



## Application of planar optodes to measure CO<sub>2</sub> gradients in the rhizosphere of unsaturated soils

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Soil CO<sub>2</sub> efflux is a major pathway in the global C cycle and comprises root respiration as well as microbial respiration. Soil respiration is therefore tightly linked to root activity and C release from roots as root exudates are rapidly decomposed by soil microorganisms. As the rhizosphere is characterized by high C turnover rates and high spatial variability of processes, a method is needed to account for this spatial and temporal variability of CO<sub>2</sub> production in the rhizosphere. Planar optodes were previously applied to measure the distribution of pH and O<sub>2</sub> in the rhizosphere. So far, CO<sub>2</sub> measurements were only a few times successfully conducted in saturated soils, although the importance of soil CO<sub>2</sub> respiration is usually higher under aerobic conditions. We therefore tested whether planar optodes can be applied to measure CO<sub>2</sub> in the rhizosphere of unsaturated soils.

Maize (*zea mays*) plants were grown in 5 rhizoboxes filled with sandy soil. After four weeks, planar optodes sensitive to CO<sub>2</sub> (PreSens GmbH, SF-CD2R - range: 0 – 1 % pCO<sub>2</sub>) were attached to the rhizosphere surface. We selected 2-5 positions with visibly growing roots per box and attached 20-40 cm<sup>2</sup> optodes at each position. The CO<sub>2</sub> concentration was measured at three different volumetric soil water contents (VWC), a) 21%, b) 28% and c) after saturation at 38%. The change of CO<sub>2</sub> signal was monitored over a period of 15 hours after saturation of samples to test the optode equilibration time. The obtained images were calibrated and analyzed using ImageJ v. 1.51 (NIH).

Gradients of CO<sub>2</sub> were clearly visible around root tips. Around mature root parts, the increase of CO<sub>2</sub> was only half as strong as for young root parts, likely due to high root respiration in the growing root parts and due to microbial decomposition of root exudates. The equilibration time for the rhizosphere was 10 hours, while in bulk soil equilibrium was not reached after 15 hours. This suggests that optodes should be attached to samples and equilibrated for at least 15 hours prior to measurements. Soil moisture had a strong effect on the measured CO<sub>2</sub> concentration. For 21% VWC the average CO<sub>2</sub> concentration was 0.23 μmol CO<sub>2</sub> l<sup>-1</sup> while it was 0.38 μmol CO<sub>2</sub> l<sup>-1</sup> for 28% VWC and increased to 1.47 μmol CO<sub>2</sub> l<sup>-1</sup> after saturating the samples to 38% VWC. For 28% and 38% VWCs, CO<sub>2</sub> gradients were clearly visible around the roots, while no gradients were detectable at VWC of 21%. This is likely due to the increased diffusion of CO<sub>2</sub> into the optodes under higher VWC. Statistical comparisons between VWC levels were highly sensitive, indicating that small changes in soil WC will greatly affect the measured CO<sub>2</sub> concentration and thus may be a strong confounding variable for treatment comparisons in unsaturated soils. However, provided that the soil moisture is kept constant in all samples, optode measurements of CO<sub>2</sub> can be used in moist soil samples to quantify relative differences in CO<sub>2</sub> concentration between treatments.