New Phytologist

Research

Dynamics of oxygen and carbon dioxide in rhizospheres of *Lobelia dortmanna* – a planar optode study of belowground gas exchange between plants and sediment

Nikola Lenzewski[1]*, Peter Mueller[1]*, Robert Johannes Meier[2], Gregor Liebsch[2], Kai Jensen[1] and Ketil Koop-Jakobsen[3]

[1]Applied Plant Ecology, Biocenter Klein Flottbek, Universität Hamburg, Ohnhorststrasse 18, 22609 Hamburg, Germany; [2]PreSens, Precision Sensing GmbH, Am BioPark 11, 93053 Regensburg, Germany; [3]MARUM – Center for Marine Environmental Sciences, University of Bremen, Leobener Strasse 8, 28359 Bremen, Germany

Author for correspondence:
Peter Mueller
Tel: +49 0 40 42816 272
Email: peter.mueller@uni-hamburg.de

Received: *20 September 2017*
Accepted: *21 November 2017*

New Phytologist (2018) **218:** 131–141
doi: 10.1111/nph.14973

Key words: freshwater lakes, isoetids, plant–soil interaction, radial oxygen loss, rhizobox, rhizosphere imaging, soil biogeochemistry.

Summary

- Root-mediated CO_2 uptake, O_2 release and their effects on O_2 and CO_2 dynamics in the rhizosphere of *Lobelia dortmanna* were investigated.
- Novel planar optode technology, imaging CO_2 and O_2 distribution around single roots, provided insights into the spatiotemporal patterns of gas exchange between roots, sediment and microbial community.
- In light, O_2 release and CO_2 uptake were pronounced, resulting in a distinct oxygenated zone (radius: *c.* 3 mm) and a CO_2-depleted zone (radius: *c.* 2 mm) around roots. Simultaneously, however, microbial CO_2 production was stimulated within a larger zone around the roots (radius: *c.* 10 mm). This gave rise to a distinct pattern with a CO_2 minimum at the root surface and a CO_2 maximum *c.* 2 mm away from the root. In darkness, CO_2 uptake ceased, and the CO_2-depleted zone disappeared within 2 h. By contrast, the oxygenated root zone remained even after 8 h, but diminished markedly over time.
- A tight coupling between photosynthetic processes and the spatiotemporal dynamics of O_2 and CO_2 in the rhizosphere of *Lobelia* was demonstrated, and we suggest that O_2-induced stimulation of the microbial community in the sediment increases the supply of inorganic carbon for photosynthesis by building up a CO_2 reservoir in the rhizosphere.

Introduction

Lobelia dortmanna (Campanulaceae; *Lobelia* in the following) is a common representative of isoetid plants, a group of small and slow-growing amphibious or submerged macrophytes. *Lobelia* is native to Northern and Central Europe, and North America, where it inhabits shallow parts (<2 m) of oligotrophic soft water lakes with sandy sediments and low organic content (Farmer & Spence, 1987; Farmer, 1989; Pedersen & Sand-Jensen, 1992; Smolders *et al.*, 2002).

The soft water lakes inhabited by *Lobelia* are characterized by their limited availability of inorganic carbon. As resistance at the boundary layer is higher, and diffusivity of gases is lower, in water than in air, submerged living plants usually require high concentrations of CO_2 to saturate their photosynthetic demand (Maberly & Spence, 1989; Madsen, 1993). Furthermore, *Lobelia* exclusively utilizes CO_2 as an inorganic carbon source, whereas bicarbonate cannot be utilized (Wium-Andersen, 1971; Raun *et al.*, 2010). However, the amount of CO_2 in the sediment of soft water lakes is markedly higher than in the water column, and many isoetids have the ability to utilize this plentiful reservoir of inorganic carbon via root uptake (Wium-Andersen, 1971; Boston *et al.*, 1987; Winkel & Borum, 2009). The leaves of *Lobelia* have a thick cuticle lacking stomata that prevents gas loss through the leaves and promotes O_2 and CO_2 exchange across the rhizodermis, which has significantly higher gas permeability (Pedersen & Sand-Jensen, 1992). This causes a tight coupling of aboveground photosynthesis and belowground gas exchange between the plant and sediment.

CO_2 is taken up by the roots and transported, via a pronounced lacunae system in the roots and the two large lacunae in every leaf (Wium-Andersen, 1971; Madsen *et al.*, 2002; Winkel & Borum, 2009), to the chloroplasts in the leaves, which are located close to the lacunae (Raven *et al.*, 1988). In this way, *Lobelia* acquires the majority of its inorganic carbon for photosynthesis, recovering >98% of its CO_2 requirements by sediment uptake (Richardson *et al.*, 1984; Winkel & Borum, 2009). In contrast with several other isoetid species, *Lobelia* does not carry out its assimilation of inorganic carbon via a CAM (Crassulacean acid metabolism)-like mechanism (Boston & Adams, 1983; Raven *et al.*, 1988).

O_2 produced in the leaves is transported via the lacunae system to the roots, where it is released into the surrounding sediment

*These authors contributed equally to this work.

along the entire root (Sand-Jensen & Prahl, 1982; Møller & Sand-Jensen, 2008), promoting aerobic decomposition and thereby higher CO_2 concentrations at depth in the sediment (Sand-Jensen *et al.*, 1982). The extent of the oxygenated zone is dependent on the O_2 demand of the microbial community in the sediment and decreases with increasing concentrations of sediment organic matter (Sand-Jensen *et al.*, 2005a; Raun *et al.*, 2010; Møller & Sand-Jensen, 2011).

O_2 and CO_2 dynamics in the rhizosphere of *Lobelia* are strongly affected by diurnal cycles as *Lobelia* roots absorb CO_2 for photosynthesis and release O_2 in the light. This leads to a distinct diel pattern in the rhizosphere pore-water chemistry with low CO_2 concentrations in the light, as a result of root-facilitated CO_2 uptake, and high CO_2 concentrations in the dark, as the uptake ends and CO_2 from root and sediment respiration is released (Pedersen *et al.*, 1995). By contrast, the O_2 concentration in the rhizosphere increases in light as a result of plant-mediated sediment oxygenation resulting from photosynthesis. In darkness, less O_2 is released as no O_2 is produced by photosynthesis (Pedersen *et al.*, 1995; Sand-Jensen *et al.*, 2005a,b; Møller & Sand-Jensen, 2011; Ribaudo *et al.*, 2017).

The investigation of O_2 and CO_2 dynamics inside *Lobelia* rhizospheres without exerting damage to the belowground biomass or disturbing the spatial gradients in the pore-water chemistry arising from root–sediment interactions is challenging. Consequently, most studies in *Lobelia* rhizospheres rely on micro- and mini-electrode profiling or point measurements at different depths over time (Pedersen *et al.*, 1995; Risgaard-Petersen & Jensen, 1997; Sand-Jensen *et al.*, 2005b; Møller & Sand-Jensen, 2008). These measurements demonstrate CO_2 removal and O_2 enrichment in the isoetid rhizosphere in light. However, information on the direct connection between the roots and sediment processes as well as the spatial and temporal impact of the individual roots is sparse.

The use of planar optode technology, in combination with rhizoboxes, facilitates the quantitative monitoring of temporal and spatial dynamics of O_2 and CO_2 around individual roots with minimal disturbance of the biomass and biogeochemical gradients (Blossfeld *et al.*, 2012). In the last two decades, planar O_2 optodes have rendered possible the recording of O_2 distribution in sediments and soils (Glud *et al.*, 1996; Holst *et al.*, 1998), and planar optode technology is slowly gaining a foothold in research on rhizosphere O_2 dynamics (Jensen *et al.*, 2005; Frederiksen & Glud, 2006; Askaer *et al.*, 2010; Minett *et al.*, 2013; Jovanovic *et al.*, 2015; Koop-Jakobsen & Wenzhöfer, 2015; Larsen *et al.*, 2015; Han *et al.*, 2016; Koop-Jakobsen *et al.*, 2017). In comparison, planar optode investigations of CO_2 are still in their infancy (Santner *et al.*, 2015), and studies on CO_2 dynamics in rhizospheres are still very sparse. Blossfeld *et al.* (2013) investigated the CO_2 dynamics in the rhizosphere of *Viminaria juncea* (Fabaceae), showing that growing roots exhibit a large zone of influence (millimeter-scale) on soil CO_2 content, thereby demonstrating the usefulness of the technology to assess CO_2 dynamics in terrestrial rhizospheres.

The current study investigated the root–sediment interactions in the rhizosphere of *Lobelia* using a novel planar optode system: the VisiSens TD optode system from PreSens GmbH, Regensburg, Germany. The spatial variation of O_2 and CO_2 around roots of *Lobelia* was recorded as two-dimensional images, and the impact of light exposure of the leaves was investigated.

We hypothesized, first, large fluctuations in plant-mediated sediment oxygenation between light and dark periods, as the low gas permeability of *Lobelia* leaves makes photosynthesis the primary O_2 source for belowground O_2 transport. Second, we hypothesized a high spatial and temporal variability in the sediment CO_2 levels of *Lobelia* rhizospheres as a result of active root uptake of CO_2 for photosynthesis combined with O_2-stimulated CO_2 production.

Materials and Methods

Plant and sediment material

Lobelia dortmanna L. plants and sediments for this study were collected from the Ihlsee near Bad Segeberg in Schleswig-Holstein, Germany. The mesotrophic lake has a surface area of 29 ha, a mean depth of 7.4 m and a maximum depth of 21.5 m. Ihlsee has a catchment area of 89 ha, which is dominated by forests in the western and southern parts and by single detached houses with private gardens in the northern and eastern parts. As a former oligotrophic lake, typical isoetid plant species, such as *Isoetes lacustris* (Isoetaceae), *Littorella uniflora* (Plantaginaceae) and *Lobelia*, colonize the shallow water zones of the Ihlsee. Samples of *Lobelia* were collected from the northern, shallow water zones during the summer of 2016 (53°57′36″N, 10°18′0″E). Sediments of the sampling sites are sands with < 2% contributions of silt and clay, and a low organic matter content of *c.* 1%. The sediment pH of the site was pH 6.3 during sampling. The samples were placed in plastic bags filled with lake water and transported to the laboratory, where they were planted in aquaria with sediment and water collected at the field site. The samples were cultured for 3 months in a climate chamber (12 h : 12 h, light : dark; *c.* 200 μmol m^{-2} s^{-1}; 18°C) until the start of the measurements. Sediment pH was checked after the measurements and remained within a range of pH 6.3–6.7, close to *in situ* conditions.

Preparation of a rhizobox for optode investigations

A rhizobox (Fig. 1), an aquarium for the visualization of rhizosphere processes (Neumann *et al.*, 2009), was custom-made from Makrolon® plates, a transparent polycarbonate sheet material. Rhizoboxes consisted of a U-shaped inner frame (10-mm Makrolon®) with detachable front and back plates (5-mm Makrolon®), making a cubic aquarium (10 × 10 × 10 cm^3). Front and back plates were attached with 10 screw threads, and a 2-mm neoprene round cord was mounted along the edges of the U-shaped inner frame (Fig. 1). This made the front and back plates detachable, and yet water tight when attached.

Preparation of *Lobelia* samples for optode investigations

To prepare the *Lobelia* samples for planar optode studies, the rhizobox was filled halfway with sediment, and water was added to fill the rhizobox. The sediment was mixed and subsequently allowed

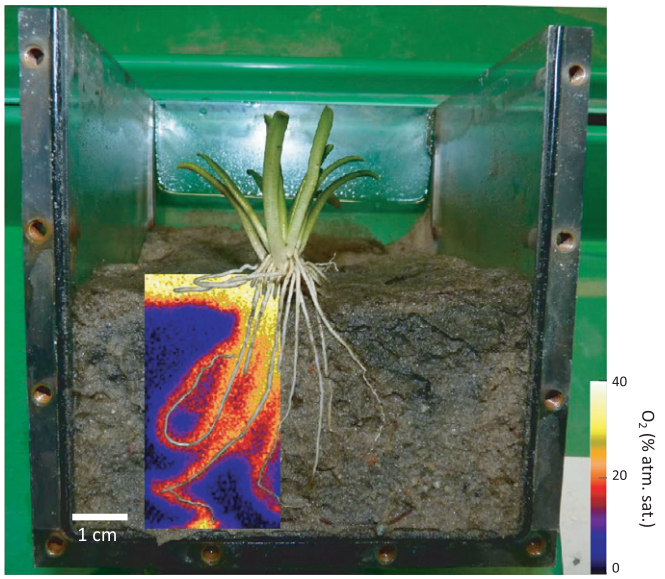

Fig. 1 Experimental set-up: *Lobelia dortmanna* in an opened and drained rhizobox with multiple roots placed for optode analysis. Part of the corresponding O_2 optode image is inserted as an overlay image showing the O_2 distribution relative to the location of the roots under submerged conditions during measurements. This study reports on the results of measurements conducted on single roots of individual plants; in this photograph, the effect of several roots of a single plant is shown.

to settle, ensuring a uniform distribution. Subsequently, the rhizobox was slowly drained, placing it on its side. The rhizobox was moved to an upright position, and a small hole was dug in the sediment at a 1-cm distance from the front plate. The *Lobelia* sample for investigation was selected and planted in the hole. Roots selected for measurements remained on the sediment surface, facing the front plate. Subsequently, the rhizobox was placed at a 45° angle, and the front plate was detached. The selected roots were placed on the sediment in a position lining up with the front plate (Fig. 1). An optode foil (O_2 or CO_2 sensitive) was attached to the front plate. Under water, the optode foil was slowly pressed against the front plate, and both items were gently lifted out of the water. This generated a uniform and air-bubble-free water film between the foil and front plate, holding the foil in place. The front plate with the foil attached in a position covering the root was mounted on the rhizobox, and the rhizobox was slowly filled with water, adding water along the back plate, whilst the box was still at a 45° angle. In this way, the sediment was slowly saturated with water, preventing the entrapment of air bubbles along the optode foil. This approach was only possible because of the high permeability of the sandy substrate, which allowed rapid percolation of the added water, and the high stability of the sediment, which allowed it to be placed at an angle without collapsing. Finally, for measurements, the rhizobox was placed in an upright position and completely filled with water.

Optode investigation of O_2 and CO_2 in *Lobelia* rhizospheres

Planar optode investigations of O_2 and CO_2 dynamics in *Lobelia* rhizospheres were conducted using the planar optode system VisiSens TD (a novel optode system for two-dimensional mapping of O_2, pH and CO_2 from PreSens – Precision Sensing GmbH). The VisiSens TD system is a fluorescence imaging-based optode system. For a detailed description of the planar optode imaging principles and the theoretical background behind the VisiSens optode system, the reader is referred to Wang *et al.* (2010), Tschiersch *et al.* (2011, 2012) and Kumari & Gupta (2017). The VisiSens TD system is an integrated system with image acquisition hardware and software (VisiSens ScientifiCal) for image processing. It facilitates the read-out of optode sensor foils with a high spatial resolution of up to 25 µm. The optode investigations were conducted with O_2-sensitive optode foils (PreSens GmbH product code: SF-RPSu4; size, 4×4 cm^2 and 10×10 cm^2; range, 0–100% O_2 atm. sat.) and CO_2-sensitive optode foils (PreSens GmbH product code: SF-CD1R; 4×4 cm^2; 1–25% CO_2).

Image acquisition was conducted with a camera resolution of 1292×964 pixels. The camera and LED light sources were positioned at a distance of *c.* 20 cm from the rhizobox. The acquired optode images had a resolution of eight to nine pixels per millimeter. Optode measurements were conducted continuously over a light–dark cycle with a temporal resolution of one image per 10 min, resulting in time series of >200 images. To facilitate optode recordings during illumination of the plants, the light illuminating the aboveground biomass was set on a timer, turning off the light for a 3-min period every 30 min, synchronized with the time of image acquisition. Consequently, the actual temporal resolution was one image per 10 min during darkness and one image per 30 min during light.

Calibration and initial image processing were conducted using the integrated VisiSens ScientifiCal software. Further quantitative data acquisition of specific features in the optode images, such as measurements of profiles across the roots, was performed using the image processing software ImageJ (version: Fiji).

To visualize the exact position of the root relative to the O_2 and CO_2 distribution shown in the optode images, the georeferencing tool in Qgis v.2.18.1 was used for the generation of overlap images of a root photograph of the selected root in the rhizobox with the associated optode image. Subsequently, the root was made visible in the optode image using the software Gimp v.2.8.18.

Calibrations and units of measurement

In this study, O_2 measurements are expressed as the percentage O_2 saturation in freshwater at atmospheric equilibrium (% atm. sat.). The O_2 optode foils were calibrated by the application of a two-point calibration with anoxic sediment for 0% O_2 and air-bubbled water for 100% atmospheric equilibrium O_2. CO_2 is measured as CO_2 partial pressure (pCO_2) and is expressed here as percentage CO_2 at atmospheric pressure (abbreviated as % CO_2 in the following). For CO_2 optode foils, the calibrations were conducted using four concentrations between 0.01 and 17% CO_2 in water-saturated air, in accordance with the manufacturer's guidelines. CO_2 foils were calibrated in a closed chamber with a small inlet and outlet, and the flow-through gas stream

was controlled by a high-precision air pump (ADC, Hoddesdon, Hertfordshire, UK). The foil was exposed to each CO_2 concentration until the read-out stabilized (5–15 min). Calibrations were conducted at the same temperature as the actual measurements. Planar CO_2 optode foils are sensitive only for CO_2. The other components of the carbonic acid equilibrium (i.e. carbonate and bicarbonate) are not measured, but can be calculated if the pH and temperature are known. However, as *Lobelia* exclusively utilizes CO_2 as an inorganic carbon source (Wium-Andersen, 1971), the direct measurement of CO_2 is sufficient for the present study on the belowground CO_2 dynamics in the *Lobelia* rhizosphere.

Experimental design and spatial and temporal analysis

This study investigates the interaction between individual roots of *Lobelia* and their immediate environment. In order to investigate the spatial and temporal variation of O_2 and CO_2 around the roots of *Lobelia*, planar optode measurements were conducted on single roots of five individual plants ($n = 5$). Selected roots were manually placed on the sediment, lining up the front plate to allow for direct contact with the optode foil (Figs 1, Supporting Information S1). Optode measurements were conducted continuously for three 8-h periods, alternating between dark and light conditions (dark–light–dark).

The spatial extent of the effect exerted by the roots on the O_2 and CO_2 concentration in the surrounding sediment was demonstrated in optode images. The extent of the spatial variation was quantified by measurement of the pore-water concentration profiles of O_2 and CO_2. Concentration profiles were measured perpendicular to the roots at the location at which the root-affected zone was widest. Data were extracted from these profiles to account for the extent of the affected zones and the maximum and minimum concentrations of O_2 and CO_2 in the pore-water. The radii of the root-affected zones were measured in the profiles as the distance from the root surface to the point at which an effect on O_2 or CO_2 could no longer be detected. To account for the effect of light exposure of the leaves, the spatial variation was recorded at the end of the light phase and at the end of the second dark phase.

The temporal variation of the O_2 and CO_2 concentrations in the sediment surrounding the roots was measured continuously over time during alternating light and dark periods. After the sample had been prepared for analysis in the rhizobox, it was first exposed to an 8-h dark period (dark 0–8 h). This preparatory period was introduced in order to re-equilibrate the sediment condition after sample preparation, and to generate a starting point without any effects of light exposure. This period is not shown in the results. Subsequently, the samples were analyzed during subsequent dark (8–16 h)–light (16–24 h)–dark (24–32 h) periods.

Average O_2 and CO_2 concentrations were followed within a designated area around the roots, which was manually determined on the basis of the O_2 and CO_2 patterns in the respective optode images. The area was selected as the whole affected area, which was visually different from the bulk sediment in optode images. The area was selected from the optode image showing the largest area affected in the recorded time series. This method to measure temporal variation is a feature included in the VisiSens ScientifiCal 1.0 image processing software. In addition, for CO_2, there is a distinct area in the immediate vicinity of roots in which CO_2 uptake affects the CO_2 concentration patterns in the sediment surrounding the roots. The temporal variation of CO_2 within this area was also recorded as described above. To test for effects of illumination (light vs dark period) on the size and O_2 or CO_2 concentrations of the designated areas, paired *t*-tests with size and concentration values measured at the end of light vs dark periods were conducted.

Results

Spatial rhizosphere O_2 and CO_2 distribution

The spatial O_2 and CO_2 distribution around the roots of *Lobelia* under light and dark conditions is shown by example for one root in Fig. 2. Altogether, five roots of individual plants were investigated. The optode images of all replicates under light and dark conditions are shown in Fig. S1. Measures of the spatial distribution of O_2 and CO_2, as well as maximum and minimum concentrations, are given in Table 1. *Lobelia* roots affected the O_2 and CO_2 content of the sediment surrounding the roots, which is clearly shown in the optode images. Three distinct rhizosphere zones can be identified: the oxic root zone (O_2 release from the root to the sediment results in an oxygenated zone in the immediate vicinity of the root; Fig. 2a–d); the CO_2-enhanced zone (respiratory processes are stimulated by root presence, which is demonstrated by an enhanced CO_2 concentration in a wider area around the root; Fig 2e–h); and the CO_2 uptake zone (CO_2 uptake via the roots results in a CO_2-depleted zone in the immediate vicinity of the root inside the CO_2-enhanced zone; this zone is only present in light; Fig. 2e,g).

O_2 spatial distribution

Under both light and dark conditions, O_2 was released along the entire root of *Lobelia* into the sediment, resulting in a zone of oxidized sediment around the root, which is clearly shown in the optode images and cross-sectional profiles (Fig. 2a–d). O_2 concentrations declined from the root surface into the anoxic bulk sediment. The maximum O_2 concentration was measured at the root surface at the end of the light period, where it reached an average maximum concentration of $29.8 \pm 2.3\%$ O_2 atm. sat. (Table 1). At the end of the first dark period, O_2 was still present in the oxic root zone, but the maximum concentration was significantly lower in darkness than in light, reaching an average of $6.6 \pm 1.2\%$ O_2 atm. sat. ($P < 0.001$; Table 1). The oxic root zone extended further into the bulk anoxic sediment in the light than in the dark, with average radii of 3.1 ± 0.3 mm and 1.0 ± 0.4 mm under light and dark conditions, respectively ($P < 0.01$; Table 1). In one of the five plants investigated, no oxic root zone was detected in the dark (Fig. S1; LOB#1). This replicate was omitted from the measurements in Table 1.

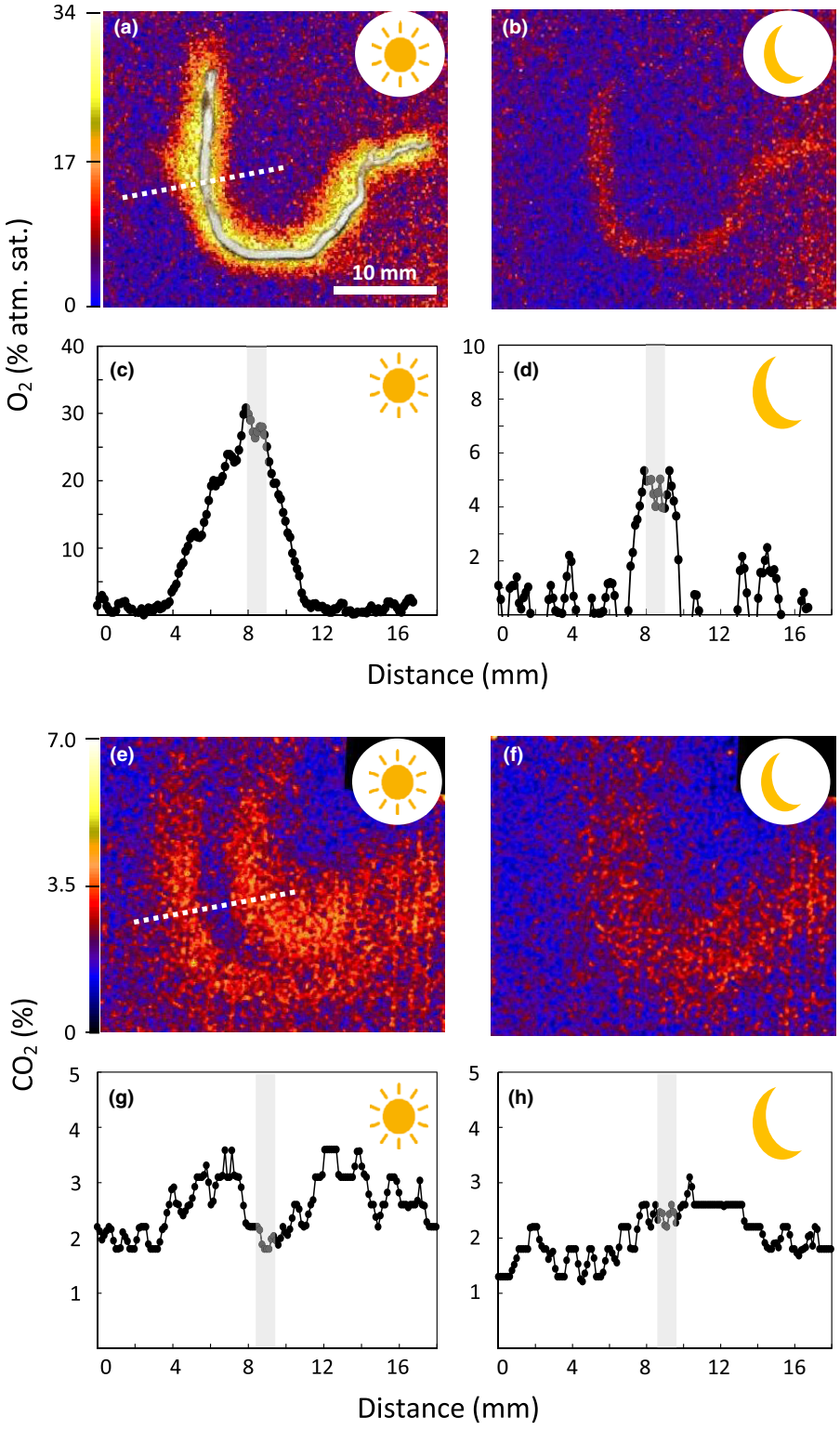

Fig. 2 Spatial variation in O_2 and CO_2 concentrations around a single root of *Lobelia dortmanna* (additional replicates are shown as Supporting Information Fig. S1). (a, b, e, f) Optode images of the spatial distribution of O_2 and CO_2 concentrations during light (left) and dark (right) conditions. The exact location of the root is shown in (a). (c, d, g, h) Cross-sectional concentration profiles of O_2 and CO_2 during light and dark conditions; gray bars indicate root position and width. The profile locations are shown as a dashed line in (a, e). O_2 is expressed as the percentage of O_2 saturation in freshwater at atmospheric equilibrium (% atm. sat.). CO_2 is measured as pCO_2 and is expressed as the percentage CO_2 at atmospheric pressure.

CO_2 spatial distribution

In the CO_2-enhanced zone, CO_2 accumulation occurred in light and partly also during the dark period. This resulted in CO_2 concentrations markedly higher than in the bulk sediment unaffected by root presence, which is clearly shown in the optode images and cross-sectional profiles (Figs 2e–h, S1).

The CO_2-enhanced zone had a maximum radius of 9.8 ± 3.6 mm (Table 1; observed in the second dark period). The maximum pCO_2 was measured to be $5.3 \pm 0.2\%$ CO_2 on the root surface, whereas the bulk sediment unaffected by root presence had an average pCO_2 of $2.0 \pm 0.3\%$ CO_2 (Table 1).

As the root initiated its CO_2 uptake in light, a CO_2-depleted zone appeared around the roots inside of the CO_2-enhanced

Table 1 Radius, maximum and minimum concentration of oxic root zones in light and dark, the CO_2 uptake zone (CO_2 is depleted in the immediate vicinity of the roots in light) and the CO_2-enhanced zone (covering a larger zone around roots in which CO_2 accumulates) in rhizospheres of *Lobelia dortmanna*

Oxygen	Radius (mm)	Max (% atm. sat.)	Min (% atm. sat.)
Oxic root zone light	3.1 ± 0.3	29.8 ± 2.3	0
Oxic root zone dark	1.0 ± 0.4	6.6 ± 1.2	0
Carbon dioxide	**Radius (mm)**	**Max (%)**	**Min (%)**
CO_2 uptake zone	2.0 ± 0.7	5.1 ± 0.3	2.5 ± 0.3
CO_2-enhanced zone	9.8 ± 3.6	5.3 ± 0.2	2.0 ± 0.3

The extent of zones was measured from cross-sectional profiles across the root at the widest position. Radius was measured from the root surface to the point in bulk sediment at which the measured effect (O_2 release, CO_2 uptake or CO_2 accumulation) becomes undetectable. In the oxic zone, concentration maxima were measured on the root surface and minima in the bulk anoxic sediment. In the CO_2 uptake zone (detectable in light only), concentration minima were measured at the root surface and maxima were measured in the bulk sediment in the vicinity of the root. In the CO_2-enhanced zone, maxima were measured in darkness at the root surface and the minima in the bulk sediment away from the root. Five roots of individual plants were investigated. In one of the five investigated plants, the CO_2 uptake zone (light) and oxic root zone (dark) were not detectable in the optode images. Thus, the data provided here refer to mean values and standard deviations of four replicates ($n = 4$). O_2 is expressed as the percentage of O_2 saturation in freshwater at atmospheric equilibrium (% atm. sat.). CO_2 is measured as pCO_2 and is expressed as percentage CO_2 at atmospheric pressure.

zone, which is clearly shown in the optode image and cross-sectional profile (Fig. 2e,g). This resulted in a local CO_2 minimum with an average pCO_2 of 2.5 ± 0.3% CO_2 located on the root surface, which is comparable with the concentration in the bulk sediment, which was 2.0 ± 0.3% CO_2. The CO_2 maximum during light had an average pCO_2 of 5.1 ± 0.3% CO_2 and was located at an average distance of 2.0 ± 0.7 mm from the root surface (Table 1). In one of the five plants investigated, no CO_2 uptake zone was detected in light (Fig. S1; LOB#1). This replicate was omitted from the measurements in Table 1. In darkness, the CO_2 uptake zone is not present and the CO_2 maximum is located at the root surface (Fig. 2f,h).

Temporal rhizosphere O_2 and CO_2 dynamics

O_2 dynamics: Over consecutive dark (8 h)–light (8 h)–dark (8 h) periods, the development of the O_2 concentration was followed within a predetermined area covering most of the oxic root zone (Fig. 3). At the entry of the first dark period (8–16 h), the O_2 concentration of the oxic root zone had reached a stable level, where the O_2 concentration was in the range of 2–7% atm. sat., which was low and yet distinguishable from the anoxic background (Figs 3, S1). One sample (LOB#1) responded differently during both dark phases by completely eliminating the oxic root zone within 1 h, leaving the root in anoxic conditions. However, in general, the oxic root zone persisted throughout the first dark period (8–16 h). During light exposure (16–24 h), O_2 release was immediately enhanced, and the oxic root zone expanded (Fig. 3; time-series images). The O_2 concentration of the oxic root zone increased continuously throughout the light period, before leveling off and approaching a steady state towards the end of the 8 h of light exposure (Fig. 3). During the subsequent dark period (24–32 h), the O_2 concentration declined continuously, leveling off and approaching a steady state with constant average O_2 concentration of *c.* 5–7% atm. sat. after 8 h of darkness. This level is similar to the level at the entry point to the first dark period (8–16 h), where the sample had been in darkness for 8 h.

CO_2 dynamics: Over consecutive dark (8 h)–light (8 h)–dark (8 h) periods, the development of the CO_2 concentration was followed around five roots of individual plants within two predetermined areas covering the CO_2-enhanced zone (Fig. 4a) and the CO_2 uptake zone (Fig. 4b). In the latter, one of the five plants showed no CO_2 uptake zone in light (Fig. S1; LOB#1); for clarity, this replicate was omitted from Fig. 4(b).

At the entry to the first dark period (8–16 h), the pCO_2 of the CO_2-enhanced zone had reached a stable level of 3.3–5.0% CO_2. Throughout the first dark period (8–16 h), the CO_2 concentration remained at this level or showed a tendency to decrease slightly (Fig. 4a). The concentration of the CO_2-enhanced zone varied strongly among samples, yet all replicates showed higher CO_2 concentrations around the roots than in the bulk sediment, and the CO_2-enhanced zone was clearly distinguishable in the optode images (Fig. 4; time-series images 1 + 2).

In light (16–24 h), the CO_2 concentration was immediately depleted in the CO_2 uptake zone, where it remained stable at a lower level throughout the light period (Fig. 4b). The CO_2 concentration in the CO_2 uptake zone was markedly lower than in the surrounding sediment, and the CO_2 uptake zone is clearly distinguishable in the optode images (Fig. 4; time-series images 3 + 4). Despite CO_2 uptake in the immediate vicinity of the roots throughout the light period (16–24 h), the CO_2 concentration continuously increased in the CO_2-enhanced zone in a larger zone around the roots (Fig. 4a). In the subsequent dark period (24–32 h), the CO_2 uptake zone immediately began to diminish and, within 2 h (Fig. 4b), it was no longer distinguishable inside the CO_2-enhanced zone (Fig. 4; time-series images 5 + 6). The CO_2 concentration in the enhanced zone continued to increase for 1–4 h in the dark, reaching a level of 3.6–6.3% CO_2. Subsequently, the CO_2 concentration remained at this level or showed a tendency to decrease slightly throughout the rest of the dark period (Fig. 4a).

Discussion

This study is the first to combine planar optode measurements of O_2 and CO_2 in rhizospheres. It demonstrates a tight coupling between photosynthetic processes in *Lobelia dortmanna* during light exposure of the leaves and the spatiotemporal dynamics of O_2, CO_2 and their interaction in the rhizosphere pore-water.

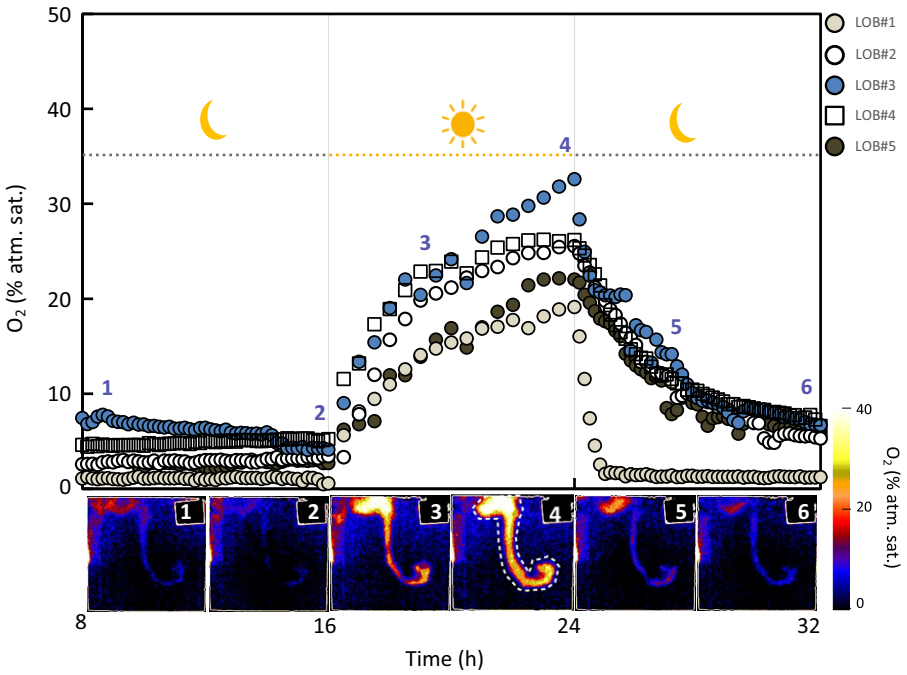

Fig. 3 Temporal variation in O_2 around single roots of *Lobelia dortmanna* shown for five individual plants during consecutive dark–light periods. Graph shows mean O_2 concentration (as percentage O_2 saturation at atmospheric equilibrium) of the oxic root zone over time (top). O_2 optode images show the spatial distribution of O_2 and development of the oxic root zone at selected time points 1–6 (bottom; example shown for replicate LOB#3). The O_2 concentration was measured in a designated area covering the largest extent of the oxic root zone, which was manually selected for each root in the optode image captured at the end of the light period (shown as a dashed line in optode image 4).

O_2 dynamics

In accordance with our first hypothesis, we demonstrate large fluctuations in plant-mediated sediment oxygenation between light and dark periods in the rhizosphere of *Lobelia*. O_2 release across the root surface was pronounced along the entire root in light, and O_2 contents in the resulting oxygenated root zone increased throughout the light phase. In darkness, the O_2 supply was significantly reduced, resulting in a drastic decline in the sediment O_2 content around the roots (Fig. 3). These findings support previous studies showing strong diurnal changes in O_2 release from the roots of *Lobelia* and other isoetids (Sand-Jensen *et al.*, 1982, 2005a,b; Pedersen *et al.*, 1995; Møller & Sand-Jensen, 2011; Ribaudo *et al.*, 2017).

Furthermore, we demonstrate that *Lobelia* is capable of maintaining oxic root zones around its roots even during prolonged dark periods without input from photosynthetic O_2 production. This O_2 release to the sediment in darkness has been observed previously in *Lobelia* (Sand-Jensen *et al.*, 1982; Risgaard-Petersen & Jensen, 1997). However, as roots and the microbial community will continue to respire O_2, the oxic root zone would be completely depleted of O_2 unless there was continuous supply of O_2 from aboveground sources. In the dark, photosynthetic O_2 production is excluded. Hence, the O_2 release either originates from exchange with the overlying water or from O_2 stored as a reservoir in the plant. Both the land and water forms of *Lobelia* have short stiff leaves, which lack stomata and are covered by a thick cuticle providing high resistance to gas exchange between the aboveground biomass and the water column (Pedersen & Sand-Jensen, 1992). Consequently, O_2 leaking out of the roots during dark periods most probably originates from stored O_2 resources. *Lobelia* possesses large air-filled lacunae between the leaf and root tips (Pedersen & Sand-Jensen, 1992), which may buffer the O_2 depletion in darkness. In support of this concept, Pedersen *et al.* (1995) found that *Lobelia* was capable of maintaining oxic root zones over an 8-h dark period, but not during extended dark periods of 24 h.

CO_2 dynamics and O_2–CO_2 interaction

In accordance with our second hypothesis, we demonstrate high spatial and temporal variability in the sediment CO_2 content of *Lobelia* rhizospheres as a result of enhanced CO_2 production facilitated by O_2 release, combined with root uptake of CO_2 for photosynthesis (Figs 3, 4). These two counterbalancing processes gave rise to a distinct pattern in the CO_2 profile in light, with a CO_2 minimum at the root surface and a CO_2 maximum on both sides of the roots (Table 1). In the dark, the profile was reversed, and the CO_2 maximum was located at the root surface. Overall, root-facilitated CO_2 production affected a larger zone than CO_2 uptake.

Following the CO_2 concentration in the CO_2 uptake zone during consecutive light and dark periods (Fig. 4b) clearly showed that CO_2 uptake is dependent on the light exposure of the leaves. CO_2 uptake was investigated on five roots of five individual plants (Fig. S1). Three of these roots clearly demonstrated that CO_2 uptake can occur along the entire length of the root. In one of the five replicates, however, CO_2 uptake was not visible. This may be the cause of the natural variation in morphology-determined gas-exchange ability of the root, or lower photosynthesis. In this regard, it is noteworthy that this root also showed the lowest O_2 concentration in the oxic root zone. Furthermore, a lack of a CO_2 uptake zone could be caused by damage to the root exerted during handling. However, as the root generated both an oxic zone and a CO_2-enhanced zone around the root, we find this to be the least likely explanation.

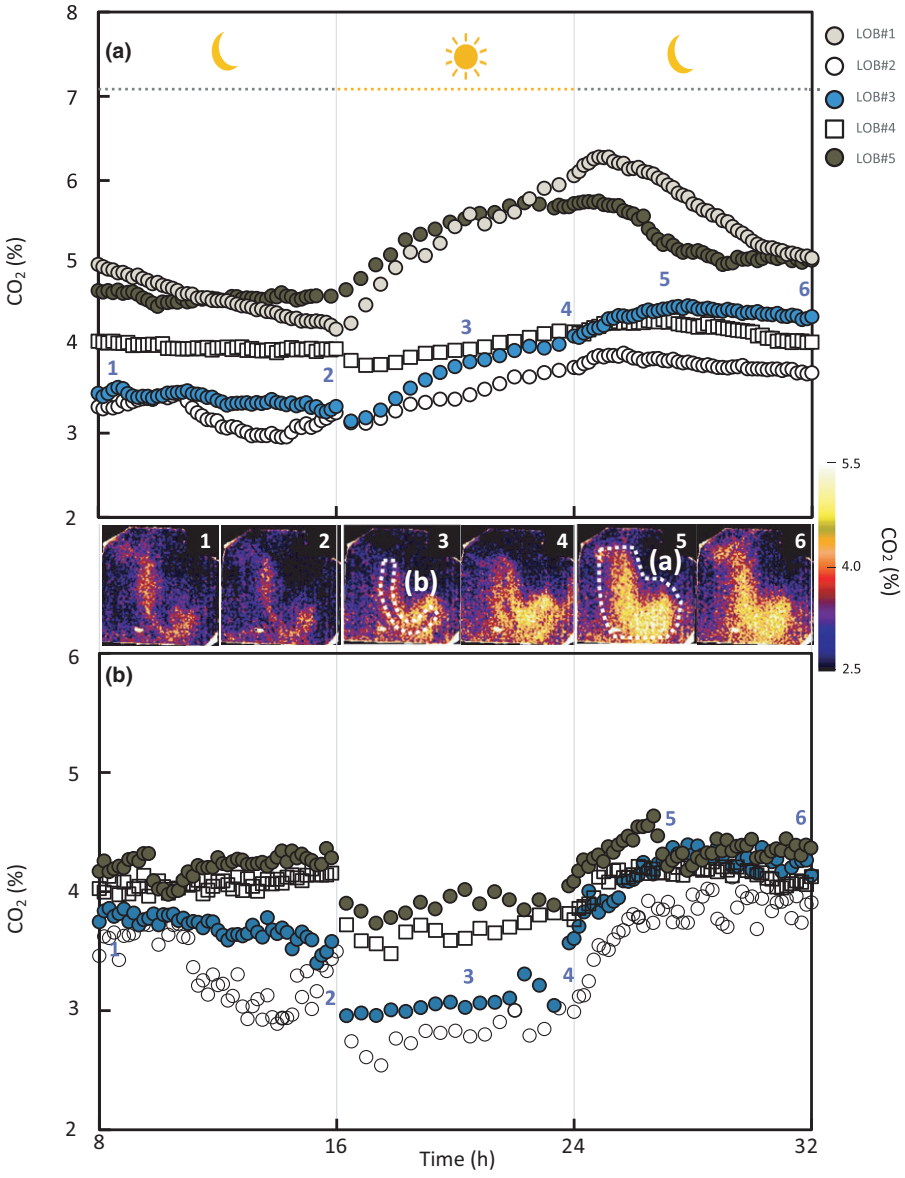

Fig. 4 Temporal variation of CO_2 around single roots of *Lobelia dortmanna* during consecutive dark–light periods. The series of CO_2 optode images shows the spatial distribution of CO_2 at selected time points (1–6; shown for replicate LOB#3). The top graph shows the average CO_2 concentration measured in the larger vicinity of the root (radius *c.* 1–2 cm), where CO_2 is enhanced as a result of increased respiration. The bottom graph shows the average CO_2 concentration measured in the immediate vicinity of the root (radius *c.* 0–0.5 cm), covering the area in which CO_2 is depleted as a result of root uptake (replicate LOB#1 was omitted as no CO_2 uptake was detected; Supporting Information Fig. S1). The designated areas were manually selected for each root in the optode image capturing the largest effect. The designated areas for measurement in (a) the CO_2-enhanced zone and (b) the CO_2 uptake zone are shown as dashed lines in optode images 5 and 3, respectively. CO_2 is measured as pCO_2 and is expressed as the percentage CO_2 at atmospheric pressure.

The CO_2 concentration in the CO_2-enhanced zone continued to increase beyond the light period and continued into the subsequent dark period, where the CO_2 concentration peaked after 1–4 h. We demonstrate a strong positive correlation between the O_2 concentration of the oxic root zone at the end of the light period and the time it takes for the CO_2 concentration to peak in the subsequent dark period (Fig. 5). This shows that the sustained CO_2 production beyond the light period relied on the reservoir of O_2 built up in the sediment and lacunae tissue during the light phase. As the stored O_2 supply was depleted, CO_2 production decreased, and the CO_2 concentration in the CO_2-enhanced zone reached its maximum (Fig. 4a).

Roots stimulate microbial activity in the sediment

Although CO_2 was taken up by the roots in light, the impact was restricted to a small area in the immediate vicinity of the roots and, in general, there was a net increase in the CO_2 concentration around the root throughout the light period. As CO_2 was taken up by the roots during the light period, but there was still a net increase in CO_2 observed around the roots, the CO_2 production resulting in this increase must be exclusively generated by the microbial community. Hence, we can hereby demonstrate that roots in *Lobelia* rhizospheres stimulate microbial respiratory processes. The high degree of sediment oxygenation was the primary cause of the stimulated microbial activity. As shown in Fig. 5, there is a close correlation between sediment oxygenation and CO_2 production. However, in addition to an O_2-induced stimulation of microbial activity, the release of root exudates as labile organic substrates could have contributed to the observed effect (Mueller *et al.*, 2016). Our observations of increased microbial activity are supported by Karjalainen *et al.* (2001), who demonstrated that *Lobelia* enhances microbial activity and biomass in the sediment through sediment oxygenation and excretion of dissolved organic carbon.

Cumulative effects

In this study, we focused primarily on single roots and their impact on the surrounding sediment through sediment oxygenation and CO_2 uptake. However, inside a natural *Lobelia* rhizosphere, with multiple closely located roots simultaneously exerting their impact on the sediment, the cumulative effect may be more pronounced than the effects of single roots. In this study, we recorded an optode image showing O_2 release from multiple roots (Fig. 1). In this image, the cumulative effect was clearly shown in the top part of the sediment, resulting in a higher and more uniform O_2 distribution compared with the bottom part of the sediment, where the roots were separated with less overlap between oxic root zones. It is possible that the higher O_2 concentration obtained by this cumulative effect can increase the O_2 concentration to a level at which it can importantly contribute to sustained night-time respiration of the belowground biomass, as suggested by Sand-Jensen *et al.* (2005b).

Moreover, the average CO_2 concentration in the rhizosphere can be affected in this way, as the roots are closely located and the CO_2 uptake zones overlap. Pedersen *et al.* (1995) measured CO_2 concentrations inside *Lobelia* rhizospheres and found that the concentration in pore-water decreased in light and increased in darkness. Our study reveals that the effect of root CO_2 uptake is restricted to the immediate vicinity of roots, and that, overall, CO_2 production is stimulated around the root. However, when multiple roots from each plant are located closely together and simultaneously exert their impacts on the sediment, it could result in a net uptake of CO_2 from the rhizosphere. Indeed, the study by Pedersen *et al.* (1995) reported that CO_2 uptake during light exposure was most pronounced in the top 3 cm of the sediment, corresponding to the depths with the highest root density.

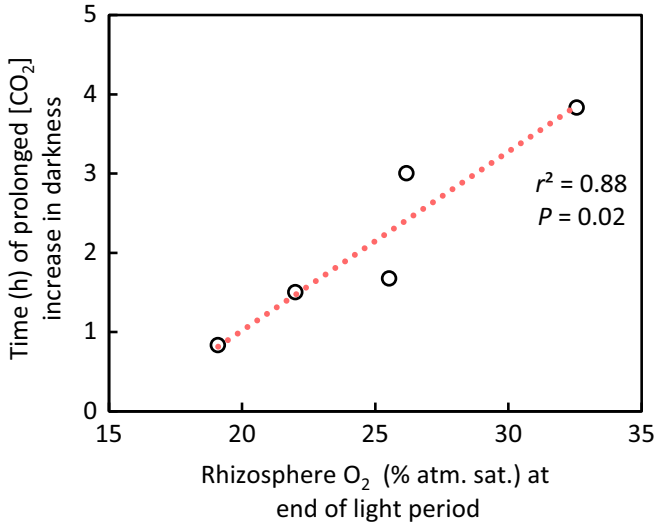

Fig. 5 Relationship between O_2 concentration in the oxic root zone at the end of the light period (at 24 h) and the duration (h) of prolonged CO_2 concentration increase in darkness around single roots of *Lobelia dortmanna* (compare Fig. 4a; $n = 5$).

Planar optodes – methodological considerations

Planar optode technology was used to visualize O_2 and CO_2 dynamics around individual roots. In the samples investigated, the O_2 concentrations were within a range of *c.* 0–35% atm. sat., and pCO_2 was within a range of 1–7% CO_2. The analytical performance of the optode foils was sufficient to capture the distinct concentration patterns around the individual roots for both analytes. However, in the anoxic parts of the sediment, the pixel values for O_2 range between *c.* +2 and −2 (% atm. sat.; Fig. 2). In the image processing of O_2 optode images, this range occasionally leads to a misleading color-coding of the bulk sediment, as the high values are colored as being different from zero, although the sediment is completely anoxic. The optode images were recorded with a spatial resolution of eight to nine pixels per millimeter, which proved to be sufficient to capture processes going on in the immediate vicinity of the roots on a submillimeter scale. At the same time, the foil size (4×4 cm^2) was large enough to capture the entire zones of O_2 and CO_2 accumulation around the roots.

For technical reasons, planar optode measurements in rhizospheres usually need to be conducted *ex situ* and involve the transplantation of plant samples into rhizoboxes for investigation. This may alter the rhizosphere conditions and processes relative to natural conditions in the field. For instance, the root-associated microbial community would temporarily be disturbed, as would the interaction of roots and mycorrhizal fungi (Moora *et al.*, 2016). This may affect the magnitude of rhizosphere oxygenation and CO_2 accumulation, causing a divergence between the experimental and natural conditions. However, our observation of the dynamics of CO_2 uptake, CO_2 accumulation and rhizosphere oxygenation corresponds well with findings of field studies or studies on undisturbed rhizospheres of *Lobelia* (Pedersen *et al.*, 1995; Sand-Jensen *et al.*, 2005a,b; Ribaudo *et al.*, 2017).

The planar optode system has individual foils for O_2 and CO_2, and, consequently, the foils must be exchanged between measurements, which requires the rhizobox to be drained and opened. This procedure must be performed with great care to ensure that the root remains in the same position. In this study, all measurements were conducted serially and the switching of optode foils between measurements occasionally resulted in slight changes to the root position (Fig. S1; LOB#4).

Planar optode measurements have a systematic drawback causing the extent of zones with increased levels of O_2 and CO_2 around the roots to be overestimated. That is, the side of the rhizobox against which the roots are placed (here the front plate) is an impermeable barrier, which restricts the dispersion of dissolved gases. This causes an enlargement of the zone of the gaseous analyte compared with normal conditions, as the gas builds up against the side of the rhizobox (Polerecky *et al.*, 2006). Despite this systematic drawback, the planar optode technique still provides a unique opportunity to visualize the spatial heterogeneity of the O_2 and CO_2 distribution inside natural sediments, capturing the net impact of the interaction between plant processes and microbial and chemical processes in the sediment.

Conclusion

Belowground sediment oxygenation is a mechanism by which aquatic and amphibious plants can improve nutrient uptake (Bradley & Morris, 1990; Lai *et al.*, 2012) and reduce exposure to phytotoxins, such as H_2S and reduced Fe(II) and Mn(II) species (Rozema *et al.*, 1985; Pezeshki, 2001; Lee, 2003). In this study, we have demonstrated a tight coupling between root presence and its O_2 release and the availability and uptake of CO_2 in the rhizosphere of *Lobelia dortmanna*. O_2-induced stimulation of microbial activity resulted in the accumulation of CO_2 around the roots, and root uptake of this inorganic carbon resource was demonstrated during light exposure of the leaves. Hence, for small freshwater plants, such as isoetids, which retrieve their inorganic carbon from the sediment, belowground oxygenation is also a mechanism that can increase the supply of sediment CO_2 to sustain the plant's photosynthetic requirements.

Acknowledgements

The work of Nikola Lenzewski was supported by the Landesamt für Landwirtschaft, Umwelt und ländliche Räume Schleswig-Holstein and the Untere Naturschutzbehörde Kreis Segeberg within the framework of the project 'Maßnahmen zur Förderung und Entwicklung der Strandlingsrasen in schleswig-holsteinischen Seen'. The work of Ketil Koop-Jakobsen was supported by MARUM – Center for Marine Environmental Sciences, and partly by Helmholtz Association, Contract HA-304, Robotics Explorations of Extreme Environments – ROBEX, for initial sensor optimization for aquatic research. Peter Mueller was funded by the Bauer-Hollmann Stiftung and the Rudolf und Helene Glaser Stiftung within the framework of the INTERFACE project.

Author contributions

N.L., P.M. and K.K-J. designed and conducted the study, analyzed the data and wrote the manuscript. K.J. was involved with the study design and commented on the manuscript. R.J.M. and G.L. provided methodological assistance and commented on the manuscript.

ORCID

Peter Mueller http://orcid.org/0000-0001-6974-5498
Ketil Koop-Jakobsen http://orcid.org/0000-0002-1540-6594

References

Askaer L, Elberling B, Glud RN, Kühl M, Lauritsen FR, Joensen HP. 2010. Soil heterogeneity effects on O_2 distribution and CH_4 emissions from wetlands: *in situ* and mesocosm studies with planar O_2 optodes and membrane inlet mass spectrometry. *Soil Biology and Biochemistry* **42**: 2254–2265.

Blossfeld S, Gansert D, Mancuso S. 2012. The use of planar optodes in root studies for quantitative imaging. In: Mancuso S, ed. *Measuring roots*. Berlin, Heidelberg, Germany: Springer, 83–92.

Blossfeld S, Schreiber CM, Liebsch G, Kuhn AJ, Hinsinger P. 2013. Quantitative imaging of rhizosphere pH and CO_2 dynamics with planar optodes. *Annals of Botany* **112**: 267–276.

Boston HL, Adams MS. 1983. Evidence of crassulacean acid metabolism in two North American isoetids. *Aquatic Botany* **15**: 381–386.

Boston HL, Adams MS, Pienkowski TP. 1987. Utilization of sediment CO_2 by selected North American isoetids. *Annals of Botany* **60**: 485–494.

Bradley PM, Morris JT. 1990. Influence of oxygen and sulfide concentration on nitrogen uptake kinetics in *Spartina alterniflora*. *Ecology* **71**: 282–287.

Farmer AM. 1989. *Lobelia dortmanna* L. *Journal of Ecology* **77**: 1161–1173.

Farmer AM, Spence DHN. 1987. Environmental control of the seasonal growth of the submerged aquatic macrophyte *Lobelia dortmanna* L. *New Phytologist* **106**: 289–299.

Frederiksen MS, Glud RN. 2006. Oxygen dynamics in the rhizosphere of *Zostera marina*: a two-dimensional planar optode study. *Limnology and Oceanography* **51**: 1072–1083.

Glud RN, Ramsing NB, Gundersen JK, Klimant I. 1996. Planar optrodes: a new tool for fine scale measurements of two-dimensional O_2 distribution in benthic communities. *Marine Ecology-Progress Series* **140**: 217–226.

Han C, Ren J, Tang H, Xu D, Xie X. 2016. Quantitative imaging of radial oxygen loss from *Valisneria spiralis* roots with a fluorescent planar optode. *Science of the Total Environment* **569–570**: 1232–1240.

Holst G, Kohls O, Klimant I, Konig B, Kuhl M, Richter T. 1998. A modular luminescence lifetime imaging system for mapping oxygen distribution in biological samples. *Sensors and Actuators B–Chemical* **51**: 163–170.

Jensen SI, Kuhl M, Glud RN, Jorgensen LB, Prieme A. 2005. Oxic microzones and radial oxygen loss from roots of *Zostera marina*. *Marine Ecology – Progress Series* **293**: 49–58.

Jovanovic Z, Pedersen M, Larsen M, Kristensen E, Glud RN. 2015. Rhizosphere O_2 dynamics in young *Zostera marina* and *Ruppia maritima*. *Marine Ecology Progress Series* **518**: 95–105.

Karjalainen H, Stefansdottir G, Tuominen L, Kairesalo T. 2001. Do submersed plants enhance microbial activity in sediment? *Aquatic Botany* **69**: 1–13.

Koop-Jakobsen K, Fischer J, Wenzhöfer F. 2017. Survey of sediment oxygenation in rhizospheres of the saltmarsh grass—*Spartina anglica*. *Science of the Total Environment* **589**: 191–199.

Koop-Jakobsen K, Wenzhöfer F. 2015. The dynamics of plant-mediated sediment oxygenation in *Spartina anglica* rhizospheres—a planar optode study. *Estuaries and Coasts* **38**: 951–963.

Kumari A, Gupta KJ 2017. VisiSens technique to measure internal oxygen and respiration in barley roots. In: Jagadis Gupta K, ed. *Plant respiration and internal oxygen: methods and protocols*. New York, NY, USA: Springer, 39–45.

Lai W-L, Zhang Y, Chen Z-H. 2012. Radial oxygen loss, photosynthesis, and nutrient removal of 35 wetland plants. *Ecological Engineering* **39**: 24–30.

Larsen M, Santner J, Oburger E, Wenzel WW, Glud RN. 2015. O_2 dynamics in the rhizosphere of young rice plants (*Oryza sativa* L.) as studied by planar optodes. *Plant and Soil* **390**: 279–292.

Lee RW. 2003. Physiological adaptations of the invasive cordgrass *Spartina anglica* to reducing sediments: rhizome metabolic gas fluxes and enhanced O_2 and H_2S transport. *Marine Biology* **143**: 9–15.

Maberly SC, Spence DHN. 1989. Photosynthesis and photorespiration in freshwater organisms: amphibious plants. *Aquatic Botany* **34**: 267–286.

Madsen TV. 1993. Inorganic carbon assimilation and growth of aquatic macrophytes. In: Jackson MB, Black CR, eds. *Interacting stresses on plants in a changing climate*. Berlin/Heidelberg, Germany: Springer, 267–285.

Madsen TV, Olesen B, Bagger J. 2002. Carbon acquisition and carbon dynamics by aquatic isoetids. *Aquatic Botany* **73**: 351–371.

Minett DA, Cook PLM, Kessler AJ, Cavagnaro TR. 2013. Root effects on the spatial and temporal dynamics of oxygen in sand-based laboratory-scale constructed biofilters. *Ecological Engineering* **58**: 414–422.

Møller CL, Sand-Jensen K. 2008. Iron plaques improve the oxygen supply to root meristems of the freshwater plant, *Lobelia dortmanna*. *New Phytologist* **179**: 848–856.

Møller CL, Sand-Jensen K. 2011. High sensitivity of *Lobelia dortmanna* to sediment oxygen depletion following organic enrichment. *New Phytologist* **190**: 320–331.

Moora M, Öpik M, Davison J, Jairus T, Vasar M, Zobel M, Eckstein RL. 2016. AM fungal communities inhabiting the roots of submerged aquatic plant *Lobelia dortmanna* are diverse and include a high proportion of novel taxa. *Mycorrhiza* **26**: 735–745.

Mueller P, Jensen K, Megonigal JP. 2016. Plants mediate soil organic matter decomposition in response to sea level rise. *Global Change Biology* **22**: 404–414.

Neumann G, George T, Plassard C. 2009. Strategies and methods for studying the rhizosphere—the plant science toolbox. *Plant and Soil* **321**: 431–456.

Pedersen O, Sand-Jensen K. 1992. Adaptations of submerged *Lobelia dortmanna* to aerial life form: morphology, carbon sources and oxygen dynamics. *Oikos* **65**: 89–96.

Pedersen O, Sand-Jensen K, Revsbech NP. 1995. Diel pulses of O_2 and CO_2 in sandy lake sediments inhabited by *Lobelia dortmanna*. *Ecology* **76**: 1536–1545.

Pezeshki SR. 2001. Wetland plant responses to soil flooding. *Environmental and Experimental Botany* **46**: 299–312.

Polerecky L, Volkenborn N, Stief P. 2006. High temporal resolution oxygen imaging in bioirrigated sediments. *Environmental Science & Technology* **40**: 5763–5769.

Raun ANEL, Borum J, Sand-Jensen KAJ. 2010. Influence of sediment organic enrichment and water alkalinity on growth of aquatic isoetid and elodeid plants. *Freshwater Biology* **55**: 1891–1904.

Raven JA, Handley LL, Macfarlane JJ, McInroy S, McKenzie L, Richards JH, Samuelsson G. 1988. The role of CO_2 uptake by roots and CAM in acquisition of inorganic C by plants of the isoetid life-form: a review, with new data on *Eriocaulon decangulare* L. *New Phytologist* **108**: 125–148.

Ribaudo C, Bertrin V, Jan G, Anschutz P, Abril G. 2017. Benthic production, respiration and methane oxidation in *Lobelia dortmanna* lawns. *Hydrobiologia* **784**: 21–34.

Richardson K, Griffiths H, Reed ML, Raven JA, Griffiths NM. 1984. Inorganic carbon assimilation in the Isoetids, *Isoetes lacustris* L. and *Lobelia dortmanna* L. *Oecologia* **61**: 115–121.

Risgaard-Petersen N, Jensen K. 1997. Nitrification and denitrification in the rhizosphere of the aquatic macrophyte *Lobelia dortmanna* L. *Limnology and Oceanography* **42**: 529–537.

Rozema J, Luppes E, Broekman R. 1985. Differential response of salt-marsh species to variation of iron and manganese. *Vegetatio* **62**: 293–301.

Sand-Jensen KAJ, Borum J, Binzer T. 2005a. Oxygen stress and reduced growth of *Lobelia dortmanna* in sandy lake sediments subject to organic enrichment. *Freshwater Biology* **50**: 1034–1048.

Sand-Jensen KAJ, Pedersen OLE, Binzer T, Borum J. 2005b. Contrasting oxygen dynamics in the freshwater isoetid *Lobelia dortmanna* and the marine seagrass *Zostera marina*. *Annals of Botany* **96**: 613–623.

Sand-Jensen KAJ, Prahl C. 1982. Oxygen exchange with the lacunae and across leaves and roots of the submerged vascular macrophyte, *Lobelia dortmanna* L. *New Phytologist* **91**: 103–120.

Sand-Jensen K, Prahl C, Stokholm H. 1982. Oxygen release from roots of submerged aquatic macrophytes. *Oikos* **38**: 349–354.

Santner J, Larsen M, Kreuzeder A, Glud RN. 2015. Two decades of chemical imaging of solutes in sediments and soils – a review. *Analytica Chimica Acta* **878**: 9–42.

Smolders AJP, Lucassen ECHET, Roelofs JGM. 2002. The isoetid environment: biogeochemistry and threats. *Aquatic Botany* **73**: 325–350.

Tschiersch H, Liebsch G, Borisjuk L, Stangelmayer A, Rolletschek H. 2012. An imaging method for oxygen distribution, respiration and photosynthesis at a microscopic level of resolution. *New Phytologist* **196**: 926–936.

Tschiersch H, Liebsch G, Stangelmayer A, Ljudmilla B, Rolletschek H. 2011. Planar oxygen sensors for noninvasive imaging in experimental biology. In: Minin I, ed. *Microsensors*. Rijeka, Croatia: Intechopen, 281–294.

Wang Xd, Meier RJ, Link M, Wolfbeis OS. 2010. Photographing oxygen distribution. *Angewandte Chemie International Edition* **49**: 4907–4909.

Winkel A, Borum J. 2009. Use of sediment CO_2 by submersed rooted plants. *Annals of Botany* **103**: 1015–1023.

Wium-Andersen S. 1971. Photosynthetic uptake of free CO_2, by the roots of *Lobelia dortmanna*. *Physiologia Plantarum* **25**: 245–248.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Optode images of CO_2 and O_2 distribution around single roots of *Lobelia dortmanna* after dark and light periods (n = 5).

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.