Title
Planar optodes: promising tools for non-invasive 2D imaging of rhizospheric dynamics of pH, O2 and CO2.

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To improve the understanding of soil bioprocesses, especially nutrient flow and carbon allocation between roots and rhizosphere, knowledge of the spatial and temporal dynamics of the physical and chemical conditions of the soil is essential. Especially the physico-chemical parameters pH, redox potential (E_h), oxygen and carbon dioxide partial pressure (pO_2; pCO_2) are of key importance, because they characterize the environmental conditions for the entire soil biota (plants, fungi, microorganisms, etc.). However, these parameters are neither stable over time, nor are they homogeneously distributed in the soil. Therefore, quantitative high-resolution analyses of radial pH, oxygen and carbon dioxide gradients from the roots towards the bulk soil and axial gradients along the roots are essential for an advanced understanding of plant-mediated effects on soil biochemistry and biology. Furthermore, to avoid any disturbance of the natural conditions of the biogeochemical micro pattern in the soil by the measuring technique itself, such as infiltration of oxygen or increased turbation of the soil substrate by moving e.g. pH or oxygen electrodes, new non-invasive techniques for accurate high-resolution investigations of soil bioprocesses are required.

To overcome these methodical limitations, a novel rhizobox-based non-invasive 2D imaging system was constructed, which allows high-resolution optical measurements of the spatial and temporal dynamics of pH, oxygen and carbon dioxide in the soil and in the root-soil interface without any disturbance of the biological and physico-chemical conditions caused by the method itself. The optical measurement of pH, O_2 and CO_2 by so called planar optodes is based on the measurement of the fluorescence decay time of pH, O_2 and CO_2 -sensitive indicator dyes, which are fixed in a sensor foil (Gansert & Blossfeld 2008). The analyte, e.g. molecular oxygen, shows proportionality between the analyte concentration and the fluorescence decay time of the corresponding indicator. Thus, optical measurement of molecular oxygen is based on the effect of dynamic luminescence quenching by oxygen as the quenching molecule (Fig. 2). The collision between the O_2 sensitive luminescent indicator molecule (O_2 luminophore) in its excited state and the quencher (molecular oxygen) results in radiation less deactivation, and is called collisional or dynamic quenching. After collision, energy transfer takes place from the excited indicator molecule to oxygen which consequently is transferred from its ground state (triplet state) to its excited singlet state.

To meet these specific methodical demands, this system consists of a conventional x-y stepper motor frame, holding a rhizobox equipped with the planar optodes (PreSens Precision Sensing GmbH, Regensburg, Germany). These planar optodes are placed inside of the rhizobox in direct contact with the soil and the growing roots. A polymeric optical fiber (POF) is connecting an optical phase detection device of the planar optode (e.g. pH-1 mini; PreSens GmbH) to this stepper motor device and by this is transferring the optical sensing signal to the planar optodes from outside of the rhizobox (Fig. 1). The moving of the POF allows the scanning of defined sections of the planar optodes and the creation of 2D images of the measured parameters.

This novel technique was firstly used to investigate the effect of roots of selected wetland plants on the dynamics of pH patterns in submerged soils (Fig. 3), revealing considerable diurnal pH changes of about 1 pH unit along single roots of *Juncus effusus* L., closely linked to the onset of photosynthesis (Blossfeld & Gansert 2007). Furthermore, so called planar pH-O_2 hybrid optodes were effectively used for analyzing rhizospheric pH and O_2 dynamics of three different wetland plants (*Juncus effusus* L., *Juncus inflexus* L., *Juncus articulatus* L.), revealing a species specific diurnal pattern of oxygen release (up to almost 200 µmol O_2 L^{-1}), which was even detectable for lateral roots (Blossfeld 2008). Further applications were tested successfully with regard to pH dynamics along growing roots in trace metal contaminated soils, revealing strong effects of trace metal tolerant and intolerant plant species on trace metal availability (Blossfeld et al.; submitted), as well as for visualizing for the first time the impact of urea hydrolysis on soil pH under non submerged or waterlogged soil conditions (additional contribution to the IPNC XVI; publication in prep.). First tests of application of planar CO_2 optodes are in progress.

Therefore, planar optodes show the potential for becoming a powerful tool for non-invasive and quantitative mapping of the key environmental parameters in the root-rhizosphere-soil interface.

References

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Figures

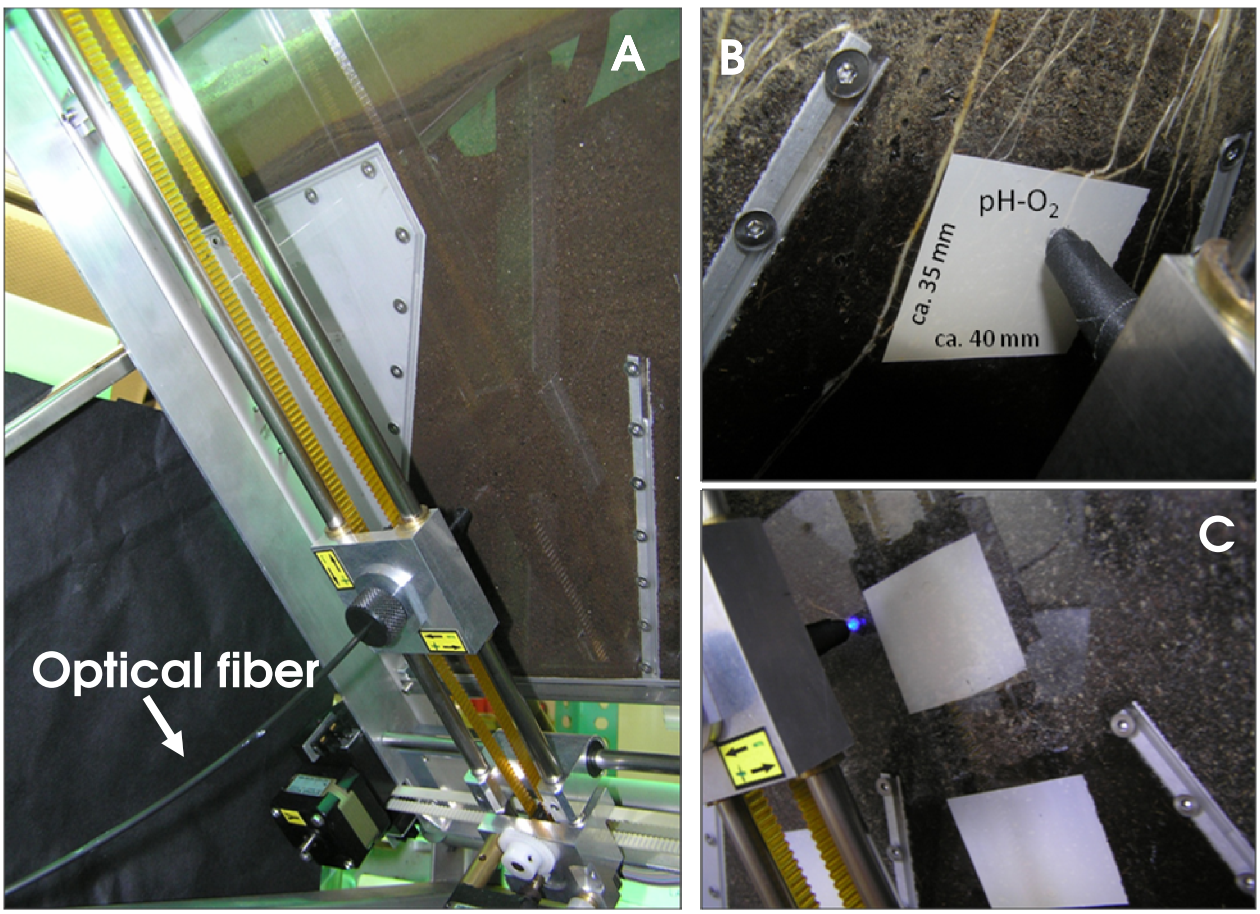

Figure 1. Optical scanning of a planar pH-O_2 hybrid optode via an optical fiber. The planar optode (sensor foil) is in direct contact with the soil and the roots inside of a rhizobox.

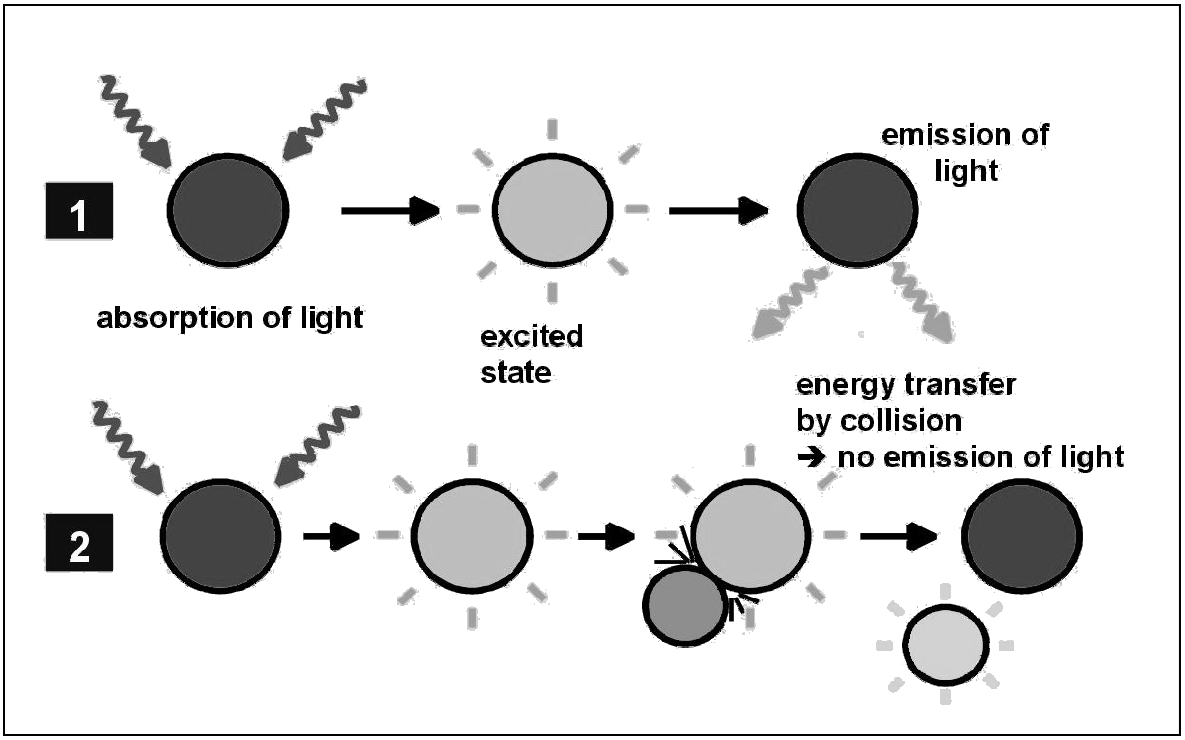

Figure 2. Principle of dynamic luminescence quenching of an excited oxygen-sensitive indicator dye (luminophore) by molecular oxygen. (1) Luminescence of the excited luminophore in absence of oxygen. (2) Luminescence quenching by energy transfer from the excited luminophore to oxygen as the quenching molecule (Figure provided by PreSens Precision Sensing GmbH, Regensburg, Germany)

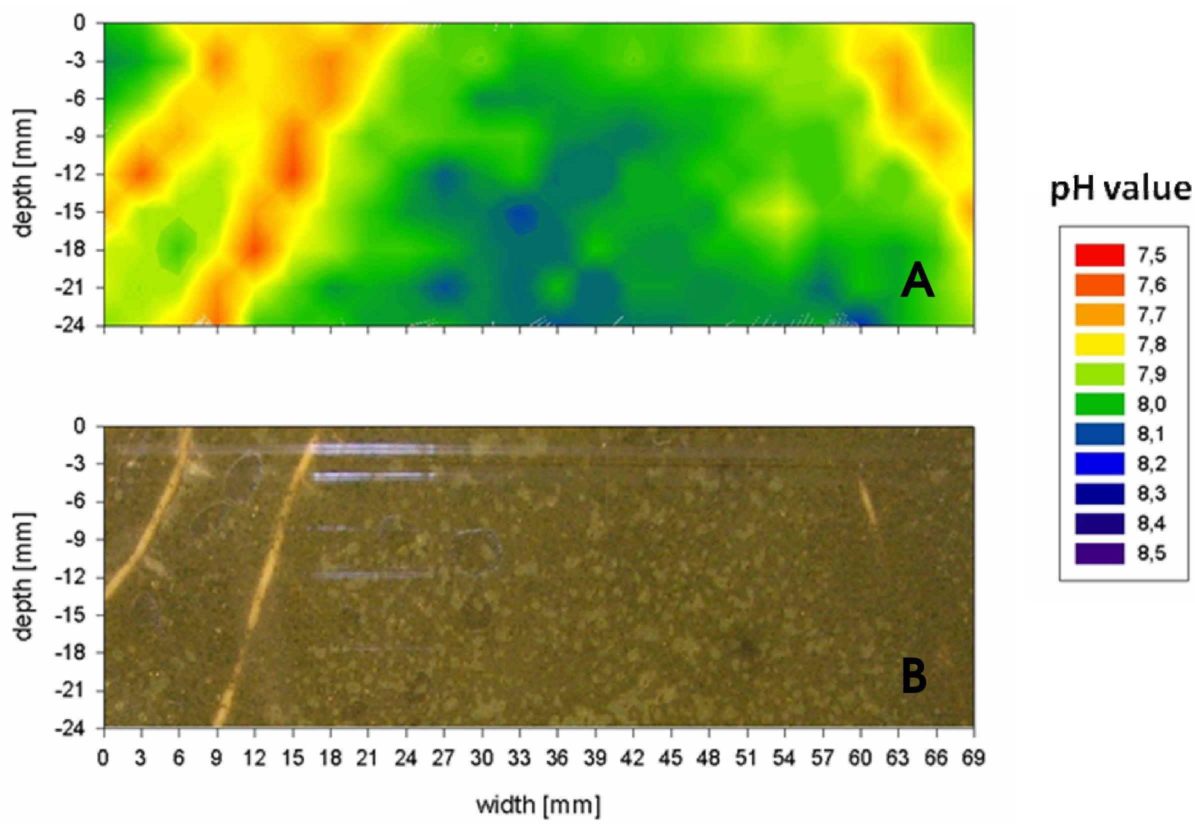

Figure 3. Two dimensional visualisation (A) of the non-invasively measured bulk soil and rhizospheric pH of *Juncus effusus* growing in a fully submerged soil (B).