The Beta Rhizotron: a Dynamic Beta Particle Imaging Detector Equipped Rhizotron

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- **Project title:** The Beta Rhizotron: a Dynamic Beta Particle Imaging Detector Equipped Rhizotron
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- **NOFO Number:** N/A
- **Funding Opportunity Announcement:** National Laboratory Funding Opportunity (2025)
- **DOE/SC Program Office:** DOE Biological Environmental Research (BER)
- **DOE/SC Program Office Technical Contact:** Amy Swain, Email: Amy.Swain@science.doe.gov
- **DOE Award Number:** N/A
- **PAMS Pre-application tracking number:** N/A
- **Research area or areas as identified in FOA:** Bioimaging Research and Approaches for Bioenergy and the Environment
- **Senior/Key Personnel:**
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Institution	Year 1	Year 2	Year 3	Total
Jefferson Lab (Lead Institute)	**$1,487,851**	**$1,471,315**	**$1,333,351**	**$4,292,518**
U.C. Santa Cruz (subaward)	($170,573)	($275,000)	($300,000)	($745,573)
Michigan State University (subaward)	($175,000)	($275,000)	($300,000)	($750,000)

Funding Requested by Institution including subaward

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1 Introduction

The ability to study plant root systems in a non-invasive manner is crucial for understanding root physiology, nutrient uptake, and plant-soil interactions. Traditional root imaging techniques, such as X-ray computed tomography (CT), magnetic resonance imaging (MRI), and rhizotrons/rhizoboxes with optical imaging, provide valuable structural information but are limited in tracking the real-time dynamics of nutrient absorption and transport within root systems [1, 2, 3, 4, 5, 6]. Beta particle imaging, using beta particle emitting radioisotopes such as Carbon-14 (^{14}C), Calcium-45 (^{45}Ca), and Iron-59 (^{59}Fe), offers a unique opportunity to visualize and quantify nutrient fluxes directly within living plants [7, 8, 9, 10, 11]. However, existing beta imaging technologies such as phosphor imaging plates, scintillator-based imagers, and autoradiography with X-ray film are often constrained by low spatial resolution, high background noise, low dynamic range, and insufficient detection efficiency, making them often unsuitable for detailed root studies in complex soil environments [12, 13, 14, 15, 16]. To address these limitations, this research proposes the development of a "**Beta Rhizotron**," a beta (β) particle dynamic imaging system equipped with a Micro-Pattern Gaseous Detector (MPGD). MPGDs are widely used for their high spatial resolution, fast response time, and good signal-to-noise performance for particle detection in nuclear and high energy physics research [17, 18]. By integrating a MPGD into a rhizotron/rhizobox system and utilizing ultra-thin rhizotron and detector entrance windows to minimize attenuation of low-energy beta particles, we expect to achieve *in-situ* high-resolution, real-time imaging of plant root dynamics, enabling researchers to visualize nutrient uptake pathways and metabolism. The ability to track beta-emitting radioisotopes at the microscale will provide new insights into root function, nutrient cycling, and plant adaptation strategies, beneficial to fields such as agricultural sciences, environmental biology, and plant physiology. By leveraging nuclear imaging techniques for plant research, the Beta Rhizotron project represents a significant advancement in plant imaging technology, bridging the gap between nuclear physics and plant science for agricultural and ecological applications.

Regarding Buy America Requirement, the work proposed in this project will NOT involve the construction, alteration, maintenance and/or repair of public infrastructure in the United States.

2 Background and Motivation

2.1 Review of Current Root Imaging Techniques

Understanding plant root systems and their interactions within soil is fundamental for advancements in plant biology, sustainable agriculture, and environmental sciences. Current imaging methods, including structural, optical, radiographic, and neutron-based techniques, have significantly enhanced root visualization. Nevertheless, each method exhibits inherent limitations regarding isotopic specificity, penetration, practicality, and capacity for dynamic functional imaging.

X-ray CT provides three-dimensional images of roots in soil without destructive sampling, allowing detailed morphological analysis of root architecture [1, 19]. However, it predominantly captures static structural information rather than physiological processes. X-ray CT is limited by low sensitivity to nutrient uptake dynamics and poor contrast resolution between fine roots and surrounding soil particles, producing artifacts and obscuring finer structures [20, 3]. MRI facilitates non-destructive visualization of root structures and soil moisture distributions [3, 21]. Yet, MRI has

typically spatial resolutions limited to millimeter scales, which is inadequate for detailed analysis of fine root systems. As with X-ray CT, MRI is insensitive to isotopic signals and metabolic processes, limiting its usefulness in tracking nutrient uptake or isotopic tracing studies [22]. The complex and expensive instrumentation required further limits the widespread adoption. Optical imaging techniques, including visible-light photography and fluorescence imaging, deliver high-resolution root imaging under controlled laboratory settings [23, 24, 25, 26]. However, their reliance on visible wavelengths drastically limits their application in opaque substrates such as soils, making them ineffective for observing natural root systems or underground physiological processes in realistic field conditions [27]. Functional imaging approaches utilizing radioisotopes, such as in Positron Emission Tomography (PET) and Single-Photon Emission Computed Tomography (SPECT), overcome some limitations by enabling tracking of isotopically labeled nutrients [4, 28, 29]. However, these techniques are constrained by relatively low spatial resolution of typically millimeter or more, which prevents detailed imaging of fine root structures.

Recently, neutron imaging has emerged as a promising tool for visualizing root-soil-water interactions due to the high sensitivity of neutrons to the hydrogen atoms in water [30, 31]. This sensitivity allows effective visualization of water distribution around roots, facilitating studies on root water uptake patterns. Despite these strengths, neutron imaging methods typically require specialized neutron sources available only at large-scale research facilities, limiting accessibility and portability. Moreover, neutron imaging lacks isotopic specificity for tracking nutrient uptake processes, rendering it unsuitable for detailed physiological or metabolic studies involving labeled isotopes. Ground Penetrating Radar (GPR) is a non-invasive, rapid method that has been explored for root imaging *in-situ* , particularly suitable for field applications due to its portability and rapid data acquisition [32, 33]. However, the spatial resolution of GPR is severely limited (often centimeters) and heavily dependent on soil conditions, particularly moisture and texture. Additionally, differentiation between roots, rocks, soil features, and other underground objects remains challenging, and fine roots ($<$2 mm diameter) are typically undetectable, significantly reducing its applicability for detailed root system studies.

In summary, current root imaging techniques do offer unique strengths, yet all possess limitations in spatial resolution, sensitivity, isotopic specificity, and applicability under realistic environmental conditions. The ideal root imaging method should integrate the advantages of high-resolution structural imaging, isotopic tracking capabilities, dynamic physiological visualization, and applicability in realistic soil environments. This provides the primary motivation for developing the Beta Rhizotron—to address existing limitations and introduce a new aspect of root imaging that enables dynamic, functional visualization of root systems.

2.2 Review of Isotopes Used in Plant Biology

Radioactive isotopes have become essential tools in plant biology research, allowing detailed examination of nutrient uptake, metabolic pathways, and internal transport mechanisms. These radioisotopes are typically classified by the type of radiation emitted when they under go radioactive decay, including gamma rays, positrons, alpha particles, and beta particles, each with unique advantages and applications in plant physiology studies [34, 35, 36].

Gamma-emitting radioisotopes have been widely employed in plant research due to their penetrating ability and ease of detection. They are particularly valuable for studying nutrient distribution patterns and movement throughout plant tissues using imaging methods such as SPECT. However,

the relatively low spatial resolution and high background noise of gamma imaging restrict detailed visualization of fine-scale physiological processes within roots and surrounding soil environments [16]. Positron-emitting isotopes, detected using PET imaging, provide functional insights into plant metabolism, allowing researchers to visualize real-time physiological processes like carbon assimilation, nutrient transport, and water flow within living plants [4, 37, 28]. Although PET imaging delivers valuable metabolic and transport data, its application is constrained due to moderate spatial resolution which complicates detailed analysis at the microscale. Alpha-emitting radioisotopes, which emit helium nuclei (alpha particles), have limited applications in plant research because of their short range, high energy, and potentially causing biological damage. Their use is typically restricted to highly specialized tracer studies requiring extremely high spatial resolution and precision, where short-range detection is advantageous [38, 39]. Beta-emitting isotopes, which emit electrons during radioactive decay, are widely applied in autoradiography and tracer studies to investigate localized nutrient dynamics and metabolic activities within plant tissues. Beta imaging approaches offer higher spatial resolution than gamma and positron imaging, allowing for detailed localization of nutrients and metabolic products within roots and other plant structures [40, 41].

Nevertheless, existing beta imaging methods often face challenges due to energy attenuation and limited penetration, particularly in realistic root-soil imaging scenarios. Since our focus is beta imaging, we will provide more reviews on common beta emitting isotopes for plant research that will be used in this proposed research.

2.2.1 Carbon-14 as Carbon Tracer

Tracking carbon flow in plants is essential for understanding photosynthesis, carbon allocation, respiration, and nutrient interactions—core processes in plant physiology, ecology, and agricultural productivity. Among various radiotracers, ^{14}C has been extensively used in plant research due to its stable labeling capability, low-energy beta emission, and long half-life (5,730 years), making it ideal for both short- and long-term biological studies. ^{14}C labeling enables researchers to investigate how photoassimilated carbon is transported from source tissues (e.g., leaves) to sink organs (e.g., roots, tubers, or seeds). Studies using $^{14}CO_2$ have provided key insights into carbon partitioning under environmental stresses and developmental stages. For example, Farrar and Williams (1991) used $^{14}CO_2$ to quantify assimilate distribution in barley, revealing how sink strength and environmental factors influence carbon flow [42]. Kuzyakov and Domanski (2000) conducted a comprehensive review of ^{14}C-based studies in soil-plant systems, emphasizing its value in rhizosphere research and root exudation analysis [43]. Modern applications extend to studying root–microbe interactions, carbon turnover, and belowground carbon cycling. Pausch and Kuzyakov (2011) applied ^{14}C labeling in continuous and pulse-labeling modes to differentiate root-derived carbon from microbial and soil carbon pools, highlighting ^{14}C's precision in root tracking [44]. Unlike gamma-emitting isotopes, ^{14}C's low beta energy ($\sim$156 keV) allows for high-resolution, low-background imaging but requires careful detector design to minimize energy loss in experimental setups. Therefore, ^{14}C imaging cannot typically be performed *in-situ* within the soil due to the strong attenuation of low-energy beta particles, which requires the removal or thinning of soil barriers during imaging. The use of ^{14}C remains indispensable in plant carbon studies, and its utilization with the Beta Rhizotron system will enable real-time, spatially resolved tracking of carbon movement in roots under dynamic conditions.

2.2.2 Other Isotopes as Tracking Essential Nutrients

Tracking carbon pathways offers valuable insights into photosynthesis and carbon allocation under varying environmental conditions. In parallel, fungi and bacteria in the rhizosphere play a crucial role in mobilizing micronutrients—such as calcium (Ca), iron (Fe), and copper (Cu)—which are typically bound in soils as insoluble mineral forms [45, 46, 47]. Calcium supports cell wall structure and signaling, iron is essential for photosynthesis and respiration, and copper is involved in redox reactions and oxidative stress responses but can become toxic at high concentrations [48, 49, 50]. Microorganisms enhance nutrient bioavailability through various mechanisms. Mycorrhizal fungi expand root access to soil microdomains and secrete organic acids like citric and oxalic acid to solubilize mineral-bound Ca, Fe, and Cu [51, 52]. Plant growth-promoting rhizobacteria (PGPR) and fungi also release siderophores—specialized Fe-chelating molecules that enhance iron uptake under deficiency [53]. These include catecholates, phenolates, and hydroxamates, which show varying specificity to metal forms [54]. Additionally, microbial phosphatases liberate phosphate and calcium from organic complexes, further contributing to plant nutrient access [55]. These interactions intensify during early plant growth when nutrient demand is high, however the specific pathways for metal uptake and microbe–plant interactions remain incompletely understood.

While iron mobilization via siderophores is well-documented, mechanisms for microbially mediated uptake of other metals are less clear. Distinguishing bioavailable organic forms from mineral-bound species remains a challenge, as microbial transformations in soil pH, redox potential, and organic matter content influence metal solubility and accessibility. Unraveling these processes is crucial for developing strategies to enhance nutrient use efficiency and plant resilience in low-fertility soils.

A major bottleneck in advancing this understanding is the lack of non-destructive, spatially resolved tools for tracking micronutrient movement in the rhizosphere. Radiotracing offers a powerful method, as radioactive emissions provide direct quantitative data on tracer concentrations in soil and plant tissue. However, current radiotracing technologies are limited by the short half-lives of positron-emitting isotopes used in PET imaging. For example, the longest-lived PET isotope of calcium is ^{39}Ca ($t_{1/2} < 1$ s), and ^{52}Fe ($t_{1/2} = 8.3$ h) is marginally useful for iron studies but still too short-lived for tracking over days or weeks. In contrast, beta-emitting isotopes such as ^{45}Ca ($t_{1/2} = 163$ d) and ^{59}Fe ($t_{1/2} = 44$ d) are well-suited for longer-term studies, but no *in-vivo* imaging system currently supports their use. Consequently, researchers must rely on destructive sampling: excising, digesting, and counting plant tissue to obtain uptake data, which prevents longitudinal monitoring of individual plants.

The **Proposed Beta Rhizotron** aims to overcome these limitations by enabling *in-vivo* , non-destructive imaging of beta-emitting radiotracers such as ^{45}Ca, ^{59}Fe, and ^{64}Cu within rhizosphere systems over extended periods. This will facilitate a deeper understanding of nutrient uptake dynamics, microbe–plant interactions, and spatial distribution patterns of essential micronutrients in real-time and under realistic soil conditions. Below in Table 1 is list of radioisotopes used in the proposed research.

Table 1: Properties of selected isotopes in this proposed research.

Element	**Isotope**	**Half-life**	**Endpoint (keV)**	**Other decay info**
C	^{14}C	5730 y	156 (100%)	–
Ca	^{45}Ca	163 d	256 (99.998%)	–
Cu	^{64}Cu	12.7 h	579 (38.5%)	β^+: 278 keV (17.5%)
Fe	^{59}Fe	44 d	273 (45%), 466 (53%)	γ:1099 keV (56.5%), 1292 keV (43.2%)

2.3 Beta Imaging for Plant Biology

2.3.1 Existing Beta Imaging Tools

Beta imaging techniques have long been used to track radiotracer movement in biological samples, including plant tissues. Several detector systems have been developed to visualize beta-emitting isotopes such as ^{14}C, ^{32}P, and ^{45}Ca. Each system differs in sensitivity, spatial resolution, real-time capability, and suitability for biological applications—especially in complex root environments.

One of the most common imaging platforms is the scintillator-based beta imager in which beta particles interact with a scintillating material (typically plastic or ZnS:Ag) and emit visible scintillation light that is captured by a photodetector [56, 57, 58]. These imaging systems provide moderate resolution ($\sim$1 mm) and allow for faster readouts compared to film-based methods. However, they often suffer from parallax error, relatively low spatial resolution, and bulky hardware resulting in less than ideal results for microscale root imaging [59]. Phosphor imaging plates, also known as storage phosphor screens, have been widely used in plant autoradiography [14, 60]. These screens accumulate energy from impinging beta particles and release the energy as luminescence upon laser stimulation which is recorded as a digital image. Phosphor-based systems can achieve high resolution (50$\sim$100 μm) and good sensitivity, but they require long exposure times (often several hours), followed by offline image processing, limiting their use for dynamic or real-time studies [61].

Traditional autoradiography using X-ray film involves placing photographic film in direct contact with a beta-emitting sample. Over time, the emitted particles expose the film to produce an image of the tracer distribution. This method is inexpensive and provides high resolution, but it is time-consuming, lacks quantitative accuracy, and involves hazardous chemical development steps [13]. Semiconductor-based beta detectors, such as silicon strip detectors and cadmium telluride (CdTe) arrays, offer excellent resolution and direct electron detection without conversion layers. These systems are capable of sub-millimeter precision and can be used for real-time imaging. However, they are often expensive, have limited active areas, and may require cooling systems or vacuum environments thus restricting their application in plant biology research [62, 63].

Recent advances have introduced CMOS sensor-based imagers, often combined with thin scintillator layers to convert beta particles into visible light. These systems benefit from low noise, high frame rates, and compact design. While they are promising for portable or integrated imaging solutions, their efficiency in detecting low-energy beta particles is limited without careful material optimization and calibration [64]. Despite the progress made across these platforms, most existing beta imaging tools lack a combination of high spatial resolution, real-time operation, and compatibility with live plant environments. These limitations create a gap that the proposed MPGD-based Beta Rhizotron system aims to fill, offering real-time functional imaging with enhanced spatial detail and system adaptability.

2.3.2 MPGD as a Beta Imaging Detector for Plant Study

MPGDs are a type of gas-based particle detectors designed to detect ionizing radiation with high spatial resolution and fast response time. Initially developed for particle physics experiments, MPGDs have more recently been adapted for applications in medical imaging, dosimetry, and biological radiation detection [17, 65, 66]. MPGDs operate by amplifying the ionization signal created when a charged particle travels through a gas volume. Electrons produced through the ionization of gas atoms by the incident charged particle drift toward a micro-patterned amplification structure—such

as in a Gas Electron Multiplier (GEM) or Micro-Mesh Gaseous Structure (Micromegas)—which creates a cascade of secondary ionizations. This results in a strong, localized electrical signal that can be read out with high precision read out digitizing systems [67, 68].

Over the past decade, the versatility of MPGDs has extended their use into fields such as biology, medical diagnostics, materials science, and dosimetry. In biomedical imaging, MPGDs have been adapted for autoradiography of tissue sections labeled with beta-emitting isotopes, providing high-resolution visualization of molecular tracers [69]. In materials science, MPGDs are used for beam profiling and radioactive contamination mapping. These applications exploit MPGDs' ability to detect low-energy electrons with fine spatial detail and minimal electronic noise [70, 71]. Commercial beta imaging systems such as BeaQuant-s (AI4R, France) and iQID (scintillator-based with CCD/CMOS readout) have made beta imaging more accessible for biomedical and pharmacological studies [72, 73]. These platforms offer sub-millimeter resolution and are suitable for fixed samples, thin sections, or *in-vitro* assays. However, they are not optimized for *in-situ* biological imaging, especially within soil or hydroponic plant systems since the system requires the placement of the sample in the gas volume. Limitations include poor environmental tolerance (e.g., moisture and humidity), non-adaptability to irregular sample geometries, and lack of use in biological growth chambers. Moreover, many commercial systems are based on solid scintillators or indirect detection schemes that degrade in sensitivity for low-energy beta emitters like ^{14}C, especially when the source is embedded in complex biological matrices.

As of now, there are no reported instances of an *in-vivo* non-intrusive beta imaging system being applied to plant roots. These limitations present a clear opportunity to develop a dedicated MPGD-based beta imaging platform tailored for plant science. The Beta Rhizotron concept aims to leverage the high resolution and real-time capabilities of MPGDs in a system specifically designed for *in-situ* imaging of living root systems. By integrating a thin-window rhizotron with a planar MPGD, this platform will allow direct visualization of radiolabeled nutrient transport and metabolic dynamics in the root.

2.4 Collaboration

2.4.1 Jefferson Lab Detector Development Background

The Jefferson Lab Radiation Detector and Imaging (RD&I) Group for over 25 years has leveraged nuclear physics radiation detection technologies for applications in the radiotracer imaging modalities of positron emission tomography (PET) and single photon emission computed tomography (SPECT). The Group in collaboration with academia, industry and other national laboratories has been developing application-specific radiation detector systems for preclinical (small animal), clinical and plant biology. Figure 1 shows Jlab developed PET system and MPGD system.

The RD&I Group designed and built a plant PET system called PhytoPET to use the PET radioisotope ^{11}C to study $^{11}CO_2$ to study *in-vivo* carbohydrate transport in plants in the Duke University Phytotron [28, 74]. The PhytoPET system is composed of several compact detector modules based on scintillator crystals coupled to position sensitive photomultiplier tubes (PSPMTs) with active areas of 5 cm^2. The RD&I Group built a similar system for the University of Regina that has been used to image [^{18}F]fluoro-2-deoxy-D-glucose (FDG) in soil microbe research at the University of Saskatchewan, Saskatoon, Canada [75]

The Group also developed a standalone beta imaging system with a small active imaging area of 4.5

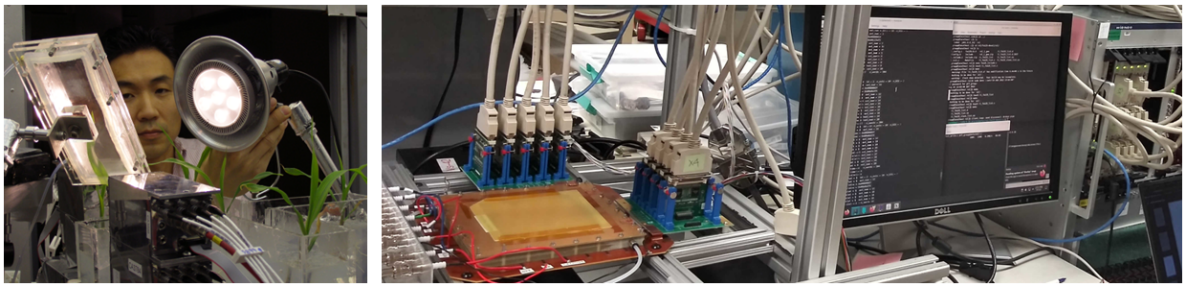

Figure 1: JLab developed PhytoPET system for plant imaging *(Left)*. $^{11}CO_2$ was introduced by a custom-made leaf cuvette. MPGD cosmic ray test stand at JLab being utilized for characterization of a GEM detector with strip readout *(Right)*. The readout system consists of a set of preamplifiers mounted to the detector readout printed circuit board (PCB) with signal cables routed from the preamps to a JLab custom designed Flash ADC boards (FA125s) housed in a VSX crate. The data acquisition computer is running the JLab designed CODA data acquisition software.

cm x 4.5 cm. Plants typically have very thin leaves, thus little medium is present for the emitted positrons to undergo an annihilation event. Given that emitted positrons (beta+ particles) from ^{11}C have a range of approximately 4 mm of water equivalent material before positron annihilation, many of the emitted positrons do not annihilate inside the leaf, resulting in limited sensitivity for PET imaging. To address this problem the Group developed a compact, beta+/beta- particle imager (PhytoBeta) for $^{11}CO_2$ leaf imaging. The detector is based on the same PSPMT used in the PhytoPET module but coupled to a thin plastic scintillator appropriate for beta particle detection [40].

In addition to the PhytoPET and PhytoBeta plant imaging systems, the RD&I group has experience developing advanced Micro-Pattern Gaseous Detector (MPGD) systems. The group is contributing to the DOE Electron-Ion Collider (EIC) project through the design and implementation of MPGD's based on micro-Resistive WELL (μRWELL) technology. Additionally, the RD&I group is collaborating with the Hampton University Proton Cancer Institute (Hampton, VA) on developing proton imaging devices with Gas Electron Multiplier (GEM) technology. This combined expertise makes the team uniquely qualified to effectively apply advanced MPGD technologies to new root imaging applications.

2.4.2 U.C. Santa Cruz Radioisotope/Plant Research Background

The Radiological Instrumentation Laboratory in the Department of Electrical and Computer Engineering at the University of California, Santa Cruz (UCSC) lead by Dr. Shiva Abbaszadeh is very active in radioisotope detector development research spanning the entire detection and imaging process, including the development and fabrication of detectors; characterization and imaging techniques; data modeling, processing, and validation; computational problem solving; and the quantitative characterization of biological processes. Dr. Abbaszadeh is an expert in PET imaging, is collaborating with researchers and clinicians at multiple institutions.

Dr. Abbaszadeh has teamed up with Dr. Weixin Cheng a researcher in the Department of Environmental Studies at UCSC with research projects occurring over decades and covers root-soil interactions and on to the effects of global change factors (e.g., elevated CO_2, nitrogen deposition, and global warming) on belowground processes, and to global patterns of social-ecological entanglement (e.g., biospheric carrying capacity and ecological foundation of human societies). Dr.

Cheng's lab group engages in research on ecological processes at varying scales from the rhizosphere to the landscape. The research projects utilize stable isotopes as well as radioisotopes to achieve tracing/labeling and rhizosphere imaging.

Together Drs. Abbaszadeh and Cheng use carbon radioisotopes to advance the rhizosphere imaging studies from (i) *in-vitro* to *in-situ* , (ii) indirect to direct, (iii) intermittent to continuous, (iv) 2-dimensional (2D) to 3-dimensional (3D), and (v) fragmentary to scalable. Monitoring root growth non-invasively is challenging for conventional techniques largely due to soil opacity. Thus measuring dynamic molecular phenomena, such as root-microbe interactions is difficult for the same reason. Optically transparent soil substitutes have limited utility because not all plants can be cultured in artificial substrates and some plants are strongly impacted by the growth medium. Consequently, leveraging higher energy photon imaging modalities (e.g., X-ray computed tomography, SPECT and PET) have been the focus of Drs. Abbaszadeh and Cheng for functional 3D imaging of the rhizosphere under natural conditions.

Additionally, in 2023 the UCSC team formed a collaboration with the Jefferson Lab RD&I Group in which UCSC is using the Jefferson Lab developed PhytoPET modules to supplement their research by adding another PET imaging methodology to take full advantage of their use of ^{11}C to research various pathways in which plant roots affect soil organic carbon (SOC) and the mechanisms that regulate SOC dynamics in the rhizosphere. Possible pathways include root-driven aggregate turnover and exudate-driven microbial turnover, which involves microbial activities fueled by plant exudate. Understanding the various pathways offer valuable insights for improving strategies to enhance soil carbon sequestration. Additionally, these findings hold direct relevance for global soil carbon modeling efforts and have the potential to address the well-documented uncertainty and inconsistencies in existing models.

2.4.3 Michigan State University Radioisotope/Plant Research Background

At the Department of Plant, Soil and Microbial Sciences at Michigan State University (MSU), Dr. Gregory Bonito leads a research program focused on the ecology and evolution of plant-fungal-bacterial associations, with a focus on belowground interactions in the rhizosphere. Fungi and bacteria in the rhizosphere provide important links between plants and soils, and are functionally important to plant health and nutrition as well as nutrient cycles and the flow of energy through terrestrial ecosystems. Dr. Bonito's research leverages high-throughput sequencing and other -omics tools, as well as isotope tracer and microscopy techniques - to visualize and better understand environmental and genetic factors that structure root microbiome communities and functions.

Dr. Gregory Severin is a radiochemist in the Department of Chemistry at MSU. Dr. Severin leads the development of "isotope harvesting" methods for collecting and purifying byproduct radionuclides produced by the DOE Facility for Rare Isotope Beams (FRIB) at MSU. FRIB is capable of providing intense beams of rare isotopes for basic nuclear physics research, as well other applications such as in nuclear medicine and biosystems radiotracing. Dr. Katharina Domnanich is a radiochemist in the Department of Chemistry at MSU. Dr. Domnanich's research is focused on radionuclide production development for application-based research with a particular emphasis on the radiochemistry of 'non-standard' isotopes. This work also includes the extraction and use of short-lived isotopes, which have the potential for translational understanding of plant and soil biology.

Dr. Bonito, Dr. Severin and Dr. Domnanich have been collaborating informally nearly a decade and have carried out collaborative research using radioisotopes of copper, calcium and arsenic to understand metal uptake by fungi, fungi and bacteria, as well as intact plant microbiomes consisting

of plants, fungi and bacteria. Dr. Severin and Dr. Domnanich have been collaborating and publishing together since before 2020 and have been collaboraing with Dr. Bonito. For instance, together they have used ^{45}Ca, which has a half-life 162.6 days, in fungal-bacterial calcium transport studies. Bonito and Severin are co-authors on a perspective on the utility of short-lived radioisotopes for scientific discovery.

3 Project Objectives:

Goal of the Proposed Research

The primary goal of this research is **to develop and validate a novel beta particle dynamic imaging system - termed the Beta Rhizotron - capable of non-invasive, high-resolution, real-time visualization of plant root systems using beta-emitting radioisotopes (e.g., ^{14}C and ^{45}Ca) *in-situ*** .

Addressing limitations of conventional imaging techniques, our project strategically employs cutting-edge MPGD technology, integrated within a specially designed rhizotron setup, to facilitate advanced functional visualization. The outcome of this project will combine the investigative power of MPGD based beta imaging system for functional visualization in plant-microbial research in facilities having access to beta-emitting radioisotopes such as ^{14}C or ^{45}Ca, commercially available or produced onsite, as at the UCSC and MSU.

To ensure the systematic achievement of the Beta Rhizotron's expected capabilities, we have defined **four specific aims** and established **seven critical milestones** (described later in Section 4.5) to guide our progress across the three-year performance period. These aims are as follows:

Specific Aims:

(1) Build 10 cm x 10 cm imaging field of view MPGDs with ultra-thin entrance window (less than 10 μm) that allows low energy (∼50 keV) beta particle penetration.

(2) Build Beta Rhizotrons equipped with MPGDs that allow direct contact of plant roots and soil with the MPGD entrance window.

(3) Evaluate Beta Rhizotron performance in environmental growth chambers.

(4) Demonstrate real-time dynamic imaging capability for nutrient uptake visualization at U.C. Santa Cruz and Michigan State University.

The successful completion of these specific aims will establish the Beta Rhizotron as an essential investigative tool, providing opportunities for novel insights into plant biology, environmental science, and rhizosphere processes. Importantly, this innovative imaging platform will significantly enhance our understanding of fundamental plant root physiological processes and plant-microbe interactions, potentially transforming research methodologies in these critical biological and ecological fields.

In the following sections, we will discuss in detail the selection and integration of each subsystem comprising the Beta Rhizotron system based on MPGD technology. Additionally, we will describe explicitly how the integration of Rhizotrons with MPGD technology uniquely enables high-resolution plant beta imaging within controlled plant growth environments.

4 Proposed Research and Methods

4.1 Overview of Beta Rhizotron

The Beta Rhizotron is a specialized imaging system designed to capture low/mid-energy ($\approx$ 50 keV - 2 MeV) beta emissions from radioactive tracers within plant root systems using a high-resolution MPGD. Technically, the system is built around the principle of planar beta particle detection, where roots labeled with beta-emitting radioisotopes—such as ^{14}C for carbon metabolism studies or ^{59}Fe for Iron uptake studies— are positioned adjacent to a sensitive gas-filled detection plane. The MPGD, operating with either a GEM (Gas Electron Multiplier) or μRWELL (micro-Resistive WELL) amplification stage, amplifies ionization signals generated by beta particles traversing the gas medium. These signals are read out through a finely segmented anode structure (sub millimeter pitch), enabling precise spatial localization of particle interactions within the detector gas with sub-millimeter resolution.

The Rhizotron window and MPGD cathode (also serving as beta particle entrance window) are optimized to minimize the beta particles' travel distance through media, thereby preserving energy and maximizing detection efficiency. A root observation window made of thin material such as low-Z material like Mylar or Polyethylene film, separates the growing roots from the detector without significantly attenuating the beta particles. Coupled with fast data acquisition electronics and real-time processing, the Beta Rhizotron delivers dynamic imaging of beta activity, at intervals of every minute or hour depending upon the radioisotope which will reveal temporal changes in the tracer distribution. Environmental parameters such as humidity, light, and temperature are controlled to ensure natural root growth conditions. This technical configuration allows for continuous, *in-situ* monitoring of nutrient fluxes and root function in a living plant, transforming conventional Rhizotron studies from static imaging into a quantitative, temporally resolved, radiographic platform. The overall concept of Beta Rhizotron is depicted in Figure 2.

4.2 Detector Development for Beta Rhizotron

4.2.1 Study for Efficiency and Imaging Capabilities of the Beta Rhizotron System

The proposed Beta Rhizotron would provide 2-D position intensity maps of emitted betas - proportional to the density of radionuclides in plant roots - as well as temporal information on these density maps on the scale of tens of minutes to hours per time slice, depending on the particular radionuclide being studied. The distribution of kinetic energy for betas emitted from various radioisotopes of ^{45}Ca and ^{64}Cu, are illustrated in Figure 3. Also indicated by the vertical lines are the energy thresholds for betas with kinetic energies of 40 keV (blue) and 100 keV (red). The Beta-Rhizotron would count the number of beta particles ionizing the MPGD drift gas above some threshold in ionization pairs produced. Typically, the types of MPGD configurations being considered are better than 90% efficient for the detection of minimum ionizing particles, such as cosmic ray muons and high energy electrons (β). Furthermore, due to the increase in the ionization cross section at lower energies, we expect that more ionization will be produced for the betas of interest relative to such energetic particles. Therefore, we expect that the intensity of detectable betas in a given area of the MPGD will be proportional to the density of radionuclides in this area times the integral of the energy distribution for beta particles above some energy loss threshold.

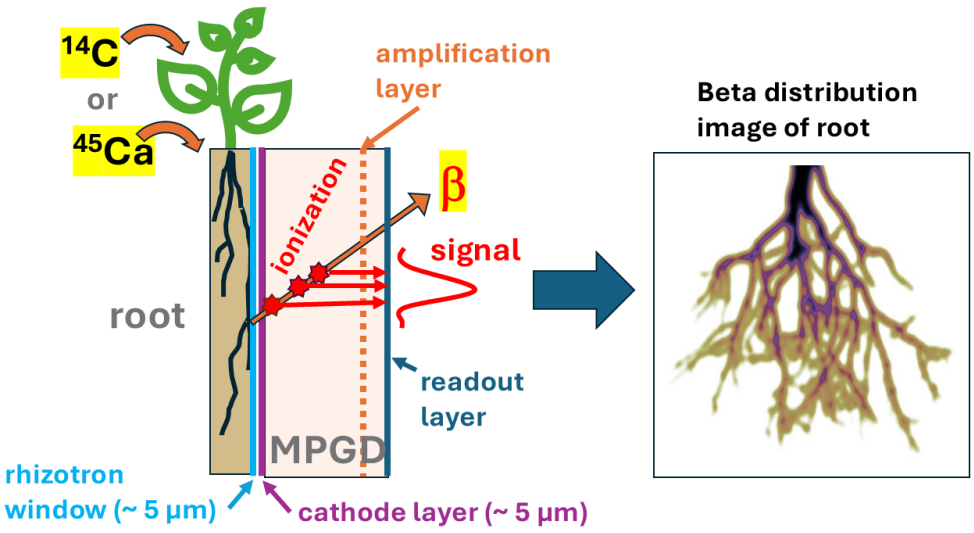

Figure 2: Illustration of the Beta Rhizotron utilizing an MPGD (*not to scale*). Beta particles emitted from plant roots ionize the detector gas and the electron cloud is drifted towards an amplification stage and then to the readout electrodes. Ultra-thin films for the rhizotron window and cathode layer are used to minimize beta particle attenuation. The induced electrical current signal on the electrodes is then passed to front-end electronics.

Estimates were made of the rates for betas entering the MPGD gas valume assuming the radioisotope uptake of 100 μCi of material. For this it was assumed that the radioisotope was uniformly distributed within the root system and that the cross sectional area of the roots was 2% of the 10 cm x 10 cm detector area. For simplicity it was also assumed that only 50% of the beta spectrum will have high enough energy to enter the detector gas. We note that this is a cautious estimation for most of the nuclei to be studied, which we will return discussion to shortly. Based on these assumptions the estimated rate per area is 27,000 /min/mm^2 or 1000 /min for (200 μm)^2, resulting in less than 1% statistical fluctuations in a 10 minute time slice.

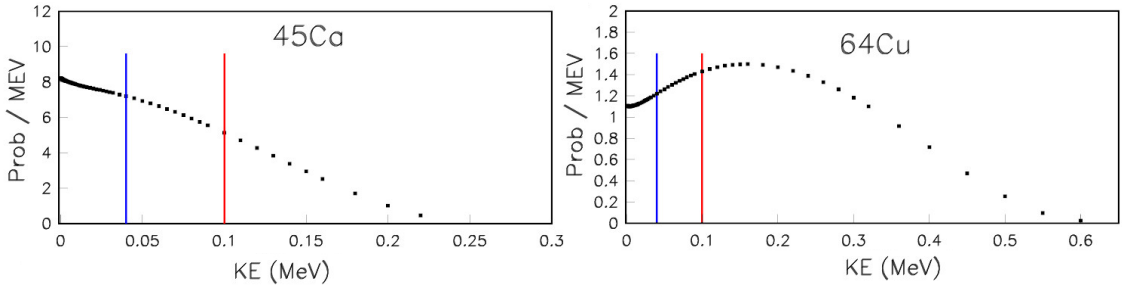

Figure 3: Spectra for various beta emitters: ^{45}Ca *(Left)*, and ^{64}Cu *(Right)*. The vertical lines indicate threshold beta-particle energies of 40 keV (blue) and 100 keV (red).

Thus far we have ignored a couple of important considerations for the operation of the Beta Rhizotron: i) the angular distribution of betas entering the detector emanating from a small root volume, and ii) the intensity reduction due to the beta-particle energy loss in the additional material traversed from root volumes further from the window. The former results in both more material being traversed and the average ionization position being shifted relative to the X-Y point of origin.

The latter also results in more material traversed, resulting to a reduction in observed intensity.

4.2.2 Selection of Rhizotron window

First estimates of the energy threshold for beta particle detection in the MPGD were performed utilizing the NIST EStar [76] average range and stopping power calculator for electrons. For single materials we take the average stopping energy as an estimate of the energy threshold, ignoring the energy straggling width. Readily available candidate materials for the Beta-Rhizotron window and the MPGD window/cathode are 1 mm thick Rohacell PMI foam and 6 μm thick Aluminized Mylar polyester film, respectively. The average stopping energy for betas in these materials were found to be 40 keV for Rohacell and 19 keV for Mylar. The average stopping energy for 50 μm liquid water was 40 keV. For a root pressed up against the Beta-Rhizotron window we take the total energy threshold to be $\approx$ 60 keV and with an additional 50 μm layer of water between the root and window to be 100 keV.

For ^{64}Cu, with an endpoint energy of around 0.65 MeV, a threshold of 40 keV (100 keV) leads to an estimated reduction in intensity of less than 20% (33%). For mapping tracer labeled nutrient density with the proposed detector in thin roots up against the Beta-Rhizotron window the more critical parameter is the variation in the material thickness as seen by betas, which will result in variations in the observed intensities. Even 15% variations in the material thickness results in only a few percent change in the total intensity. For ^{14}C the situation is much different, with small variations in material thickness having a much larger impact on the intensity uniformity. It is here that investigations into reducing the material budget will be critical.

In order to get a more realistic estimate for the expected 2-D intensity histograms, a made use of a we made use of a Monte Carlo simulation package known as GEANT4 [77]. The GEANT4 simulation was performed based on the following configuration: an 8 μm Beta-Rhizobox Kapton window, a 6 μm Mylar MPGD cathode / window, a 3 mm thickness of the MPGD drift gas volume, and a narrow (1 mm x 1 mm cross sectional area in X-Y) rectangular column of 7 cm in length containing a uniform spatial distribution of the Beta-particle emitter to approximate a root. The 2-D intensity map for betas that produce ionization in the gas is shown in the Left panel of Figure 4. The rectangular column is quite visible over ionization positions reconstructed far from the beta-particle emission point. The latter stems from two sources: 1. beta-particle emissions at large angles ($\approx$ 45 degrees or greater) and 2. beta-particles that undergo large angle Coulomb scattering. Such events pass through significantly more material, resulting in larger energy losses and significantly larger energy deposition in the MPGD gas. Exploiting these differences is but one possible avenue to explore to improve image quality.

The Right panel of Figure 4 shows histograms of kinetic energies for positrons that ionize the MPGD gas, both at the generation point in the root column (black) and in the MPGD gas (red). The energy loss results in an average shift of $\approx$50 keV for this configuration (consistent with our earlier estimation), with a longer tail towards lower energies due to the energy straggling distribution.

4.2.3 MPGD Design and Configuration

Selection of MPGD type

MPGDs offer several design options, each with unique advantages and operational mechanisms suitable for beta detection. Among the leading candidates are Gas Electron Multipliers (GEM), Micromegas, and the Micro-Resistive WELL (μRWELL). MPGDs are capable of high spatial resolution

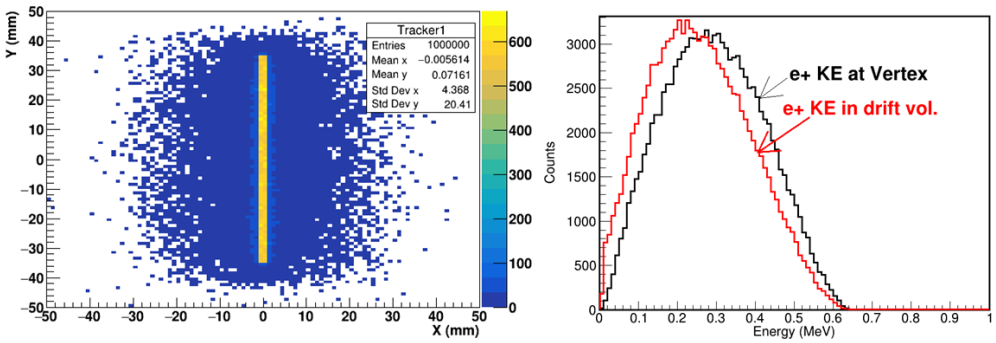

Figure 4: 2-D histogram of the intensity of betas in MPGD gas based on GEANT [77] simulation of a narrow column of emission for ^{64}Cu, as described in the text (*Left*). The positions in X,Y are given by the average position of all ionization in the gas for each beta. Shift in kinetic energy due to energy loss for positrons making it into the MPGD gas based on the simulation (*Right*).

(<100 µm). The selection of a suitable MPGD architecture is critical for achieving the required spatial resolution, signal-to-noise ratio, stability, and environmental robustness in the rhizotron-based beta imaging systems.

Gas Electron Multiplier (GEM): GEM detectors [17] operate using a series of thin polymer foils (typically Apical) coated with conductive material and perforated with microscopic holes. When a voltage is applied, a high electric field inside the holes leads to gas amplification via electron avalanches. GEMs can be cascaded (e.g., triple-GEM) to increase gain [78, 79].

Micro MEsh GAseous Structure (Micromegas): Micromegas detectors [80] consist of a micro metallic mesh suspended ∼100 microns above a readout plane, creating a narrow amplification gap. Electrons drift into this gap and undergo avalanche multiplication. Micromegas are known for fast timing, high gain, and simplicity in construction compared to GEMs.

Micro-Resistive WELL (µRWELL): The µRWELL [81] is a relatively recent development that combines elements of GEM and Micromegas technologies into a compact, spark-protected, and mechanically simple structure. It features a single amplification stage in which a resistive layer is deposited over the readout PCB, integrated with a WELL-shaped amplification structure (essentially a single GEM foil bonded to the resistive layer) [82, 83, 84]. This resistive layer suppresses discharges, improves gain stability, Its single amplification stage also simplifies the fabrication and reduces

Table 2: Materials for rhizotron thin windows for beta particle imaging

Name	**Material Type**	**Density (g/cm^3)**	**Tensile Strength (MPa)**	**Gas Permeability**	**Typical Thickness**	**Remarks**
Kapton	Polyimide Polymer	1.42	231	Low (excellent)	25–50 µm	Common in MPGD detectors
Mylar	PET (Polyester)	1.39–1.4	172	Low (good)	6–50 µm	Economical, easy handling
Beryllium	Metal	1.85	∼370	Negligible	10–50 µm	Excellent transmission, toxic handling
Rohacell	PMI Foam	∼0.032	0.4–3.5	Moderate	1–5 mm	Lightweight, moderate permeability

mechanical complexity compared to multi-GEM stacks.

Fabrication of MPGD prototype

The Jefferson RD&I Group has extensive experience with GEM and μRWELL detector technologies [79, 85, 86, 84].

The RD & I group recently introduced the GEM-μRWELL hybrid detector concept [87] which combines a GEM foil used for pre-amplification with μRWELL layer as second amplification to provide robustness and gain flexibility for high detection efficiency and excellent spatial resolution capabilities when combined with low channel count capacitive-sharing 2D-strip (or pad) readout structure [84, 88]. Figure 5 is a diagram of a cross sectional view of a GEM-μRWELL hybrid detector (left) and a photograph of a prototype developed by the detector group and tested for tracking application in future particle physics experiments. The detector will have an active area

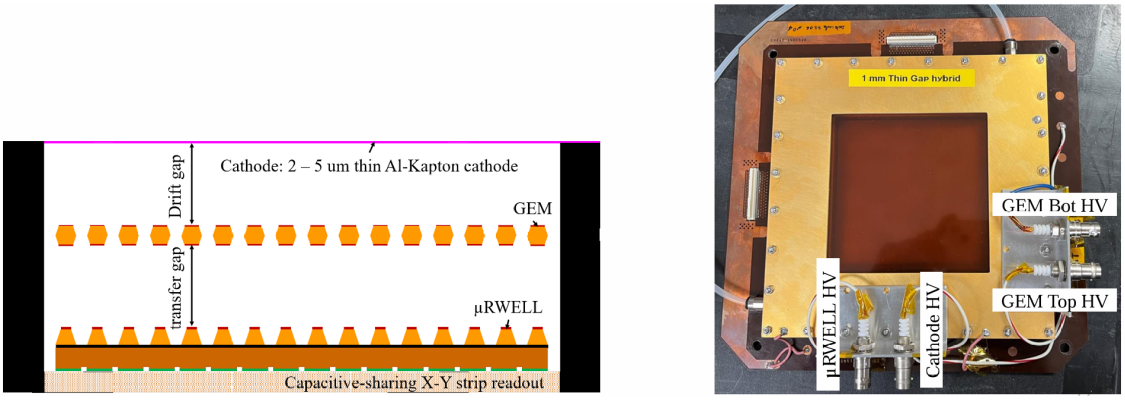

Figure 5: Cross-sectional view of the GEM-μRWELL hybrid detector *(Left)*. Picture of a 10 cm × 10 cm GEM-μRWELL hybrid prototype fabricated and tested at Jefferson Lab *(Right)*.

of 10 cm × 10 cm optimized for low energy beta-particle detection with adjustable gain capability thanks to the dual amplification and ultra low mass entrance window to minimize energy loss of the impinging particle. The effort will primarily focused on the minimization of entrance window material which will serve both as gas window and the cathode layer required to establish the electric field in the detector drift gas volume. We will study the mechanical strength and handling of ultra thin (2 to 5 μm) aluminized Kapton (or Mylar) foils for the delicate step of framing during the detector assembly.

Two prototypes will be developed, one with 800 μm-pitch X-Y strip anode readout and the second with 6.4 mm × 6.4 mm pad readout will be developed for spatial resolution performance comparison. The two readout types will be based on the novel concept of capacitive-sharing readout structures to minimize the number of strips (or pads) to be read out by the readout electronics and DAQ while maintaining the excellent spatial resolution capabilities. The concept of capacitive-sharing readout is described in great detail in [84]. Figure 6 shows a μRWELL PCB coupled with 800 μm-pitch X-Y strip capacitive-sharing anode readout. Minimizing the number of readout channels is critically important for cost saving, reduction of data volume and detector packaging consideration specially for applications outside the Nuclear Physics and High Energy Physics environment.

The Jefferson Lab RD&I group will be in charge of the full design and fabrication of the prototypes. The detectors will be assembled inside the RD&I group's MPGD clean room and the preliminary tests for the characterization of the prototypes will be performed on the RD&I Group MPGD

cosmic-ray test stand. The critical elements of the detector such as the GEM amplification foil and the µRWELL PCB will be procured from the CERN Micro Pattern Technologies Workshop (CERN, Switzerland).

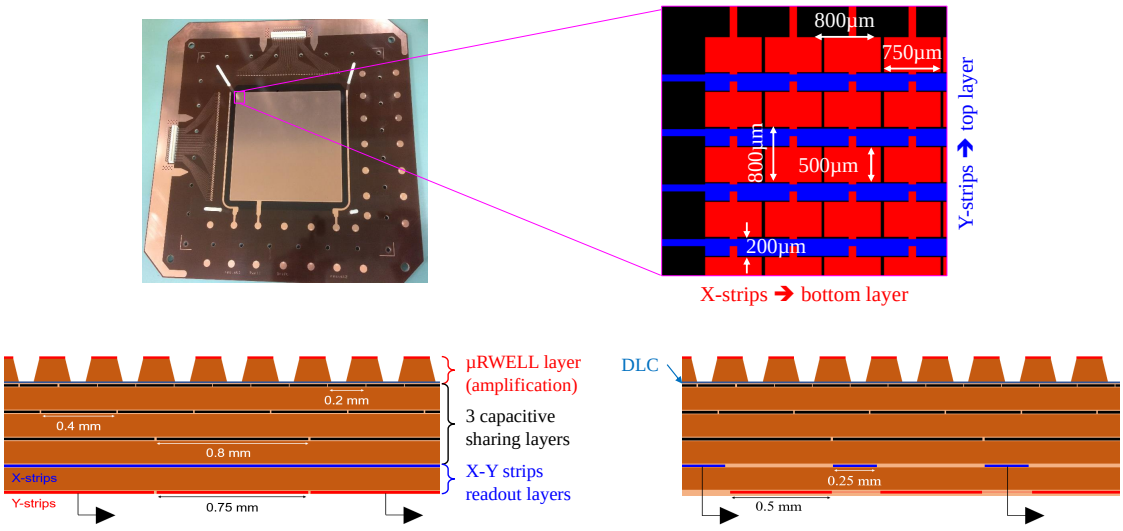

Figure 6: Concept of a µRWELL with capacitive-sharing 800 µm-pitch X-Y strip anode readout multilayer PCB

GEM-µRWELL hybrid detector is the technology of choice for the Beta-Rhizotron detector because of it's simple design, the high-voltage flexibility for gain optimization, the high spatial and timing resolution performance and operation stability in harsh conditions.

4.2.4 Data Acquisition and Signal Processing

The Data AcQuisition (DAQ) envisioned for reading out the rhizotron not only needs to meet the technical specifications for detecting ionization signals produced by beta particles with good efficiency (>90%) in the MPGD drift gas, but must also be available to match the project schedule outlined below in Section 4.4. With that in mind, we propose our *default* front-end electronics system to be based on ongoing development work already in progress at Jefferson Lab. This system is already planned as an upgrade to the readout of nuclear physics detectors in Jefferson Lab's Experimental Hall D, with members of the RD&I Group (led Dr. Christy) already collaborating on testing this system for use with other MPGD systems. The electronics consists of an *analog* electronics card providing signal amplification and shaping and a *digital* card providing a flash analog to digital (ADC) unit and a field programmable gate array (FPGA) integrated circuit (IC) for signal pulse processing. The flash ADC provides waveform sampling of the current signal produced on the readout electrodes at 166 mega samples per second (MSPS) with 14 bit precision, and a time window of up to 10 μs. While this system is more expensive per channel than other possible systems based on ASICs, it has the advantages of being both robust for optimization and low risk in terms of availability. This system is very similar to the current 125 MSPS flash ADC (FA125) system that is currently in use in Hall D. If funded, we would tag onto an already planned requisition of cards in order to lower the costs per channel, which is reflected in the proposed budget.

It is envisioned that the DAQ system deployed to the university groups would run in self-triggering mode, with initial tests at Jefferson Lab possibly utilizing a random triggering mode up to ≈

100 kHz. In the self-triggering mode the readout of all MPDG signal channels would be initiated if at least one of the channels is above a set threshold programmed into the FPGA, with the charge and time recorded for all pulses in the time window above a noise suppressing threshold. The proposed DAQ work aligns well with both the expertise and ongoing projects of the Jefferson Lab RD&I Group, providing significant synergy and efficiency.

4.3 Integration of MPGD with Rhizotron

4.3.1 Design of Rhizotron

Window Material Selection for the Rhizotron

In the development of a beta-imaging rhizotron system using MPGDs, selecting the optimal thin window material is crucial. The window material must enable maximum beta particle transmission, particularly for low-energy isotopes like ^{14}C, while maintaining mechanical strength, chemical stability, and compatibility with plant growth over time. Beta particles are highly sensitive to material thickness and density—higher density or thicker windows attenuate beta particles more significantly, especially at energies below 200 keV. Additionally, as the window will be in contact with moist soil or humid environments, hygroscopicity (the tendency to absorb water) must be minimal to avoid warping, swelling, or degradation. Our first choice for the rhizotron window material is Kapton, due to its exceptional mechanical strength, thermal stability, and extensive use in radiation environments, making it ideal for initial integration with the MPGD system. However, if hygroscopicity or long-term moisture exposure becomes an issue, alternative films such as polypropylene (PP)—which has significantly lower water absorption—will be considered. Additionally, non-hygroscopic barrier coatings, such as Parylene C, may be applied on top of Kapton to improve moisture resistance while preserving its structural benefits. Table 3 summarizes the key physical and chemical characteristics of candidate materials.

Table 3: Comparison of candidate thin-film materials for rhizotron window.

Material	**Density (g/cm^3)**	**Thickness (µm)**	**Tensile Strength**	**Hygroscopicity**	**Notes**
Kapton	1.42	20–50	Very High	Very Low (≤0.7%)	Good chemical resistance
Mylar	1.39	12–50	High	Low (0.8–1.2%)	Moderate Flexiblility
Polyethylene	0.94–0.96	10–50	Low	Moderate(0.3–0.5%)	Poor tear resistance
Polypropylene	0.90–0.92	10–50	Moderate	Low (∼0.05%)	Stronger than PE
Parylene C	1.29	5–20	Moderate	Negligible (<0.01%)	Applied as conformal coating

Design and Test of Rhizotron

To demonstrate the feasibility of MPGD-based beta imaging for plant root studies, a custom-designed rhizotron prototype—Beta Rhizotron will be developed and tested. The primary purpose of this rhizotron is to create a biologically viable, mechanically stable, and beta-transparent interface between the plant root environment and the MPGD. The design will prioritize both root

growth compatibility and beta particle transmission efficiency. The rhizotron will be constructed as a rectangular growth chamber with dimensions of 10 cm × 10 cm in lateral area, matching the active imaging area of the MPGD system. The thickness of the chamber will be adjustable between 5 and 10 cm, depending on the root architecture of the target plant species. A thinner profile will be used for small-rooted model plants like Arabidopsis thaliana, while thicker versions may be used for crop seedlings with more aggressive root systems. The chamber will be fabricated from clear plastic materials (e.g., acrylic or polycarbonate) to allow optical inspection during preliminary trials and ensure chemical stability in various soil conditions. One side of the rhizotron will remain open to allow the insertion of a thin beta-transparent film, such as Mylar or Kapton (∼5 μm thick), which serves as the interface between the plant roots and the MPGD. This film must be mechanically supported yet thin enough to allow low-energy beta particles to pass through with minimal attenuation. The chamber will be sealed with this film during assembly and mounted onto the MPGD during imaging experiments. While thinner rhizotron designs (∼5 mm) are desirable to encourage root growth close to the film (thus maximizing detection efficiency), they also present practical limitations. A rhizotron that is too thin may restrict root development, increase stress on the plant, and reduce the representativeness of natural root architecture [89]. Moreover, extremely shallow soil volumes may lead to rapid desiccation, nutrient gradients, and limited microbial activity—all of which could affect experimental reproducibility.

To validate the design, a series of biological and mechanical tests will be performed using target plants grown under controlled conditions. The thin-film window will be tested across different soil types (e.g., potting mix, sandy loam, hydroponic gel) to evaluate its ability to hold:

- Moisture and soil mass without tearing or leaking,
- Root pressure and expansion over time,
- Chemical compatibility with fertilizers, pH shifts, and microbial activity.

In addition, root growth behavior will be monitored and measured to assess whether roots are naturally guided toward and along the thin-film window. These initial tests are not intended to generate imaging data but to establish the structural and biological feasibility of the Beta Rhizotron design. Results will be incorporated into integration with the MPGD for beta imaging experiment.

4.3.2 Environmental Control System for MPGD and Rhizotron

Integrating a MPGD to a soil-based rhizotron requires a robust environmental control system that balances the operational needs of the detector with the biological requirements of plant growth. Key parameters include temperature, humidity, gas purity, and isolation between the detector and soil, all of which can affect imaging performance, system stability, and root physiology. Temperature is critical for both MPGD function and plant health. Detectors like GEMs or Micromegas require a stable temperature range to maintain gain stability and minimize electronic noise. Simultaneously, plant root growth and microbial activity are temperature-sensitive. To maintain consistency, the Beta Rhizotron will include thermal regulation via heating mats or Peltier elements, coupled with internal sensors for feedback control. The entire system may be enclosed in a growth chamber to reduce ambient fluctuations. Humidity presents a dual challenge. While root zones must retain moisture for healthy growth, high humidity in the detector housing can lead to electrical instability or gas contamination. To address this, the detector volume will be hermetically sealed from the soil using a thin, beta-particle transparent barrier as mentioned before (e.g., Mylar or Kapton). Inside the sealed chamber, a continuous flow of dry gas (such as Ar/CO_2) will maintain low humidity

(<10%), regulated with humidity sensors and feed back control system. Gas flow and pressure stability are essential for MPGD operation. A dedicated gas delivery system will maintain appropriate pressure and flow rate, ensuring consistent detector response while protecting sensitive components from atmospheric intrusion or over-pressurization. Light management will also be considered. Since roots grow in darkness, the rhizotron will include light shielding, while allowing optional integration of controlled lighting for studies involving photomorphogenic responses. In summary, the environmental control system will ensure thermal and moisture isolation, gas purity, and physical separation between the root environment and detector electronics. This will allow stable, long-term imaging of radiotracer-labeled nutrient uptake in realistic soil conditions without compromising detector performance or biological relevance.

There is a final important item to be mentioned. Though it appears the various support technologies needed for the operation of MPGDs such as DAQ electronic and gas systems. If the project demonstrates the basic goal of an operational MPGD based rhizotron/rhizobox which provides a powerful tool for rhizosphere research constructing less expensive and less cumbersome Beta Rhizotrons would not be difficult. One would know exactly what sort of DAQ electronics capabilities would be needed and the gas volume could be filled and sealed requiring replacement gas only periodically.

4.4 Test of Beta Rhizotron with Plant

To validate the performance of the Beta Rhizotron system under biologically relevant conditions, collaborative experiments will be conducted at two partner institutions—U.C. Santa Cruz (UCSC) and Michigan State University (MSU)—each contributing distinct radiotracer setups and expertise.

At U.C. Santa Cruz, the rhizotron system will be tested using Carbon-14 -labeled compounds, such as $^{14}CO_2$ or ^{14}C-sucrose, to investigate carbon allocation and root metabolic activity. Given that ^{14}C emits low-energy beta particles (maximum 156 keV, average 50 keV), the detector configuration as mentiojned above will emphasize ultra-thin entrance windows and minimal material between root and detector surface to reduce energy loss. Plants will be exposed to ^{14}C tracers in a controlled gas or hydroponic delivery system following standard protocol used in previous studies [90]. The beta imaging setup will be optimized for long integration times to capture slow dynamics of carbon transport and partitioning in the root system.

At Michigan State University, the rhizotron will be configured for use with ^{45}Ca, ^{59}Fe, and ^{64}Cu, a mid-energy beta emitter commonly used for studying nutrient uptake and translocation. The mid-energy beta emission allows for imaging through denser root-soil interfaces, making it suitable for experiments involving soil-grown plants. The detection setup at Michigan State University will be tailored to handle mid-energy beta deposition, requiring careful shielding and calibration. Plants will be labeled with nutrients through soil-root feeding, and imaging will focus on capturing dynamic uptake and spatial redistribution over time.

These dual experimental tracks will allow comparative evaluation of the Beta Rhizotron system's performance across different isotopes and soil/root conditions, supporting flexible adaptation of the platform for various biological imaging applications. Data from both setups will contribute to refining detector parameters and validating imaging capabilities in functional root phenotyping.

Figure 7 shows the design overview and operational steps for the rhizobox system integrated into the Beta Rhizotron. Initially, an open-faced rhizobox (10 cm $\times$ 10 cm $\times$ 1 cm) is prepared (Fig.

7a). This rhizobox is filled with soil and then sealed with a thin beta-transparent film, which facilitates minimal attenuation of beta emissions from radiotracers (Fig. 7b). During the seed germination phase, an external support wall is attached to reinforce structural integrity and protect the delicate transparent film (Fig. 7c). Once the plant has established sufficient root growth, the external support is carefully removed to expose the root zone without disturbing the roots (Fig. 7d). Subsequently, the MPGD is installed directly against the transparent film surface, enabling precise beta particle detection following radiotracer application (Fig. 7e). Finally, the MPGD remains securely in place, allowing continuous or time-lapse imaging of root system activity as required for the duration of the experiment (Fig. 7f).

Along with beta imaging using the MPGD, it is important to incorporate structural imaging to provide context and anatomical details of the root system. However, dynamic structural imaging during beta imaging sessions is currently impractical because once the MPGD is attached to the beta rhizotron window, removing it becomes difficult due to the fragile nature of the rhizotron window film. Therefore, structural optical imaging will be performed only at the final stage, after all MPGD-based beta imaging has been completed.

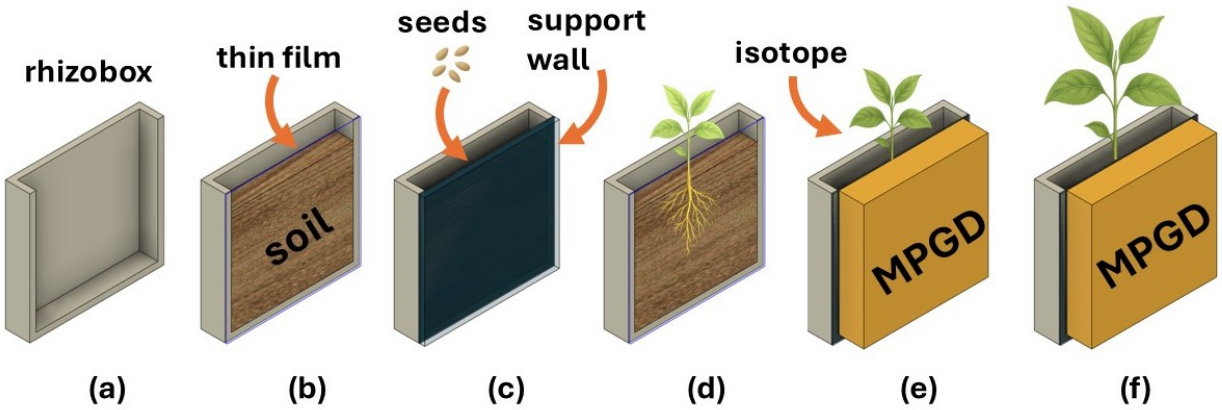

Figure 7: Design overview of the rhizobox used in the Beta Rhizotron system. Schematic of an open-faced rhizobox (10 cm × 10 cm × 1 cm) (a). Rhizobox filled with soil and sealed with a thin beta-transparent film (b). Seed germination phase with an external support wall attached for structural reinforcement (c). Support wall removed after plant establishment to expose root zone (d). MPGD detector installed against the film surface, followed by radiotracer delivery (e). MPGD remains in place for continuous or time-lapse imaging as desired (f). At the end of each experiment, MPGD will be removed and optical image will be acquired to capture root structure.

4.4.1 Test of Beta Rhizotron at UCSC for Carbon Tracking

UCSC will evaluate the capabilities of the new MPGD-based Beta Rhizotron for high-resolution, dynamic imaging of carbon processes in the rhizosphere using ^{14}C radiolabeling. Two central research questions guide this effort:

1. How do different plant-soil systems influence photosynthate carbon allocation to root growth, respiration, and exudation over time scales from minutes to weeks?

2. How are fast, micro-scale metabolic processes (e.g., root exudation and microbial uptake) linked

to environmental variables, soil carbon storage, and plant growth across broader temporal scales?

Background for Question 1: Soil-surface CO_2 efflux is widely used to estimate total belowground respiration, which includes both root and microbial contributions. Globally, belowground respiration is the second-largest carbon flux after plant photosynthesis and contributes more CO_2 than fossil fuel emissions [91, 92]. A significant portion of this flux is driven by rhizospheric activity, which also plays a key role in soil organic carbon (SOC) dynamics [93]. Approximately 27% of photosynthetically fixed carbon is allocated to root exudates, which fuel rhizosphere microbial respiration [94]. Partitioning the sources of rhizosphere CO_2-root respiration, microbial respiration, and decomposition of soil organic matter—remains a major challenge due to overlapping processes and methodological limitations [95]. We will use ^{14}C labeling to quantify carbon allocation to roots, exudates, and respiratory fluxes in a soil-grown plant system housed within the MPGD Beta Rhizotron. The system's high spatial and temporal resolution will allow us to distinguish root- and microbe-derived $^{14}CO_2$ in situ and generate data scalable to canopy-level carbon fluxes. Cheng and colleagues have previously used ^{14}C partitioning to study rhizosphere fluxes [96, 97, 90], though with lower spatiotemporal resolution than the Beta Rhizotron now offers.

Background for Question 2: We also aim to understand how fast rhizospheric processes at microscales affect slower, long-term pathways of carbon stabilization in soils. Root exudates are rapidly metabolized by rhizosphere microbes [96], and much of persistent SOC is thought to originate from microbial necromass and transformation products [98, 99]. Using the Beta Rhizotron, we will track $^{14}CO_2$ production from microbial respiration at 10-minute intervals. Root- and microbe-derived $^{14}CO_2$ will be distinguished using a glucose-trapping method [96, 97]. To complement dynamic imaging, destructive sampling will be used to assess microbial carbon use efficiency and stabilization via ^{14}C-labeled microbial biomass, mineral-associated organic matter (MAOM), and microbial residues. Microbial turnover will be estimated using the ^{18}O-water method [100, 101]. These integrated measurements will allow us to scale rhizosphere carbon processing from minutes to weeks [102].

Protocol for ^{14}C-imaging of the rhizosphere using pulse labeling: The ^{14}C experiments will be completely carried out in a fume hood at the Radiological Instrumentation Laboratory (RIL) at University of California Santa Cruz. CO_2 is not adsorptive to the inner surface of ducting systems and RIL is certified to perform $^{14}CO_2$ imaging within fume hood. The fume hood assures that all traces of $^{14}CO_2$ released during the experiments will be sucked out of the fume hood and exhausted out of the building. Preparation of the pulse labeling set-ups: The schematic illustration of the system set-ups is given in Figure 8 . The top labeling chamber containing the entire plant canopy forms a completely sealed air space, which is also physically connected to the bottom soil chamber using an acrylic adapter (not shown), but the air circulation is completely separated from the bottom soil chamber. The closed air circulation of the top labeling chamber is driven by an inline air pump which connects with the $^{14}CO_2$ injection module and an infra-red CO_2 analyzer for monitoring CO_2 concentration inside the top chamber. Another air pump is used to flash the $^{14}CO_2$ (produced by root and soil respiration) out of the bottom soil chamber instantly and continuously during the experiment.

Pulse labeling: After testing the functionality of the setups, the plant's aboveground portion would then be exposed to a pulse of $^{14}CO_2$ by injecting 0.5 mCi of NaH14CO3 solution into the flask containing 10 percent phosphoric acid. Based on our previous experiments, the injected $^{14}CO_2$ inside the top labeling chamber is expected to be completely taken up by plant photosynthesis within five minutes of the injection [96]. After the radioactive injection, live plant tissue and wet

soil can release the radioactive source in the form of $^{14}CO_2$. Therefore, these materials are processed within the fume hood to ensure safety. To minimize the release of residual $^{14}CO_2$ into the fumehood, a small Ascarite II column (sodium hydroxide-coated non-fibrous silicate, Thomas Scientific) will be incorporated into an additional circulation pump line. Once the injected $^{14}CO_2$ within the top labeling chamber is fully assimilated by the plant through photosynthesis, the Ascarite II column will absorb any residual $^{14}CO_2$ remaining in the plant chamber. Air exiting the lower soil chamber will pass through a $Na(OH)CO_2$ trap assemblage composed of a tall glass test tube with a glass airstone connected to air inlet tubing submerged in 0.2 M Na(OH); this is to be exchanged with a "fresh" trap at a 10-20 minute interval for scintillation counting of $^{14}CO_2$ thru time. Once the experiment has concluded, all plant and soil materials will be destructively sampled in the fumehood, and then plant materials will be dried in a sealed container at 80 °C to kill the plant tissues. This rapid "baking" process is designed to ensure no radioactivity is released during the drying phase. After work has finished, all radioactive materials and wastes (soil, plant, Ascarite II columns) will be stored in the proper locations as standard protocol.

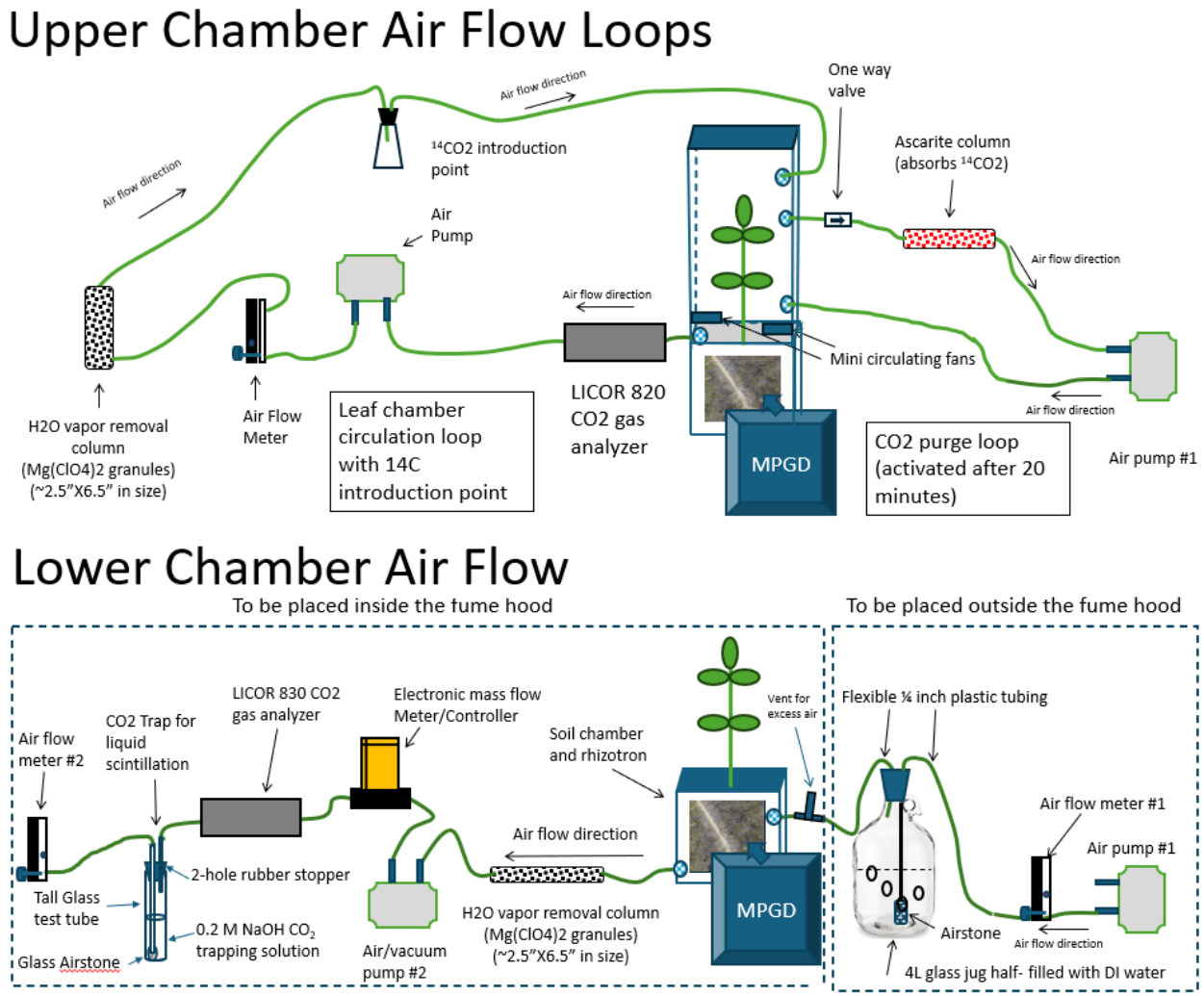

Figure 8: Experimental setup diagram showing the airflow through the airtight upper ^{14}C labeling chamber and the lower ^{14}C rhizo-imaging chamber.

4.4.2 Test of Beta Rhizotron at MSU for Nutrient Tracking

At Michigan State University, we will test the Beta Rhizotron in a series of experiments involving micronutrient transport in the bioenergy sorghum (Sorghum bicolor), one of DOE Joint Genome Institutes Flagship Plant species. The goal of these experiments will be to evaluate whether or not we can get comparable or better information with the rhizotron vs. *ex-situ* liquid scintillation counting (LSC) and phosphorimaging. All experiments will be based upon tracking 2D distributions of radiotracers on the rhizotron wall that has the imaging detector attached to it. We will focus on stable bioenergy sorghum crosses for these experiments given their rapid growth and importance in bioenergy and microbiome sciences [103, 104].

Experiment 1: We will start by evaluating the rhizotron and beta imaging setup through a simple baseline experiment. For this, we will introduce a known quantity of radiotracer, with activities ranging from tens to hundreds of µCi, at a defined location within the rhizotron. A thin cellulose filter paper, adhered to the rhizotron wall by surface tension, will serve as the delivery medium. Beforehand, the selected radionuclides, ^{45}Ca, ^{59}Fe, and ^{64}Cu (see Table 1), will be quantified using gamma spectroscopy or LSC. This experiment will be conducted with all selected radioisotopes in various chemical forms. Citrates and oxalates will be selected as they are common natural chelating agents that enhance nutrient availability for plants, while carbonates and phosphates will be used to represent less-soluble mineral forms typically found in geological soil materials [105]. Additionally, these rhizotron experiments will be conducted both with and without the soil growing medium, which, for each of these experiments will consist of sand. We will utilize the beta imager to monitor changes in the distribution and concentration of the radiolabeled mineral over time. From an imaging perspective, these experiments will provide insights into: i) how different radioisotopes and their varying beta energies affect imaging quality, and ii) the impact of the growing medium on imaging performance. From a plant science standpoint, it will enhance our understanding of how different chemical forms impact nutrient distribution within the rhizotron setup. Additionally, to benchmark our beta imaging approach against an established method, we will perform phosphor imaging by exposing the rhizotron wall to a film. Data collection will be established across a time course and maintained consistently, ranging from several hours to a few weeks. However, with the phosphor imaging method, temporal resolution is limited to these predefined time points. The resulting images will be compared with data from beta imaging in terms of spatial resolution and overall image quality. In addition, ^{64}Cu has unique decay properties, as it also emits positrons, making it suitable for PET imaging. This will enable a cross-comparison not only with phosphor imaging, but also with PET, next to beta imaging. The inclusion of PET imaging will allow for comparison with a method that is not limited to only *ex-situ* measurements.

Experiment 2: In this experiment, we will build upon the initial setup and insights gained on radiotracer distribution by using the same rhizotron filled with growth media (sand) and radiotracer, while additionally introducing a fungal colony to assess microbial transformation and transport. For these experiments we will choose to work with *Fusarium* and *Mortierella* fungal isolates that were obtained from the sorghum rhizosphere. These rhizosphere fungi are characterized by rapid growth, are known to interact with bacteria in the rhizosphere, and have been shown to produce siderophores (Bonito, unpublished data). Mycelial colonies of a single species will be introduced with colonized substrate in a patch along the rhizosphere wall, and adjacent to the thin cellulose sheet blotted with labelled mineral forms. As the fungal colony spreads, it will develop hyphae throughout the growth medium, which we hypothesize will impact the diffusion and accumulation of metals in the rhizobox. We will evaluate this hypothesis by comparing the radiotracer distribution to the data obtained in Experiment 1. We will maintain the same chemical forms of the radiotracers (citrate,

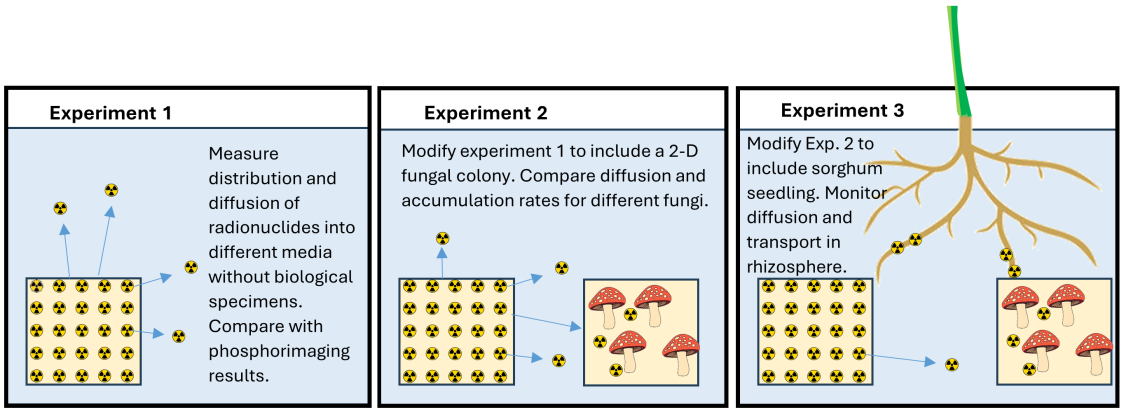

Figure 9: Schematic representation of a three-step experimental design to evaluate radionuclide distribution and transport using the Beta Rhizotron system. Experiment 1 establishes baseline radionuclide distribution and diffusion characteristics in abiotic media, serving as a control and validation against phosphorimaging results *(Left)*. Experiment 2 expands upon this by introducing a two-dimensional fungal colony, allowing for comparative studies of radionuclide diffusion and fungal accumulation rates *(Middle)*. Experiment 3 incorporates a sorghum seedling into the fungal-radionuclide system to monitor complex interactions, specifically diffusion and transport processes within the rhizosphere environment *(Right)*.

oxalate, carbonate, and phosphate) and the activity levels previously determined as suitable. Data acquisition will be performed using the beta imager and cross-checked with traditional phosphor imaging, as well as PET imaging for ^{64}Cu. These experiments will provide i) a deeper insight into the impact of fungal colonies on nutrient distribution within the rhizotron setup, ii) possible dependencies of the nutrient distribution on the administered chemical form in conjunction with fungal colonialization, and iii) a comparative assessment of the beta imaging method against traditional imaging techniques, evaluating potential improvements in spatial resolution and image quality.

Experiment 3: Here, we will further modify the rhizotron setup by introducing a sorghum seedling. Sorghum is an ideal plant for this study, given its rapid growth and importance as a bioenergy crop. Building on insights from previous experiments, where we examined the influence of fungal colonies on nutrient distribution in the growing media and the potential role of different chemical forms, this next experiment will investigate how these factors collectively impact micronutrient uptake by the sorghum plant. Nutrient uptake will be tracked using radiotracers at activity levels previously determined as suitable for imaging, applied in the same chemical forms used in prior experiments. Appropriate controls will be included to ensure that the activity levels do not interfere with plant growth. Data acquisition will be conducted using the beta imager, with cross-checking through phosphor imaging and PET imaging for the ^{64}Cu studies. Iterations of this experiment will be carried out to simulate low and high rhizosphere diversity conditions. Low rhizosphere diversity treatments will be based on individual fungal inoculations (e.g. *Fusarium*, *Mortierella*), whereby more complex high diversity treatments will use sorghum field soil as inoculum to simulate more natural field conditions with diverse fungi, bacteria and soil fauna. This set of experiments will specifically give insights into how the combination of the sorghum plant, fungal and microbial colonization influences radioisotope uptake and distribution. Additionally, the enhanced temporal resolution of beta imaging will inform the hypothesis on how uptake dynamics evolve as the seedling progresses through different developmental stages.

4.5 Project Organization and Milestones

Jefferson Lab is responsible for the overall management of the project and coordination of the tasks and roles of the other two teams (UCSC and MSU). Jefferson Lab will lead the design, fabrication, and testing of the Micro-Pattern Gaseous Detector (MPGD) and its integration into the Beta Rhizotron platform. The team will also be responsible for developing and validating the data acquisition (DAQ) system, ensuring compatibility with radiotracer imaging requirements, and delivering a fully functional prototype to UCSC and MSU for biological deployment. Jefferson Lab will oversee risk management, schedule adherence, and quality assurance across the project. The significant events that present 7 milestones are:

- M1 — Completion of MPGD and Rhizotron design specification and documentation.
- M2 — Completion of MPGD fabrication and standalone detector performance testing.
- M3 — Completion of integrated Data Acquisition (DAQ) system and imaging software.
- M4 — Assembly and validation of the first Beta Rhizotron prototype.
- M5 — Delivery and installation of the Beta Rhizotron units at UCSC and MSU.
- M6 — Completion of radiotracer preparation and isotope safety approvals at UCSC/MSU.
- M7 — Execution of root imaging experiments using the Beta Rhizotron with plants and radiotracers.

U.C.Santa Cruz is responsible for designing a dedicated rhizotron chamber tailored for ^{14}C isotope studies. The UCSC team will develop a carbon radiotracer delivery system suitable for gas-phase administration to plants and ensure proper containment and shielding. UCSC will lead experiments involving ^{14}C beta emitters for imaging root carbon allocation dynamics, in coordination with Jefferson Lab for imaging data processing and analysis.

Michigan State University is responsible for designing and implementing rhizotron systems optimized for ^{45}Ca, ^{59}Fe, ^{64}Cu and other medium-energy beta emitters. MSU will develop soil-compatible isotope delivery methods and handling protocols for longer-lived radiotracers. The team will lead biological experiments focused on nutrient uptake, root–microbe interactions, and spatial mapping of calcium, iron and copper transport in soil-grown plants using the Beta Rhizotron.

The Table 4 outlines the project timeline with key milestones. Note: A total of three DAQ systems will be built, but not all by the end of Year 2. In Year 2, two completed systems will be deployed—one to UCSC and one to MSU.

Risks

The Beta Rhizotron project involves several key risks that could impact technical feasibility and scientific outcomes. A primary challenge is the attenuation of beta particles in soil, which may limit spatial resolution or sensitivity, especially for low-energy isotopes like ^{14}C. Additionally, confining plant roots within the detector's geometry could alter natural growth patterns or root–microbe interactions, reducing biological relevance. Imaging artifacts from soil heterogeneity, moisture, or electronic noise may further degrade data quality. Integration risks also exist, as detector components must operate reliably in soil-rich, humid environments over extended periods. Procurement of specialized Micro-Pattern Gaseous Detector (MPGD) components—such as GEM foils delays or availability issues, particularly if sourcing from European Organization for Nuclear Research (CERN). Similarly, delays in acquiring or configuring a compatible data acquisition (DAQ) system could impact project timelines and initial testing. Addressing these risks through robust calibration

protocols, modular hardware design, early procurement planning, and multidisciplinary collaboration will be essential to ensure project success.

Table 4: Project Timeline with Milestones

	Year 1				Year 2				Year 3			
Jefferson Lab												
Procurement for MPGD and DAQ	X	X	X	X	X	X						
Design of MPGD and Rhizotron	X	X	M1									
Build MPGD Prototype		X	X	M2								
Build DAQ System			X	X	X	M3						
Test Beta Rhizotron for Deployment						X	M4					
Deploy Beta Rhizotron to Collaborators							M5					
Experiment and Data Analysis							X	M7	X	X	X	X
UC Santa Cruz												
Design & Test of Rhizotron for UCSC	X	X	M1									
Prepare Isotope Delivery System (^{14}C)			X	X	X	M6						
Setup Experiment and Test Rhizotron					X	X						
Setup Beta Rhizotron							M5					
Plant Study with Beta Rhizotron							X	M7	X	X	X	X
Michigan State University												
Design & Test of Rhizotron for MSU	X	X	M1									
Prepare Isotope Delivery System (^{45}Ca)			X	X	X	M6						
Setup Experiment and Test Rhizotron					X	X						
Setup Beta Rhizotron							M5					
Plant Study with Beta Rhizotron							X	M7	X	X	X	X

Appendices

Appendix A BIBLIOGRAPHY & REFERENCES CITED

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