

Supplementary File 1: Text S1. Fabrication and calibration of O₂ planar optode

O₂ Planar optodes can be fabricated by coating the “cocktail” includes an analyte-sensitive indicator dye, an analyte-insensitive reference dye and an analyte-permeable polymer on a transparent dust-free polyethylene terephthalate [1]. The analyte concentrations are quantified by the ratio of indicator and reference signal through calibration. In this study, the O₂ optode was prepared by two steps: first, the sensor “cocktail” was prepared by dissolving 20 mg reference fluorophore coumarin C545 (C545T) and 10 mg Platinum(II)5,10,15,20-tetrakis-(2,3,4,5,6-pentafluorophenyl) porphyrin (PtTFPP), 500 mg polystyrene in 10 mL toluene. The “cocktail” was then gently stirred and sprayed on a transparent dust-free polyethylene terephthalate using ultrasonic spray equipment. Second, a thin layer of silicone rubber (Dow Corning 3140) was coated on top of the dry sensing layer as a protective layer, and the silicone layer was further cured for 24 h in a hood.

The calibration process was performed using deionized water with varying O₂ concentrations at 25°C. The fluorescence intensity ratio of Red and Green was applied for measuring O₂ concentration. A modified Stern–Volmer equation was used to measure the O₂ concentration according to the fluorescence intensity ratio as follows [2]:

$$\frac{R}{R_0} = \alpha + (1 - \alpha) \frac{1}{1 + K_{SV}C} \quad (1)$$

where R and R_0 are the fluorescence intensity ratio (Red/Green) in the present and absence of O₂, respectively, C is the O₂ concentration, K_{sv} is the Stern-Volmer quenching constant, α is the non-quenchable fraction of the fluorescence signal, and it is temperature independent.

The two-dimensional (2D) distribution of O₂ in the rhizosphere was used to calculate the ROL per area of root surface (ROL rate) according to the O₂-gradient around roots [3,4], the calculation equation was shown below:

$$ROL = \varphi \times R_S \times L \times \left(\frac{L}{2A} + 1 \right) \quad (2)$$

where ϕ is the sediment porosity which can be determined by measuring the weight loss of sediments dried overnight at 110 °C to constant weight^[5]. ϕ was 0.85 in this study; R_s is the average volume-specific O₂ consumption in sediment which can be calculated by the diffusive O₂ uptake and O₂ penetration depth^[6,7], R_s was 3.73 $\mu\text{mol}/\text{m}^3/\text{s}$ in this study; L is half the width of the oxygenated zone, which was measured by planar optodes imaging; A is half the width of the root diameter which was obtained with WinRHIZO image analysis software, A was 0.15 mm in this study.

Text S2. Preparation of HR-ZCA DGT

The HR-ZCA DGT was fabricated according to the following steps: 0.4 g Chelex-100 resin was ground thoroughly. 0.153 g AgNO₃ was dissolved in 0.45 mL deionized water. The ground Chelex-100 resin and dissolved AgNO₃ solution were then mixed with a 4.22 mL polyacrylamide solution, followed by the addition of 10 μL of 10 % w/w APS and 2.5 μL TEMED to form the gel solution. The gel solution was pipetted between two preheated glass plates separated by 0.40 mm thickness plastic spacers. After a 30 min polymerization period, the gel was immersed in 500 mL of 0.2 mol/L KI over 12 h. Then the gel was immersed in a solution of 16.10 g ZrOCl₂ · 8H₂O dissolved in 500 mL of deionized water for 2 h, followed by immersion in 500 mL of 0.05 mol/L MES (pH = 6.70) for 40 min to form the binding gel. The binding gel was immersed in deionized water to remove all residual chemicals and then maintained in 0.01 mol/L NaNO₃ before use. The DGT-labile flux (F) can be measured as follows:

$$F = \frac{M}{At} \quad (3)$$

Where M is the accumulated mass of elements in the DGT gel, A is the exposed surface area of the DGT gel, t is the deployment time of DGT. The accumulated mass was calculated according the signals of a series of standard gels of accumulated mass^[8]. To improve analytical precision, the ¹³C was used as an internal normalization standard.

Text S3. DNA extraction and sequencing

The extracted DNA stored at -20°C prior to further analysis. The quantity and quality were measured using a NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively.

PCR amplification of the bacterial 16S rRNA genes V4–V5 region was performed using 515F/907R primer (5'-GTGCCAGCMGCCGCGGTAA-3' and 5'-CCGTCAATTCMTTTRAGTTT-3'). Sample-specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. The PCR components contained 5 μl of buffer (5 \times), 0.25 μl of Fast pfu DNA Polymerase (5U/ μl), 2 μl 2.5 mM dNTPs, 1 μl 10 μM primer, 1 μl DNA Template, and 14.75 μl ddH₂O. Thermal cycling consisted of initial denaturation at 98°C for 5 min, followed by 25 cycles consisting of denaturation at 98°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for 45 s, with a final extension of 5 min at 72°C . PCR amplicons were purified with Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, amplicons were pooled in equal amounts, and pair-end 2 \times 250 bp sequencing was performed using the Illumina NovaSeq platform with NovaSeq 6000 SP Reagent Kit (500 cycles) at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China).

Text S4. Quantitative PCR of As-cycling genes

PCR amplifications of *aioA*, *arrA*, *arsC* and *arsM* were performed with the primers AroAdeg2F/AroAdeg2R, AS1F/AS1R, amlt-42-F/amlt-376R, and arsMF1/arsMR2 (Table S3), respectively, according to the previous study^[9,10]. PCR amplification was performed in the reaction mixtures contained 2 μL buffer (10 \times), 0.2 μL Taq DNA Polymerase (5U/ μL), 0.8 μL dNTPs, 1.6 μL primer, 2 μL DNA Template, and 13.4 μL ddH₂O. Thermal cycling consisted of initial denaturation at 98°C for 5 min, followed by 40 cycles at 95°C for 10 s, 55°C for 45 s, 72°C for 1min, with a final extension of 10 min at 72°C .

The abundance of As-cycling genes was estimated by quantitative real-time polymerase chain reaction (qPCR) performed by a Roche LightCycler 480 II. The assays used the following reaction chemistry: 10 μ L 2 \times SYBR real-time PCR premixture, 0.4 μ L primer F, 0.4 μ L primer R, 2 μ L DNA template and 6.8 μ L ddH₂O in a final volume of 20 μ L. qPCR thermal cycling parameters were: 95°C for 5 min followed by 40 cycles at 95°C for 15 s, 60°C for 30 s, hold on at 4°C.

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