Xylem sap flow as a major pathway for oxygen supply to the sapwood of birch (*Betula pubescens* Ehr.)

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ABSTRACT

The role of xylem sap flow as an aqueous pathway for oxygen supply to the wood parenchyma of *Betula pubescens* saplings was investigated. Using micro-optode sensors the oxygen status of the sapwood was quantified in relation to mass flow of xylem sap. Sax flow was gradually reduced by an increasing oxygen depletion in the root space. The effect of sap flow on radial O₂ transport between stem and atmosphere was assessed by a stoichiometrical approach between respiratory CO₂ production and O₂ consumption. Restriction of sap flow set in 36.5 h after the onset of O₂ depletion, and was complete after 71 h. Interruption of sap flow drastically increased the O₂ deficit in the sapwood to 70%. Sax flow contributed about 60% to the total oxygen supply to the sapwood. Diurnal O₂ flow rates varied between 3 and 6.3 nmol O₂ m⁻² leaf area (LA) s⁻¹ during night- and daytime, respectively. Maximum O₂ flow rates of 20 nmol O₂ m⁻² LA s⁻¹ were reached at highest sap flow rates of 5.7 mmol H₂O m⁻² LA s⁻¹. Sax flow not only affected the oxygen status of the sapwood but also had an effect on radial O₂ transport between stem and atmosphere.

Key-words: aquaporins; hypoxia; respiration; roots; sap flow; stem heat balance.

INTRODUCTION

It has long been recognized that the transpiration stream plays a vital role in aeration of living tissues surrounded by the cambium of woody plants (Bailey 1913; Haberlandt 1914). The importance of xylem sap flow for oxygen supply to and removal of carbon dioxide from parenchymatous tissues of the sapwood was clearly recognized by Bailey (1913) when he stated that the xylem of arborescent plants has an important function for transport of gases dissolved in water from the roots to the cambium and leaves. Axial oxygen transport in flood-tolerant plant species from aerial parts to the roots has been a subject of broad interest (Hook, Brown & Wetmore 1972) with special reference to physical processes involved in gaseous diffusion of oxygen through intercellular gas-spaces such as thermo-osmosis or Graham’s law of diffusion (Armstrong 1979; Grosse, Frye & Lattermann 1992; Grosse 1997). However, long-distance gaseous oxygen transport primarily takes places in the intercellular gas-space continuum of the outer cortex surrounded by the phellogen (Hook et al. 1972), which has been identified as a thermo-osmotically active partition in several tree species (Eschrich 1995; Grosse 1997).

Radial gaseous diffusion of atmospheric oxygen across the cambial sheath towards the wood parenchyma can only be effective if continuity through cambium pores is maintained. In trees of *Betula pendula* Roth radial influx of oxygen into the sapwood has been suggested as a gaseous pathway mainly effective at night, by which xylem sap will be enriched with dissolved oxygen (Gansert, Burgdorf & Lösch 2001). With respect to species-specific wood anatomy, oxygen supply to the various types of wood parenchyma (Braun 1970; Grosser 1977; Schweingruber 1978; Eschrich 1995) principally depends on both the abundance of intercellular spaces and their effectiveness for gaseous diffusion (i.e. the gaseous path), and xylem sap flow – the aqueous path – that primarily supports those parenchyma strands that shield water transport from embolism and regulate secretion of osmotica and hormones into the xylem sap. Oxygen concentrations measured in sapwood of different anatomical structure and metabolic activity vary over a broad range from pronounced hypoxia to near saturation (Ekland 1990, 1993, 2000; Gansert et al. 2001; Del Hierro et al. 2002; Mancuso & Marras 2003).

New types of miniaturized, high-sensitive oxygen sensors allow improved quantitative analyses of the oxygen status and oxygen flow rates in different compartments of the woody cormus on a high-resolution time scale. Previous efforts on the measurement of oxygen concentrations in the sapwood by use of these sensors focused on the preclusion from interference with atmospheric oxygen, and maintenance of xylem sap flow by avoidance of embolism. Thus, underwater access to the sapwood was used as a methodical approach for *in situ* measurements of diurnal and seasonal variations of endogenous oxygen concentrations in woody plants (Gansert et al. 2001). This approach may contribute to identifying circumstances and metabolic dependencies that are responsible for the different oxygen status of plant shoots under varying ontogenetic, environmental, and seasonal conditions. Using the new type of micro-optode oxygen sensors, the purpose of this study was: (1)
to investigate the role of xylem sap flow as an aqueous pathway for oxygen supply to the sapwood parenchyma of young mountain birch (*Betula pubescens* Ehr.); (2) to quantify its effect on the oxygen status of the sapwood; and (3) to evaluate the potential effect of xylem sap flow on oxygen transport between stem and atmosphere during stem respiration.

**MATERIALS AND METHODS**

**The experimental set-up**

In early June 2002, two potted plants of 5-year-old field-grown *B. pubescens* saplings about 1.7 m tall were brought into a climate chamber and preconditioned to the experimental light and temperature regime for 10 d. The soil substrate was natural loess (volume $=8.3 \times 10^{-3} \text{ m}^3$) in which the roots grew undisturbed for 1 year. In order to simulate a near-daylight spectrum, HRI-T 400 W/D lamps (Radium; Osram, München, Germany) were used as the light source during a 13 h day period (0700 to 2000 h). Prevention of upper crown parts from excessive warming was achieved by insertion of a glass pane (4 mm thick) 8 cm beneath the light unit. Horizontal air ventilation between the lamps and the glass pane efficiently reduced the effect of excessive heat production on leaf gas exchange. Thus, air temperature measured at 27 cm beneath the lamps fluctuated between 21 and 23 °C during night-time and between 23 and 27 °C during daytime. Because air ventilation started about 30 min prior to the onset of light a drop in air temperature of 2–3 °C always marked the end of the night.

Each pot was packed in a polyethylene bag leaving the top open, and immersed in the water bath of a cryostat (Type MCM0; Colora Messtechnik GmbH, Lorch, Germany). In each pot three copper–constantan thermocouples were inserted at different soil depths (7, 13 and 17 cm from the surface) 8 cm radially off the stem base. A mean soil temperature of $17 \pm 0.3 \text{ °C}$ at a depth of 13 cm was maintained by permanent temperature control of the water baths. Figure 1 presents the experimental set-up for both trees schematically. Air temperature and relative humidity were simultaneously measured at 27 and 95 cm beneath the lamps by use of two Vaisala sensors (HMP 35 A; Vaisala, Helsinki, Finland). Photosynthetic photon flux density (PPFD) was measured at half crown length, 55 cm beneath the lamps by use of three sensors (SKP 215; Skye Instruments Ltd, Llandrindod Wells, Powys, UK) which were evenly positioned over a distance of 80 cm. Global radiation (Pyranometer SKS 1110; Skye Instruments Ltd) was also measured at the same height as the PPFD sensors. On both trees two Plexiglass cuvettes (own construction) were positioned on the stem at 96 and 101 cm above the stem base, respectively. At those heights the stems showed nearly the same diameter of about 8 mm. The upper cuvette was designed for measurement of $O_2$ concentration in the cuvette atmosphere and in the sapwood, whereas the lower one was prepared for measurement of $CO_2$ efflux rates. Both cuvettes are described in detail in the following section. To preclude interference in $CO_2$ and $O_2$ gas exchange due to bark photosynthesis, all internodes and the cuvettes were shielded from light by strips of black silk cloth wrapped around the woody plant parts. The data of all microclimatic variables were synchronously recorded at 10 s intervals and averaged over 5 min intervals by data loggers (Squirrel 1250 series; Grant Instruments Ltd, Barrington Cambridge, UK).

**Cuvettes for measuring stem gas exchange**

Cuvettes for the measurement of $O_2$ and $CO_2$ gas exchange between ambient air and the stem were made out of Plexiglass as shown schematically (Fig. 2). Each cuvette consisted of two semi-cylinders (wall thickness $=5 \text{ mm}$, thickness of top and bottom $=6 \text{ mm}$) which enclosed a stem internode of up to 8 mm in diameter and 20 mm in length. A rubber seal fixed in a groove (2 mm wide, 1.5 mm deep) along the plane side of one semi-cylinder ensured a leak-proof seal when both semi-cylinders were pressed onto each other by two steel strings. Silicon rubber (Loctite no. 5910; Loctite GmbH, München, Germany) was used to seal the remaining gap between the stem and the cuvette. Each cuvette was equipped with four Plexiglass tubes ($d_{int} = 10 \text{ mm}$, $l = 62 \text{ mm}$) with different inner diameters to be connected to different gas analysing instruments. The cuvette being used for $O_2$ measurements was sealed from ambient air by insertion of a silicon septum (3 mm thick) into each Plexiglass tube. The one being used for $CO_2$ measurements was run in a permanent open airflow modus (see below).

**$O_2$ cuvette**

The concentrations of molecular oxygen were simultaneously measured both, in the cuvette atmosphere ($V_{air} = 5.28 \text{ cm}^3$), and in the aqueous phase of the sapwood at a depth of 2.9 mm from the stem surface. Measurements of molecular oxygen were performed by fibre-optical micro-optode sensors (see below). Two opposite tubes were used as mechanical guides for the plastic syringes and the attached cannulae that penetrated through the silicon septa. The third tube was filled with soda lime as the $CO_2$ absorbent (Fig. 2). Thereby, during the experiments total pressure in the $O_2$ cuvette remained near constant at atmospheric pressure because an increase of $CO_2$ partial pressure due to continuous $CO_2$ efflux from the stem, was compensated by an equivalent chemical binding of $CO_2$. Maintenance of atmospheric pressure in the $O_2$ cuvette was confirmed by use of a calibration manometer (DP 205; Ametek Precision Instruments GmbH, Meerbusch, Germany) attached to one of the tubes. The air temperature in the cuvette was measured by a Pt-100 temperature sensor ($2 \times 2.3 \times 0.5 \text{ mm}$; S105PD4A; Telemeter Electronic, Donauwörth, Germany), inserted into the cuvette through the fourth tube, and connected to the MICROX® oxygen analysing system (PreSens GmbH, Regensburg, Germany). Measurements of oxygen concentration and temperature were carried out at 5 min intervals.

The optical measurement of molecular oxygen was performed by an improved MICROX system with a micro-optode sensor (sensor type B; PreSens GmbH). The MICROX system consists of a pulsing laser light source (excitation wavelength 505 nm) to excite the \( \text{O}_2 \) sensor, an optical fibre as signal transducer, a photodetector and the optical sensor. The micro-optode consists of a plastic syringe \((d = 7 \text{ mm})\) which houses the optical fibre with the \( \text{O}_2 \) sensor – a luminophor – on its tapered tip \((d \leq 30 \mu \text{m})\). In order to move the sensor out of the cannula \((d_{\text{out}} = 0.5 \text{ mm}, l = 37 \text{ mm})\) the fibre is fixed to the plunger. The MICROX system measures the luminescence lifetime of an immobilized \( \text{O}_2 \) sensitive luminophore which is inversely proportional to the number of oxygen molecules that reversibly react with the luminophor. By this method no oxygen is consumed during the measurement, and the signal is independent of changes in sap flow velocity. Details of this optic–chemical \( \text{O}_2 \) measurement have been described elsewhere (Gansert et al. 2001).

**CO\textsubscript{2} cuvette**

Measurements of \( \text{CO}_2 \) concentration were performed by use of LCA-4 porometers (ADC BioScientific Ltd, Hoddesdon, UK) each being attached to the \( \text{CO}_2 \) cuvettes of the trees as shown in Fig. 2. A permanent airflow at a rate of 0.2 L min\(^{-1}\) was maintained throughout the experimental period. Air temperature in the \( \text{CO}_2 \) cuvettes was measured by the same type of Pt-100 sensor mentioned above, which was connected to the temperature input side of the porometer. Due to continuous operation of two porometers (one for either tree), porometer-specific drifts in \( \text{CO}_2 \)
analysing accuracy were minimized by re-calibration at 3 d intervals. Because the O₂ and CO₂ cuvettes were positioned close to each other on internodes of the same diameter it was assumed that the rate of CO₂ efflux in the O₂ cuvette was nearly the same as measured in the CO₂ cuvette. Measurements of CO₂ efflux were synchronized with measurements of O₂ concentration and also recorded at 5 min intervals.

**Leaf photosynthesis and transpiration**

Rates of photosynthesis, dark respiration, and transpiration of leaves were measured by use of a third LCA-4 porometer with a non-climatized leaf cuvette. Measurements were carried out in two modes. In the random mode 10 leaves per tree were selected and all parameters necessary for calculating the CO₂ and H₂O flux rates were recorded several times throughout the day. During all measurements the leaves were handled in the same order. In the automatic record mode one leaf of the upper crown of a tree, that showed a representative photosynthetic rate, was inserted into the leaf cuvette and single measurements were automatically recorded at 5 min intervals for 1 week.

**Xylem sap flow**

Xylem sap flow was measured by use of Dynagage® stem flow sensors (SGA-10; Dynamax Inc., Houston, TX, USA) combined with a ‘Flow2’ sap flow monitor system (Dynamax Inc.). The gauges, one per tree, were positioned about 20 cm above the stem base at stem diameters of 12.2 and 12.6 mm for saplings ‘A’ and ‘B’, respectively. Tight contact between the flexible heater and the stem surface was ensured by use of four cable ties per gauge in addition to the velcro straps.

The manufacturer’s calibration procedure recommends that the Kₗ values, namely the effective thermal conductance of the sheath of materials surrounding the heater, should be measured under conditions of no sap flow. For this purpose, the gauges were mounted around a dry stick of birch wood. Wrapping the gauges in several layers of aluminium foil ensured sufficient thermal insulation from ambient temperature variations. The measured Kₗ values at zero sap flow gave 0.8349 (resistance of gauge 1 = 132.5 W) and 0.812 W mK⁻¹ (resistance of gauge 2 = 134.6 W). Following Smith & Allen (1996) the voltage across the heater was adjusted to 3.58 V for both gauges. Thereby the risk of overheating the stems at low sap flow rates was removed. All voltages necessary for calculating mass flow of xylem sap were logged synchronously with CO₂ and O₂ measurements at 5 min intervals (CR10; Campbell Scientific, Inc., Logan, UT, USA).

The leaf area specific sap flow rate (Fₗₐ) was calculated using the total leaf area of a tree as the reference unit. Hence, at the end of the experiment the area of each leaf was measured using a LI-3100 area meter (Li-Cor Inc., Lincoln, NE, USA) and summed. The total leaf area of saplings ‘A’ and ‘B’ was 0.365 and 0.451 m², respectively.

For reasons of quantitative analysis of the role of xylem sap flow for endogenous oxygen transport the experimental set-up used in this study focused on the simultaneous measurement of several ecophysiological parameters of relevance for the interpretation of oxygen transport pro-
cesses. Limited resources of equipment confined the investigations to only two tree individuals of the same phenological state.

Experimental restriction of xylem sap flow

Xylem sap flow was gradually reduced by an increasing oxygen depletion in the root space that lasted for several days. For that purpose, atmospheric oxygen supply to the soil and root system was interrupted by sealing the pots with the polyethylene bags and warming them up to 32 °C in the water baths of the cryostats. It was assumed that the temperature-dependent exponential increase in respiration of roots and soil organisms should cause a corresponding oxygen deficiency and even hypoxia in the soil atmosphere. This in turn should lead to an interruption of water uptake by the roots. Recovery from restriction of xylem sap flow was induced by subsequent re-aeration of the soil atmosphere.

Calculations

According to Henry’s law, the concentration of dissolved oxygen in water saturated with air, \( C_{O_2} \) (mol L\(^{-1}\)), was calculated as:

\[
C_{O_2} = \frac{P_{O_2} C_w}{K_{O_2}} \tag{1}
\]

where \( P_{O_2} \) is the partial pressure of oxygen in air at atmospheric conditions (21 278 Pa), \( C_w \) the number of moles of \( \text{H}_2\text{O} \) per litre (55.6), and \( K_{O_2} \) (Pa × 10\(^{-6}\)) the solubility coefficient of oxygen in water. Within the temperature range \( T \) from 0 to 30 °C the temperature dependence of \( K_{O_2} \) was calculated as:

\[
K_{O_2} = 0.0729 \cdot T + 2.694 \tag{2}
\]

derived from linear regression analysis of \( K_{O_2} \) values determined at different temperatures (Von Willert, Matyssek & Herppich 1995). The relative concentration of dissolved oxygen (% water saturation) at a given temperature could then be converted into the absolute concentration in the aqueous solution, \( [O_2] \), given in \( \mu \text{mol L}^{-1} \). The oxygen flow rate (\( \mu \text{mol O}_2 \text{ m}^{-2} \text{ leaf area s}^{-1} \)) in the sapwood was then calculated from the known values of \([O_2]\) and the leaf area specific sap flow rate \( F_{LA} \) (mmol \( \text{H}_2\text{O} \text{ m}^{-2} \text{ s}^{-1} \)).

The temperature dependence of CO\(_2\) efflux from the stems was quantified by use of the Arrhenius-equation:

\[
J_{CO_2}^a (T) = a \exp[-b \cdot 1000 / (R \cdot T)] \tag{3}
\]

where \( J_{CO_2}^a \) is \( \text{CO}_2\) efflux rate, here given as \( \mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \) (the superscript ‘st’ stands for ‘stem’), \( T \) = temperature (°K), and \( R = 8.314 \text{ J K}^{-1} \text{ mol}^{-1} \). The coefficients \( a \) and \( b \) were calculated by non-linear regression analysis according to the database available (SigmaPlot 6.00; SPSS Inc., Chicago, Il, USA). The coefficient \( b \) is the activation energy (kJ mol\(^{-1}\)); namely the amount of heat energy necessary to activate respiration metabolism (Schöpfer & Brennicke 1999).

RESULTS

Xylem sap flow and leaf gas exchange under control conditions

In mid-June, the experiment started after 10 d of acclimatization of the plants to the prevailing light regime (530 \( \mu \text{mol} \text{ photons m}^{-2} \text{ s}^{-1} \) on average in the upper crown), and room temperature conditions (20 °C at the end of the night to maximum values of between 26 and 28 °C at the end of the day), and lasted for 2 weeks. The mean rate of leaf net CO\(_2\) gas exchange (\( J_{CO_2} \)) in the upper crown of both trees was 6–8 \( \mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \) with little variation throughout the day (Fig. 3a). Mean stomatal conductance \( g_s \) of the leaves ranged from 100 to 150 mmol \( \text{H}_2\text{O} \text{ m}^{-2} \text{ s}^{-1} \) indicating open stomata. Thus, the leaves transpired at mean rates between 1.2 and 1.8 mmol \( \text{H}_2\text{O} \text{ m}^{-2} \text{ s}^{-1} \). Dark respiration rates were nearly constant at 1 mmol \( \text{CO}_2 \text{ m}^{-2} \text{ s}^{-1} \) during night-time. These data of leaf gas exchange were measured at constant soil temperature conditions of 17 °C.

When sap flow was unimpeded, an increase of \( g_s \) during night-time was observed several times, as on 14 June (Fig. 3a) or 18 June (day 6 of the experiment, Fig. 4b). Stem sap flow under these control conditions was in a range between 1.1 and 1.6 mmol \( \text{H}_2\text{O} \text{ m}^{-2} \text{ LA s}^{-1} \) in the light period, and 0.6–0.9 mmol \( \text{H}_2\text{O} \text{ m}^{-2} \text{ LA s}^{-1} \) on average in darkness.

Restriction of xylem sap flow

Oxygen depletion in the root space started on day 4 of the experiment from 2100 h onward, when the pots were sealed and warmed up to 32 °C (T-soil, Fig. 4a). In the following light period leaf area specific sap flow rate \( F_{LA} \) increased by 34% compared with previous rates at 17 °C soil temperature (Fig. 4c). Thus, highest rates of \( J_{CO_2} \) (10 \( \mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \)) and \( g_s \) (150 mmol \( \text{H}_2\text{O} \text{ m}^{-2} \text{ s}^{-1} \)) were also measured (Fig. 4b). On the second day of oxygen depletion (day 6), restriction of sap flow was observed by a decrease of \( F_{LA} \) from 930 h onward towards 0.3 mmol \( \text{H}_2\text{O} \text{ m}^{-2} \text{ s}^{-1} \) at the end of the day (Fig. 4c). Due to reduced sap flow, mean leaf transpiration rates \( E \) dropped from 1.94 to 0.96 mmol \( \text{H}_2\text{O} \text{ m}^{-2} \text{ s}^{-1} \), and \( J_{CO_2} \) was reduced by half. On day 7, from 2000 h onward, no sap flow was recorded by the Dynagage sensors, which was 71 h after the onset of \( O_2 \) depletion (Fig. 4c). Cessation of transpiration and net CO\(_2\) uptake by the leaves indicated that interruption of sap flow caused a complete closure of the stomata from the fourth day of oxygen depletion onward. The majority of the leaves could not tolerate such water shortage so that trees ‘A’ and ‘B’ lost 92 and 87% of their total leaf area, respectively, within the following days.

After re-aeration of the roots on day 8 of the experiment from 2100 h onward, \( F_{LA} \) rose to 2.3 mmol \( \text{H}_2\text{O} \text{ m}^{-2} \text{ s}^{-1} \) within 20 min, in spite of the darkness. It gradually increased further to a final maximum rate of 5.7 mmol \( \text{H}_2\text{O} \text{ m}^{-2} \text{ s}^{-1} \) (Fig. 4e). Thus, at its maximum, \( F_{LA} \) showed a near four-fold increase after re-aeration. Continuous poromeric measurements also revealed recovery from water stress.

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The effect of sap flow restriction on changes in oxygen concentration in the sapwood

The concentrations of molecular oxygen in the O₂ cuvette atmosphere were always clearly different between day and night. During daytime, the O₂ concentration was reduced to about 94% of the atmospheric concentration (100% air saturation corresponds to 21% O₂ concentration of the atmosphere). During night-time, the O₂ concentration was in the range between 96 and 100% of air saturation. This diurnal pattern was measured with no ongoing bark photosynthesis and seemed not to be affected by the gradual interruption of sap flow (Fig. 4d).

Under conditions of unimpeded sap flow, the O₂ concentration measured in the sapwood also showed a clear alternating diurnal pattern, but at a level considerably below concentrations of an aqueous solution of the same temperature saturated with air. During the day, [O₂] was reduced to 75% of water saturation, whereas it increased to 82% on average at night (Fig. 4d). Thus, the sapwood of the B. pubescens saplings showed a permanent oxygen deficit. This O₂ deficit alternated between 15% during the night, rising to 30% during the day.

The gradual reduction of sap flow caused a corresponding decrease of the O₂ concentration in the sapwood. As a result of the first decrease of \( F_{LA} \) on day 6 of the experiment, [O₂] decreased from 75 to 67% at the end of the day. During the following night, recovery towards concentrations of previous nights took place when \( F_{LA} \) also rose. However, the increase in [O₂] was greater than might be expected from the sap flow increase. During the next day, [O₂] dropped further to 58% of water saturation. When sap flow ceased completely on day 8, [O₂] also reached its minimum of 30% of water saturation at the end of the light period (Fig. 4d). Hence, corrected for differences in temperature, sap flow rates between 1 and 1.5 mmol H₂O m⁻² s⁻¹ corresponded to an oxygen concentration of about 119 mmol O₂ L⁻¹ which is 61% of the initial concentration of dissolved oxygen in the sapwood at unimpeded sap flow (Fig. 5). A similar result was obtained from the second tree, in which sap flow contributed 58% to the O₂ concentration in the sapwood during daytime.

After re-aeration of the roots and recommencement of sap flow to 2.3 mmol H₂O m⁻² s⁻¹ [O₂] initially increased...
from 30 to 48% (77–134 μmol O$_2$ L$^{-1}$) within 3.5 h (Fig. 4d). Under these conditions the specific oxygenation rate was 2 nmol O$_2$ L$^{-1}$ s$^{-1}$ per mmol H$_2$O m$^{-2}$ LA s$^{-1}$ sap flow. However, the process of O$_2$ re-loading of the sapwood was retarded so that it took 42 h longer to approximate the initial [O$_2$] of 200 μmol L$^{-1}$.

**Leaf area-based oxygen flow rates in the sapwood**

From a knowledge of both, the temperature-corrected absolute concentration of dissolved oxygen and the $F_{LA}$, the oxygen flow rate in the sapwood can be calculated using the

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**Figure 4.** (a) Diurnal temperature regime in the climate chamber (T-room), in the porometer cuvette (T-cuv), and at 13 cm soil depth (T-soil) of potted *B. pubescens* saplings (sapling ‘B’) during the experimental period from 15 to 22 June 2002 (days 3–10). (b) Continuous record of net CO$_2$ exchange rate ($J_{CO_2}$) and stomatal conductance ($g_s$) of a single leaf before, during, and after recovery from gradual interruption of xylem sap flow. Daily means of $J_{CO_2}$ (squares) and standard deviation of 10 leaves randomly distributed in the upper crown are also indicated. (c) Leaf area specific sap flow rate ($F_{LA}$) and transpiration rate of a single leaf ($E$) as well as mean values ($n = 10$) of upper crown leaves (dots) indicate restriction of the transpiration stream on day 6 of the experiment from 0930 h onward. (d) Daily variations of the relative O$_2$ concentrations in the cuvette atmosphere (O$_2$ cuvette), and dissolved in the sapwood at 2.9 mm depth from the stem surface (diameter = 7.6 mm). On day 4, from 2100 h onward, O$_2$ depletion in the root space was induced (indicated by arrow).
tree-specific total leaf area (LA) as the reference unit. During the light periods and under conditions of unimpeded sap flow, the oxygen flow rate in the sapwood reached 5–6.3 nmol O$_2$ m$^{-2}$ LA s$^{-1}$ in saplings ‘B’ and ‘A’, respectively (Fig. 5). In line with the diurnal variation of $F_{LA}$, the oxygen flow rate decreased to about 3 nmol O$_2$ m$^{-2}$ LA s$^{-1}$ at night. Recomencement of sap flow after re-aeration of the roots caused a rapid increase in oxygen flow to rates of about 6 nmol O$_2$ m$^{-2}$ LA s$^{-1}$ within the first 20 min of re-aeration. Highest oxygen flow rates of 20 nmol O$_2$ m$^{-2}$ LA s$^{-1}$ were reached at highest sap flow rates of 5.7 mmol H$_2$O m$^{-2}$ LA s$^{-1}$.

Stoichiometrical relations between CO$_2$ efflux and O$_2$ exchange across the stem surface

The rates of CO$_2$ efflux from the stem ($J_{CO2}^H$) of either tree sapling were not affected by the gradual decrease of sap flow. For example on day 8 of the experiment, when no sap flow was measured, $J_{CO2}^H$ was 2.4 μmol m$^{-2}$ s$^{-1}$ on average during daytime. Similar rates were also measured on days 4–6, when sap flow occurred (Fig. 6). A correlation of $J_{CO2}^H$ versus cuvette temperature showed a characteristic exponential temperature-dependent relationship of CO$_2$ release, as described by the Arrhenius-equation with corre-
A temperature regime between 21 and 27°C from 10 June to 6 July 2002. Correlation coefficients (r) of 0.74 and 0.8 indicate that CO₂ efflux was primarily due to respiration of stem parenchyma. Reversible interruption of xylem sap flow by oxygen depletion in the roots

At conditions of unimpeded sap flow the leaf area-specific flow rates of *B. pubescens* saplings (1–1.5 mmol H₂O m⁻² s⁻¹) were highly consistent with flow rates derived from mature *B. pendula* trees (1.2 mmol H₂O m⁻² s⁻¹), naturally grown in a mixed birch-pine forest (Backes 1996). Similar rates of *F_LA* of 1.8–3.6 mmol H₂O m⁻² s⁻¹ at maximum on sunny days were also reported for mature *B. pendula* trees (Ladefoged 1963).

In this study xylem sap flow was reversibly reduced, and even stopped, by a combined treatment of warming and oxygen depletion in the root space. Three experimental findings are of interest: (1) warming up to 32°C caused an initial increase in *F_LA* by 34% compared with previous rates at 17°C, followed by an increase of stomatal conductance and photosynthesis; (2) restriction of sap flow occurred first after 36.5 h from the onset of oxygen depletion; and (3) sap flow recommenced within 20 min after re-aeration of the roots.

Because the apoplastic path of radial water transport across the root cortex towards the xylem is interrupted or at least drastically reduced at the endo- and often at the


Figure 7. Temperature dependence of CO₂ efflux from the stems (*J_{CO₂}^s*) of two 5-year-old *B. pubescens* saplings (sapling ‘A’: d = 7.6 mm; sapling ‘B’: d = 7.8 mm) grown in a climate chamber at a temperature regime between 21 and 27°C from 10 June to 6 July 2002. Correlation coefficients (r) of 0.74 and 0.8 indicate that CO₂ efflux was primarily due to respiration of stem parenchyma.

This equivalence was used as the reference level for respiration-dependent oxygen consumption which partially contributed to the O₂ deficit usually measured in the cuvette atmosphere. The remaining portion of the O₂ deficit could then be attributed to radial O₂ influx from the cuvette atmosphere into the stem. With regard to the alternating decrease and increase of oxygen concentration in the cuvette atmosphere between day and night, respectively, the stoichiometrical approach provided an estimate of radial O₂ flux in the stem during the light and dark periods (Fig. 6). It revealed that nocturnal O₂ deficits in the cuvette atmosphere, namely the difference between CO₂ efflux and O₂ exchange rate (bold line), were considerably smaller than by day. This is due to both temperature-dependent reduction of respiratory oxygen consumption, and higher O₂ efflux from the stem. At unimpeded sap flow (days 4–6) the mean O₂ efflux rate was +0.33 µmol O₂ m⁻² s⁻¹, and thus, the O₂ deficit in the cuvette atmosphere was smaller than expected from respiration during the day. The gradual reduction of sap flow caused a change from O₂ efflux into an increasing O₂ influx into the stem (day 7, −0.31 µmol O₂ m⁻² s⁻¹; day 8, −0.54 µmol O₂ m⁻² s⁻¹). Therefore, the O₂ deficit in the cuvette atmosphere increased above that expected from respiration. When sap flow recommenced, reversion from oxygen sink to source towards a rate similar to that measured at normal sap flow took place. Thus, according to the stoichiometrical relations applied, xylem sap flow also affected radial oxygen exchange between stem and atmosphere in *B. pubescens* saplings. At rates from 1 to 1.5 mmol H₂O m⁻² LA s⁻¹ source to sink relations were in a range of up to 0.9 µmol O₂ m⁻² stem surface s⁻¹.

**DISCUSSION**

Reversible interruption of xylem sap flow by oxygen depletion in the roots

If sap flow contributes to the oxygen supply of the sapwood, one may question whether sap flow also affects radial oxygen transport between stem and atmosphere? Respiratory O₂ consumption of wood parenchyma interferes with physico-chemically driven radial O₂ transport. To examine this implication, rates of O₂ exchange between stem and cuvette atmosphere, and of CO₂ efflux can be related to each other on the basis of three assumptions: (1) the respiratory quotient (RQ) is close to unity. This is the case when glucose is the primary substrate respired; (2) no O₂ deficit occurs in the cuvette atmosphere, that is, the O₂ concentration in the cuvette reaches 100% air saturation; (3) no sap flow is measured at that time. The second and third assumptions were fulfilled during night-time on day 8 of the experiment (Fig. 4d). At that time, the stem respired at a near constant rate of 1.35 µmol CO₂ m⁻² s⁻¹. It was therefore concluded that O₂ consumed by respiration originated from the stem at a rate equivalent to the rate of respiratory CO₂ production (Fig. 6). This equivalence was used as the references.
exodermis, all water molecules must pass cell membranes. Thus, the modes of water movement across membranes are of major importance for water uptake by the roots (Steudle 1997, 2000a). Aquaporins contribute considerably to the hydraulic conductivity of plant cell membranes (Maurel 1997; Steudle 1997; Schöffner 1998; Tyerman et al. 1999). Here, water supply to the shoot is under metabolic control, and becomes affected by temperature and oxygen supply. Regulation of radial water flow by aquaporins is based on opening or closing of existing aquaporins or by variation of their density in membranes (Steudle 2000b). The rapid recommencement of sap flow in P. pubescens saplings within 20 min after re-aeration points to a reversible metabolic regulation of transmembrane water flow rather than an increase in aquaporine density.

Oxygen deficiency not only affects radial water transport by a changed activity of aquaporins – lack of phosphorylation de-activates water channels (Johansson et al. 1996; Kjellbom et al. 1999) – but also reduces energy-dependent active ion pumping. This affects the passage of water by changing the osmotic gradient across plasma membranes. In primary roots of Zea mays that were grown hydroponically anoxia caused a decrease of the ATP/ADP ratio by 64% after 15 h of deoxygenation (Birner & Steudle 1993). These authors stated that under anaerobiosis the reduction in energy charge of the root tissues causes a successive switching off of ion pumps located at the xylem and at the cortical plasmalemma, respectively.

The experimental procedure of oxygen depletion in the root space applied in this study provides some evidence that radial movement of water towards the xylem in roots of P. pubescens is primarily under metabolic control. The increase in temperature from 17 to 32 °C caused an overall increase in root metabolism including those processes being involved in water transport as long as oxygen availability did not limit respiratory ATP supply. The gradual depletion of oxygen in the root space must have affected root respiration and thus, the energy charge of the cells most likely decreased as well. Due to a low energy charge both the de-activation of aquaporins, and loss of active ion pumping are probably the key-processes for the gradual interruption of xylem sap flow. The fact that about 90% of the leaves of either tree could not survive the water-stress conditions induced by root hypoxia may prove that interruption of sap flow was complete. It may indicate further that water transport bypassing the root endodermis was negligible. Finally, the rapid and full recommencement of sap flow after re-aeration of the roots within 20 min, and the resulting stomatal opening of remaining leaves also provides evidence for a metabolically induced interruption of water transport which was neutralized when oxygenation set in.

The role of xylem sap flow for the oxygen status of the sapwood

In June, the sapwood of P. pubescens saplings showed a high oxygen status during unimpeded sap flow. At standard temperature and pressure (\( T = 20 °C, \ P = 100 \text{kPa} \)) daily maxima of \([O_2]\) of up to 245 μmol O₂ L⁻¹ occurred. Such values are in line with those reported for different tree species when the growing season begins (Table 1). Even the daily minimum of \([O_2]\) was high (about 210 μmol O₂ L⁻¹), whereas in spring, in mature B. pendula the minimum value dropped to 70 μmol O₂ L⁻¹ (Gansert et al. 2001). Similar minima were also reported for Picea abies, Quercus robur or Acer platanoides during the growing season in July and August (Table 1). Although the diurnal span of concentration of dissolved oxygen in young P. pubescens was less distinct than in mature B. pendula, the daily course with an early morning maximum and minimum in the late afternoon was basically the same. However, due to constant temperature conditions in the climate chamber, the nocturnal rise in \([O_2]\), typically observed under natural conditions, was missing.

The gradual reduction of sap flow provided a quantitative assessment of its effect on oxygen concentrations and flow rates in the sapwood of P. pubescens saplings. Because the day/night temperature regime variation was nearly constant over the 3 d period from day 6–8 of the experiment, the decrease in daytime \([O_2]\) from 75% (188 μmol O₂ L⁻¹) at unimpeded sap flow to a minimum of 30% (77 μmol O₂ L⁻¹) when sap flow was interrupted could not be attributed to an increase in respiratory oxygen consumption. Rather, this provides evidence that sap flow accounts

### Table 1. Concentrations of molecular oxygen (min – max) dissolved in the sapwood of different tree species cited in literature

<table>
<thead>
<tr>
<th>Tree species</th>
<th>d.b.h. (cm)</th>
<th>Tree height (m)</th>
<th>Tree age (year)</th>
<th>Season</th>
<th>([O_2]) (μmol L⁻¹)</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercus robur</td>
<td>15</td>
<td>10</td>
<td>n.s.</td>
<td>May – Sept</td>
<td>68 (Jul)—231 (May)</td>
<td>Eklund (1993)</td>
</tr>
<tr>
<td>Betula pendula</td>
<td>10</td>
<td>8</td>
<td>n.s.</td>
<td>Mar – Apr</td>
<td>71–171</td>
<td>Gansert et al. (2001)</td>
</tr>
<tr>
<td>Laurus nobilis</td>
<td>7 (stem base)</td>
<td>21</td>
<td></td>
<td>Dec</td>
<td>172–258</td>
<td>Del Hierro et al. (2002)</td>
</tr>
<tr>
<td>Olea europaea</td>
<td>1.5–1.8</td>
<td></td>
<td>4</td>
<td>Apr</td>
<td>28–100</td>
<td>Mancuso &amp; Marras (2003)</td>
</tr>
</tbody>
</table>

d.b.h., diameter at breast height. For compatibility, the data presented by the authors were corrected for an aqueous solution saturated with air at \( T = 20 °C \) and atmospheric pressure (285 μmol O₂ L⁻¹).
for some 60% of the oxygen concentration in the sapwood. Undoubtedly, sap flow rate, oxygen loading of xylem sap and oxygen withdrawal from it by oxidative respiration of living cells of the shoot, are factors which synergistically influence the extent of oxygen available from the aqueous transport path. The observed oxygen depletion down to 77 μmol O₂ L⁻¹ when sap flow ceased is comparable with the low value of 42 μmol O₂ L⁻¹ observed in B. pendula during flushing; namely when sap flow was negligible (Gansert et al. 2001). From wood anatomical studies of B. pendula and B. pubescens (Braun 1970; Grossex 1977; Schweinigruber 1978) it can be assumed that, with the exception of multisieriate rays, the parenchymatous tissues of birch sapwood have little or no contact with the intercellular gas-space continuum. The drastic reduction of [O₂] in sapwood have little or no contact with the intercellular gas-spaces of the wood. Rather, it appears to allow the passage of gases preferentially through the intercellular gas-spaces of the rays (Larson 1994).

The oxygen relations in the sapwood of B. pubescens saplings presented here substantiate the importance of sap flow for oxygen supply to the sapwood as has been shown earlier in the field for mature B. pendula (Gansert et al. 2001). It also supports the concept of a dual transport system that supplies wood parenchyma of trees with oxygen via radial gas diffusion and axial flow of oxygen dissolved in the xylem sap as suggested earlier (Hook et al. 1972; Gansert et al. 2001). During daytime, the xylem sap flow appears to be the major path for transport of dissolved oxygen axially through the sapwood. During night-time, when sap flow approximates zero, the gaseous path for O₂ transport, driven by diffusion gradients radially through intercellular gas-spaces prevails (Gansert et al. 2001). The water-saturated cell walls of the apoplast function as the primary absorbing matrix for gaseous oxygen supplied by radial gaseous diffusion. Thus, xylem sap can be enriched with oxygen using the overall surface of the above-ground woody corium as the absorbing area. On the other hand, xylary diffusion namely the axial transport of dissolved oxygen in the xylem sap of tracheids and vessels only by diffusion, can hardly be considered as an efficient pathway for oxygen supply from the roots to the above-ground sapwood when there is no sap flow at night. According to Fick’s second law, movement of oxygen in water over a distance of 1 m driven by diffusion alone takes several years, depending on the concentration gradient, so that this pathway can only be effective over short distances smaller than 100 μm (von Willert et al. 1995).

Respiration of wood parenchyma represents an intrinsic biogenous factor which complicates quantitative estimates of diurnal oxygen supply to the sapwood from measurements of the local [O₂] inside the stem. Respiratory oxygen consumption of parenchymatous tissues of the wood strongly depends on their quantity and actual metabolic conditions with respect to growth, storage or secretion activities. During the day, respiratory oxygen consumption exponentially increases with a rise in temperature, and the reverse pattern is shown at night. Thus, O₂ consumption approximates the daily minimum before dawn. The oxygen status of the sapwood is therefore a resultant of oxygen consumption (O₂ sink) by respiration of wood parenchyma as a function of temperature and metabolic condition, and oxygen supply (O₂ source) via the aqueous and gaseous pathways. The contribution of the aqueous path in the form of xylem sap flow depends above all on canopy transpiration which is a function of light incident on the leaves and the leaf-to-air water vapour pressure deficit (VPD). Gaseous diffusion is driven by concentration, temperature and pressure gradients. Therefore, the diurnal variation of the source–sink relation of oxygen in the sapwood is differentially affected by abiotic variables. The stoichiometrical approach on the relation between CO₂ efflux and O₂ consumption applied here, provides an estimate of oxygen exchange across the stem surface which seems to be physico-chemically coupled with xylem sap flow. Principally, in the absence of bark photosynthesis the stem was never a net source for oxygen. However, enhanced nocturnal O₂ efflux contributed to smaller O₂ deficits in the cuvette atmosphere than during daytime. This observation is in line with a nocturnal increase in [O₂] of xylem sap measured under field conditions in spring and summer (Gansert et al. 2001). Conversely, oxygen deficits higher than those solely due to respiratory oxygen consumption mark the existence of an increasing endogenous oxygen sink as was measured in the sapwood when sap flow was interrupted. These results may indicate that xylem sap flow not only affects the oxygen status of the sapwood itself, but also has an effect on radial oxygen transport across the stem surface. Exposure of mature stems of B. pendula to a hypoxic atmosphere and prevention of bark photosynthesis caused an oxygen efflux.
from the stem which was coupled with xylem sap flow (Gansert & Burgdorf, unpublished). Hence, the cambium of birch species (B. pubescens, B. pendula, and B. ervalantii) is not an impervious sheath, but allows radial fluxes of O2 and CO2 into and out of the stem through the intercellular gas-space continuum.

Although Bailey (1913) clearly recognized the importance of the transpiration stream as an aqueous pathway for gases 90 years ago, the quantitative analysis of the role of xylem sap flow for endogenous source-sink relations of O2 and CO2, and gas exchange between woody plant parts and atmosphere has just begun. The causal understanding of these relations will depend on the success in quantitative differentiation between biogenous processes such as respiration and dark photosynthesis, and physico-chemical processes such as diffusion, solubility and dissociation or flow in the gaseous and aqueous phase in woody parts of arborescent plants.

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