

Development of a Biomimetic Root Oxygen Bioavailability Sensor

D. M. Porterfield, S. S. French, A. R. DeCarlo

ABSTRACT. *Low oxygen availability in soil can impair root function, thereby decreasing agronomic productivity. Without oxygen to support mitochondrial respiration, energy levels in roots may limit mineral nutrient (N, P, K) transport. Traditional methods for measuring soil oxygenation include the use of redox electrodes and polarographic oxygen sensors. These approaches are limited to measuring oxygen concentrations at specific locations and cannot determine the bio-availability of that oxygen to a growing root. An innovative approach has been developed for direct measurement of oxygen bioavailability. Instead of building the sensor to measure oxygen concentrations, it is possible to construct an electrochemical system in a manner that makes it sensitive to changes in oxygen transport and availability in the soil. Furthermore, it is even possible to construct the sensor to match the oxygen consumption characteristics of a specific root tip, which is important because most metabolism and nutrient uptake occurs here. This is accomplished by using a conductive-gel membrane system that allows sensor profiles to be engineered to match the metabolic profiles of specific species. If constructed to these standards, the sensor will biomimetically replicate biophysical oxygen depletion profiles that are analogous to those found in the rhizosphere of a growing root tip. The biomimetic root oxygen bio-availability (ROB) sensor integrates all biotic and abiotic factors in the soil that limit oxygen transport to the root, while providing real-time sensing. This new sensor technology could be used as a research tool or as a closed-loop control system for field and greenhouse irrigation.*

Keywords. *Aeration, Anoxia, Biomimetic, Biosensor, Hypoxia, Oxygen, Rhizosphere, Root, Soil.*

With regard to the root soil system, the most important parameter we want to relate to root physiology and metabolism is not necessarily the absolute oxygen concentration but instead the root oxygen bio-availability. While it is true that the oxygen concentration contributes significantly to ROB, we have to consider other factors that may limit the plant's ability to utilize the oxygen that is present. These include the pore size, water content, and microbial activity in the soil. For example, the soil atmosphere may have ambient O₂ levels, but because of soil compaction and small pore size, the percent volume of soil gas per unit volume of soil may be very low (Scott and Erick-

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The authors are **David Marshall Porterfield, ASABE Fellow**, Associate Professor, Department of Agricultural and Biological Engineering, Department of Horticulture and Landscape Architecture, Weldon School of Biomedical Engineering, Bindley Bioscience Center-Physiological Sensing Facility, Purdue University, West Lafayette, Indiana; **Steven S. French**, Undergraduate Research Assistant, Department of Mechanical Engineering, University of Texas Pan-Am, Edinburg, Texas; and **Andrew R. DeCarlo, Graduate Research Assistant**, Department of Agricultural and Biological Engineering, Bindley Bioscience Center-Physiological Sensing Facility, Purdue University, West Lafayette, Indiana. **Corresponding author:** D. Marshall Porterfield, Department of Agricultural and Biological Engineering, Department of Horticulture and Landscape Architecture, Weldon School of Biomedical Engineering, Bindley Bioscience Center-Physiological Sensing Facility, Purdue University, 225 S. University St., West Lafayette, IN 47907; phone: 765-494-1190; fax: 765-496-1115; e-mail: porterf@purdue.edu.

son, 1964). We must also consider the diffusion of oxygen in the unstirred layer near the root surface, as well as the movement of oxygen induced by buoyancy-driven thermal convection (BDTC) within the soil and rhizosphere. Metabolic heat produced by the root itself can drive BDTC within the rhizosphere, while heterogeneity of the soil and light hitting the soil surface can drive BDTC within the bulk soil. This is a very complex system, and we can hardly measure all of these factors in addition to absolute oxygen concentration.

The question, then, is how to monitor these changes with sensors so that these effects can be better described and measured. Standard electrochemical and optrode (optical fiber with oxygen specific fluorescent dye) oxygen sensors will not work in this application because they are designed to measure and report exact oxygen concentrations, which is only one component of ROB. It is possible that one could measure the result of decreasing ROB with a standard sensor, but that approach is limited by where the sensor is placed in the soil relative to the root. The plant root system is heterogeneous, and a single active root will have regions that differ drastically with regards to nutrient uptake and metabolic oxygen consumption, with the majority of these activities being localized within the root tip. The only way that a standard oxygen concentration sensor could be used to detect resultant changes related to a decrease in ROB is to directly measure the tissue or possibly the microscopic rhizosphere region for oxygen concentration changes. While this can be accomplished in a specialized experimental setup, it would be next to impossible to do in a soil system. What is needed is a sensor that will facilitate real-time long-term monitoring *in situ* and will measure the ROB within the system. If the sensor were to simulate the rhizosphere profiles associated with root oxygen demand, then it could simulate responses to any of the biotic and abiotic factors that can limit oxygen bioavailability to a root.

The solution to all of these problems is to use a biomimetic approach to simulate the activity of a living root in a way that provides an electronic signal that can be measured relative to optimal oxygen bioavailability. Instead of building a polarographic sensor to measure only oxygen concentration, the sensor should be constructed to report both oxygen concentration and the oxygen activity in the soil media. If the sensor was approximately the same size as and consumed oxygen at levels that matched an actual root tip, then the sensor output could be used to approximate ROB. Mimicking and simulating the root tip profiles is important here because the bulk of metabolic activity and nutrient absorption occurs in the root tip.

Our objective is to develop a sensor that will measure ROB based on the concept of biomimetic sensing. The adaptation of biomimetics to sensing will allow us to directly monitor physiological responses to environmental change. Our approach is based on the use of electrochemistry to simulate respiratory oxygen consumption by mitochondria in the root tissue. Traditional oxygen electrodes also work by reducing oxygen to water on the cathode, but the movement of oxygen to the cathode is diffusion-limited by a resistive membrane, which renders the sensor stir-insensitive. Therefore, the measured current from the sensor is controlled both by the characteristics of the membrane and the size and shape of the cathode (Bard and Faulkner, 1980). Important properties of the membrane include size, shape, and oxygen permeability, which all contribute to control the resistance of the flow of oxygen from regions outside of the electrode to the cathode where it is reduced. Therefore, by controlling the size, shape, and composition of the membrane as well as the size and shape of the cathodic surface, we can construct a sensor with the capacity to simulate the activity of a living root. If constructed to these standards, the ROB sensor will consume oxygen to produce oxygen-depletion profiles in the medium outside the sensor that simulates the rhizosphere profiles around growing root tips. If anything changes the bioavailability of oxygen (e.g., direct changes in oxygen

concentration, inhibition of BDTC, soil compaction, soil microbial activity, or soil flooding), the sensor will register a change in output (amps) comparable to the change in ROB experienced by a living root.

This sensor design was conceived originally for work in the development of advanced life support systems for NASA, which include engineered crop production systems as a foundation for bioregenerative systems. Previous research has shown that plant roots respond to microgravity exposure by inducing a hypoxic stress response (Porterfield et al., 1997; Porterfield, 2002a; Liao et al., 2004), and the ROB sensor was used to show that this was associated with reduction in BDTC, which is physically limited in microgravity (Liao et al., 2004). On earth, areas of application of the ROB sensor include basic research, environmental monitoring, and precision agriculture. The advantage of using this approach is that the sensor accurately integrates all biotic and abiotic factors in the soil that may modify biological oxygen transport to the root, providing for a more accurate measure of root system oxygenation. Specific applications of the technology in agriculture would be to control irrigation of crops. Over-irrigation commonly leads to transient soil flooding and root zone hypoxia, which decreases crop productivity. The sensor could be used to monitor ROB in relation to soil water saturation, thereby preventing over-irrigation and water logging-induced crop productivity decline. Added benefits of preventing over-irrigation would be to: (1) conserve valuable water resources in arid regions, (2) prevent the runoff of agrochemicals into the surrounding environment, and (3) prevent the accumulation of groundwater salts in the soil.

Materials and Methods

Measuring ROB Profiles

In order to approximate root oxygen demand, two 10-day-old maize roots were scanned using the self-referencing oxygen optrode system (Porterfield et al., 2006). The self-referencing technique (Porterfield, 2007) allows us to measure directly the oxygen diffusion gradients and flux patterns along an actual root. The optrode was constructed based on modifications to previously described methods (Kuhl and Jorgensen, 1992). Briefly, we used a 100/140 μm step index silica glass fiber optic patch cable with a standard ST connector. The fiber is cut in half and the protective PVC is removed to expose approximately 10 cm of exposed glass fiber. This is then pulled to a diameter of approximately 20 μm using a microprocessor-controlled laser puller (Sutter P-2000). The pulled fiber is then modified to function as an oxygen sensor based on the application of platinum tetrakis (pentafluorophenyl) porphyrin (PtTFPP) in a polystyrene membrane according to previously published protocols (Lee and Okura 1997). PtTFPP was obtained from Frontier Scientific (Logan, Utah), and the polystyrene was obtained from Sigma Aldrich. After tip coating, the optrode was allowed to cure overnight. The optrode was then operated as a frequency domain fluorescent lifetime sensor (Bambot et al., 1994; Gewehr and Delpy, 1993; Liao et al., 1997; Papkovsky et al., 1993; Rodriguez et al., 1999; Thar et al., 2001) and calibrated against known oxygen solutions. These calibration solutions were made from deionized water that had been bubbled with gas mixtures with oxygen concentrations ranging from 100% to 0%, with the remainder of the gas mixture being nitrogen. Since we are using an optical sensor, this approach was used to scan similar profiles at the surface of the ROB sensor without fear of there being any electrical interference. The data obtained can then be used to compare measured root biological oxygen demand with biomimetic profiles associated with the activity of the ROB sensor.

Biomimetic ROB Sensor

The generic ROB sensors have been constructed according to the diagram in figure 1. This design is a modification of a Clark-style electrode, but with some significant innovations. Both the Clark- and Whalen-style electrodes are constructed to be stir-insensitive by application of a gas-permeable membrane over a very small cathode. This effectively increases the resistance of the electrode to a level where the consumption of oxygen in the electrode can no longer significantly impact the concentration of oxygen in the medium that is being measured (Schneiderman and Goldstick, 1978). For the ROB sensor, our design required that we increase the size of the cathode and decrease the resistance of the membrane so that the sensor does significantly change the oxygen levels in the rhizosphere media, to levels comparable to a living root. A gold wire with a diameter of 500 μm is used as the cathode, and a silver wire with a diameter of 250 μm , electroplated with chloride ions, is used as a reference or counter electrode. This electrode assembly is then placed into a mold that approximates the size and shape of the root tip. In initial stages of development, we chose to build and test a generic ROB sensor for proof-of-concept testing. Once the electrode assembly was placed into the mold, the mold was filled with a 12% acrylamide hydrogel (40% w/w acrylamide solution 29:1, 47.9% w/w diH₂O, 2.1% w/w ammonium persulfate, 10% 1 mM KCl, and 7.5 μL tetramethylethylenediamine). The (29:1) acrylamide solution was made from 29g acrylamide, 1 g N, N methylene bisacrylamide, and diH₂O to 100 mL. The 12% acrylamide hydrogel matrix was chosen because it has low resistance to oxygen, is mechanically stable, and it approximates the water content of a living root.

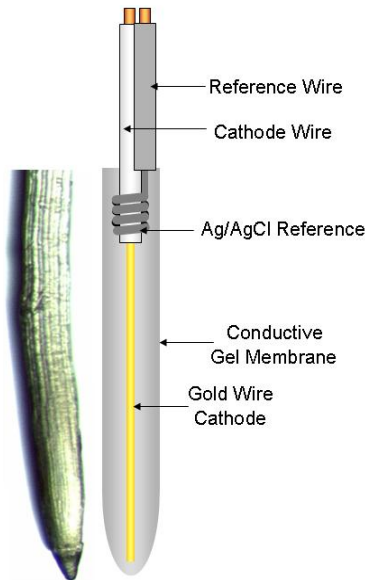


Figure 1. Construction of a root oxygen bioavailability sensor. The design is similar to a Clark-style oxygen electrode in that both the reference and cathode are contained within a common solution and membrane. This allows the sensor to operate in both aqueous and aerial environments. The gel membrane system and the geometry of the cathode are designed to allow the sensor to consume oxygen in a manner that simulates the activity of an actual root. The construction parameters of the sensor can be modified to produce a sensor that has the same size and oxygen consumption characteristics as a defined species.

The ROB sensor was operated using a BAS 100b electrochemistry workstation (BioAnalytical Systems, West Lafayette, Ind.). During operation, the cathode is polarized to -750 mV and the resulting current is related to oxygen transport and availability in the surrounding media. This current is directly converted into oxygen consumption (moles per second) with the formula: $I/(2qn)$, where I is the current (amps), q is the number of amps per electron (1.602×10^{-19}), and n is the number of electrons per mole (6.022×10^{23}). The denominator is multiplied by 2 to account for the number of electrons required to reduce an O_2 molecule to water.

Results

The basic ROB sensor design is based on simulating the micro-gradient flux profiles of an actual growing root. Our initial work (Liao et al., 2004) focused on a generic sensor design that was used for microgravity experiments concerned with BDTC. Here we have advanced the technology to begin to approach a true biomimetic corn-specific version of the ROB. The microenvironment profiles of the rhizosphere are important because the oxygen activity in this diffusion-limited space ultimately influences and limits the ROB.

A fundamental design criterion for the ROB sensor is the recreation of diffusion boundary layer conditions in the rhizosphere. We used the self-referencing oxygen optrode technique (Porterfield et al., 2006) to scan to the $20 \mu\text{m}$ rhizosphere boundary layer profile of a corn root. Corn seeds (FR27 \times FRM017, grade 24RD) were provided by Illinois Foundation Seeds, Inc. (Champaign, Ill.; www.ifsi.com). The seeds were surface sterilized (Andrews et al., 1993; Johnson et al., 1994) and grown using aeroponic cultivation as previously described (Porterfield and Musgrave, 1998). All experiments were conducted on roots of plants in early vegetative growth with 3 to 5 leaf blades. A representative plot of the oxygen profiles measured along a growing root tip can be seen in figure 2. Similarly, oxygen consumption profiles were measured along an ROB sensor, as shown in figure 2. The scans of the oxygen flux from a corn root revealed the basic characteristic pattern associated with the developmental zones of the growing root. This is similar to the basic patterns previously measured using the self-referencing amperometric oxygen electrode (Porterfield, 2002b), where the quiescent center and zones of cell expansion are associated with a stark decrease and increase in oxygen flux as the sensor scans from the root tip to the zone of maturation. The average profiles were very similar in magnitude (root = 73.06 ± 16.41 ; ROB = 71.51 ± 2.57), although the root had more variability associated with the growth zone, which was absent in the biomimetic ROB sensor.

We also tested the ROB sensor for basic response changes in bioavailability by changing the level of convection or purging oxygen by bubbling with nitrogen (fig. 3). We provided variable levels of convection by varying the speed of a 5 mm magnetic stir bar in a standard electrochemistry fluid chamber. The ROB sensor reported varying levels of oxygen bioavailability based on the mechanical convection driven at 800, 400, and 200 rpm. Moderate air bubbling of the media using an aquarium pump and an air-stone also increased the ROB sensor output almost to the level of output associated with 800 rpm of mechanical convection. In addition to the introduction of 21% oxygen from the air, air bubbling also drives mechanical convection in the system. With air bubbling, the oxygen concentration is in equilibrium, with 21% partial-pressure oxygen and approximately 78% nitrogen gas. Air bubbling with pure nitrogen gas purges the oxygen out of the solution, to be replaced by nitrogen, which is chemically inert to the sensor at these polarization potentials because of the chemical stability of triple-bonded N_2 gas. When the oxygen is purged in this standard way, the sensor output drops to zero.

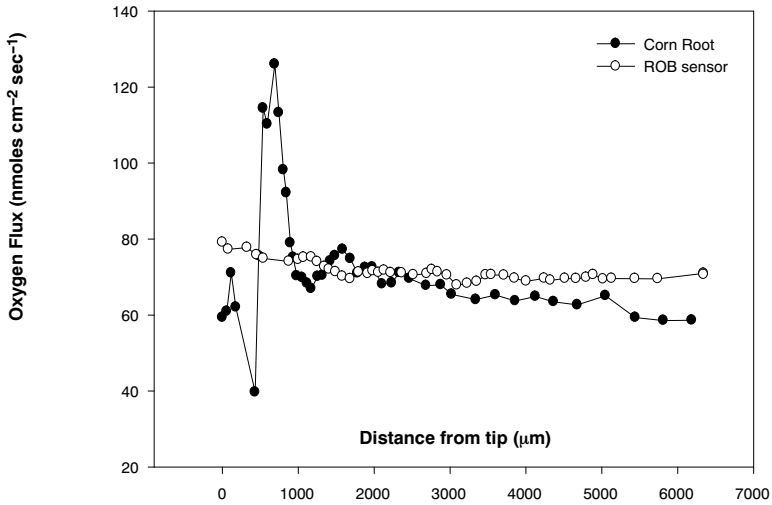


Figure 2. Flux patterns measured using a self-referencing oxygen optrode (excursion 20 μm at 0.1 Hz). The near pole of the optrode was at the interfacial surface and only measured the unstirred layer of a thickness of 20 μm . We scanned and measured a corn root (black symbols) and a biomimetic ROB sensor (white symbols). The scan started at the tip and extended back beyond 6 mm from the tip. The growing tip of a root is heterogeneous as it contains young tissues that are divided into the root cap, apical meristem, and the zone of elongation.

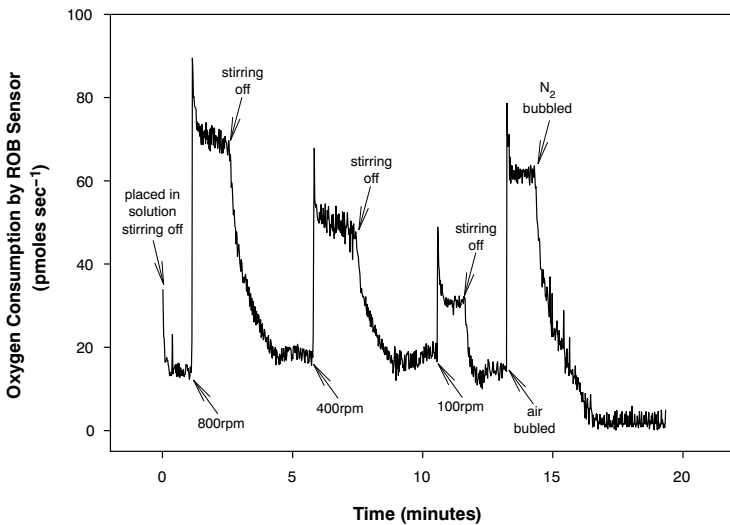


Figure 3. The use of a root oxygen bioavailability sensor to measure and report changes in convection-based oxygen transport in an aqueous medium. Note the difference between oxygen availability in stirred (rapid mechanical convection) and air bubbled (moderate mechanical convection) conditions.

Conclusion

Unlike traditional oxygen concentration measurements, the root oxygen bioavailability sensor integrates all biotic and abiotic factors in the soil that may modify biological oxygen transport to the root. The sensor technology is based on the construction of a sensor that acts as a “metabolic robot” that spatially and temporally mimics the process of oxygen consumption by the root, while providing real-time output signals of what the oxygen bioavailability actually is. This is accomplished by the use of a novel gel-conductive membrane system that allows sensor construction profiles to be modified in such a way as to allow specific sensors to be constructed to match the actual size, shape, and metabolic profiles of roots of specific species of plants.

The ROB sensor was also tested to characterize the response of the sensor to changes in ROB caused by convective transport or oxygen concentration (fig. 3). Because the sensor is stir-sensitive, it cannot be calibrated reliably in terms of oxygen concentration. Therefore, the output is converted into oxygen consumption values. These output values should be similar to that of a growing root based on the average flux values measured using the self-referencing optrode (fig. 2). While the ROB sensor did not have all of the developmental features associated with root growth, the overall performance reliably recreated the root rhizosphere activity over the major portions of the root. Considering the relatively small discrepancy between the root and the ROB, this would most likely not result in any significant performance reduction in terms of biomimicry, but this could be tested in future physiological calibration experiments. Despite small difference in developmental heterogeneity, the ROB sensor can reliably detect differences in convection and gas bubbling in tests that were comparable to responses of plant roots grown in hydroponic systems.

The promise of this technological approach is real-time sensors that are engineered to match specific crops. Here we have targeted *Zea mays* as an important crop. Corn is typically considered to be an upland plant with limited ability to withstand waterlogging and flooding that induces hypoxia. In response to hypoxia, the root will activate various stress response genes for fermentative metabolism, to tolerate and survive the stress event over the short term. The induction of fermentative metabolism is metabolically costly in comparison with aerobic mitochondrial metabolism, and this can have a direct impact on crop productivity. This also affects nutrient uptake and metabolism, which can affect long-term reproductive development in the plant.

The next stage of development of this sensor technology is to engineer the sensors to match specific plant roots with physiological calibration. Physiological calibration would be based on a series of tests to compare measured ROB values with hypoxic gene stress induction over a range of induced oxygen regimes. These regimes would range from moderate transient hypoxia, to full anaerobic conditions to allow the sensor output to be correlated with physiological stress that would limit crop productivity. These experiments would provide the calibration data needed to interpret the sensor output values. With the physiological calibration data set, each uniquely designed crop-specific ROB sensor will need to be tested to fall within a design criteria range, but not necessarily calibrated individually. This data would also be used to determine how much the developmental heterogeneity of the root would impact the biomimetic performance of the sensor. Based on these data, the cathode and conductive-gel membrane could be modified to simulate this feature of the root.

In addition to applications for advanced controls in hydroponically cultivated greenhouse crops or for field-irrigated crops, the biomimetic ROB sensor also has application in phytoremediation research. In wetland systems, the mobility of heavy metals can be reduced by rhizosphere processes mediated by the plants growing in these

flooded soils. These plants typically tolerate long-term flooding by the development of aerenchyma tissue in the root to transport oxygen to the growing root tip, but also to the soil where it is chemically available to form metal-oxide complexes. The exact role of root exudates and rhizosphere oxygen is not well understood. The ROB sensor could potentially be used to simulate the rhizosphere activity wetland plants by driving the electrochemical oxidation of water to produce oxygen, by polarizing the gold cathode to a positive working potential.

In summary, the work we have presented here represents the first stages of the development of a large class of biomimetic sensors for measuring rhizosphere/soil/root interactions. Our sensor reproduces the basic microscale oxygen consumption profiles associated with a growing corn root. The next stages of development are to physiologically calibrate the sensor relative to hypoxic stress in corn, so as to use this as a tool to manage and improve crop productivity. Later, different species-specific versions of the sensor will be designed and tested for different crops in both greenhouse and field cultivation systems.

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