

OXYGEN EXCHANGE WITH THE LACUNAE AND ACROSS LEAVES AND ROOTS OF THE SUBMERGED VASCULAR MACROPHYTE, *LOBELIA DORTMANNI* L.*

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SUMMARY

The oxygen dynamics of *Lobelia dortmanna* were examined by continuous oxygen measurements in the lacunae and in the water around leaves and roots during light/dark cycles. *Lobelia* exchanges about six times more oxygen over the root than the leaf surface. The exchange rates per unit of surface area are two or three times higher across roots than leaves in accordance with a similar difference in permeability constants. The delay in reaching constant rates of exchange across roots is about 45 min after switching to dark and about 25 min after switching to light. This delay corresponds to the time necessary to change the oxygen concentration in the lacunae to a new steady state gradient with the water around roots. The oxygen pools in lacunae and root-water change at the same rate at equilibrium. During incubation in a closed system it is necessary to correct for changes in the lacunal oxygen pool when it is significant relative to the oxygen pool in the external water. The lacunae of *Lobelia* are an effective pathway for gas movements between leaves and sediment. The extensive lacunae, and the diffusional barrier for gases in *Lobelia*, should facilitate recycling of oxygen, and probably carbon dioxide, within the plant–sediment system.

INTRODUCTION

Submerged vascular macrophytes have a network of air chambers within their tissues (Arber, 1920; Sculthorpe, 1967). This lacunal system gives buoyancy to the plant and may serve as a gas reservoir and an effective pathway for gas movements within the plant (Sculthorpe, 1967; Wetzel, 1975). Hartman and Brown (1967) showed pronounced diurnal fluctuations of gas concentrations in the lacunae with peak values occurring prior to peak values in the surrounding water. They suggested that oxygen produced during photosynthesis accumulated in the lacunae and served as a respiratory oxygen pool during dark periods. However, they made no calculations to demonstrate quantitatively the importance of the changes in the oxygen pool in the lacunae relative to the metabolic rates and rates of oxygen exchange with the surrounding water. Westlake (1978) found only slight changes in the oxygen concentration in the lacunae during light/dark cycles and suggested that the results of Hartman and Brown (1967) were due to the unstirred experimental conditions, which increase the resistance to oxygen exchange with the surrounding water.

The lacunae are important as a pathway for gas movements, e.g. transfer of oxygen to rhizomes and roots to meet respiratory requirements or transfer of carbon dioxide from roots to leaves of isoetid species like *Lobelia dortmanna* L.

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(Wium-Andersen, 1971) and *Littorella uniflora* (L.) Aschers (Søndergaard and Sand-Jensen, 1979a, b). In many submerged vascular plants (Sand-Jensen, Prahl and Stokholm, 1982) and bog plants (Armstrong, 1964) the rate of transfer of oxygen to the below-ground parts exceeds their respiratory demand, resulting in a release of oxygen to the sediment.

The aim of the present study was to evaluate the storage and transport function of the lacunae and to determine how oxygen exchange with the lacunae affects calculations of metabolic rates made from changes of oxygen concentrations in the external water. Therefore, it was essential to measure continuously and simultaneously the oxygen exchange with both the lacunae and the surrounding water. All experiments were made with *L. dortmanna*. *Lobelia* has extensive lacunae and a high oxygen exchange across the roots, and thus a significant exchange of oxygen via the lacunal system.

MATERIALS AND METHODS

L. dortmanna was collected in Lake Almind, Denmark (Sand-Jensen and Søndergaard, 1981). The plants were rinsed in lake water and kept for not more than a week in aerated, filtered lake water (Whatman GF/C) in dim light (10^{19} quanta $m^{-2} s^{-1}$, 400 to 700 nm) at 10 °C. The epiphyte covering was removed with soft paper.

Lobelia exclusively uses free carbon dioxide for photosynthesis (Wium-Andersen, 1971). To ensure optimum carbon dioxide supply, the experimental water was enriched by bubbling with carbon dioxide to give 1 mM free CO₂ adjusted to pH 6·0.

Plant morphology

The surface area and volume of leaves and roots were determined microscopically by measuring the length and cross-sectional parameters. The roots have no root hairs. Lacunal volume was determined by measuring the diameters of the cylindrical lacunal channels (two in leaves, one in roots) at 1 cm intervals along the length of leaves and roots. Plant dry wt was measured after drying at 105 °C to constant weight.

Experiments with intact plants

The plants were mounted in two-chambered Perspex cylinders with the leaves in one chamber and the roots in the other (Wium-Andersen, 1971). The chambers were separated by a waterproof seal of Vaseline and cocoa butter around the plant stem. A two-channel peristaltic pump circulated the water from each plant chamber through independent probe chambers (see below). Each pump channel was equipped with two T-tubes, allowing switches from internal to external flow in order to shift in water of different oxygen concentrations. Oxygen diffusion through the seal, the chamber walls, and the tubing (butyl rubber) was negligible for the oxygen gradients used. Mixing of the water in the incubation system was complete in about 30 s (measured with injected dye).

The plant and probe chambers were submerged in a thermostatic water bath, and the oxygen exchange rates across leaf and root surfaces were measured during several light/dark cycles each of about 2 h duration. Two projector lamps (Philips 12 V, 75 W, type 6853) provided the light (8×10^{20} quanta $m^{-2} s^{-1}$, 400 to 700 nm).

For calculations, oxygen concentration is used instead of partial pressure in the lacunae and in the water. Temperature changes are small, oxygen and carbon dioxide exchanges are balanced under steady state and there was no release of bubbles from cut leaves and roots during active photosynthesis.

Oxygen measurements

The probe chambers [Fig. 1(a)] were made of Perspex. Water was pumped in through the side and out at the top. A fast-response Clark type oxygen electrode (Kier *et al.*, 1976) and an IC temperature transducer (Analog Devices AD 590) were mounted in the wall. A winged magnetic stirrer ensured high water velocities over the electrode membrane, thus making the electrode insensitive to changes in flowrate. Oxygen and temperature signals were amplified and recorded continuously on a four-channel recorder.

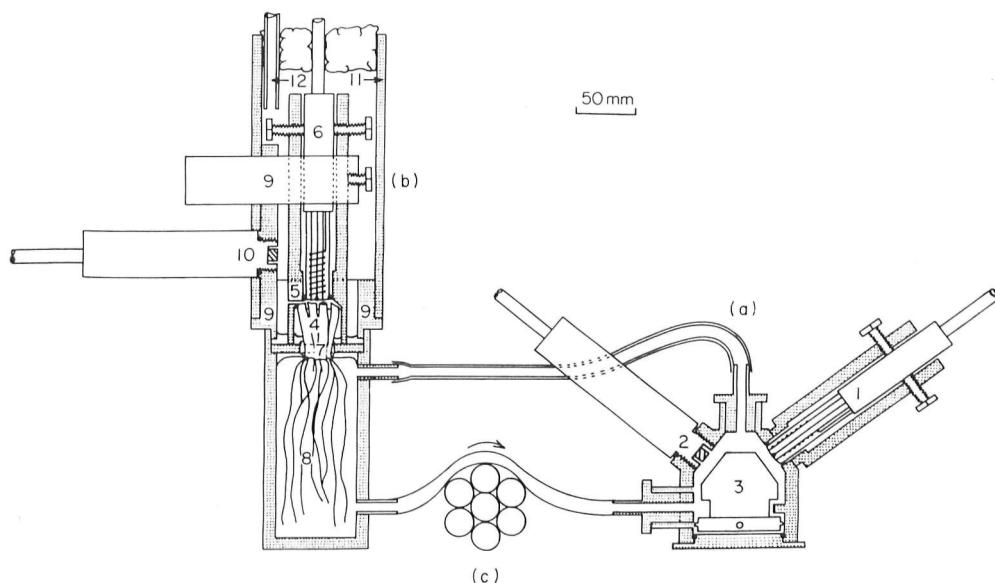


Fig. 1. (a) Probe chamber used in all experiments for measurement of oxygen and temperature in the leaf and/or root water. 1, Oxygen electrode; 2, temperature transducer; 3, disc-shaped magnetic stirrer with Perspex wing. 4, *Lobelia* with cut leaf tips; 5, electrode/leaf tube with sealed hole for outlet of air while mounting the electrode; 6, oxygen electrode; 7, waterproof seal; 8, root chamber; 9, Perspex supporting frame; 10, temperature transducer; 11, cooling jacket; 12, tube for cooling air. (c) Peristaltic pump (schematic).

The oxygen electrodes were calibrated by Winkler titrations, and signal changes caused by minor changes in temperature were corrected for, using the previously established relationship between probe signal and temperature: $E_1 = E_2 e^{0.048(t_1-t_2)}$ where E_1 and E_2 are the probe signals in the reference and measuring situations, respectively, and t_1 and t_2 are the associated temperatures.

Experiments with open lacunae

Leaves cut at the base, or root systems with the associated basal parts of cut leaves (0.5 cm), were mounted with a waterproof seal in a perforated plate, separating the two chamber compartments. Oxygen exchange with the water

around leaves or roots, was measured as described above, whereas the cut basal parts of leaves projecting into the other chamber were exposed to gas mixtures of different oxygen tensions. Oxygen concentration and temperature were measured with the usual probes working in the gas phase. The gas mixture was humidified by bubbling through gas wash bottles placed in the water bath before passing through the plant and probe chamber. The oxygen electrode working in air was calibrated against gas mixtures of different oxygen tensions and was perfectly linear within the range of measured oxygen tensions.

Oxygen measurements in the leaf lacunae

To obtain continuous and simultaneous measurements of oxygen in the leaf lacunae and in the water around the roots, the plants were mounted with a waterproof seal in the plant chamber [Fig. 1(b)]. The root chamber was filled with water, and oxygen and temperature were measured as usual. The leaf chamber was removed, and most leaves were cut to equal length near the tips to expose the lacunae. A cylindrical Perspex tube was pushed down over the leaves, and the space between leaves and tube wall was sealed with Vaseline and cocoa butter. To avoid gas exchange over the leaf surface, heavy castor oil was syringed into the Perspex tube until the oil surface was just below the open leaf lacunae. An oxygen probe was pushed through the tube from the top, leaving as small a dead-air space as possible (about 0.06 cm³ or 4 % of the lacunal volume) between the membrane and the leaf tips. A narrow hole in the tube wall allowed the air to escape while mounting the probe. This hole was finally sealed, and a Perspex supporting frame with a temperature transducer and a cooling jacket were placed over the electrode and leaf tube. Temperature changes during the experiments were minimized by pumping air, at the same temperature as the water bath, through the jacket.

RESULTS

Morphology of Lobelia

Lobelia is a rosette plant with stiff leaves, a short stem and cylindrical, unbranched roots. The lacunal system is well developed with two cylindrical lacunae in each leaf and one in each root. The outer diameter of a single root and the diameter of the lacuna are almost constant along the root. Apart from the youngest leaves, the air connection is continuous from the leaf lacunae to the root lacunae. The total dry wt of the leaves is in the same range as the total dry wt of roots and stem, but the total root surface area of the plant is higher than the leaf surface area (Table 1).

Oxygen exchange rates across leaf and root surfaces

The constant rates of oxygen exchange in the light were 4.8 to 7.8 times higher across the root surface than across the leaf surface, apart from plant 5, where the entire exchange was across the root surface (Table 2). The exchange rates per unit of surface area were 1.9 to 3.6 times higher across the roots than across the leaf surface in the light. The dark uptake rates were also much higher across the roots than the leaves. The net exchange rates in the light and dark were independent of the oxygen concentration within the range tested: 3 to 20 mg O₂ l⁻¹.

Figure 2 shows the time course of external oxygen concentrations following switches between dark and light. The response in the leaf-water phase was almost immediate, whereas it was much delayed in the root-water phase. This delay of

Table 1. Biometric and morphological characteristics of Lobelia

| Numbers per plant (range, $n =$ 7 plants) | Dry wt ratio: leaves/roots | | Surface area ratio: leaves/roots | | Mean length per plant (cm) (range, $n =$ 7 plants) | | Cross sectional area of lacunae (cm^2) (mean \pm s.d., $n =$ 7 plants) | |
|---|-------------------------------|-----------------|---------------------------------------|---------|--|-----------------|--|-----------------|
| | Leaves | Roots | (mean \pm s.d., $n =$ 11 plants) | Leaves | Roots | Total | per leaf | Total |
| 10-17 | 46-77 | 1.06 \pm 0.28 | 0.62 \pm 0.18 | 3.7-5.0 | 5.3-6.3 | 0.27 \pm 0.07 | 0.0197 \pm 0.0045 | 0.16 \pm 0.04 |

The mean specific surface areas were $0.48 \pm 0.04 \text{ cm}^2 \text{ mg}^{-1}$ leaf dry wt (\pm s.d., $n = 11$) and $1.08 \pm 0.10 \text{ cm}^2 \text{ mg}^{-1}$ root dry wt (\pm s.d., $n = 11$) and the mean outer diameter of roots was $0.073 \pm 0.004 \text{ cm}$ (\pm s.d., $n = 151$).

Table 2. Oxygen exchange rates with the external water phase around leaves and roots in the light and dark

| <i>Lobelia</i> | Light incubations | | | | | | Dark incubations | | | | | |
|-------------------------|---|-----------------|-----------------|--|------------------|------------------|---|------------------|------------------|--|--------|-------|
| | $\mu\text{g O}_2 \text{ mg}^{-1} \text{ plant dry wt h}^{-1}$ | | | $\mu\text{g O}_2 \text{ cm}^{-2} \text{ surface h}^{-1}$ | | | $\mu\text{g O}_2 \text{ mg}^{-1} \text{ plant dry wt h}^{-1}$ | | | $\mu\text{g O}_2 \text{ cm}^{-2} \text{ surface h}^{-1}$ | | |
| | Leaves | Roots | Leaves | Roots | Leaves | Roots | Leaves | Roots | Leaves | Roots | Leaves | Roots |
| 1 | 0.08 | 0.62 | 0.37 | 0.89 | 0 | -0.27 | 0 | -0.27 | 0 | 0 | -0.40 | -0.40 |
| 2 | 0.07 | 0.51 | 0.23 | 0.78 | -0.06 | -0.21 | -0.21 | -0.21 | -0.21 | -0.21 | -0.33 | -0.33 |
| 3 | 0.06 | 0.48 | 0.32 | 1.17 | -0.07 | -0.12 | -0.12 | -0.12 | -0.12 | -0.37 | -0.29 | -0.29 |
| 4 | 0.11 | 0.53 | 0.60 | 1.14 | -0.02 | -0.09 | -0.09 | -0.09 | -0.09 | -0.19 | -0.57 | -0.57 |
| 5 | 0 | 0.63 | 0 | 2.01 | 0 | -0.09 | -0.09 | -0.09 | -0.09 | 0 | -0.57 | -0.57 |
| Overall mean \pm s.d. | 0.064 \pm 0.04 | 0.55 \pm 0.05 | 0.30 \pm 0.21 | 1.20 \pm 0.48 | -0.03 \pm 0.03 | -0.16 \pm 0.08 | -0.13 \pm 0.16 | -0.13 \pm 0.16 | -0.13 \pm 0.16 | -0.36 \pm 0.14 | | |

Five plants were examined. The mean exchange rate for each plant was determined for similar oxygen concentrations in the leaf and root water phases.
Temperature: 10 °C.

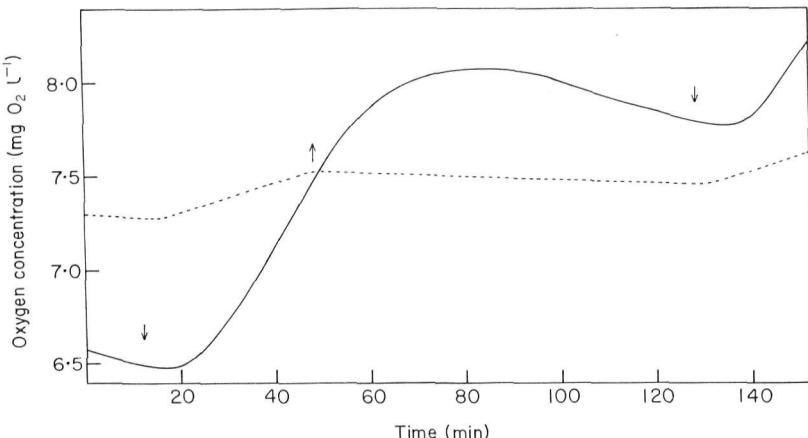


Fig. 2. The time courses of changes of oxygen concentration in the leaf (---) and root–water phase (—) during switches from light to dark (↑) and from dark to light (↓). Temperature: 10 °C.

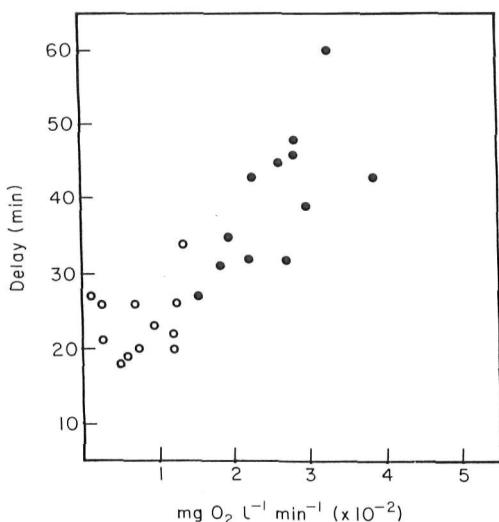


Fig. 3. The time delay to reach a new constant rate of exchange with the root water phase after switches to dark (●) or light (○) as a function of the constant rate of change in the root water concentration before the switch. Temperature: 10 °C.

oxygen changes in the root–water phase was about twice as long after a light to dark switch (40–45 min) than a dark to light switch (about 25 min). Figure 3 shows a positive linear relation between the constant rates of change in the root–water phase before a switch and the time delay to reach a new constant rate. The constant rates of change before and after a switch are in opposite directions.

When the oxygen concentration around the roots was suddenly changed by replacement of the water, the rates of change in the oxygen concentration also changed (Fig. 4). The replacement created a new gradient of oxygen between the root lacunae and the root–water phase, and the following exchanges served to level off this gradient, until the gradient before the replacement was re-established.

The results from Figure 4 and similar experiments can be used to estimate (i)

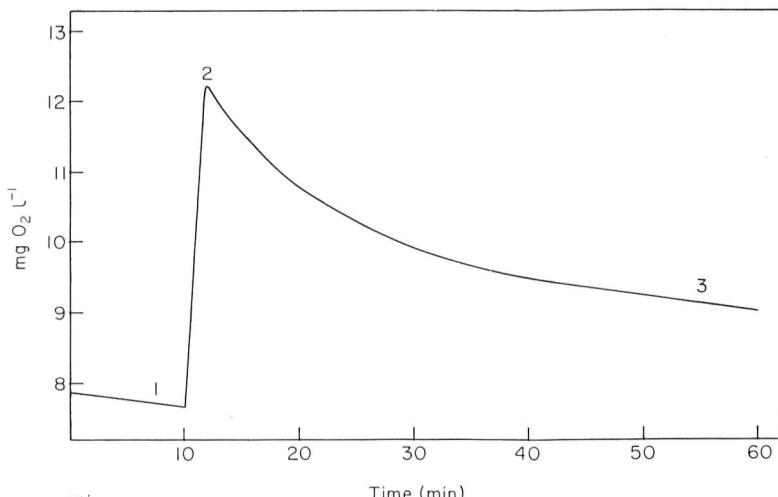


Fig. 4. The time course of change of oxygen concentration in the root water phase in the dark after a swift replacement of the root water. Index 1 and 3 show the periods of constant rates of exchange before and after the shift, respectively, and index 2 shows the time when the replacement is complete. Temperature: 10 °C.

the permeability constant of oxygen exchange across the root surface (K_r) and (ii) the lacunal volume (V'_L) with which the root–water concentration equilibrates. If we assume that the rate of exchange (the flux, F) follows Fick's 1st law and that the oxygen gradient along the length of the root lacunae is negligible, we have:

$$F = K_r(C_{L,r} - C_{w,r}), \quad (1)$$

where $C_{L,r}$ and $C_{w,r}$ are the oxygen concentrations in the root lacunae and in the root–water phase, respectively. Since the water replacement is rapid (about 30 s) we can assume that $C_{L,r}$ is the same immediately before (index 1) and after (index 2) the replacement (i.e. $C^1_{L,r} = C^2_{L,r}$) thus

$$F^1 = K_r(C^1_{L,r} - C^1_{w,r}) \text{ and } F^2 = K_r(C^2_{L,r} - C^2_{w,r}). \quad (2)$$

These equations combine to give equation (3), from which K_r can be calculated

$$K_r = \frac{F^1 - F^2}{C^1_{w,r} - C^2_{w,r}}. \quad (3)$$

The mean values and range of K_r for five plants were 22.0 (17.9 to 24.7) ng O₂ cm⁻² (root surface) min⁻¹ mg⁻¹ O₂ l concentration difference between the root lacunae and the root–water phase (Table 3).

The lacunal volume (V'_L), which equilibrates with the root–water, can be calculated from a mass balance for oxygen during the period from just after the replacement (t_2) until a new constant rate of exchange is obtained (t_3), i.e. the changes in the oxygen pool in the lacunae and in the root–water phase are equal to the metabolism (changes in the oxygen pool in the leaf–water phase are negligible). During periods of constant rates of exchange (at t_1 and t_3), the gradient is constant along the lacunae and between the lacunae and the root–water phase. We have:

$$V_{w,r}(C^3_{w,r} - C^2_{w,r}) + V'_Lf(C^3_L - C^1_L) = \Delta C_{w,r}(t_3 - t_2)(V_{w,r} + fV'_L). \quad (4)$$

Table 3. *The permeability constant for oxygen exchange across the root surface (K_r) estimated from the type of experiment of Figure 4 according to equation (3)*

| Plant | Mean K_r , with ranges in parentheses [ng O ₂ cm ⁻² (root surface) min ⁻¹ mg ⁻¹ O ₂ l] |
|--------------|---|
| 1 | 24.1 (17.1–29.4, n = 4) |
| 2 | 17.9 (15.2–20.5, n = 3) |
| 3 | 24.6 (21.5–28.5, n = 4) |
| 4 | 24.7 (17.6–30.8, n = 8) |
| 5 | 18.5 (17.0–21.1, n = 4) |
| Overall mean | 22.0 |

The unit of K_r is ng O₂ cm⁻² (root surface) min⁻¹ mg⁻¹ O₂ l concentration difference between the root lacunae and the root water phase. The oxygen concentrations in the root lacunae are converted to the equilibrium concentrations in water.

Table 4. *The lacunal volume (cm³) per plant calculated from the type of experiment shown in Figure 4 and from direct microscopic examination (further details see text)*

| Plant | Equilibration experiment | | | Microscopy |
|-------|--------------------------|-----------|---|------------|
| | Mean | Range | n | |
| 1 | 1.28 | 1.05–1.72 | 5 | 1.52 |
| 2 | 1.65 | 0.95–2.07 | 4 | 1.60 |

$V_{w,r}$ and V'_L are the volumes and $C_{w,r}$ and C_L are the oxygen concentrations in the root–water phase and in the lacunae respectively. $\Delta C_{w,r}$ is the constant rate of oxygen change in the root–water phase at t_1 and t_3 , and f is the ratio of oxygen solubility per unit volume of air relative to water. Since the rate of exchange at t_1 and t_3 is the same, then $F^1 = F^3 = K_r (C_L - C_{w,r}) = K_r (C_L^3 - C_{w,r}^3) \Rightarrow C_L^3 - C_L^1 = C_{w,r}^3 - C_{w,r}^1$, and equation (4) reduces to

$$V_{w,r} [(C_{w,r}^3 - C_{w,r}^1) - \Delta C_{w,r}(t_3 - t_2)] = V'_L f [(C_{w,r}^1 - C_{w,r}^3) + \Delta C_{w,r}(t_3 - t_2)] \quad (5)$$

The calculated values of V'_L correspond to the total lacunal volume (V_L) determined by microscopy (Table 4), so the major part of the lacunae equilibrates with the root water.

Due to the much slower rate of exchange across the leaves compared to the roots, this type of experiment was not successful for estimation of K_l (permeability constant across the leaf surface) and V'_L .

Oxygen exchange in plants with open leaf lacunae

The rate of oxygen exchange across the roots as a function of the difference between the oxygen concentrations of the air above the open leaf lacunae and the water in the root phase is shown in Figure 5. Since the system is not a true steady state system (i.e. the oxygen concentrations in the water around roots change), each point represents a simultaneous reading of the rate of exchange for a certain difference in oxygen concentration. The almost perfect linear relation, however, shows that the system behaves like a steady state system.

The linear relation also indicates that the difference in oxygen concentration

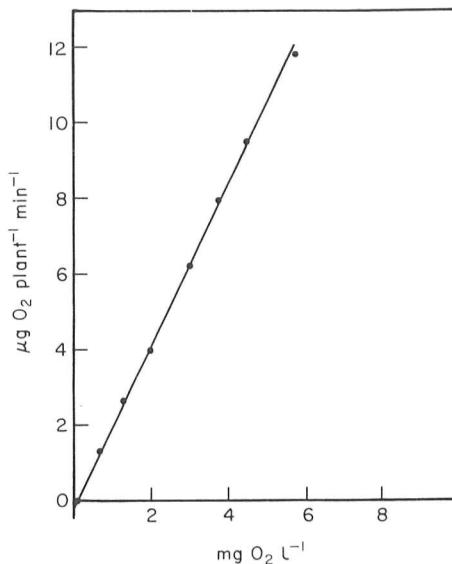


Fig. 5. The rate of oxygen exchange between the plant and the water around roots (y axis: $\mu\text{g O}_2 \text{ min}^{-1}$ per plant) as a function of the difference between the oxygen concentration in the air above the open leaf lacunae and in the water around roots (x axis: $\text{mg O}_2 \text{ l}^{-1}$). The oxygen concentration in air was converted to the equilibrium concentration in water at 17 °C. The regression line is: $y = -0.15 + 2.09x$ ($r = 0.999$). The slope of the regression line corresponds to $20.1 \text{ ng O}_2 \text{ cm}^{-2}$ (root surface) $\text{min}^{-1} \text{ mg}^{-1} \text{ O}_2 \text{ l}$. In a similar experiment with another plant the regression line was: $y = -0.13 + 1.34x$ ($r = 0.98$, $n = 14$) and the slope corresponded to $22.3 \text{ ng O}_2 \text{ cm}^{-2}$ (root surface) $\text{min}^{-1} \text{ mg}^{-1} \text{ O}_2 \text{ l}$.

along the root lacunae (or actually from the basal part of the open leaf lacunae to the root lacunae at the root tips) is small, relative to the difference in oxygen concentration across the root wall and the stationary surface film, to the bulk solution. (A simple calculation of the concentration difference in the root lacunae that is necessary to drive the net flux measured, will also show this.) That the oxygen gradient along the root lacunae in fact is very small is further supported by two sets of facts.

(1) A shift in the oxygen concentration in the air rapidly changes the rates of oxygen change in the root–water phase to a new quasi steady state in about 3 to 4 min (Fig. 6). The time course of oxygen concentration in the water after such a shift can be described by the following equation if we assume that C_L is constant in the lacunal system.

$$\ln \frac{C_{w,r}^0 - C_L}{C_{w,r}^t - C_L} = K_r t. \quad (6)$$

We neglect respiration which has a small effect in this case where the rate of exchange is high. $C_{w,r}^0$ and $C_{w,r}^t$ are the oxygen concentrations in the root–water phase at time zero and t respectively. Figure 7 shows that the data fit perfectly a linear regression of

$$\ln \frac{C_{w,r}^0 - C_L}{C_{w,r}^t - C_L}$$

versus the time with K_r as the slope. The assumption of the lacunal system as a totally mixed container with respect to oxygen is therefore supported. (A more complex description which includes respiration is omitted here for simplicity, since

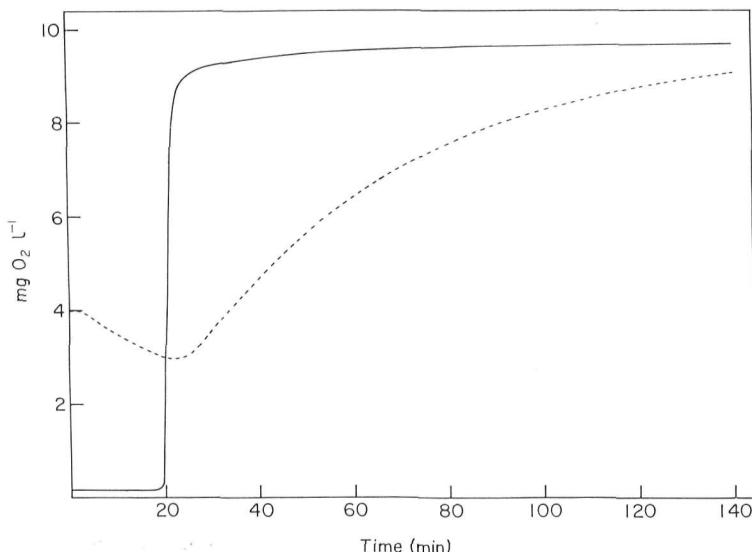


Fig. 6. The time courses of changes of oxygen concentration in the air above the open leaf lacunae (—) and in the root–water phase (---) before and after a change in oxygen concentration in the gas phase. The gaseous oxygen concentrations were converted to the equilibrium concentration in water at 17 °C.

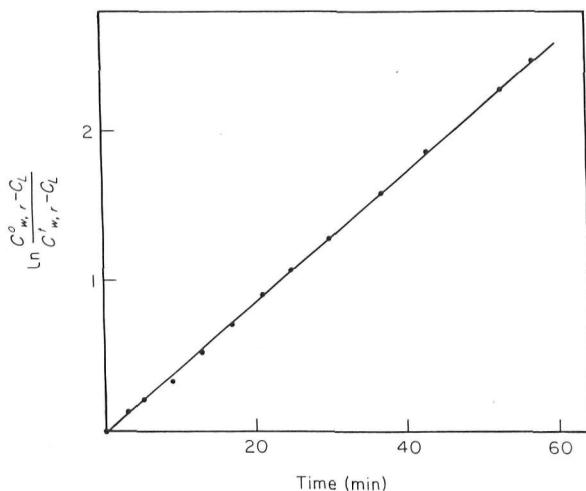


Fig. 7. The relation between

$$\ln \frac{C^{\circ}_{w,r} - C_L}{C'^{'}_{w,r} - C_L}$$

and time during the period 30 to 90 min in Fig. 6. The regression line was $y = -0.018 + 0.0436x$ ($r = 0.9998$). The slope (K_r) corresponds to $21.4 \text{ ng O}_2 \text{ cm}^{-2} (\text{root surface}) \text{ min}^{-1} \text{ mg}^{-1} \text{ O}_2 \text{ l}$.

it involves computer fitting of the respiration rate and of K_r . However, the results show a similar perfect fit, the same value of K_r is found and K_r is very insensitive to the respiration rate.)

(2) Jensen, Stolzy and Letey (1967) used a root tube model with a similar morphology to *Lobelia* to calculate the difference in oxygen concentration in the

root lacunae along the root relative to the difference in oxygen concentration between the lacunae at the upper end of the root and the root-water phase. They did not consider respiration. A calculation based upon *Lobelia* is shown in Table 5. The calculated difference in oxygen concentrations along the root lacunae is only 0·065 of the difference in oxygen concentrations from the upper end of the root lacunae and into the bulk solution around roots.

Table 5. The partial pressure of O_2 within a cylindrical root model at different distances along the root at steady state (according to Jensen et al., 1967)

$$\frac{p_z - p_{w,r}}{p_e - p_{w,r}} = \frac{\cosh \delta (L-z)}{\cosh \delta L},$$

where

p_e = the partial pressure of O_2 (mmHg) at the upper end of the root

p_z = the partial pressure of O_2 (mmHg) at a distance z from the upper end

$p_{w,r}$ = the partial pressure of O_2 (mmHg) in the bulk solution around roots

z = the distance (cm) from the upper end of the root

L = the length of the root (cm)

$\delta = (\beta K_r / \pi r^2 D)^{1/2} (cm^{-1})$

β = a constant $RT/M = 1\cdot9487 T \times 10^{-3}$ (mmHg $cm^3 \mu g^{-1}$)

K_r = the oxygen permeability of the root wall, including a stationary surface film of water ($\mu g mmHg^{-1} min^{-1} cm^{-1}$ of root length)

r = the radius of the root (cm)

D = the apparent diffusion coefficient of O_2 along the root ($cm^2 min^{-1}$)

With the morphology of *Lobelia* from Table 1 and with the value of K_r from Fig. 5 we have

$\beta = 0\cdot573 \text{ mmHg } cm^3 \mu g^{-1}$ (at $17^\circ C$)

$K_r = 20\cdot1 \text{ ng } O_2 \text{ cm}^{-2} \text{ min}^{-1} \text{ mg}^{-1} O_2 l = 0\cdot214 \text{ ng } O_2 \text{ min}^{-1} \text{ mmHg}^{-1} \text{ cm}^{-1}$ of root (at $17^\circ C$)

$D = 12\cdot2 \text{ cm}^2 \text{ min}^{-1}$ (oxygen diffusion in still air at $17^\circ C$)

$\bar{r} = 0\cdot028 \text{ cm}$ (mean inner radius of root lacunae)

$\bar{L} = 5\cdot8 \text{ cm}$ (mean length of roots)

$\delta = 0\cdot0639 \text{ cm}^{-1}$

$$\frac{p_L - p_{w,r}}{p_e - p_{w,r}} = 0\cdot935, \frac{p_e - p_L}{p_e - p_{w,r}} = 0\cdot065$$

With *Lobelia* the oxygen gradient along the short distance in the leaf lacunae (only basal part) to the root lacunae (about 0·5 cm) is negligible. Since respiration is not included in this model it is only applicable when the respiration effects are negligible, i.e. at high exchange rates.

The permeability constant for oxygen transport from the basal part of the leaf lacunae across the root wall and stationary surface film into the bulk solution is given by the slope of the regression line in Figure 5. This is a mean permeability constant, since we cannot detect any differences between the roots or along the length of the root. Since the oxygen gradient within the lacunae is small, this permeability constant is also close to K_r . The respiration does not affect the linearity of the relation, not even at the lowest exchange rates measured. K_r is $20\cdot1 \text{ ng } O_2 \text{ cm}^{-2}$ (root surface) $\text{min}^{-1} \text{ mg}^{-1} O_2 l$ in Figure 5 and almost the same in Figure 7 (21·4) with the same plant. Similar values of K_r were found in experiments with other plants (see legend to Fig. 5), and by the more simple experiments and calculations in Table 3.

The regression line in Figure 5 intersects the x -axis at $0\cdot07 \text{ mg } O_2 l^{-1}$. This is the concentration difference that is necessary to ensure a net flux of oxygen to the root tissues to cover their respiratory demand (at $17^\circ C$). If there was no respiration, the line should pass the origin.

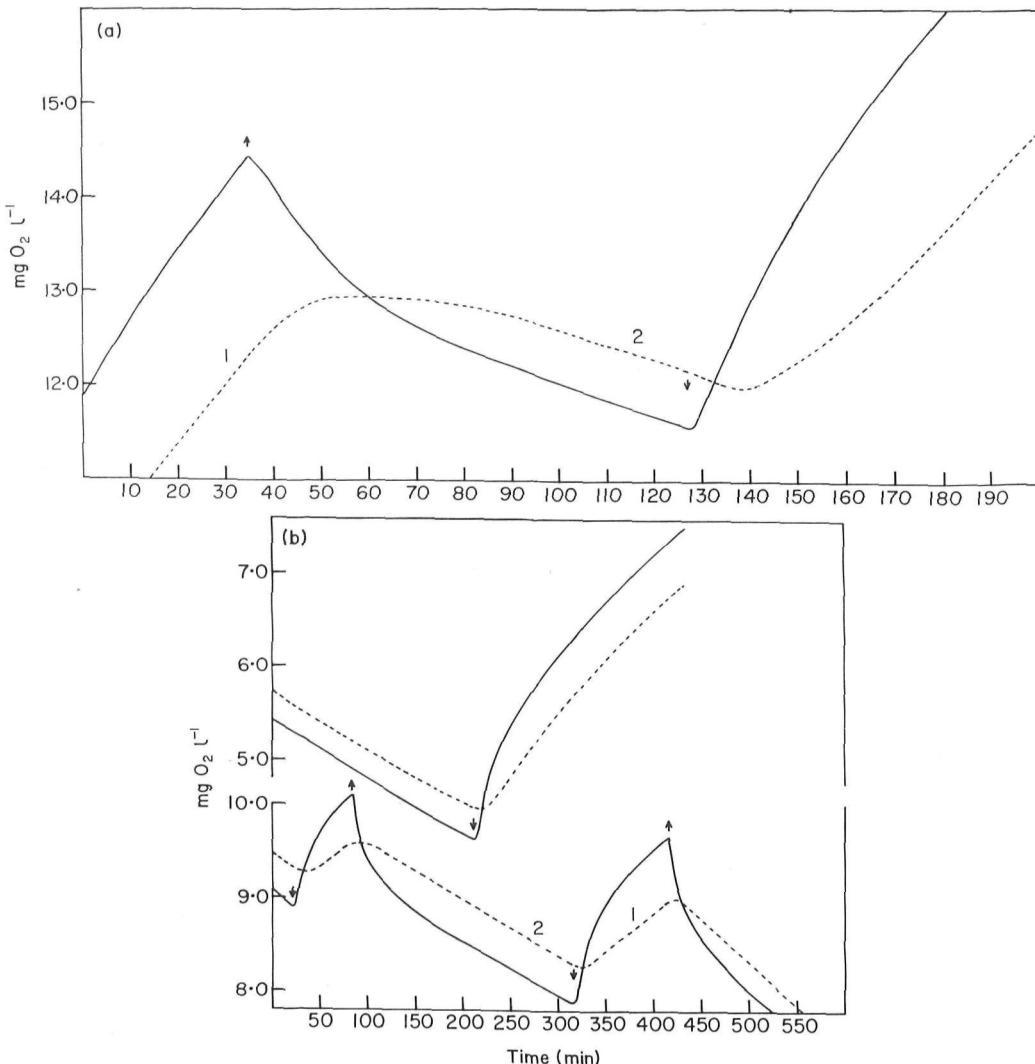


Fig. 8. The time courses of changes in oxygen concentration in the leaf lacunae (—) and in the root–water phase (---) during switches from light to dark (↑) and from dark to light (↓). The oxygen concentration in the leaf lacunae was converted to the equilibrium concentration in water at 17 °C.

When the lacunae in the basal part of the leaves were open and in contact with air, while the outer leaf surface was in contact with water, the experiments showed a similar pattern to Figures 5 and 7. However, the difference in oxygen concentrations between the air and the water at zero net flux was higher, 0·1 to 0·2 mg O₂ l⁻¹, and the slope of the regression line (K_l) was lower, 6·2 to 10·1 ng O₂ cm⁻² (leaf surface) min⁻¹ mg⁻¹ O₂ l ($n = 4$).

Continuous measurements of oxygen concentrations in the leaf lacunae and in the root water phase

During prolonged light or dark periods the oxygen concentrations in the leaf lacunae and in the root–water phase changed linearly and in parallel [Fig. 8(a), (b)].

The oxygen concentration in the leaf lacunae was higher than in the root–water phase during the light period and lower in the dark. These differences in oxygen concentrations between the two compartments created a net flux of oxygen downward into the root–water phase in the light and upward into the lacunae in the dark. The constant rates of oxygen exchange were close to those predicted from the regression line in Figure 5 for the same oxygen concentration differences: Fig. 8(a)1 38·9 ng O₂ cm⁻² (root surface) min⁻¹ (predicted: 40·8 for 2·1 mg O₂ l⁻¹); Fig. 8(a)2 minus 11·1 (predicted minus 12·7 for minus 0·56 mg O₂ l⁻¹), Fig. 8(b)1 8·9 (predicted 11·8 for 0·66 mg O₂ l⁻¹) and Fig. 8(b)2 minus 5·1 (predicted minus 9·8 for minus 0·42 mg O₂ l⁻¹).

After switches to light or dark the effect on the oxygen concentration was almost immediate in the leaf lacunae, whereas it was much delayed in the root–water phase [Fig. 8(a), (b)]. The response in the root–water phase was identical to the results shown in Figure 2. After a switch from light to dark the decrease in the lacunal oxygen concentration was slow during the first 2 to 4 min, probably due to a reversal of the oxygen concentration gradient between the chloroplasts and the leaf lacunae. After about 4 min the decrease was more rapid, and then levelled off gradually to a constant linear decrease after about 45 min. This pattern was due to the loss of oxygen from the lacunae and into the root–water phase which gradually became zero as the concentration difference diminished. A mass balance on the changes in the oxygen pools in the lacunae and in the root–water phase from the time of switch until a new linear decrease of oxygen concentrations was reached corresponded exactly to the expected respiratory oxygen consumption during that period (Table 6).

Table 6. *An oxygen mass balance during the period from the switch from light to dark [time 35 min, Fig. 8(a)] until the state of linear oxygen concentration changes [time 80 min, Fig. 8(a)]*

| Phase | Volume (cm ³) | Oxygen changes ($\mu\text{g O}_2$) | Oxygen consumption ($\mu\text{g O}_2$) |
|------------|------------------------------|---|---|
| Root water | 51·0 | 29·6 | -35·0 |
| Lacunae | 1·81 | -106·5 | -41·5 |
| Total | | -76·9 | -76·5 |

The changes of the oxygen pools in the lacunae and the root–water are calculated from the changes in oxygen concentrations, the volumes and the ratio of oxygen solubility in air relative to water. (29·3 at equilibrium at 17 °C and standard pressure.) The oxygen consumption is calculated from the rate of linear oxygen concentration changes [beyond time 80, Fig. 8(a)]. Changes in the oxygen pool in the tissues are negligible (Table 7).

A switch from dark to light created a complementary pattern of the above, except that linear changes in oxygen concentrations were reached more rapidly as already described in Figure 2.

Due to the substantial oxygen pool in the lacunae relative to the external medium, a calculation of the metabolic rate from oxygen changes in the external medium would lead to a serious underestimation. During the periods of constant rates of change in the oxygen concentrations in the lacunae and in the root–water phase in Figure 8(a), it is possible to calculate the rates of change in the oxygen pools from the concentration changes, the volumes, and the solubility of oxygen in air relative to water (Table 7). In this example the changes in the oxygen pools

in the lacunae and in the root-water (51 to 55 % and 43 to 47 % of the total change respectively) are about the same, whereas the changes in the oxygen pool in the tissues are negligible (about 2 % of the total).

Since the linear changes of oxygen concentrations in the lacunae and in the root-water are parallel, it is also possible to calculate the true metabolic rate of the plant (M_c) in experiments where only the rates of oxygen change are registered

Table 7. *Changes in the oxygen pool in the water, in the lacunae, and in the tissues of Lobelia in the light and dark during linear changes in the oxygen concentrations [calculated from the experiment in Fig. 8(a)]*

| Phase | Volume (cm ³) | Oxygen changes (μg O ₂ min ⁻¹ and % of total) | |
|---------|------------------------------|---|---------------|
| | | Light | Dark |
| Water | 51.0 | 2.45 (42.8 %) | 0.86 (47.2 %) |
| Lacunae | 1.81 | 3.16 (55.3 %) | 0.92 (50.6 %) |
| Tissues | 2.37 | 0.11 (1.9 %) | 0.04 (2.2 %) |

It is assumed that the oxygen concentration in the tissue changes at the same rate as in the lacunae and in the external water and that the solubility of oxygen is the same as in water.

in the root-water. To include the small changes in the oxygen pool in the tissues we assume that: (1) the solubility of oxygen in the tissues is the same as in the external medium and (2) the changes of oxygen concentrations are parallel to those in the external water (see Table 7). Then:

$$M_c = \Delta C_{w,r} (V_{w,r} + V_{tis} + V_L f), \quad (7)$$

where V_{tis} , V_w and V_L are the volumes of tissue water, external water and lacunal air respectively.

During oxygen diffusion from the leaf lacunae to the root-water phase the major resistance is the root wall, including the stationary surface film. Figures 5 and 7 show that the respiration effect on the calculation of the permeability constant (K_r) was small. Setting the coefficient of oxygen diffusion across the root wall equal to that in water we can calculate the thickness (s) of the root wall plus the stagnant surface liquid film. In cylindrical coordinates we have (see Letey and Stolzy, 1964):

$$F = \frac{D(C_{L,r} - C_{w,r})}{r_L [\ln(r_L + s) - \ln r_L]}, \quad (8)$$

$$s = r_L \left[\exp \left(\frac{D}{r_L + K_r} \right) - 1 \right], \quad (9)$$

where r_L is the radius of the root lacunae, 0.028 cm; D is the coefficient of oxygen diffusion in water, 0.12 mm² min⁻¹. K_r is the permeability constant, 20.1 ng cm⁻² (outer root surface) mg⁻¹ O₂ l or 26.2 ng O₂ cm⁻² (inner lacunae surface) mg⁻¹ O₂ l.

This calculation shows a thickness of the stationary surface film of 0.107 cm (the thickness of the root wall, 0.0087 cm is subtracted). Therefore, with these assumptions the major resistance to oxygen diffusion should be liquid film diffusion. From the thickness of the surface film it is possible to calculate the concentration difference across the film for a certain flux. For instance, for the dark exchange of minus 11.1 ng O₂ cm⁻² min⁻¹ in Figure 8(a) the concentration

difference calculated from equation 8 will be $0.42 \text{ mg O}_2 \text{ l}^{-1}$. This value is close to the concentration difference of $0.56 \text{ mg O}_2 \text{ l}^{-1}$ measured between the lacunae and the bulk solution. Therefore, the oxygen concentrations at the inner and outer surface of the root wall are probably very close in the dark during periods of linear changes in the oxygen concentrations.

DISCUSSION

Lobelia is exceptional, since it is the only plant investigated that exchanges most oxygen with the external medium over the root surface (Table 2). Some bog plants (Armstrong, 1964) and submerged vascular plants (Sand-Jensen *et al.*, 1982) also release oxygen from the roots, but only a minor fraction of the amount released from the leaves in the light. The major root exchange of *Lobelia* is due to the small resistance to downward diffusion in the lacunae from leaves to roots followed by passage over an extensive root surface through a thin root wall. The permeability constant for oxygen exchange per unit of surface area was two or three times higher across the roots than the leaves. This difference corresponds to the 1.9 to 3.6 times higher release of oxygen per unit of surface area from roots than leaves in the light (Table 2).

Oxygen is produced in cells surrounding the leaf lacunae, so the resistance to oxygen diffusion towards the lacunae is very small relative to the resistance towards the leaf surface. *Lobelia* has a prominent cuticle on the leaf surface, which is probably responsible for the high resistance to oxygen diffusion. The cuticle and especially the cuticular waxes are known to be very resistant to water diffusion (Schönherr, 1976) and the same applies to gases (i.e. oxygen) diffusing in the water phase (A. Melzer, pers. comm., 1981).

The permeability constant for oxygen release by roots of *Lobelia* is high [$0.214 \text{ ng O}_2 \text{ min}^{-1} \text{ cm}^{-1}$ (of root) mmHg^{-1} calculated from Fig. 5] when compared to estimated values for terrestrial crop plants (Jensen *et al.*, 1967): *Zea Mays L.* (0.147 to 0.184), *Hordeum vulgare L.* (0.076 to 0.102) and *Oryza sativa L.* (0.014 to 0.021). For terrestrial crop plants growing in waterlogged soils (e.g. *Oryza*) the oxygen supply to the root system will be optimal when the resistance to downward diffusion is low and the resistance to oxygen release to the sediment is high. A high resistance to gas diffusion along the roots and across the root surface of *Lobelia*, on the other hand, would impede uptake of sediment carbon dioxide and increase accumulation of lacunal oxygen in the light.

The major resistance to oxygen diffusion towards the root–water phase is the root wall and the surrounding stationary surface film (Table 5). Assuming a diffusion coefficient through the root wall like that in water it was calculated that the resistance was mainly confined to a surface film about 0.1 cm thick. Therefore, the permeability constants determined will probably be especially sensitive to reductions in the surface film thickness achieved by increased velocity and turbulence in the root–water phase. Westlake (1967) demonstrated that the rate of oxygen exchange by *Ranunculus pseudofluitans* (Syme) Baker was highly dependent on the flow rate, presumably via its effect on the thickness of the surface film.

Measurements of the time course of oxygen exchange after rapid changes of the external oxygen concentrations were successfully used for calculations of permeability constants and lacunal volumes (Figs 4, 6 and 7). These methods should be considered in future work. For instance, the lacunal volume and its eventual

light/dark dependent changes are difficult to determine by the conventional destructive methods.

The long delay of oxygen exchange to the root–water phase following a light or dark switch (Figs 2 and 8) can be due to a physiological (Ried, 1968; Walker, 1973; Dromgoole, 1979) and/or a physical delay (diffusion equilibration). Several facts show that the physiological delay is of minor importance. (i) The oxygen concentration in the leaf lacunae and in the leaf water phase responded immediately upon switches indicating that photosynthetic and respiratory activities were not delayed. (ii) The time delay was not affected by repeated light/dark cycles of variable duration, which could be expected if the physiological delay was important (i.e. Dromgoole, 1979). (iii) The changes in the oxygen pool in the lacunae and in the root water phase during the delay exactly balanced the expected metabolic activities (Table 6). Therefore, the pronounced time delay is mainly due to diffusion equilibration of oxygen concentrations in the extensive lacunae and in the root–water phase. Since the change in the lacunal oxygen concentration that is needed to reach a new equilibrium is about the same from light to dark as from dark to light (Fig. 8), it is reached more rapidly in the light where the metabolic activity is higher (Table 2). Accordingly, the delay also increases when the rate of exchange before the switch is higher (Fig. 3). A high rate of exchange is due to a large difference in oxygen concentrations between the lacunae and the root water phase (Fig. 8), and it takes a longer time to reverse the concentration difference to reach a new steady state. The time delay for leaf exchange is much shorter for other submerged vascular macrophytes under stirred conditions (2 to 20 min, Kelly, Moeslund and Thyssen, 1981) due to smaller lacunal volumes, higher metabolic rates and different resistance to oxygen exchange within the plant and with the external water. A longer time delay after switches from light to dark than from dark to light was found by Kelly *et al.* (1981), probably for the same reason as for *Lobelia*, although no explanation was presented.

Measurements of oxygen changes in the external medium in a closed system lead to serious underestimates of metabolic rates of vascular plants when the oxygen capacity in tissues or lacunae is significant, relative to that in the external medium (Table 7). If the three oxygen pools change at the same relative rate, which is probably so under well-stirred conditions after a certain time delay (e.g. Fig. 8), the true metabolic rate can be calculated from equation 7. These corrections are avoided in true steady state experiments (e.g. flow-through systems), where oxygen concentrations are constant in all three phases except in connection with changes in external variables. True steady state experiments are generally preferable, but they are technically more difficult and often not so sensitive as batch experiments. For many purposes batch experiments with vascular plants are satisfactory, but continuous registration of the external oxygen concentration during incubation is needed if special precautions are not taken (e.g. Sand-Jensen, 1978).

The oxygen concentration in the lacunae of *Lobelia* is the result of a dynamic equilibrium with oxygen concentrations around leaves and especially roots. The function of the lacunae as potential gas reservoirs must therefore be evaluated in relation to the dissolved gas pool, especially in the sediment. If we assume that the present experiments approximate the oxygen exchange conditions in the field, simple calculations show that diurnal changes in the oxygen concentration in the rhizosphere are likely as a consequence of high rates of oxygen exchange from roots and low oxygen consumption rates in the sediment. Preliminary measurements have shown high oxygen concentrations in the rhizosphere in Danish *Lobelia*

populations whereas no oxygen was present in the sediment outside the populations. *Lobelia* populations in Lake Stejlholt, Denmark had a biomass of about 90 g dry wt m⁻², the oxygen consumption by the sediment (plant free) from the overlaying water was only 25 to 30 mg O₂ m⁻² h⁻¹ at 15 °C in the dark, and the porosity of the sediment was about 50 % (Sand-Jensen, unpublished). A rhizosphere to 15 cm depth (Wium-Andersen and Andersen, 1972a) contains about 75 l interstitial water m⁻², and the lacunal volume in the plants is about 0·9 l m⁻² (calculated from Table 1). The net production of oxygen by *Lobelia* is about double the oxygen release from roots (Table 7), i.e. about 1·1 mg O₂ g⁻¹ plant dry wt h⁻¹ at 10 °C (Table 2). If we apply this figure to field conditions, the net production of oxygen will be about 100 mg O₂ m⁻² h⁻¹. If 30 mg O₂ m⁻² h⁻¹ is consumed and lost by diffusion in the sediment, the net increase in the lacunal and rhizospheric oxygen pools during steady state at 10 °C is 17 and 53 mg O₂ m⁻² h⁻¹ respectively. The latter value corresponds to an increase of the oxygen concentration in the interstitial water of 0·7 mg O₂ l⁻¹ h⁻¹.

The oxygen release by roots of *Lobelia* ensures effective aerobic mineralization of the organic matter in the sediment and prevents reducing substances (e.g. hydrogen sulphide and ferrous ions) from entering the plant. However, the very high potential of oxygen release by roots of *Lobelia* does not seem necessary considering the low reducing capacity in the sandy sediments which are the preferred substrates (Sand-Jensen and Søndergaard, 1979). The facility of oxygen exchange by the roots is more likely a consequence of an adaptation to ensure optimum carbon dioxide supply (Sand-Jensen and Søndergaard, 1979; Søndergaard and Sand-Jensen, 1979a, b). No direct measurements of the resistance to carbon dioxide diffusion in *Lobelia* are available, but experiments by Wium-Andersen (1971) indicate that like oxygen the permeability constant for carbon dioxide exchange is higher across the roots. The concentration of carbon dioxide is only about 0·01 mm in the lake water and 1 to 4 mm in the interstitial water in the sediment of Danish *Lobelia* lakes (Wium-Andersen and Andersen, 1972b; Sand-Jensen and Søndergaard, 1979). The rate at which carbon dioxide is produced is very low in these sandy sediments to judge from the oxygen consumption rates, so adaptations which minimize carbon dioxide losses from the plant–sediment system would reduce the risk of carbon dioxide limitation of photosynthesis. A high resistance to gas exchange across the leaf surface fulfils this purpose and is a logical consequence of a low resistance to gas exchange across the root surface. The lacunal and interstitial pools of oxygen and carbon dioxide may work in close connection, relatively effectively sealed off from the lake water pools. This system would facilitate recycling of both gases within the two pools.

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REFERENCES

- ARBER, A. (1920). *Water Plants. A Study of Aquatic Angiosperms.* Cambridge, Cambridge University Press.
- ARMSTRONG, W. (1964). Oxygen diffusion from the roots of some British bog plants. *Nature*, **204**, 801–802.
- DROMGOOLE, F. I. (1979). Photosynthetic and respiratory transients of oxygen exchange in *Carpophyllum* species (Fucales, Phaeophyceae). *Aquatic Botany*, **6**, 133–147.
- HARTMAN, R. T. & BROWN, D. L. (1967). Changes in internal atmosphere of submerged vascular hydrophytes in relation to photosynthesis. *Ecology*, **48**, 252–258.
- JENSEN, C. R., STOLZY, L. H. & LETEY, J. (1967). Tracer studies of oxygen diffusion through roots of barley, corn and rice. *Soil Science*, **103**, 23–29.
- KELLY, M. G., MOESLUND, B. & THYSSEN, N. (1981). Productivity measurement and storage of oxygen in the aerenchyma of aquatic macrophytes. *Archiv für Hydrobiologie* (In press).
- KIER, F., ALLERMANN, K., FLOTO, F., OLSEN, J. & SORTKJÆR, O. (1976). Changes of exponential growth rates in relation to differentiation of *Geotrichum candidum* in submerged culture. *Physiologia Plantarum*, **38**, 6–12.
- LETEY, J. & STOLZY, L. H. (1974). Measurement of oxygen diffusion rates with the platinum microelectrode. *Hilgardia*, **35**, 545–576.
- RIED, A. (1968). Interactions between photosynthesis and respiration in *Chlorella*. I. Types of transients of oxygen exchange after short light exposures. *Biochimica et Biophysica Acta*, **153**, 653–663.
- SAND-JENSEN, K. (1978). Metabolic adaptation and vertical zonation of *Littorella uniflora* (L.) Aschers and *Isoetes lacustris* L. *Aquatic Botany*, **4**, 1–10.
- SAND-JENSEN, K., PRAHL, C. & STOKHOLM, H. (1982). Oxygen release from roots of submerged aquatic macrophytes. *Oikos* (In press).
- SAND-JENSEN, K. & SØNDERGAARD, M. (1979). Distribution and quantitative development of aquatic macrophytes in relation to sediment characteristics in oligotrophic Lake Kalgaard, Denmark. *Freshwater Biology*, **9**, 1–11.
- SAND-JENSEN, K. & SØNDERGAARD, M. (1981). Phytoplankton and epiphyte development and shading effect on submerged macrophytes in lakes of different nutrient status. *Internationale Revue Der Gesamten Hydrobiologie*, **66**, 529–552.
- SCHÖNHERR, J. (1976). Water permeability of isolated cuticular membranes. The effect of cuticular waxes on diffusion of water. *Planta*, **131**, 159–164.
- SCULTHORPE, C. D. (1967). *The Biology of Aquatic Vascular Plants.* Edward Arnold, London.
- SØNDERGAARD, M. & SAND-JENSEN, K. (1979a). Physico-chemical environment, phytoplankton biomass and production in oligotrophic, softwater Lake Kalgaard, Denmark. *Hydrobiologia*, **63**, 241–253.
- SØNDERGAARD, M. & SAND-JENSEN, K. (1979b). Carbon uptake by leaves and roots of *Littorella uniflora* (L.) Aschers. *Aquatic Botany*, **6**, 1–12.
- WALKER, P. A. (1973). Photosynthetic induction phenomena and the light activation of ribulose diphosphate carboxylase. *The New Phytologist*, **72**, 209–235.
- WESTLAKE, D. F. (1978). Rapid exchange of oxygen between plant and water. *Verhandlungen Internationale Vereinigung Für Theoretische Und Angewandte Limnologie*, **20**, 2363–2367.
- WETZEL, R. G. (1975). *Limnology.* W. B. Saunders, Philadelphia, London & Toronto.
- WIUM-ANDERSEN, S. (1971). Photosynthetic uptake of free CO₂ by the roots of *Lobelia dortmanna*. *Physiologia Plantarum*, **25**, 245–248.
- WIUM-ANDERSEN, S. & ANDERSEN, J. M. (1972a). The influence of vegetation on the redox profile of the sediment of Grane Langsø, a Danish *Lobelia* lake. *Limnology and Oceanography*, **17**, 948–952.
- WIUM-ANDERSEN, S. & ANDERSEN, J. M. (1972b). Carbon dioxide content in the interstitial water in the sediment of Grane Langsø, a Danish *Lobelia* lake. *Limnology and Oceanography*, **17**, 943–947.

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