 °C and 18 $\pm$ 1 °C during light and dark periods, respectively. Containers were watered with distilled water every day, in order to maintain 60% water holding capacity (WHC).

I. Bacteria Interactions with Soil and Plants

BIONOTE-L sensor has previously been used to analyze different soil samples and estimate the compounds present and their concentrations, providing promising results [1]. In this study, a new application of this sensor is proposed, which is aimed at analyzing the behavior of bacteria within the soil and their interaction with plants. Therefore, five measurement procedures were tested to determine the most effective of the research objectives. Each measurement procedure was tested on a different day, resulting in five days of repeated measurements.

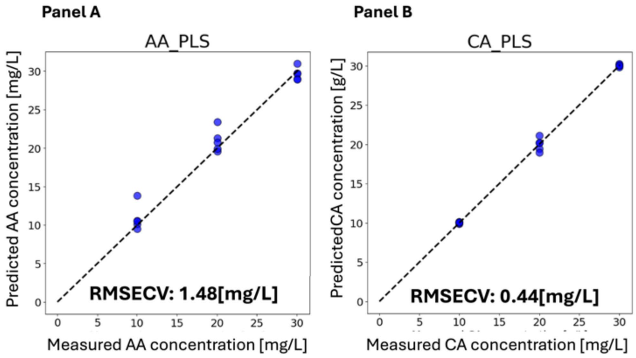
All measurements taken on the various days were carried out in the following order: $\boldsymbol{C_i}$ cup (**C**ontrol cup number i), $\boldsymbol{PA_i}$ cups (cup number i with **P**l**A**nt and soil), $\boldsymbol{B_i}$ cups (cup number i with **B**acterium and soil), $\boldsymbol{PB_i}$ cups (cup number i with **P**lant, **B**acterium and soil) in Fig. 2. All cups without bacterial inoculation were measured first, followed by those in which bacteria were placed to avoid contamination. Each cup was analyzed using the BIONOTE-L sensor for 15 min for a total of eight measurement cycles.

J. Statistics

Considering that each voltammogram registered by the BIONOTE-L is composed of 500 points and that the classification task is here performed by discriminating different profiles for each voltammogram, a multivariate data analysis technique is needed. Partial least square discriminant analysis has been used in this work. Cross validation was performed via leave-one-out criterion.

III. Results

The results are presented according to the three objectives defined in the introduction for the three measurement sessions.



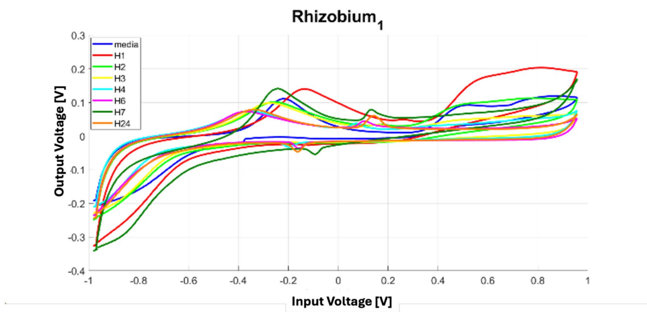


Fig. 4. Predicted citric acid concentration versus measured citric acid concentration and the associated RMSECV: (a) AA and (b) CA.

First, the performance of the BIONOTE-L sensor was evaluated using various concentrations of citric and ascorbic acid. The second part investigated the electrical properties of four bacterial genera (Rhizobium, Bacillus, Enterobacter, Kosakonia) in solution. Finally, the third part examined the electrical behavior of Bacillus strain when interacting with soil and cornplants. The BIONOTE-L sensor was used for all three parts.

A. Measurements With Standards

For both acids, the goal was to first demonstrate that the sensor would return different fingerprints corresponding to the different concentrations of the acid in solution, and then that the differences between these were due to the variations in concentration appropriately captured by the sensor.

Fig. 3(a) and (b) show the average of the last five measurement cycles (blue, red, and black) recorded for each of the three concentrations of ascorbic acid ($C_6H_8O_6$), 30, 20, and 10 mM, and the average of the last five measurement cycles (blue, red, and black) recorded for each of the three concentrations of citric acid (C_6H_8O7), 30, 20, and 10 mM, respectively [36], [37].

As mentioned, the measurements of ascorbic acid and citric acid were intended to test the sensor's ability to monitor microorganism-mediated transformations in soil. However, the concentrations tested are much higher than those expected to be detected in the bacteria-soil-plant system, so the observed saturation is not a concern. If it were, the BIONOTE-L can be adjusted in sensitivity by modifying the control electronics. The fingerprints obtained at different acid concentrations were distinct from one another. Moreover, these differences are proportional to the variations in acid concentration in the solution; in fact, the peaks detected increase in amplitude proportionally with increasing acid concentrations.

To demonstrate sensor's ability in sample discrimination and concentration identification, the voltammograms have been treated as multidimensional data records. Indeed, each voltammogram is composed of 500 points. The sensor's ability to display specific fingerprints based on both the type and concentration of acid is further confirmed by the results obtained through principal component analysis (PCA) applied to a dataset containing the values of the three average voltammograms for both acids. The results are represented along the first two principal components with their corresponding variance values; the data related to the concentrations of the two acids are indicated using different symbols (by shape or color). In Fig. 3(c), each of these form distinct clusters, which are also sufficiently compact and well-separated along both directions (particularly along PC1, which accounts for the greatest variance), effectively demonstrating the sensor's ability to provide different fingerprints based on both the acid type and its concentration.

To demonstrate that the differences between the recorded fingerprints were indeed attributable to the variations in acid concentration in solution, the values of the average voltammograms for the measurements of the three citric acid concentrations and the values of the average voltammograms for the measurements of the three ascorbic acid concentrations were used as two multidimensional datasets, and partial least squares discriminant analysis (PLS-DA) was applied to construct an ad hoc predictive model (cross-validated using the "leave one out" criterion). In Fig. 4(a), the graph shows the predicted citric acid concentration versus the measured citric acid concentration and the associated RMSECV. In Fig. 4(b), the graph shows the predicted ascorbic acid concentration versus the measured citric acid concentration and the associated RMSECV.

In both panels of Fig. 4, it can be observed that the BIONOTE-L sensor demonstrates an excellent ability to detect the concentration of citric acid in solution with an RMSECV of 0.4414 g/L (round 2% of the tested interval) and a good ability to detect the concentration of ascorbic acid in solution with an RMSECV of 1.48 g/L (round 7% of the tested interval).

B. Measure of Bacteria in Culture

All data collected using the BIONOTE-L device were analyzed using PLS-DA, with O.D. measurements used as a reference. This procedure has been performed for each bacteria culture.

1) Rhizobium Repeatability: Fig. 5 shows the voltammetry results for the Rhizobium in "Rhizobium medium 1 and then again after 1, 2, and 3 hours. These consistent readings demonstrate good repeatability. The Supplementary Materials (from S2.1 to S2.4) contain all voltammetry data for the remaining hours of the first day (up to 24 hours) and for the following four days of the measurement.

a) Growth Monitoring: Fig. 6 demonstrates the ability of the sensor to monitor bacterial growth of Rhizobium by showing how the voltammetry readings changed over a 24-hour period. The readings were taken at 1, 2, 3, 4, 6, 7, and 24 hours after the start of the experiment.

The *Supplementary Material* (from S3.1 to S3.3) shows that the same pattern of change occurred over each of the other four days of measurement.

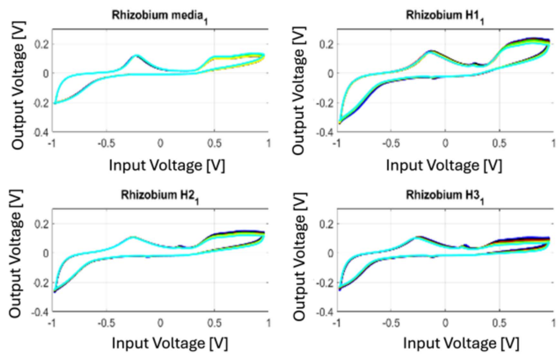


Fig. 5. Voltammograms recorded on the first day for media and for Rhizobium at 1, 2, and 3 hours from the beginning of the experiment. Distinct voltammetric shapes are observed at different time points. In addition, good reproducibility is evident among the voltammograms recorded at the same time.

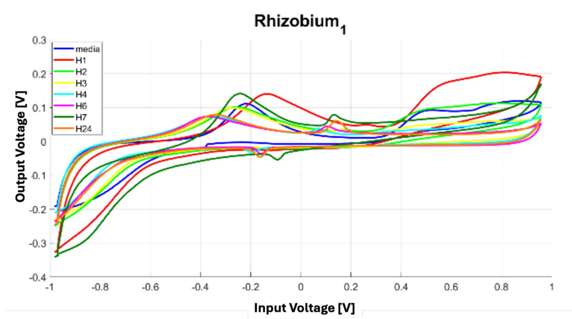


Fig. 6. Voltammograms registered for Rhizobium on the first day after 1, 2, 3, 4, 6, 7, and 24 hours from the beginning of the experiment.

The resulting PLS-DA model confirmed that the changes seen in Fig. 7 reflected an increase in bacterial concentration due to culture growth. As shown in Fig. 10, the strong correlation between the predicted and measured O.D. values, with RMSECV values ranging from 0.007 to 0.33, further supports this conclusion.

2) Bacillus. Repeatability: Fig. 8 shows the voltammetry results for a Bacillus culture on the first day. Six consecutive measurements were taken, starting with Bacillus in "Bacillus media 1 and then again after 1, 2, and 3 hours. The consistent readings demonstrate good repeatability. The Supplementary Materials (from S4.1 to S4.4) contain all the voltammetry data for the remaining hours of the first day (up to 24 hours) and for the following four measurements.

3) Growth Monitoring: Fig. 9 demonstrates the sensor's ability to monitor bacterial growth of Bacillus by showing how the voltammetry readings changed over a 24-hour period. Readings were taken at 1, 2, 3, 4, 6, 7, and 24 hours after the start of the experiment. The Supplementary Material (S5.1) shows that the same pattern of change occurred over the other two days of measurement. The resulting PLS-DA model confirmed that the changes seen in Fig. 12 reflected an increase in bacterial concentration due to culture growth. As shown in Fig. 10, the strong correlation between predicted and measured O.D. values, with RMSECV values ranging from 0.004 to 0.007, further supports this conclusion.

4) Enterobacter. Repeatability: Fig. 11 shows the voltammetry results for the *Enterobacter* culture on the first day. Six consecutive measurements were taken, starting with *Enterobacter* in Enterobacter media 1 and then again after 1, 2, and 3 hours. The consistent readings demonstrate good repeatability. The Supplementary Materials (from S6.1 to S6.4) contain all voltammetry data for the remaining hours of the first day (up to 24 hours) and for the following two days of measurement.

a) Growth monitoring: Fig. 12 demonstrates the ability of the sensor to monitor the bacterial growth of Enterobacter by showing how the voltammetry readings changed over a 24-hour period. Readings were taken at 1, 2, 3, 4, 6, 7, and 24 hours after the start of the experiment.

The Supplementary (S7.1) Material shows that the same pattern of change occurs over each of the other two days of measurement. The resulting PLS-DA model confirmed that the changes seen in Fig. 12 reflected an increase in bacterial concentration due to culture growth. As shown in Fig. 13, the strong correlation between the predicted and measured O.D. values, with RMSECV values ranging from 0.011 to 0.016, further supports this conclusion.

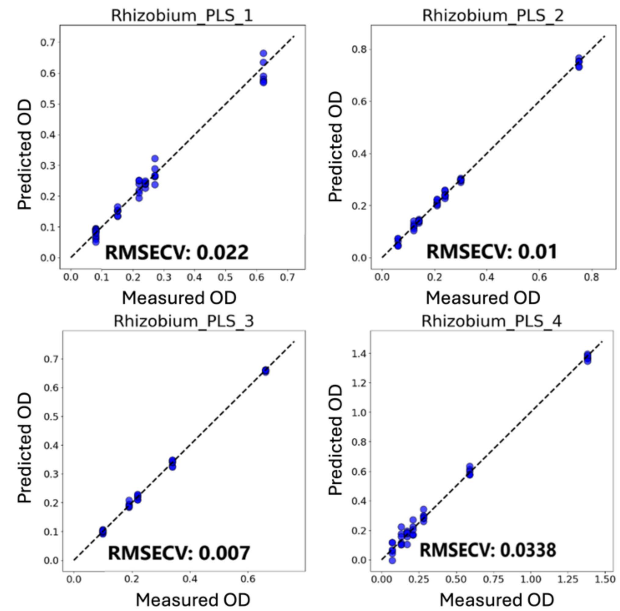


Fig. 7. Predicted versus measured value of O.D. for rhizobium measured at day 1, 2, 3, 4.

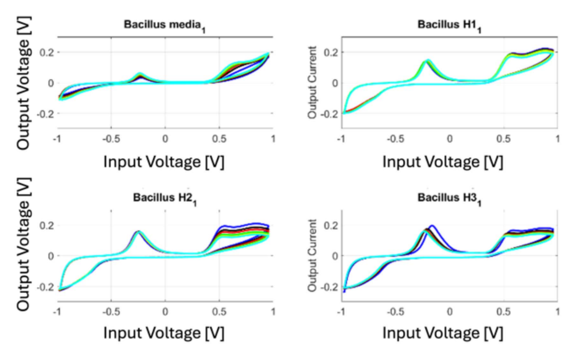


Fig. 8. Voltammograms recorded on the first day for bacillus media and for Bacillus at 1, 2, and 3 hours from the beginning of the experiment. Distinct voltammetric shapes are observed at different time points. In addition, good reproducibility is evident among the voltammograms recorded at the same time.

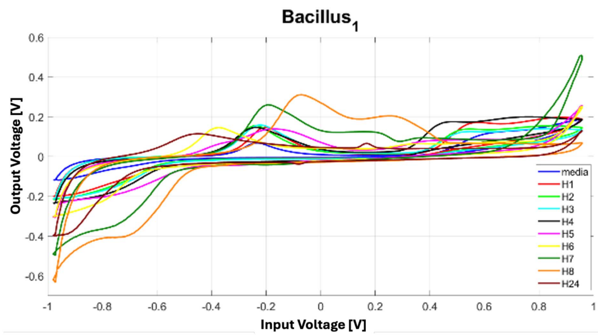


Fig. 9. Voltammograms registered for Bacillus on the first day, after 1, 2, 3, 4, 6, 7, and 24 hours from the beginning of the experiment. Distinct voltammetric shapes are observed at different time points.

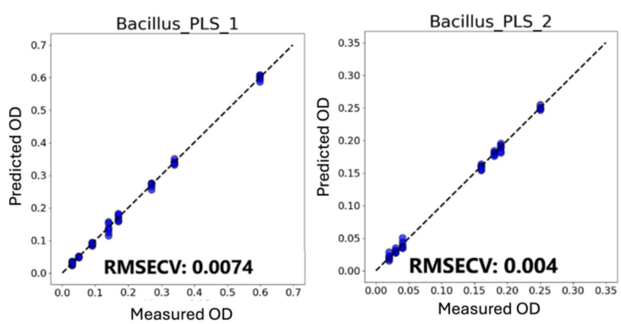


Fig. 10. Predicted versus measured value of O.D. for Bacillus measured at day 1, 2, 3, 4.

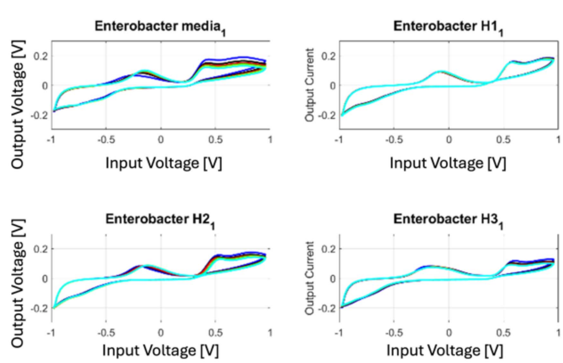


Fig. 11. Voltammograms registered on the first day for media and Enterobacter at 1, 2, and 3 hours from the beginning of the experiment. Distinct voltammetric shapes are observed at different time points. In addition, good reproducibility is evident among the voltammograms recorded at the same time.

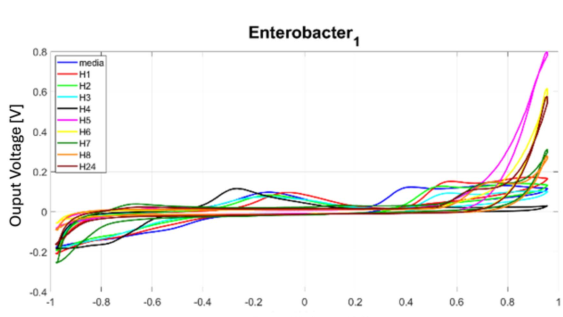


Fig. 12. Voltammograms registered for Enterobacter on day first, after 1, 2, 3, 4, 6, 7, and 24 hours from the beginning of the experiment. Distinct voltammetric shapes are observed at different time points.

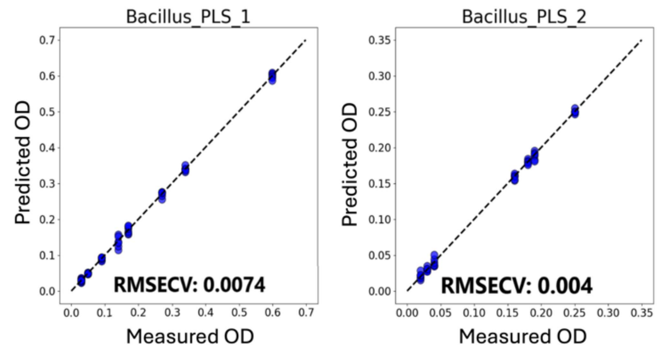


Fig. 13. Predicted versus measured value of O.D. for Enterobacter measured at day 1, 2, 3, 4.

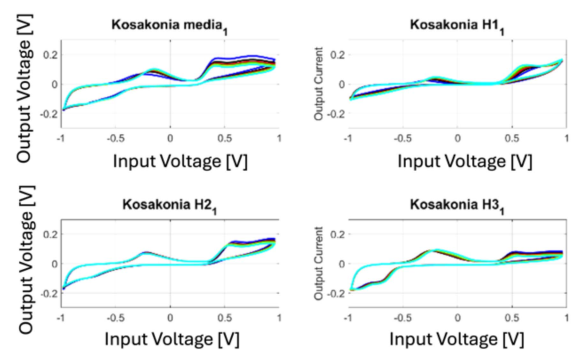


Fig. 14. Voltammograms recorded on day first for media and for Kosakonia at 1, 2, and 3 hours from the beginning of the experiment. Distinct voltammetric shapes are observed at different time points. In addition, good reproducibility is evident among the voltammograms recorded at the same time.

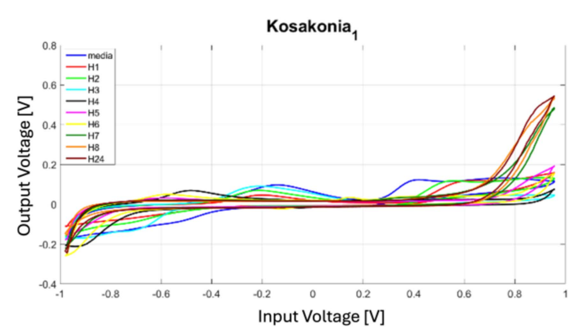


Fig. 15. Voltammograms registered for Kosakonia on the first day and 1, 2, 3, 4, 6, 7, and 24 hours after the beginning of the experiment. Distinct voltammetric shapes are observed at different time points.

5) Kosakonia. Repeatability: Fig. 14 shows the voltammetry results for the Kosakonia culture over the first day. Six consecutive measurements were taken, starting with Kosakonia in "Kosakonia media 1," and then again after 1, 2, and 3 hours. The consistent readings demonstrate good repeatability. The Supplementary Materials (from S8.1 to S8.3) contain all voltammetry data for the remaining hours of the first day (up to 24 hours) and for the following two days of measurement.

a) Growth monitoring: Fig. 15 shows the sensor's ability to monitor bacterial growth of Kosakonia by showing how the voltammetry readings changed over a 24-hour period. Readings were taken at 1, 2, 3, 4, 6, 7, and 24 hours after the start of the experiment.

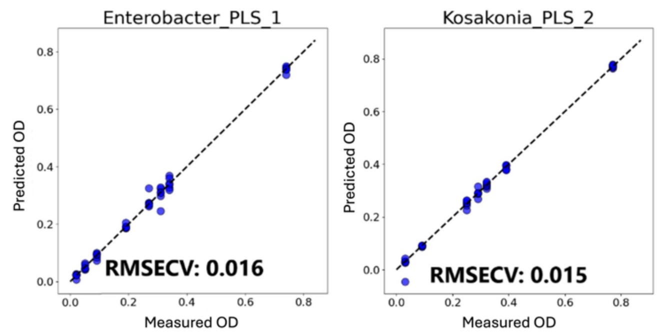


Fig. 16. Predicted versus measured value of O.D. for Kosakonia measured at day 1, 2, 3, 4.

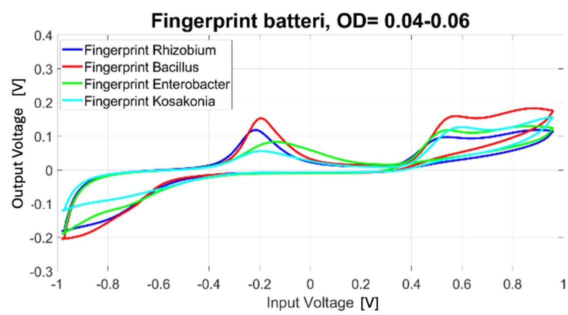


Fig. 17. Mean voltammograms of each of the four bacteria, obtained by all measurements for each type whose O.D. is in the interval between 0.04 and 0.06. Distinct voltammetric shapes are observed for different bacteria.

The Supplementary Material (S9.1) shows that the same pattern of change occurs over each of the other two days of measurement.

The resulting PLS-DA model confirmed that the changes seen in Fig. 15 reflected an increase in bacterial concentration due to culture growth. As shown in Fig. 16, the strong correlation between the predicted and measured O.D. values, with RMSECV values ranging from 0.011 to 0.016, further supports this conclusion.

6) Bacteria Discrimination: Because O.D. values vary across different days for the same measurement hour and bacterial type, we could not directly compare the hourly average voltammograms between days. Instead, we compared the voltammograms for each bacteria type across different days, acknowledging that these corresponded to varying O.D. values. This was necessary to show that the BIONOTE-L sensor could distinguish between bacterial types. Therefore, we decided to apply the following strategy: All the data relating to the voltammograms of all the bacteria were collected and ordered in increasing order based on the O.D. value, and the data of each bacterium corresponding to characteristic O.D. values common to all bacteria were chosen with an acceptable approximation (interval 0.04–0.06) and the average was calculated (for each bacterium). As shown in Fig. 17, at the same O.D. value, the sensor can provide four different fingerprints for each bacterium.

7) Overall Sensor Reproducibility: Finally, the reproducibility of the sensor on different measurement days was tested for each type of bacteria. For each bacterium, a single dataset containing the voltammogram values recorded on all measurement days was constructed, to which PLS-DA was applied.

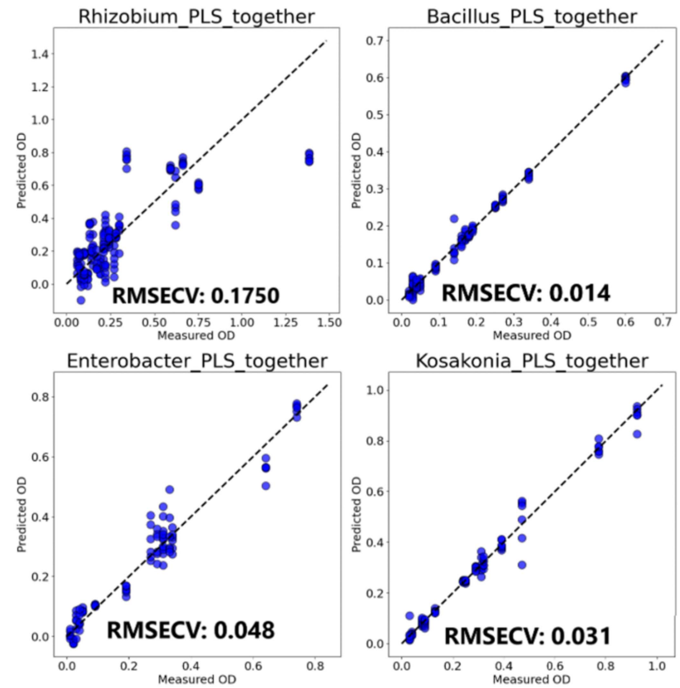


Fig. 18. Predicted versus measured O.D. for each bacterium as calculated by a PLS-DA model built on the dataset registered for all days of measurements performed for each bacterium.

In the four panels shown in Fig. 18, four graphs are reported, for each type of bacteria, with the predicted *O.D.* value versus the measured O.D. value and the associated RMSECV.

The performance of the sensor differs for different types of bacteria; in particular, for the Rhizobium measurements, an RMSECV equal to 17.5% was obtained, whereas for the Bacillus, Enterobacter, and Kosakonia measurements, an RMSECV equal to 1.4%, 4.8%, and 3.1%, respectively, was obtained. Therefore, the RMSECV obtained for the Rhizobium measurements was an order of magnitude higher than that obtained for other types of bacteria.

C. Measurements of Bacteria Interactions With Soil and Plants

For the third measurement set, we selected the best-performing bacteria based on the lowest RMSECV from PLS-DA of two days of measurements. Bacillus had the lowest errors on both days and was selected. The four samples analyzed here were: (Ci) Control Cups with soil, (Pi) Plant Cups with soil and plant, (Bi) Bacteria Cups with soil and bacteria, and (PB) Plant+Bacteria cups with soil, plant, and bacteria.

Fig. 19 shows a graph containing the average voltammograms among the different samples of the same category for each of the five types of cups (Ci, Pi, Bi, PBi). As shown, different fingerprints were obtained for each cup category. Therefore, the BIONOTE-L sensor provides fingerprints that are specific to each sample category, and the shape of the fingerprint recorded for the sample containing the soil was modified when the plant seed was added to the sample and/or bacteria were inoculated. This modification is also proportional to the compounds present in the soil sample; in fact, the peaks detected increased in the voltammograms relating to the Pi cups, which contain soil

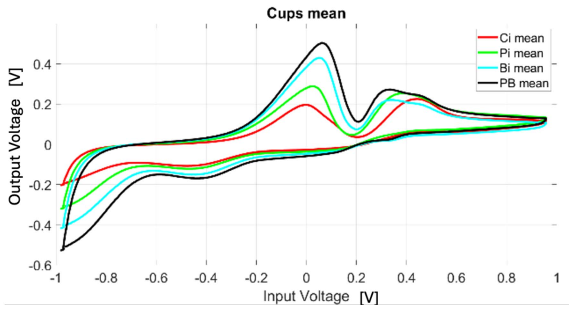


Fig. 19. Voltammograms registered for following samples: (C) Control cups with soil, (P) Plant cups with soil and plant, (B) Bacteria cups with soil and bacteria, (PB) Plant+Bacteria cups with soil, plant, and bacteria.

+ plant, then in the voltammograms relating to the Bi cups, which contain soil + bacteria, and then in the voltammograms relating to the PBi cups, which contain soil + bacteria + plant, where the most significant peaks are observed. The BIONOTE-L sensor provides reproducible fingerprints specific to each type of sample and reflects the composition of the sample. When different compounds were added to the soil samples, the response was specific to each element added and proportional to it. These results indicate that the electrical response output from the sensor shows more significant peaks when analyzing the plant + soil + bacteria sample, as the plant releases a large quantity of organic compounds into the soil, which are degraded by microbial activity, a process that contributes significantly to the production of electric current. This can be evidence of the fact that the bacterium is able to promote the growth and health of the plant: in fact, through its high activity of degradation of the compounds, it decomposes the organic macromolecules into smaller compounds, which therefore are more easily absorbed by the plant and usable for its growth, as well as making available to the plant a series of elements that contribute to its nourishment (which, in turn, increase the electrical response of the sample). Therefore, the increase in the electrical response of the samples containing soil + plant + bacteria can be evidence of beneficial microbial activity toward the plant.

IV. Discussion

From the analysis of the solutions containing the two different types of acids, it is possible to test the functioning of the BIONOTE-L sensor, which provides fingerprints specific to the type of acid, specific to the concentration of acid, and proportional to it, with peaks that increase in amplitude proportionally to the increase in acid concentration.

From the analysis of the growth curves of the four genera of bacteria, it emerges that each bacterium shows a specific growth pattern in solution: hour after hour, there is almost always an increase in the O.D. value. However, this process is never regular and reproducible; in fact, for each single bacterium, the growth curves of the different measurement days differ from each other. Moreover, the theoretically described stationary and exponential phases do not always emerge from the growth curves.

From the analysis of the electrical behavior of the four genera of bacteria in solution (Rhizobium, Bacillus, Kosakonia, and Enterobacter), the ability of the BIONOTE-L sensor to provide repeatable fingerprints (over six measurement cycles) is demonstrated, specific to the different concentration of bacteria in solution (with an RMSECV of less than 4% for Rhizobium, 1% for Bacillus, 2% for Enterobacter, and 3% for Kosakonia), specific to each genus of bacteria and with good reproducibility in the different measurement days (with RMSECV less than 20% for Rhizobium, 2% for Bacillus, 5% for Enterobacter, and 4% for Kosakonia).

From the analysis of the different types of soil samples, the ability of the sensor to provide fingerprints that are specific and reproducible for the different types of samples and that reflect their composition is evident, with more significant peaks in the case of samples containing plant + bacteria + soil.

This study demonstrates the ability of the sensor to analyze the behavior of bacteria both in solution and in soil samples, adding a piece to what was demonstrated in [1], namely, that the sensor can discriminate between different types of soil and estimate the concentration of compounds present within it.

In the present study, soil samples with a specific type of plant, in its initial growth phase, were analyzed. As the sensor has proven to work effectively with samples containing soil, plants, and microorganisms, the future development of this work could be to analyze the state of the plant-microorganism system not only in the initial growth phase of the plant, but also during the subsequent phases, not only in physiological growth conditions but also in adverse conditions such as salinity, drought, and nutrient deficiency. Thus, complete screening of the health status of the system was obtained. Furthermore, it would be possible to analyze different types of plants that usually grow and live in different temperatures and light conditions compared to those of maize plants. Small changes in the surrounding environment can trigger stimulus perception in plant cells, leading to rapid responses aimed at preventing serious damage [38]. These reactions are mediated by electrical signals and energy production. The monitoring technique presented here enables the characterization of ecosystem components—represented by voltammograms—by analyzing their energy-related information. This approach allows for the decoding of energy signals, the development of a dataset, and the creation of interpretative models to support active field monitoring.

V. Conclusion

To conclude it is important to highlight some possible implications as impact in the real-world applications.

The sensor could enable real-time, *in-situ* analysis of soil and plant health by monitoring microbial activity and compound concentrations.

This capability could support more precise and sustainable agricultural practices, such as early detection of plant stress conditions. This task could be achieved by identifying changes in the plant-microorganism-soil system under stress factors such as salinity, drought, or nutrient deficiency.

With such approach, the technology could allow farmers to take timely corrective actions, improving crop resilience and yields.

The sensor's adaptability to various plant species and environmental conditions (e.g., temperature and light) makes it a versatile tool for global agricultural applications, supporting food security in diverse climatic zones.

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