



D1.4 – Theoretical Foundations of Plants’ Digitalisation (v2)



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Executive Summary	5
1. Introduction	6
1.1. From D1.2 to D1.4.....	6
1.2. Objectives of Cycle 2.....	6
1.3. Scope of the deliverable	7
2. Consolidated Biological and System Framework.....	8
2.1. Plants as integrated biohybrid systems	9
2.2. Functional role of plant traits and ecophysiology	10
2.3. System-level interactions.....	11
2.4. Scalability and environmental constraints.....	12
3. Updated Theoretical Models	14
3.1. Plants as Antennas (PaA)	14
3.2. Plants as Energy Sources (PaES)	15
3.3. Plants as Communication Systems (PaCS)	15
3.3.1. EcoSentinel Node Design	16
3.3.2. Communication Network Design.....	17
3.4. Plants as Biosensors (PaB)	20
4. Scientific Direction for Cycle 2	23
4.1. Validation priorities	24
4.2. Integration priorities.....	25
4.3. Expected scientific contributions.....	26
4.4. Link toward WP2 and WP3	26
5. Conclusions and Future Perspectives	28
References	29
List of Figures.....	30
Abbreviations.....	31

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Executive Summary

This deliverable presents the updated theoretical foundations of plant digitalisation developed within the EcoSentinel project, building upon the conceptual framework established in D1.2 and informed by the results obtained during the first development cycle. The experimental activities conducted across the different project pillars have confirmed the feasibility of using plants as active components of future digital ecosystems, supporting sensing, communication, energy harvesting, and electromagnetic functionalities.

The knowledge generated during Cycle 1 also highlighted the need for a stronger integration between disciplines and for a more consolidated biological framework capable of explaining how these functionalities emerge from the same living system. Consequently, this updated version shifts the focus from individual technological components towards a system-level understanding of plant digitalisation, emphasizing the role of plant physiology, plant traits, environmental variability, and plant–soil–microbe interactions as common drivers across the EcoSentinel pillars.

The deliverable revisits and updates the theoretical foundations of Plants as Antennas (PaA), Plants as Energy Sources (PaES), Plants as Communication Systems (PaCS), and Plants as Biosensors (PaB), identifying the key scientific questions that will guide the second project cycle. Specific attention is devoted to integration, scalability, environmental realism, and future Proof-of-Concept developments.

Together with the complementary deliverable D1.5, which translates these concepts into design and validation strategies, D1.4 provides the scientific roadmap for Cycle 2, defining what has been learned, what challenges remain open, and where EcoSentinel research is heading in the next phase of the project.

1. Introduction

1.1. From D1.2 to D1.4

Deliverable D1.2 established the initial theoretical foundations of EcoSentinel, introducing the scientific principles underpinning the four core research pillars of the project: Plants as Antennas (PaA), Plants as Energy Sources (PaES), Plants as Communication Systems (PaCS), and Plants as Biosensors (PaB). The objective was to identify the biological, physical, and technological mechanisms through which plants could become active components of future digital ecosystems.

During the first project cycle, these theoretical foundations guided the design activities described in *D1.3 - Designs on Plants' Digitalisation* and the experimental developments performed within the different work packages. The results obtained provided the first validation of several key assumptions underlying the EcoSentinel concept and confirmed the feasibility of integrating plant-based sensing, communication, electromagnetic, and energy-harvesting functionalities.

At the same time, the first cycle revealed new scientific questions regarding biological variability, system integration, environmental realism, scalability, and the interactions among the different technological functionalities. These emerging challenges require a refinement of the original theoretical framework and a stronger integration across disciplines. D1.4 therefore updates and consolidates the theoretical foundations of plant digitalisation based on the knowledge generated during Cycle 1 and the recommendations from the project review.

1.2. Objectives of Cycle 2

The second EcoSentinel cycle aims to move beyond the validation of individual technological functions towards a more integrated understanding of plant digitalisation. Specific attention is devoted to identifying the biological mechanisms shared across the four project pillars and to understanding how plant physiology, environmental conditions, and plant–soil–microbe interactions influence system performance.

The objectives of Cycle 2 are aimed to:

- consolidate the biological framework underpinning EcoSentinel technologies;
- refine the theoretical models based on experimental evidence generated during Cycle 1;
- strengthen the integration among sensing, communication, energy harvesting, and electromagnetic functionalities;
- address scalability and environmental variability as fundamental design constraints;
- provide a scientific basis for the next generation of experiments and Proof-of-Concept developments.

1.3. Scope of the deliverable

This deliverable presents the updated theoretical foundations supporting the EcoSentinel vision of plant digitalisation. It summarizes the main conceptual advances emerging from the first project cycle, identifies the scientific challenges that remain open, and defines the research directions that will guide the second cycle of activities. D1.4 focuses primarily on the scientific rationale underlying EcoSentinel technologies. It addresses the biological principles, theoretical models, interdisciplinary interactions, and research priorities that define the project's scientific trajectory. In this sense, the deliverable explains why specific research directions are being pursued, and which scientific questions must be addressed during the second cycle. The complementary deliverable D1.5 translates these updated theoretical foundations into design implications, validation strategies, and implementation approaches. While D1.4 establishes the conceptual framework, D1.5 focuses on how these concepts will be experimentally tested, integrated, and progressively incorporated into the next generation of EcoSentinel demonstrators and Proof-of-Concept developments.

2. Consolidated Biological and System Framework

The first EcoSentinel development cycle provided important evidence supporting the feasibility of plant-based digital technologies. Experimental activities demonstrated that plants do contribute to radiofrequency transmission, support energy harvesting through Plant-Microbial Fuel Cells (P-MFCs), provide biologically relevant sensing information, and operate as integral components of autonomous communication systems. At the same time, the first cycle highlighted a key challenge identified during the project review: although the individual technological components have shown promising results, a more consolidated biological framework is required to explain how these components interact within a coherent living system.

In the initial phase of the project, the four EcoSentinel pillars (i.e., Plants as Antennas (PaA), Plants as Energy Sources (PaES), Plants as Communication Systems (PaCS), and Plants as Biosensors (PaB)), were necessarily developed through discipline-specific conceptual models. This approach was essential to establish the scientific foundations of each research area and to identify the most relevant plant functional traits and associated processes. However, the results obtained during Cycle 1 indicate that the performance of each technological function cannot be understood independently from the biological system in which it operates.

Plants should therefore not be considered passive substrates supporting isolated technological functions. Instead, they must be interpreted as integrated biohybrid systems, where morphology, physiology, water relations, metabolism, and interactions with soil microorganisms simultaneously influence energy production, electromagnetic behaviour, sensing capabilities, and communication performance. The same biological processes that regulate plant growth and environmental responses also affect dielectric properties, electrical conductivity, root exudation patterns, microbial activity, and the generation of measurable signals.

The objective of the consolidated framework presented in this chapter is therefore to establish a common biological language across disciplines (Figure 1). By identifying the biological mechanisms that are shared among antennas, biosensing, energy harvesting, and communication systems, EcoSentinel seeks to create a unified conceptual basis for experimental validation, data integration, and future Proof-of-Concept developments. By identifying the biological mechanisms that are shared among antennas, biosensing, energy harvesting, and communication systems, EcoSentinel seeks to create a unified conceptual basis for experimental validation, data integration, and future Proof-of-Concept developments.

The framework also addresses one of the major scientific challenges emerging from the first project cycle: scalability. While individual demonstrations have confirmed the feasibility of specific plant-based functionalities, future developments require

understanding how these functions behave across different species, environmental conditions, spatial scales, and deployment scenarios. Consequently, biological variability is not treated as a source of noise but as a fundamental property that must be incorporated into the theoretical models and validation strategies of Cycle 2.

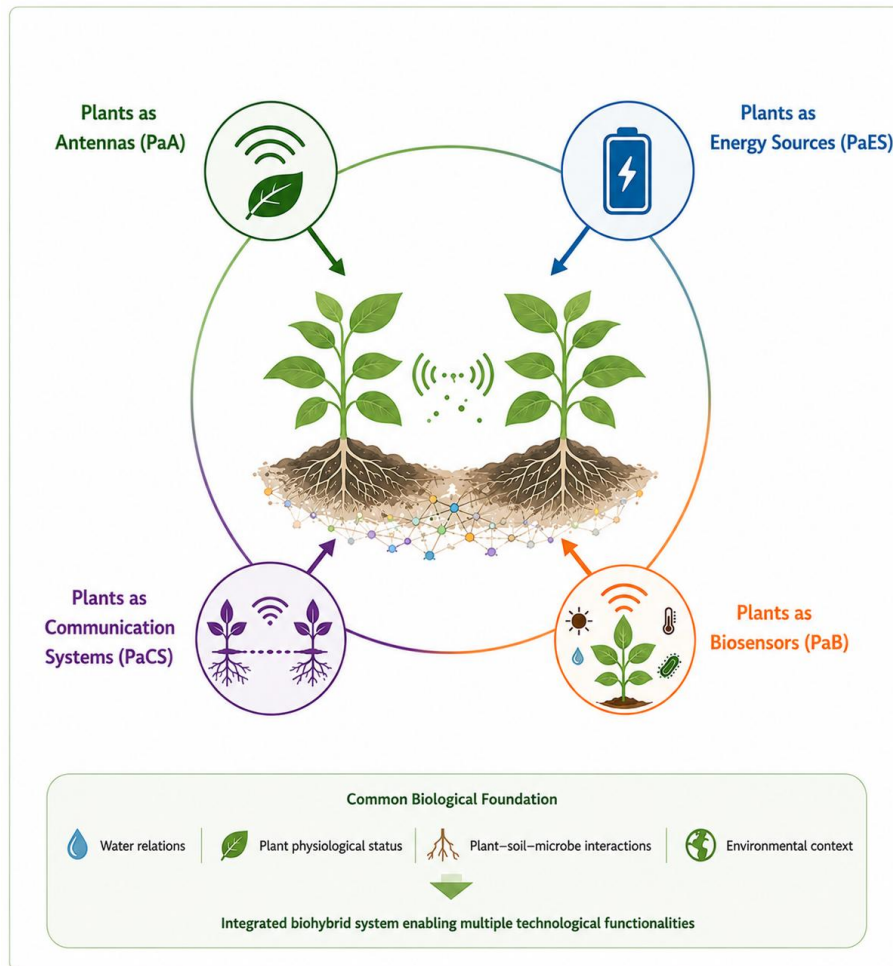


Figure 1. Conceptual representation of the four EcoSentinel technological pillars and their common biological foundation. PaA: Plants as Antennas; PaES: Plants as Energy Sources; PaCS: Plants as Communication Systems; PaB: Plants as Biosensors.

2.1. Plants as integrated biohybrid systems

Plants continuously exchange matter, energy, and information with their surrounding environment. Water uptake, nutrient acquisition, gas exchange, photosynthesis, root exudation, electrical signalling, and interactions with soil microorganisms occur simultaneously and are tightly interconnected. These processes generate physical, chemical, and electrical properties that can potentially be harnessed for technological purposes. Within EcoSentinel, plants are therefore considered biohybrid systems, where biological processes and technological components coexist and interact. We are testing that plant tissues influence electromagnetic propagation and antenna performance, root-soil-microbe interactions support energy harvesting through P-MFC, physiological

responses generate measurable sensing signals, and the resulting information can be transmitted through ultra-low-power communication systems.

The first project cycle demonstrated that these functions can be individually exploited. However, it also showed that their performance cannot be fully understood in isolation. Changes in plant physiological status may simultaneously affect dielectric properties, energy generation, sensing outputs, and communication performance. Consequently, Cycle 2 adopts a system-level perspective in which plants are no longer treated as independent technological substrates but as integrated living systems whose biological dynamics influence all EcoSentinel functionalities.

2.2. Functional role of plant traits and ecophysiology

The theoretical framework developed in D1.2 identified a set of plant traits potentially relevant to EcoSentinel applications. The first experimental cycle confirmed that several of these traits (i.e., leaf water content and succulent-related traits, such as leaf thickness and specialised water storage tissues) seem to play a role in technological performance, while also highlighting that their effects cannot be interpreted independently from plant ecophysiology and environmental conditions. Functional trait approaches have demonstrated that a limited number of key plant traits explain a substantial proportion of ecological strategies across species, providing a useful framework for interpreting biological variability within EcoSentinel [1].

Plant functional traits represent measurable expressions of underlying biological processes (e.g., photosynthesis, rhizodeposition, evapotranspiration and osmoregulation) that determine how plants interact with the environment and, in the EcoSentinel context, how they may perform across bioelectrical communication, energy harvesting, and biosensing. At the leaf level, morphological traits such as specific leaf area (SLA), tissue organisation, leaf thickness, and water content influence how plants interact with electromagnetic radiation and how bioelectrical signal propagates through them. At the root level, traits including root exudate rate and composition are investigated to evaluate how they affect both the structure of soil microbial communities and the electrochemical conditions at the root-soil interface. Physiological processes, such as photosynthesis, stomatal regulation, nutrient transport, gas exchange and stress responses, further modulate the electrical, dielectric, biochemical, and energetic properties of plant tissues, acting as indicators of plant status and potential drivers of bioelectrical signal propagation.

Among the various factors investigated so far, plant water content emerges as a central driver linking multiple EcoSentinel functionalities. Water storage and transport processes influence plant physiological stability and strongly affect several physical properties of plant tissues, making water status one of the most promising common variables across EcoSentinel applications [2]. Water content directly influences leaf dielectric permittivity and conductivity, affects plant energy balance and physiological activity by modulating stomatal conductance, photosynthetic rate, and overall metabolic

activity, regulates root exudation processes that support microbial communities, and alters the environmental conditions under which sensing and communication systems operate. Consequently, water-related processes represent a common biological denominator connecting the different technological pillars of the project.

Similarly, plant–soil–microbe interactions play a fundamental role beyond their direct contribution to energy harvesting (e.g., [3]). Root exudates influence microbial community composition and activity, directly affecting P-MFC performance, while microbial processes in turn modify soil conditions, nutrient availability, and the physiological status of the plant itself. These reciprocal feedback loops simultaneously affect sensing outputs, energy generation, and the long-term system stability.

Rather than identifying isolated variables associated with a single technological function, EcoSentinel aims to understand how coordinated biological responses influence multiple technological outputs simultaneously. This approach is expected to improve the predictive power of future models and facilitate the integration of different EcoSentinel subsystems within a unified biological framework. The increasing availability of standardized plant trait information through global databases further supports the development of transferable models applicable across species and environmental conditions [4].

2.3. System-level interactions

One of the main lessons learned during the first EcoSentinel cycle is that the four project pillars cannot be considered independent technological domains. Instead, they should be interpreted as different manifestations of the same underlying biological system.

At the core of EcoSentinel lies a network of interactions connecting plant morphology and physiology, environmental conditions, energy harvesting, sensing, and communication processes. Environmental drivers such as temperature, humidity, light availability, soil moisture, and nutrient status influence plant physiological responses. These responses modify tissue water content, dielectric properties, metabolic activity, root exudation rates and composition followed by continuous changes in microbial dynamics. In turn, these complex processes and interactions affect antenna performance, P-MFC energy production, biosensing signals, and communication capabilities.

This interconnected structure creates both opportunities and challenges. On one hand, a single biological process may generate information useful for multiple technological functions. For example, changes in water status may simultaneously affect electromagnetic properties, harvested energy, spectral responses, and communication-related indicators. On the other hand, variability originating from biological processes may propagate across subsystems, creating complex interactions that must be considered during system design and validation.

For this reason, Cycle 2 adopts a system-level approach in which EcoSentinel components are analysed not only individually but also through their mutual interactions. Specific attention will be devoted to identifying shared variables, common drivers, and potential feedback mechanisms linking energy harvesting, communication, biosensing, and electromagnetic performance.

This integrated perspective is expected to support the development of unified datasets, multimodal sensing strategies, and future digital-twin-like approaches capable of representing plant status through the combined interpretation of heterogeneous information sources (Figure 2).

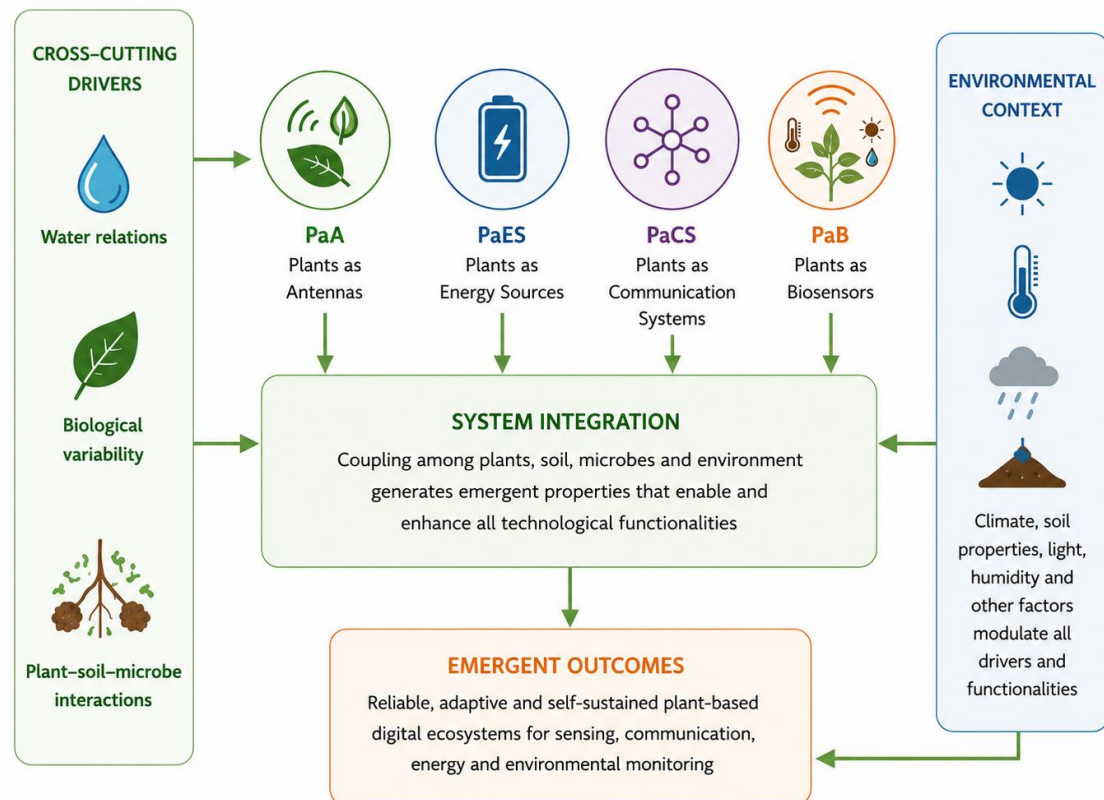


Figure 2. Conceptual framework of the EcoSentinel integrated biological system. Cross-cutting biological drivers and environmental context influence the four technological pillars, whose integration enables the development of resilient plant-based digital ecosystems. PaA: Plants as Antennas; PaES: Plants as Energy Sources; PaCS: Plants as Communication Systems; PaB: Plants as Biosensors.

2.4. Scalability and environmental constraints

A major challenge for EcoSentinel is the transition from controlled laboratory demonstrations to realistic environmental deployment scenarios. While the first project cycle successfully demonstrated the feasibility of several plant-based technological functions, future developments require understanding how these functions behave across different species, environments, and spatial scales.

Unlike conventional technological devices, biological systems are inherently variable. Plant morphology, physiological status, growth stage, and microbial communities'

composition differ substantially across species, individuals, seasons, and environmental conditions. Among the sources of variability, environmental conditions play a primary and dynamic role: temperature fluctuations, water availability, soil properties, solar radiation, and atmospheric conditions influence plant physiology, directly affecting dielectric behaviour, energy production, sensing outputs, and communication performance.

In the EcoSentinel context, these sources of variability influence the performance of all the subsystems and must therefore be incorporated into the theoretical framework, not treated as “experimental noise”, but rather recognised as properties of the biological system that deliver information about plant status and environmental dynamics.

Building this understanding is essential for developing robust and transferable EcoSentinel technologies.

Reconnecting to the previous subsection 2.2, plant functional traits represent a key operational framework for managing biological variability and addressing scalability. As measurable characteristics shaped and selected by environmental conditions, functional traits can help identify common morphological and physiological patterns across species, allowing the creation of functional groups that share ecological strategies. Rather than relying on species-specific responses, focusing on traits, such as leaf water content, root structure, exudate composition, and stomatal conductance, enables the identification of transferable relationships between biological properties and technological performance. Functional traits provide therefore a scalable approach for generalising EcoSentinel principles across biological, environmental and spatial scales.

To achieve this, scalability must therefore be considered at multiple levels. Biological scalability concerns the transferability of results across species and functional groups. Spatial scalability addresses the transition from individual plants to plant communities and ecosystem-level deployments. Technological scalability involves the integration of multiple autonomous nodes operating under heterogeneous environmental conditions and energy constraints.

Trait-based approaches and global trait databases provide an important opportunity to incorporate biological variability into transferable models, moving beyond species-specific interpretations and identifying generalizable relationships across different plant models and deployment scenarios [4]. By identifying common patterns, critical constraints, and transferable principles, EcoSentinel aims to establish the foundations for future plant-based digital systems capable of operating under realistic environmental conditions and across a broad range of application scenarios.

3. Updated Theoretical Models

3.1. Plants as Antennas (PaA)

The initial theoretical model proposed in D1.2 correctly identified that plant tissues can act as dielectric media coupled to an antenna booster, and that antenna performance is influenced by plant morphology, permittivity, dielectric losses, conductivity, hydration level, and antenna placement. The results obtained in D2.1 confirm the validity of this general direction: when the antenna booster is placed in contact with the *Aloe vera* leaf, the plant material can significantly enhance radiation efficiency compared with free-space, and this effect depends strongly on leaf geometry, antenna position, dielectric permittivity and the internal leaf tissue composition. However, the initial research focus must now be refined. The results indicate that water content should not be treated only as one plant trait among many, but rather as a dominant variable controlling the electromagnetic behaviour of plant tissues because it directly modifies the complex permittivity, dielectric losses, and effective conductivity of the plant material.

Therefore, future Plants as Antennas (PaA) theoretical models should focus on the relationship between plant water content, spatially varying dielectric properties, and antenna-booster radiation efficiency. As complex living organisms, plants exhibit a heterogeneous tissue organisation with distinct dielectric properties and should therefore no longer be modelled as homogeneous dielectric objects with a single fixed permittivity value. Atanasov et al. showed that the relative permittivity of natural plant leaves at 2565 MHz varies significantly between species and, for some crops, depends on the position of the leaf on the plant stem and its distance from the soil [5]. In addition, Luo et al. showed that water distribution inside leaves can be spatially non-uniform, with measurable variations from the leaf base to the tip and faster drying near the tip under water shortage or changes in atmospheric humidity [2]. Since plant-tissue permittivity is strongly influenced by water content, these findings support the need to represent plants in electromagnetic simulation tools as heterogeneous, frequency-dependent, and moisture-dependent biological materials, where permittivity and losses may vary along the stem, along the leaf, across the leaf section, and over time as hydration changes.

This refinement is particularly important because the coupling mechanism between the antenna booster and the plant is expected to differ across frequency bands. At lower frequencies, such as 433 MHz or 868 MHz, the plant or leaf may behave mainly as an electrically small dielectric extension or loading structure that modifies the effective antenna size and ground reference. At higher frequencies, such as 2.4 GHz, the wavelength becomes more comparable to leaf dimensions, meaning that local tissue geometry, water distribution, dielectric losses, and near-field coupling are expected to play a stronger role. Consequently, the proposed research should be expanded from the current 900 MHz frequency band validation toward a comparative study at 433 MHz, 868 MHz, and 2.4 GHz, using identical plant samples where possible, in order to determine

how plant size, leaf geometry, water content, permittivity, conductivity, and antenna placement jointly affect matching, bandwidth, radiation efficiency, and radiation pattern. To support this updated model, the project should implement an in-house open-ended coaxial probe measurement method for characterizing plant tissues. Commercially available dielectric assessment systems, such as those provided by SPEAG [6] or Keysight [7], are technically suitable but financially impractical for the project. Developing an internal measurement method will allow systematic characterization of complex permittivity, dielectric losses, and conductivity of plant tissues under controlled hydration states at the relevant frequency bands. These measured parameters can then be used to build more realistic CST Microwave Studio models of plant–antenna systems, replacing simplified homogeneous material assumptions with experimentally derived, moisture-dependent dielectric properties.

The updated theoretical foundation of PaA should therefore shift from a broad plant-trait screening approach toward a water-content-centred, frequency-dependent electromagnetic model of plant tissues. This model should combine dielectric characterization, antenna-efficiency measurements, and full-wave electromagnetic simulations to explain when the plant enhances antenna-booster efficiency through beneficial dielectric loading and when it attenuates performance through excessive dielectric loss or conductivity. This revised direction remains aligned with the original objectives of D1.2 and D1.3, but it updates the modelling assumptions based on the results obtained in D2.1 and on the need for a more realistic representation of plant material properties.

3.2. Plants as Energy Sources (PaES)

We followed the direction and the underlying framework we described in D1.2 in the design (D1.3) and experiments (D2.2) of the first cycle. From the results of these experiments, we concluded that we are in the right direction, so there is no need for refinement of the theoretical framework laid out in D1.2. We will, however, explain in more detail the improved design used for the next round of experiments and for the second version of the proof of concept, in D1.5.

3.3. Plants as Communication Systems (PaCS)

Within EcoSentinel, the Plants as Communication Systems (PaCS) pillar focuses on enabling information exchange between plant-based nodes operating under extreme energy constraints.

The first EcoSentinel development cycle, documented through Deliverables D3.1 (PoC Design), D3.2 (PoC Elements Description), and D3.3 (PoC Results), provided the initial validation of the project's core assumptions regarding plant-powered communication systems. The work demonstrated the feasibility of integrating Plant-Microbial Fuel Cells (P-MFCs), ultra-low-power electronics, sensing capabilities, and wireless communication into a unified plant-based node. Experimental activities confirmed that bio-harvested

energy can support sensing and communication tasks, while also highlighting the practical constraints imposed by limited energy availability, intermittent operation, and environmental variability.

3.3.1. EcoSentinel Node Design

Based on the initial validation and conceptual models established during the first development cycle, translating the fundamental assumptions of plant digitization into a physical device requires a complete break from traditional sensor node architectures. The EcoSentinel node cannot be conceived as a simple assembly of commercially available low-power electronic components. It must be envisioned as an integrated bio-digital organism, where energy harvesting, local processing, detection, and transmission are co-designed to dynamically adapt to the strict and highly variable constraints of the plant-microbial fuel cell (P-MFC). In a spirit of circularity and absolute respect for the monitored ecosystem, this co-design incorporates stringent requirements for eco-friendly hardware design (Green Electronics). The architecture thus seeks to minimize the device's overall environmental footprint, particularly through the selection of biodegradable or low-impact materials for the printed circuit board (PCB) and its housing.

To fully align with this ecological paradigm, the EcoSentinel node moves away from standard fossil-based substrates like traditional FR-4, opting instead for next-generation biological PCBs, utilizing technologies such as the 100% bio-based laminates developed by Virenti. Derived from fast-growing renewable biogenic feedstocks, this technology completely decouples hardware manufacturing from oil, gas, and intensive mining operations. By replacing glass-fiber and epoxy with a bio-based material, this approach achieves an estimated 5-times lower CO₂ footprint than the industry standard. Furthermore, this biological substrate is entirely halogen-free—eliminating toxic brominated chemicals from the environment—and yields a node that is 20% lighter, significantly reducing the embedded carbon and logistical footprint of deployment without compromising standard technical and industrial performance.

To translate these eco-design principles into a functional physical platform, this development cycle introduces the creation of a small-scale, miniaturized iteration of the EcoSentinel board, hereafter designated as the EcoMini. Designing the hardware to feature extremely compact dimensions and a lightweight form factor is a critical technical requirement driven by the need to place the PCB directly onto a living plant. Minimizing the physical profile of the board is essential not only to ensure it can be safely mounted on biological tissues without damaging them, but also to mitigate the severe electromagnetic detuning, signal impedance mismatches, and radiation absorption caused by the proximity of the plant's high water content. Optimizing the spatial layout and sizing of the board thus becomes a prerequisite for preserving the radiofrequency performance and radiation efficiency of the integrated antenna. This bio-digital integration of the EcoMini system (i.e., EcoSentinel board) is not limited to its material

composition and physical size; it also dictates how it interacts with its communication environment. The node's firmware and hardware are optimized to support low-power modulations tailored to the micro-energy constraints of the P-MFC. To ensure the future scalability of the project, the EcoMini hardware is designed to potentially interface with broader network topologies currently studied within the project framework. For instance, while local data extraction and routing metrics are developed to ensure data persistence despite power fluctuations, the node's architecture remains open to future deployment scenarios. These include integration with EcoSentinel-to-LPWAN gateways to leverage existing public or private long-range networks, as well as potential long-term exploration of Direct-to-Satellite LPWAN communications for extreme, isolated environments where terrestrial coverage is non-existent.

3.3.2. Communication Network Design

The aforementioned findings reinforced the need for a communication architecture specifically designed for self-sustained plant nodes, where energy efficiency becomes the primary design driver. Consequently, the lessons learned during the first development cycle establish the foundation for the Communication Network Design presented in this section, guiding the definition of network architecture, communication strategies and thus energy-aware operational mechanisms for future EcoSentinel deployments.

3.3.2.1. Communication Network Design Principles

The EcoSentinel communication network does not aim to replicate conventional Internet of Things (IoT), Wireless Sensor Network (WSN), or Low-Power Wide-Area Network (LPWAN) architectures. Instead, it explores a new class of communication systems specifically designed for bio-powered nodes operating under severe energy constraints and intermittent energy availability. Unlike traditional wireless devices powered by batteries or continuous energy sources, EcoSentinel nodes rely on Plant-Microbial Fuel Cells (P-MFCs), where the available power is measured in microwatts and may vary significantly according to plant physiological conditions, microbial activity, and environmental factors. Consequently, communication cannot be considered an always-available service but rather an opportunistic process driven by the instantaneous energy state of the system.

The communication network is therefore not an isolated technological component but a key enabler that connects the different EcoSentinel functionalities. Through communication, information generated by biosensing mechanisms, environmental monitoring systems, and plant-powered electronics can be remotely collected, exchanged, and interpreted.

Within this context, the PaCS discipline investigates the following Research Question:

Can we remotely assess the wellbeing/status of a plant or ecosystem through a self-sustained plant node?

Answering this question requires a communication infrastructure capable of operating autonomously using bio-harvested energy while supporting the transmission of information relevant to plant physiological status and environmental conditions. At the same time, the communication system must preserve the energy autonomy of the node, ensuring that sensing, processing, and communication activities remain compatible with the limited energy budget provided by the plant-soil system.

These constraints lead to an energy-first networking approach, where communication activities are subordinated to energy availability and long-term system sustainability. Rather than maximizing throughput or minimizing latency, the primary objective is to maximize the amount of meaningful information that can be extracted from the ecosystem while maintaining autonomous operation.

The resulting communication network is guided by the following principles:

- Ultra-low-power operation, ensuring compatibility with microwatt-level energy budgets.
- Intermittent node availability, accepting that nodes may temporarily disappear and later rejoin the network depending on harvested energy.
- Minimization of idle listening, reducing one of the largest sources of energy consumption in wireless systems.
- Energy-aware operation, adapting communication behaviour to the available energy resources.
- Scalability towards distributed plant networks, supporting future deployments ranging from individual plants to ecosystem-scale monitoring.
- Robustness to environmental variability, tolerating fluctuations caused by changing plant, soil, and environmental conditions.
- Simplicity over protocol complexity, minimizing control overhead and maximizing energy efficiency.

These principles establish the foundation for the EcoSentinel Communication Network Design, including the Network Architecture, Node Roles and Topology, Discovery and Network Formation mechanisms, Communication Protocol, Data Transmission Strategy, Network Maintenance procedures, and Energy-Aware Operation model.

Based on the outcomes of the first EcoSentinel development cycle, documented in D3.1, D3.2, and D3.3, the Communication Network Design is structured around three fundamental dimensions. Together, these dimensions establish the requirements, constraints, and operational principles that will guide the evolution of the EcoSentinel communication framework.

1) Ecosystem Information

The first dimension concerns the information required to assess the status of a plant or ecosystem. Since the ultimate objective is to remotely infer plant physiological conditions and environmental dynamics, it is necessary to identify which variables and indicators provide meaningful insight into ecosystem status.

This information may include environmental measurements such as temperature, humidity, soil conditions, and light exposure, together with bioelectrical parameters, energy-harvesting indicators, communication-related metrics, and other plant-derived signals. The selection of these variables defines the sensing requirements of the EcoSentinel node and determines the type and quantity of information that must be acquired, processed, and communicated throughout the network.

- Which environmental variables are relevant?
- Which plant-derived signals provide information about plant status?
- Which indicators can be directly measured, and which must be inferred?
- What is the minimum information required to characterize ecosystem status?
- How frequently must these variables be observed?

The information requirements identified in the previous dimension establish the sensing objectives of the system. However, the feasibility of acquiring and communicating this information depends on the capabilities and limitations of the EcoSentinel node itself.

2) EcoSentinel Node Capabilities and constraints

The second dimension focuses on the capabilities and limitations of the self-sustained plant node. Unlike conventional sensor nodes powered by batteries or external energy sources, EcoSentinel nodes operate using bio-harvested energy obtained from Plant-Microbial Fuel Cells (P-MFCs). Consequently, the available energy budget is extremely limited and inherently variable.

These constraints directly influence the node's sensing, processing, storage, and communication capabilities. Factors such as duty cycle, communication frequency, computational complexity, memory usage, and sensing operations must be carefully balanced against the available energy resources. Understanding these limitations is essential for defining realistic operational behaviours and ensuring long-term autonomous operation.

- What sensing capabilities can be supported by the available energy budget?
- How much computation can be performed locally?
- What communication operations are energetically feasible?
- What level of autonomy can be achieved?
- Which operational limitations arise from intermittent energy availability?
- How does harvested energy influence node behaviour and availability?

3) Information Extraction and Interpretation

The third dimension addresses how information is transformed into knowledge. Once the relevant ecosystem variables have been identified and the operational capabilities of the node have been established, it becomes necessary to define how information is acquired, processed, transmitted, stored, and interpreted.

This includes the complete information chain, from sensing and local processing at the node level to communication, data aggregation, and higher-level interpretation. The challenge is not only to transport data efficiently but also to maximize the value of the information extracted from the ecosystem while minimizing energy consumption. Communication strategies, protocol design, network topology, data processing techniques, and decision-making mechanisms must therefore be designed as part of a unified framework where information quality and energy efficiency are jointly optimized.

- Which information should be processed locally, and which should be transmitted?
- How should communication be organized to maximize information value while minimizing energy consumption?
- How can information from multiple nodes be combined to improve ecosystem-level observations?
- Which metrics can be used to estimate plant or ecosystem status?
- How can communication, sensing, and energy harvesting be jointly optimized?
- How can the resulting information support ecosystem monitoring and decision-making?

Together, these three dimensions provide the conceptual foundation for the EcoSentinel Communication Network Design. Collectively, these dimensions define the scientific and technological boundaries of the EcoSentinel communication framework. Rather than designing a communication network around connectivity requirements alone, the EcoSentinel approach derives network behaviour from ecosystem information needs, node capabilities, and the efficient transformation of observations into knowledge.

3.4. Plants as Biosensors (PaB)

Updated hypotheses

The first EcoSentinel development cycle demonstrated that electrochemical measurements obtained from Plant-Microbial Fuel Cells (P-MFCs) contain meaningful information related to plant–soil–microbe interactions. In particular, Electrochemical Impedance Spectroscopy (EIS) proved capable of revealing changes in electrode behaviour, biological activity, and operating conditions.

However, Cycle 1 also highlighted that electrochemical measurements alone are insufficient to provide a complete characterization of plant physiological status. As a result, the Plants as Biosensors (PaB) concept is extended in Cycle 2 from a single-modality biosensing approach toward a multi-modal plant-state estimation framework.

The updated hypothesis is that plant status can be estimated through the combined analysis of electrochemical, environmental, spectral, communication-related, and energy-harvesting indicators collected from EcoSentinel nodes and complementary project datasets.

Rather than relying on a single measurement, the objective is to identify correlations among multiple sensing modalities and use them to infer the overall condition of the plant.

Expected response

The proposed biosensing framework is expected to generate a simplified plant-state indicator capable of classifying the plant into a limited number of health conditions.

The initial objective is to identify three qualitative states:

- Healthy
- Intermediate / Warning
- Stressed

The resulting indicator should remain robust against variability in individual measurements and should be applicable to long-term monitoring scenarios.

Key variables

Cycle 2 will investigate the contribution of multiple sensing modalities, including:

- P-MFC electrical indicators (voltage, harvested energy, impedance-derived features, electrode stability indicators);
- Environmental measurements (temperature, humidity, pressure, and soil-related variables);
- RF information collected through the EcoSentinel sensor node;
- Communication-related indicators, including RF propagation and link-quality metrics;
- Plant physiological measurements (e.g., photosynthetic efficiency and conductivity) and complementary datasets provided by project partners;
- Time-dependent trends and derived features extracted from long-term monitoring.

Particular attention will be given to identifying which variables or combinations of variables provide the highest predictive value for plant status evaluation.

Quantitative expectations

The objective of Cycle 2 is to establish the first data-driven digital-twin-like model capable of:

- distinguishing at least three plant-state categories;
- integrating heterogeneous data sources into a unified dataset;

- operating using a limited set of measurable variables;
- supporting long-term monitoring experiments;
- providing reproducible plant-state estimation across repeated observations.

The focus is placed on feasibility, interpretability, and robustness rather than on achieving maximum classification accuracy.

Open challenges

Several scientific and technical challenges remain open:

- collection of sufficiently large and representative datasets;
- harmonization of measurements produced by different project partners;
- definition of reliable plant-state ground-truth labels;
- identification of the most informative sensing variables;
- integration of electrochemical, environmental, spectral, and communication-related indicators;
- validation of the proposed model under long-term and real-world operating conditions;
- balancing model simplicity, interpretability, and predictive capability.

Lessons Learned from Cycle 1

Main Advances

- Electrochemical measurements obtained from P-MFCs were shown to provide meaningful biosensing information related to plant–soil–microbe interactions.
- Three-electrode EIS enabled the separation of anode and cathode contributions, improving the interpretability of electrochemical signals.
- Stable operating points compatible with both sensing and energy harvesting were identified.
- Initial evidence confirmed that plant-related information can be extracted from both electrochemical and communication-related measurements.
- The first EcoSentinel PoC demonstrated the feasibility of integrating sensing, communication, and energy harvesting functionalities within a single platform.

Main Limitations

- EIS measurements require laboratory-grade instrumentation and are difficult to directly integrate into ultra-low-power autonomous nodes.
- Electrochemical measurements alone provide only a partial representation of plant physiological status.
- Long-term datasets linking plant condition and measured variables are currently limited.
- Ground-truth characterization of plant status remains challenging.

- The relationship between individual sensing variables and plant condition is not yet fully understood.

Open Scientific Questions

- Which sensing modalities provide the strongest indicators of plant condition?
- How can electrochemical, environmental, spectral, and communication-related measurements be effectively combined?
- What is the minimum sensing configuration required to estimate plant status reliably?
- Which indicators remain stable and informative during long-term deployment?
- Can a simplified digital-twin-like model provide meaningful plant-state estimation while remaining interpretable and computationally lightweight?

4. Scientific Direction for Cycle 2

The first EcoSentinel development cycle demonstrated the feasibility of using living plants as components of biohybrid technological systems. The Proof of Concept activities confirmed that plants can simultaneously contribute to sensing, communication, energy generation, and electromagnetic interactions, while also highlighting the complexity of the biological processes underlying these functionalities.

As a result, Cycle 2 shifts the focus from feasibility demonstration towards mechanistic understanding and system integration. Rather than investigating each technological pillar independently, the project aims to identify the biological, physical, and technological principles that connect them within a common framework. Attention will be devoted to understanding how plant traits, physiological processes, environmental conditions, and technological design jointly determine system performance.

For the Plants as Antennas (PaA) pillar, research will move beyond simple trait screening approaches towards physically grounded models capable of describing the interaction between electromagnetic devices and living plant tissues. Water content, dielectric properties, tissue heterogeneity, plant morphology, and antenna placement will be investigated as interconnected variables governing electromagnetic behaviour. The objective is to develop predictive models that explain how different plant structures influence antenna performance across multiple frequency bands and environmental conditions.

For the Plants as Energy Sources (PaES) pillar, research will focus on improving the applicability of Plant-Microbial Fuel Cell technologies beyond the environmental conditions traditionally required for their operation. Emphasis will be placed on understanding the biological and electrochemical processes occurring at the root–soil–microbiome interface and on identifying strategies capable of maintaining energy generation under a wider range of environmental conditions. The long-term objective is

to support autonomous EcoSentinel nodes in ecosystems extending beyond permanently waterlogged environments.

For the Plants as Communication Systems (PaCS) pillar, the main challenge is to establish a scientifically grounded understanding of how biological information can be acquired, processed, and exchanged through highly constrained plant-based technological systems. Research will therefore focus on identifying which information is most relevant for characterising ecosystem status, how such information can be extracted from biological systems, and how it can be transmitted under the strict energy and hardware limitations imposed by EcoSentinel nodes. The objective is to define robust communication strategies based on validated requirements rather than technology-driven assumptions.

At the network level, Cycle 2 will investigate communication architectures capable of connecting local plant-based sensing systems with wider digital infrastructures while minimising energy demand, environmental impact, and deployment complexity. Different integration strategies will be explored to ensure scalability across urban, agricultural, forest, and remote environments, including scenarios where conventional terrestrial communication infrastructures may be unavailable.

Across all pillars, a central objective of Cycle 2 is to establish a stronger link between biological understanding and technological design. This requires moving beyond isolated Proofs of Concept and towards integrated models capable of explaining system behaviour across different species, environmental conditions, and deployment scenarios. The adoption of harmonised species selection criteria and trait-based approaches is expected to facilitate the development of transferable knowledge and predictive frameworks.

Ultimately, EcoSentinel aims to establish the scientific foundations for a new generation of plant-based biohybrid technologies in which sensing, communication, and energy-related functions emerge from the same underlying biological processes. Understanding these mechanisms is essential for the future development of scalable, resilient, and environmentally compatible living technological systems.

4.1. Validation priorities

The main objective of Cycle 2 is to validate the biological, physical, and technological mechanisms identified during the first development cycle. Validation activities will focus on understanding how plant condition, environmental variability, and technological design jointly affect EcoSentinel performance.

For the biosensing pillar, priority will be given to the collection of long-term multimodal datasets integrating electrochemical, environmental, spectral, and plant-related measurements. The objective is to identify variables and features that reliably reflect plant physiological status and can support future plant-state estimation models.

For the Plants as Antennas pillar, validation will focus on establishing experimentally grounded relationships between plant dielectric properties, water content, morphology, and antenna performance across different operating frequencies. Attention will be devoted to understanding the role of tissue heterogeneity and environmental variability in shaping electromagnetic behaviour.

For the Plants as Energy Sources pillar, validation activities will investigate the effectiveness of the refined Plant-Microbial Fuel Cell design proposed in D1.5 and assess its applicability under a broader range of environmental conditions than those explored during Cycle 1.

For the communication pillar, validation will focus on assessing the operation of EcoSentinel nodes under realistic energy-constrained conditions. Attention will be given to communication reliability, energy-aware operation, data continuity, and the ability of the system to maintain functionality under variable environmental and energy conditions.

Collectively, these activities aim to identify the key variables governing EcoSentinel performance and to establish the experimental evidence required for the development of predictive and transferable models.

4.2. Integration priorities

A major objective of Cycle 2 is the integration of the different EcoSentinel technological pillars within a common biological and technological framework. The project aims to move beyond the validation of individual components and towards a system-level understanding of how sensing, communication, energy generation, and electromagnetic interactions emerge from the same underlying plant-environment processes.

The first integration priority is the creation of a common cross-domain data framework combining biological, environmental, electrochemical, electromagnetic, and communication-related information generated across the consortium. This integrated data framework will provide the basis for identifying relationships among different EcoSentinel functionalities and for developing future plant-state estimation approaches.

A second integration priority concerns the development of interoperable methodologies linking biological characterization, material properties, technological performance, and system-level operation. A key aspect will be the understanding of how plant traits, physiological status, and environmental conditions simultaneously influence sensing outputs, energy generation, communication performance, and antenna behaviour.

From a technological perspective, Cycle 2 will focus on integrating the EcoSentinel node with the sensing, energy-harvesting, communication, and antenna subsystems developed within the project. This includes defining data acquisition strategies, energy-aware communication protocols, and system operation under realistic resource constraints.

The refined Plant-Microbial Fuel Cell designs developed during Cycle 2 will be incorporated into the second EcoSentinel Proof of Concept together with the biosensing, communication, and antenna technologies. This integrated demonstrator will provide the first opportunity to evaluate the performance of the complete EcoSentinel framework under realistic operating conditions.

Ultimately, the integration activities aim to establish a repeatable methodology for combining living plants, sensing technologies, communication systems, and energy-harvesting solutions into a coherent biohybrid platform.

4.3. Expected scientific contributions

The scientific contribution of Cycle 2 extends beyond the improvement of individual EcoSentinel technologies. The project aims to generate new knowledge on the interactions between plant biology, environmental processes, and technological functionalities.

A first expected contribution is a deeper understanding of how plant physiological status influences multiple sensing modalities, including electrochemical, environmental, spectral, electromagnetic, and communication-related measurements. This will support the identification of the most informative indicators for plant-state estimation and environmental monitoring.

A second contribution concerns the development of experimentally validated models linking biological processes with technological performance. These models are expected to clarify how plant traits, water status, plant–soil–microbe interactions, dielectric properties, and environmental conditions affect the operation of biosensing, energy-harvesting, communication, and antenna systems.

Cycle 2 is also expected to generate valuable experimental datasets, including multimodal sensing data, plant dielectric properties, communication performance indicators, and information related to plant–microbe interactions. These datasets will provide an important resource for future EcoSentinel developments and for the broader scientific community working on plant-based technologies.

From a technological perspective, the project will contribute to the development of energy-aware communication strategies, improved plant-integrated antenna designs, refined Plant-Microbial Fuel Cell configurations, and integrated biosensing methodologies.

Together, these advances will contribute to the establishment of a common scientific framework linking plant biology and digital technologies.

4.4. Link toward WP2 and WP3

The research directions identified in this deliverable provide the theoretical foundation for the experimental and integration activities planned in WP2 and WP3.

Within WP2, the concepts and hypotheses developed in D1.4 will be translated into experimental protocols, measurement campaigns, and validation activities. Emphasis will be placed on long-term monitoring, multimodal data collection, characterization of plant dielectric properties, assessment of Plant-Microbial Fuel Cell performance, and evaluation of communication behaviour under realistic operating conditions. These activities will provide the experimental evidence required to validate the biological and technological mechanisms identified during Cycle 1.

The results generated in WP2 will help the integration activities foreseen in WP3, while experimental observations and refined technological components will support the second EcoSentinel Proof of Concept, enabling the assessment of the complete system under realistic conditions.

The progression from D1.4 to WP2 and WP3 therefore follows a continuous pathway from theoretical foundations to experimental validation, and finally to system-level integration. This approach ensures that future EcoSentinel developments are driven by validated scientific knowledge rather than by isolated technological assumptions.

Ultimately, WP2 and WP3 will provide the framework through which the biological principles and technological concepts described in this deliverable can be translated into an operational biohybrid system capable of integrating sensing, communication, and energy-related functionalities within living plants.

The iterative interaction between WP1, WP2 and WP3 is expected to progressively reduce uncertainty, refine the theoretical framework, and improve the transferability of EcoSentinel technologies across different plant species and deployment scenarios.

5. Conclusions and Future Perspectives

The first EcoSentinel development cycle has provided important insights into scientific foundations of plant digitalisation. The results obtained across the different technological pillars confirmed that plants possess a combination of structural, physiological, and ecological characteristics that can support bioelectrical communication, energy harvesting, environmental sensing, and electromagnetic interactions. At the same time, the experimental activities highlighted the complexity of the underlying biological processes and the need for a more integrated framework capable of linking these functionalities within a common conceptual model.

One of the main outcomes of Cycle 1 is the recognition that technological performance cannot be explained by isolated plant traits alone. Instead, multiple EcoSentinel functionalities appear to be influenced by interconnected ecophysiological processes, including water relations, plant-soil-microbe interactions, nutrient dynamics, and stress responses. These findings support a transition from a technology-centred perspective towards a biologically informed systems approach.

Based on these observations, Cycle 2 will focus on understanding how coordinated biological processes influence multiple technological outputs simultaneously. Specific attention will be devoted to identifying common drivers across the different pillars, improving the mechanistic interpretation of experimental results, and developing predictive frameworks that can be transferred across species and environmental conditions. The adoption of a trait-based approach and the harmonisation of species selection among partners are expected to facilitate this objective.

The long-term ambition of EcoSentinel is not simply to optimise individual applications such as plant antennas, biosensors, energy sources, or communication systems. Rather, the project aims to establish the scientific foundations of integrated plant-based biohybrid systems in which sensing, communication, and energy-related functions emerge from the same underlying biological processes. Advancing this understanding will be essential for the future development of scalable, resilient, and environmentally compatible living technologies.

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List of Figures

Figure 1. Conceptual representation of the four EcoSentinel technological pillars and their common biological foundation. PaA: Plants as Antennas; PaES: Plants as Energy Sources; PaCS: Plants as Communication Systems; PaB: Plants as Biosensors.	9
Figure 2. Conceptual framework of the EcoSentinel integrated biological system. Cross-cutting biological drivers and environmental context influence the four technological pillars, whose integration enables the development of resilient plant-based digital ecosystems. PaA: Plants as Antennas; PaES: Plants as Energy Sources; PaCS: Plants as Communication Systems; PaB: Plants as Biosensors.	12

Abbreviations

Acronym	Description
AI	Artificial Intelligence
CAM	Crassulacean Acid Metabolism
DAK	Dielectric Assessment Kit
EcoMini	EcoSentinel Board
EM	Electromagnetic
IoT	Internet of Things
PaA	Plants as Antennas
PaBS	Plants as Biosensors
PaCS	Plants as Communication Systems
PaES	Plants as Energy Sources
P-MFC	Plant-Microbial Fuel Cell
SLA	Specific Leaf Area
TRY	TRY Plant Trait Database

EcoSentinel

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