Air pressure in clamp-on leaf chambers: a neglected issue in gas exchange measurements

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Abstract

Air pressure in leaf chambers is thought to affect gas exchange measurements through changes in partial pressure of the air components. However, other effects may come into play when homobaric leaves are measured in which internal lateral gas flow may occur. When there was no pressure difference between the leaf chamber and ambient air (\(\Delta P = 0\)), it was found in previous work that lateral CO\(_2\) diffusion could affect measurements performed with clamp-on leaf chambers. On the other hand, overpressure (\(\Delta P > 0\)) in leaf chambers has been reported to minimize artefacts possibly caused by leaks in chamber sealing. In the present work, net CO\(_2\) exchange rates (NCER) were measured under different \(\Delta P\) values (0.0–3.0 kPa) on heterobaric and homobaric leaves. In heterobarcic leaves which have internal barriers for lateral gas movement, changes in \(\Delta P\) had no significant effect on NCER. For homobaric leaves, effects of \(\Delta P > 0\) on measured NCER were significant, obviously due to lateral gas flux inside the leaf mesophyll. The magnitude of the effect was largely defined by stomatal conductance; when stomata were widely open, the impact of \(\Delta P\) on measured NCER was up to 7 \(\mu\)mol CO\(_2\) m\(^{-2}\) s\(^{-1}\) kPa\(^{-1}\). Since many other factors are also involved, neither \(\Delta P = 0\) nor \(\Delta P > 0\) was found to be the ‘one-size fits all’ solution to avoid erroneous effects of lateral gas transport on measurements with clamp-on leaf chambers.

Keywords: Air pressure, clamp-on leaf chamber, gas exchange measurement, homobaric leaves, respiration, photosynthesis, transpiration.

Introduction

Gas exchange measurements on leaves are routinely performed in physiological studies. Compared with formerly used whole-plant or whole-leaf approaches (Sestak et al., 1971) there has been a substantial shift to the measurement of clamped leaf parts. Today, over 95% of measurements of photosynthetic CO\(_2\) uptake in ISI listed journals have been performed with commercial portable photosynthesis systems using clamp-on leaf chambers (Long et al., 1996; Long and Bernacchi, 2003). This development occurred largely because of technical progress in the miniaturization and computerization of the equipment making in situ field measurements feasible. However, miniaturized clamp-on leaf chambers can cause errors in measurements that depend on the specific anatomy of the leaves (Jahnke and Krewitt, 2002; Pieruschka et al., 2006), and it is proposed here that the gas pressure in such leaf chambers needs to be addressed to evaluate the reliability of the measurements.

There has been a long-lasting debate regarding the frequently reported finding that atmospheric CO\(_2\) concentration, \(c_a\), might directly affect respiration of plants in the dark (Amthor, 1997; Drake et al., 1999). However, recent investigations using a high-resolution dual channel oxygen analyser (Davey et al., 2004) confirmed that the reported instantaneous reduction of respiration after rising CO\(_2\) concentration was probably an experimental artefact. Such artefacts can be caused either by specific technical problems when CO\(_2\) exchange is measured (Jahnke, 2001) or homobaric leaf anatomy (Jahnke and Krewitt, 2002). Homobaric leaves lack bundle-sheath extensions (Neger, 1918) and, dependent on the degree of continuity of the intercellular air spaces, leaf internal lateral gas conductivities can be high (Pieruschka et al., 2005). Lateral gas...
The observed lateral CO₂ transport was due to gas diffusion. When air pressure inside the leaf chamber, \( P_{ai} \), was the same as outside, \( P_a \) (i.e. \( \Delta P = 0 \)), indicating that the observed lateral CO₂ transport due to gas diffusion. 

Commercially available open (flow-through) gas exchange systems are generally equipped with leaf chambers in which air pressure inside the leaf chamber is not actively controlled and is mainly determined by the construction of the system. In systems where the infrared gas analysers (IRGA) for measuring gaseous CO₂ and H₂O are integrated into the leaf chamber, the gas lines between reference cell, leaf chamber, and analyser cell are extremely short; \( \Delta P \) can be considered zero in such systems (Li-Cor Inc., personal communication). In gas exchange systems in which the IRGA cells are connected to the leaf chamber by gas tubings, \( \Delta P \) depends mainly on the flow rate and the flow resistance in the outgoing gas line and can be considerably larger than zero (Walz GmbH, personal communication).

The question whether a positive air pressure in clamp-on leaf chambers may potentially affect gas exchange measurements has been considered for modern gas exchange systems only marginally. Small overpressure in leaf chambers was applied to avoid artefacts due to leakage between chamber gaskets and leaf surfaces (González-Meler and Siedow, 1999; Küppers and Häder, 1999) but, on the other hand, it was stated that insufficient chamber sealing has to be avoided (Sestak et al., 1971; Long and Häggren, 1993; Jahnke, 2001; Pons and Welchen, 2002; Long and Bernacchi, 2003). Williams (1948) observed that mass flow between the two chambers of a double chamber porometer led to artefacts in measurements of stomatal conductance when air pressure in one of the chambers was increased. Meidner (1955) reported on lateral air movement, but limited to distances of 0.5–2.5 mm and, in a review dealing with bulk flow in leaves (Shive, 1980), lateral directions were only marginally noted.

The present work focuses on the question of whether changes in air pressure inside clamp-on leaf chambers might affect the gas exchange of leaves or its measurement. To study possibly different effects of air pressure on gas exchange in the dark and light, both respiration and CO₂ assimilation rates were examined. The apical portion of attached intact homobaric or heterobaric leaves was clamped, and air pressure difference between the leaf chamber and ambient air was manipulated. Also whole leaves were measured to test whether homobaric and heterobaric leaves may show general differences in their response to variable air pressure. The goals of the present work were to examine (i) whether air pressure in leaf chambers affects gas exchange rates of either homobaric or heterobaric leaves; (ii) whether possible pressure-related effects might be different when either respiration or photosynthesis is measured; (iii) which parameters might define the possible effects; and (iv) whether a controlled overpressure can be used to avoid measurement artefacts with clamp-on leaf chambers.

**Fig. 1.** Scheme of the gas exchange system showing the control of air pressure in the leaf chamber. The concentrations of gaseous CO₂ and H₂O were measured by a differential infrared analyser (IRGA), in the incoming (reference cell) and outgoing air (analyser cell). A mass flow meter, MFM, controlled the pressure pump, GP1, by which the gas flow through the system was kept constant. A suction pump, GP2, was controlled using a differential pressure transducer, Pd, by which the pressure difference between the inside and outside of the leaf chamber (\( \Delta P \)) was kept constant at a range of levels; the differences in CO₂ concentration (\( \Delta c \)) were also experimentally altered. For further details of the gas exchange system see Jahnke (2001). \( c_{ai} \), atmospheric CO₂ concentration in ambient air; \( c_{ai} \), atmospheric CO₂ concentration in the incoming (entering) air; \( c_{ai} \), atmospheric CO₂ concentration in the leaf chamber; \( P_a \), leaf chamber gaskets; GC, experimental growth chamber; \( L_m \), leaf area outside the leaf chamber; \( L_m \), leaf area inside the leaf chamber; \( L_m \), leaf area underneath the gaskets; \( L_m \), leaf area inside the leaf chamber; LC, leaf chamber with an inner diameter of 7 cm; \( P_a \), air pressure inside the leaf chamber.

**Materials and methods**

**Plant material**

Plants of *Nicotiana tabacum* L. and *Vicia faba* L. (with homobaric leaves) and *Glycine max* (L.) Merr. and *Phaseolus vulgaris* L. (heterobaric leaves) were grown from seeds in 1.0 l pots in soil (Einheitserde, Typ P; Balster-Feuerfest GmbH, Germany) mixed with perlite (4:1 v:v). The plants were periodically watered with a nutrient solution as previously described (Pieruschka et al., 2005). Growth chambers provided controlled conditions with 14/10 h photoperiod at 400–550 μmol photons m⁻² s⁻¹ (HQI-400 W/D and Krypton lamps; Osram, München, Germany), a temperature of 23/20 °C, and relative humidity of 60/70%. Plants of *Pulmonaria officinalis* L. (homobaric leaves) were from the Botanical Garden, Universität Duisburg-Essen, potted at least 2 weeks before starting an experiment and kept in the growth cabinet as well.

**Gas exchange system**

Gas exchange measurements were performed with an open gas exchange system previously described by Jahnke (2001). The incoming and outgoing gaseous CO₂/H₂O concentrations were measured by a differential infrared gas analyser (IRGA; LI-7000, Li-Cor Biosciences GmbH, Bad Homburg, Germany). The part of the system responsible for the control of air pressure in the leaf chamber is shown in Fig. 1. The incoming gas flow was measured by a mass flow meter, MFM (Tylan FM-360; Millipore, Eschborn, Germany), and kept constant by the control of a pressure pump, GP1 (WISA 300; ASF Thomas Industries, Puchheim, Germany). The pressure difference...
between the leaf chamber and the atmosphere was measured by a pressure transducer, Pd (143PC05D; Honeywell, Offenbach, Germany), and controlled by suction pump, GP2 (WISA 300). Process controllers (Sipart DR20; Siemens AG, Germany) were used for the control circuits (dotted lines in Fig. 1). By changing the electric power of GP2, the pressure difference between the leaf chamber and ambient air ($\Delta P$) was experimentally altered either by distinct pressure steps or continuously along a ramp. The graphical programming language LabVIEW (National Instruments, Austin, Texas, USA) was used in combination with signal conditioning devices (SCXI, Signal Conditioning eXtension for Instrumentation; National Instruments) to operate the gas exchange system either manually or automatically and to control the system components (valves, pumps, etc.) and set points ($CO_2$ concentration, gas flow, air pressure inside the leaf chamber, etc.). Analogue and digital data were acquired, calculated online, and visualized on screen (for details see Jahnke and Proff, 2001).

**Experimental conditions**

The leaf chamber was located in an experimental growth chamber, GC (Fig. 1), with constant environmental conditions of 23.5 ± 0.5 °C and 60 ± 5% RH (VPD = 1.1 kPa). A circular leaf chamber with an inner diameter of 7 cm (Jahnke, 2001) was used and the pressure treatments were started after net CO$_2$ exchange rates (NCER) were stable. In the experiments, either a whole leaf was enclosed in the chamber or only the apical part of the leaf was clamped (Fig. 1). After measurement was finished, the leaf part inside the leaf chamber was cut along the inner edge of the sealing, scanned, and the area determined by using the software Scion Image (Scion Image Beta 4.03, Scion Corporation, Frederick, Maryland, USA, http://www.scioncorp.com). The leaf area enclosed in the leaf chamber of the different investigated plant species ranged between 10 and 35 cm$^2$. NCER in the dark were measured at different $CO_2$ concentrations inside ($c_a$) and outside ($c_c$) the leaf chamber ranging between 350, 700, and 2000 μl 1$^{-1}$. The resulting gradients in $CO_2$ concentration between the leaf chamber and ambient air ($\Delta c = c_a - c_c$) were 350 or 1650 μl 1$^{-1}$ with either positive or negative values denoting the direction of the diffusion gradient. The temperature inside the leaf chamber was 23.0 ± 0.5 °C and leaf temperature was 0–0.5 °C lower depending on transpiration rates; the plants were kept in darkness for approximately 36 h before starting an experiment in order to measure constant maintenance respiration (Jahnke, 2001; Penning de Vries, 1975). Under the light conditions, photosynthetic NCER (=A) was measured at a PPFD of 700 μmol photons m$^{-2}$ s$^{-1}$ and $\Delta c = 0$ (with $c_a = c_c = 350$ μl 1$^{-1}$) if not differently stated; in these experiments, the temperature inside the leaf chamber ranged between 24.0 and 25.5 °C and leaf temperature between 23.5 and 25.9 °C depending on transpiration rates which differed due to shortage in water supply to the investigated plants. Measurements of A were performed on plants with increasing drought stress by stopping irrigation for 1–3 d which caused a decline in stomatal conductance to $CO_2$, $g_{leaf,c}$, over time.

**Calculations and statistical analysis**

Gas exchange data were strictly plotted as net $CO_2$ exchange rates (NCER) where negative values of NCER indicate rates of respiration in the dark (for convenience termed R in the text) while positive NCER values indicate net $CO_2$ assimilation rates (A). This is to avoid confusion due to changes in the algebraic sign of measured (apparent) NCER observed under the various experimental treatments (as in Fig. 2B). Calculation of NCER was as previously described (Jahnke, 2001). The $CO_2$ concentration entering the leaf chamber, $c_{a,e}$, was measured under atmospheric pressure while the $CO_2$ concentration inside the chamber, $c_{a,i}$, was calculated considering the chamber pressure, $P_{a,i}$ (Fig. 1). The impact of changes in $\Delta P$ on photosynthesis ($\Delta A/\Delta P$; μmol $CO_2$ m$^{-2}$ s$^{-1}$ kPa$^{-1}$) was either directly measured or calculated according to the model proposed by Farquhar et al. (1980) assuming Rubisco limited conditions. The following parameters were used for the model: intercellular $CO_2$ concentrations, $c_i$, were calculated according to von Caemmerer and Farquhar (1981) from experiments with whole leaves either under ambient pressure, i.e. $\Delta P = 0$, or $\Delta P = 1.0$ kPa; $V_{c,max}$ of 74.6 and 66.3 μmol $CO_2$ m$^{-2}$ s$^{-1}$, and respiration in the light of 0.7 and 1.0 μmol $CO_2$ m$^{-2}$ s$^{-1}$ for $V. faba$ and $G. max$, respectively, were calculated from $A/c_i$ curves (von Caemmerer, 2000) performed under ambient $O_2$ concentration (21%) (data not shown); the Michaelis–Menten constants for Rubisco carboxylation, $K_c$ (404 μbar), and oxygenation, $K_o$ (247 μbar), as well as the $CO_2$ compensation point in absence of mitochondrial respiration, $\Gamma^*$ (37 μbar), were taken from von Caemmerer (2000; Table 2.3).

The impact of air pressure on transpiration under isothermic conditions can easily be calculated. The intercellular air space of leaves is generally considered to be saturated with water vapour pressure, WVP$_{leaf}$, which is independent of air pressure changes under constant temperature and was calculated according to Goff and Gratch (1946). However, an increase in pressure increases the water...
vapour pressure of the (non-saturated) ambient air, WVP<sub>air</sub>, thus reducing the leaf-to-air water vapour pressure deficit, VPD=WVP<sub>leaf</sub>-WVP<sub>air</sub> (Gale, 1972b). This reduction is proportional to the air pressure, and the impact of pressure on transpiration (E) was calculated as:

\[ E = \frac{g_{leaf} \cdot VPD}{P_{\text{ref}}} \]  

(1)

with \( P_{\text{ref}} \), the air pressure inside the leaf chamber and, \( g_{\text{leaf}} \), stomatal conductance to water vapour obtained before the pressure treatment. No significant differences between the stomatal conductance obtained before and after the pressure treatment were observed.

When CO<sub>2</sub> concentration and atmospheric pressure were the same in the leaf chamber and ambient air (\( \Delta c=0 \) and \( \Delta P=0 \)), the resulting NCER<sub>ref</sub> or \( E_{\text{ref}} \) were regarded as references and compared with the NCER or \( E \) values measured for \( \Delta P \neq 0 \). Pressure-related changes in \( A \) and \( E \) were then calculated as \( \Delta A/\Delta P=(\text{NCER}–\text{NCER}_{\text{ref}})/\Delta P \) and \( \Delta E/\Delta P=(E–E_{\text{ref}})/\Delta P \), respectively. Comparisons were made using analysis of variance (ANOVA) with the threshold of significance being \( P<0.05 \) and performed using SigmaStat (Version 2.03; SPSS GmbH Software, München, Germany).

**Results**

Respiration rates measured under different air pressure on partially clamped leaves

Respiration rates, \( R \), were measured in the dark on the apical portion of homobaric V. faba leaves while different CO<sub>2</sub> concentrations inside, \( c_{a,i} \), and outside, \( c_{a} \), of the leaf chamber were applied (Fig. 1). The experiment shown in Fig. 2 started, step 1, at low \( c_{a,i} \) and \( c_{a} \) (350 μl l<sup>-1</sup> and \( \Delta c=0 \); Fig. 2A); step 2, \( c_{a,i} \) was increased to 2000 μl l<sup>-1</sup> while \( c_{a} \) remained low (\( \Delta c=1650 \mu l^{-1} \)); step 3, both \( c_{a,i} \) and \( c_{a} \) were high at 2000 μl l<sup>-1</sup> (\( \Delta c=0 \)); step 4, \( c_{a,i} \) was lowered while \( c_{a} \) was kept high (\( \Delta c=–1650 \mu l^{-1} \)); step 5, the starting conditions were re-established with low \( c_{a,i} \) and \( c_{a} \) (\( \Delta c=0 \)). This protocol was identical with that previously presented for N. tabacum (Jahnke and Krewitt, 2002). The measured NCER (negative values indicate \( R \)), at \( c_{a,i}=c_{a}=350 \mu l l^{-1} \) and \( \Delta c=0 \) (steps 1 and 5) were regarded as references data through which regression lines were drawn (solid lines in Fig. 2B, C). When air pressure inside and outside the leaf chamber was similar (Fig. 2B; \( \Delta P=0 \) kPa), measured \( R \) were significantly affected for \( c_{a,i}>c_{a} \) (\( P=0.0002 \), \( n=5 \)); note that for \( c_{a,i}>c_{a} \) in step 2 the positive values of measured NCER indicated an apparent CO<sub>2</sub> uptake in the dark. When a positive pressure of 2.0 kPa (Fig. 2C) was provided inside the leaf chamber, no significant influence of changes in \( \Delta c \) on measured \( R \) was observed (\( P=0.837 \), \( n=7 \)).

These initial results were the starting point to study the impact of altered air pressure inside the clamp-on leaf chamber on measured \( R \) in more detail. The pressure inside the leaf chamber was stepwise increased resulting in \( \Delta P \) values of 0.0, 0.3, 0.6, 1.2, and 2.4 kPa (Fig. 3); both \( c_{a,i} \) and \( c_{a} \) were kept low (350 μl l<sup>-1</sup>; \( \Delta c=0 \)) (open circles, dotted lines) or only \( c_{a} \) was raised to 2000 μl l<sup>-1</sup> (closed circles, solid lines). Neither the pressure treatments nor changes in \( c_{a} \) significantly affected \( R \) of heterobaric Ph. vulgaris and G. max leaves (Fig. 3A, B). However, for homobaric V. faba and P. officinalis leaves, measured \( R \) showed significant differences when \( \Delta P \) was altered between 0 and 2.4 kPa even when \( \Delta c \) was zero (Fig. 3C, D; open circles) while, for N. tabacum, the differences were not significant (Fig. 3E). However, when \( c_{a,i} \) was increased (2000 μl l<sup>-1</sup>; \( \Delta c=–1650 \mu l^{-1} \)), measured \( R \) showed significant differences for all examined homobaric species.
(Fig. 3C–E; closed circles). Under this treatment, \( R \) was substantially larger (NCER more negative) when \( \Delta c \) and \( \Delta P \) were zero but, for \( \Delta P \approx 0.3 \) kPa, the differences in \( R \) measured at either \( \Delta c = 0 \) or \( \Delta c \neq 0 \) disappeared (Fig. 3C–E). The impact of air pressure on \( R \) under different \( \Delta c \) values was also studied under a continuous increase in air pressure between 0.0 and 1.0 kPa with a slope of 0.05 kPa min\(^{-1}\) (Fig. 4A, B). First, a whole \( V. faba \) leaflet was enclosed in the leaf chamber (closed circles) and, afterwards, only the apical part of the same leaflet was measured (open circles). In these experiments, the applied \( CO_2 \) gradients were smaller than in the previous experiments. For \( c_{a,i} = 350 \mu l\) l\(^{-1}\) and \( c_{a} = 700 \mu l\) l\(^{-1}\) (\( \Delta c = -350 \mu l\) l\(^{-1}\)), measured \( R \) of the clamped leaf part was larger than \( R \) of the whole leaf at \( \Delta P = 0 \) but, when \( \Delta P \) reached values of approximately 0.3 kPa, the differences disappeared (Fig. 4A). Reversing the \( \Delta c \) gradient caused the opposite response and the differences in \( R \) measured on either whole leaves or clamped leaf parts disappeared at \( \Delta P > \approx 0.3 \) kPa (Fig. 4B). When respiration rates were obtained at \( \Delta P \) values between 0.6 and 2.0 kPa and various combinations of \( c_{a} \) and \( c_{a,i} \) between 350 and 1050 \( \mu l\) l\(^{-1}\), no significant differences between measurements on whole leaves or leaf parts were observed (\( P > 0.05 \), \( n = 14 \); data not shown).

**Net \( CO_2 \) assimilation rates measured under different air pressure on whole leaves**

To examine general effects of air pressure on net \( CO_2 \) assimilation rates (\( A \)) of heterobaric and homobaric leaves, experiments were performed on whole leaves. When a homobaric \( V. faba \) leaf was measured at ambient pressure (\( \Delta P = 0 \); Fig. 5A) and the incoming \( CO_2 \) concentration (\( c_{a,e} \)) was set to 357.7 ± 0.2 \( \mu l\) l\(^{-1}\) (Fig. 5B, closed circles; step 1), measured \( A \) (positive NCER values) was 10.5 ± 0.44 \( \mu l\) CO\(_2\) m\(^{-2}\) s\(^{-1}\) (Fig. 5C); in step 2, \( A \) increased significantly to 11.0 ± 0.05 \( \mu l\) CO\(_2\) m\(^{-2}\) s\(^{-1}\) when \( \Delta P \) was raised by 3.0 kPa causing an increase in \( c_{a,i} \) to a calculated value of 368.6 ± 0.3 \( \mu l\) l\(^{-1}\); in step 3, \( c_{a,e} \) was reduced to 347.2 ± 0.2 \( \mu l\) l\(^{-1}\) (while \( \Delta P \) was still 3.0 kPa) causing a drop in \( c_{a,i} \) and \( A \) to values almost identical with those of step 1 when \( \Delta P \) was zero (Fig. 5B, C). The pressure-related changes in \( A \) (Fig. 6; \( \Delta A/\Delta P \), \( \mu l\) CO\(_2\) m\(^{-2}\) s\(^{-1}\) kPa\(^{-1}\)) were measured and compared with calculated values. The mean \( c_{a,i} \) values applied at \( \Delta P = 0 \) for both heterobaric and homobaric leaves ranged between 350 and 360 \( \mu l\) l\(^{-1}\) and the corresponding leaf internal \( CO_2 \) concentrations, \( c_{i} \), were 268.6 ± 13.0 \( \mu l\) l\(^{-1}\) for \( G. max \) and 256.1 ± 12.9 \( \mu l\) l\(^{-1}\) for \( V. faba \). These \( c_{i} \) values were used to calculate the impact of overpressure on \( A \) according to the model of Farquhar et al. (1980). Measured and calculated values of the pressure-related increase in \( A \) were not significantly different: 0.13 ± 0.04 and 0.13 \( \mu l\) CO\(_2\) m\(^{-2}\) s\(^{-1}\) kPa\(^{-1}\), respectively, for \( G. max \) (\( P = 0.819 \), \( n = 14 \)) and 0.14 ± 0.09 and 0.15 \( \mu l\) CO\(_2\) m\(^{-2}\) s\(^{-1}\) kPa\(^{-1}\), respectively, for \( V. faba \) (\( P = 0.345 \), \( n = 15 \)) (Fig. 6).

**Net \( CO_2 \) assimilation rates and transpiration rates measured under different air pressure on partially clamped leaves**

On clamped leaf parts, \( A \) was measured while air pressure inside the leaf chamber was increased between 0.0 and 3.0 kPa with a slope of 0.1 kPa min\(^{-1}\) (Fig. 7A, B). For heterobaric \( G. max \) leaves, a small linear increase in \( A \) with rising pressure was observed (Fig. 7A) corresponding to the Farquhar et al. (1980) model and the whole leaves results (Fig. 6). However, when a homobaric \( V. faba \) leaf was clamped, a substantial decrease in \( A \) was observed (Fig. 7B). This response was found to be dependent on \( g_{leaf,c} \) therefore measurements were performed on partially clamped \( V. faba \) leaves under increasing drought stress indicated by decreasing \( g_{leaf,c} \) values. The impact of overpressure in the clamp-on leaf chamber on measured \( A \) (\( \Delta A/\Delta P \)) diminished when stomata closed (Fig. 7C); note that the \( \Delta A/\Delta P \) values were negative and not positive as for the whole leaves (Fig. 6). For low \( g_{leaf,c} \), positive pressure in the clamp-on leaf chamber had only little effect on measured \( A \), but the impact was large when \( g_{leaf,c} \) was high: a decrease in \( A \) up to
rate, conductance. The pressure-related change in transpiration was dependent on stomatal activity (Fig. 8B; compare open and closed circles) in all replicates (Fig. 8C). The observed artefacts in respiration measurements due to lateral gas diffusion (Fig. 2B) seemed to be eliminated when homobaric leaves are measured with clamp-on leaf chambers. The assumption may not hold true. When respiration rates were measured in such studies using clamp-on leaf chambers on homobaric leaves of *N. tabacum* (Jahnke and Krewitt, 2002) or *V. faba* (Fig. 2B), the obtained gas exchange rates proved erroneous on a case-by-case basis. These experiments were performed under conditions in which the air pressure in the leaf chamber and ambient air were similar (*ΔP*=0). For *Δc*=0, the absolute CO2 concentration did not affect measured NCER but, for *Δc*≠0, clear effects were observed (e.g. Fig. 2B). Homobaric leaves have internal channels in which gas molecules can easily diffuse in lateral directions (Pieruschka et al., 2005) and, thus, the question arises whether this can be overlaid by a leaf internal air flow driven by *ΔP*.

The observed artefacts in respiration measurements due to lateral gas diffusion (Fig. 2B) seemed to be eliminated when *ΔP*=2 kPa was applied to a clamp-on leaf chamber (Fig. 2C). This led to the assumption that already a smaller overpressure might alleviate measurement problems on...
homobaric leaves and deliver the ‘true’ NCER independent of Δc. But even when Δc is zero, chamber pressure may affect measured NCER (Fig. 3C, D, open circles) which can be explained by regarding three different areas of a clamped leaf: the area inside the chamber, L_i (Fig. 1), the part outside the chamber, L_o, and the area covered by the gaskets, L_g. In the dark, respiration takes place in all leaf parts and the released CO₂ may escape through the stomata. This vertical path, however, is blocked under the gaskets: respired CO₂ must then accumulate within L_g or may laterally escape when there are open channels in the mesophyll. Some fraction of the CO₂ in L_g would then diffuse into leaf part L_i (Fig. 1) and artificially increase the measured respiration rate. The observed artefacts due to lateral CO₂ diffusion (at either Δc=0 or Δc≠0) were apparently attenuated by an overpressure in the leaf chamber; a ΔP of approximately 0.3 kPa was sufficient to compensate for lateral CO₂ diffusion obviously due to a pressure driven gas flux from the inside to the outside of the chamber (Fig. 3C, D). A minor but continued decrease in measured respiration rates was observed for ΔP even above 0.5 kPa (Figs 3C, D, 4) probably due to a reduction of the ‘effective surface area’ discussed later on in the context of Fig. 7B.

The partial pressure of air components such as CO₂ or H₂O is affected by atmospheric air pressure and may influence the photosynthetic activity of a leaf. The concentration of a given gas species in the liquid phase is proportional to its partial pressure in the gas phase (Henry’s law; Nobel, 1991). When the CO₂ concentration is homogenous throughout the intercellular air space, an
increase in air pressure would increase the molar fraction in the liquid phase, causing higher uptake of CO$_2$ (Terasnna et al., 1995) as described in the model of Farquhar et al. (1980). Thus, $A$ was simply dependent on CO$_2$ partial pressure in both heterobaric and homobaric leaves when whole leaves were measured (Fig. 6). Also with a clamp-on leaf chamber, a small increase in $A$ with raising $\Delta P$ was observed for heterobaric $G$. max leaves (Fig. 7A); homobaric $V$. faba leaves, however, showed a substantial decrease in $A$ which was dependent on stomatal conductance (Fig. 7B, C). For a given $g_{\text{leaf,c}}$, lateral air flow inside the leaf is proportional to the externally applied pressure gradient ($\Delta P$) when lateral conductance remains constant. Lateral air flow in the leaf mesophyll from the inner to the outer side of a leaf chamber might impair diffusive gas flow and, close to the gaskets where local gradients in $\Delta P$ are highest, this effect should be larger for the centre of the leaf chamber. Consequently, an overpressure can be considered actually to reduce the effective surface area in a clamp-on leaf chamber. When $\Delta P$ increases, pressure-driven air flow incorporates additional stomata which would further reduce the effective surface area inside the leaf chamber ($L_{\text{eff}} < L_i$; Fig. 1) and, consequently, apparent $A$. This is supported by the observation that at high stomatal conductance, even small $\Delta P$ values can cause substantial errors in measurement of net photosynthesis (Fig. 7C). The similar but minor decrease in respiration rate with increasing overpressure (Fig. 3C–E) was obviously due to the low stomatal conductance in the dark.

Effects of air pressure on transpiration have been mostly studied under low atmospheric pressure, for example, at high altitudes (Gale, 1972b; Körner, 1999). Under isothermal conditions, a decrease in air pressure may enhance $E$ by increasing the leaf-to-air water vapour pressure gradient as well as the diffusivity of water vapour in the air (Gale, 1972b). Accordingly, overpressure in the leaf chamber caused a decrease in transpiration rates which matched the calculated values in heterobaric leaves but, in homobaric leaves, they were smaller than calculated (Fig. 8A, B). This reduction in measured $E$ can also be explained by lateral air flow through the leaf by which vertical diffusive exchange of water vapour is diminished due to a reduction in the effective surface area as discussed before.

Both $A$ and $E$ are needed to interpret gas exchange measurements, for example, the CO$_2$ dependence of photosynthesis (von Caemmerer and Farquhar, 1981; Long and Bernacchi, 2003). The presented results show that the air pressure in clamp-on leaf chambers can affect the measurement of $A$ and $E$ and, when collected under conditions where stomata are widely open, $\Delta P > 0$ may lead to erroneous results. Unfortunately, no ‘one-size fits all’ solution was found to avoid possible problems when using clamp-on leaf chambers. At low stomatal conductance, lateral gas flow driven by pressure differences between the leaf chamber and ambient air is only minor (Figs 7C, 8C) because of the high flow resistance of the stomata. Lateral CO$_2$ exchange is then mainly by diffusion and, when CO$_2$ concentrations inside and outside the leaf chamber are different ($\Delta c \neq 0$), a pressure difference near zero ($\Delta P = 0$) can be the worst case in terms of potential measurement artefacts (Figs 2–4; Jahnke and Krewitt, 2002). On the other hand, when stomata are open, even a small overpressure in the chamber can substantially impair measurement accuracy. With $\Delta P$ as small as 0.14 kPa (=1.4 mbar), $A$ may be reduced by 1 μmol CO$_2$ m$^{-2}$ s$^{-1}$ under high $g_{\text{leaf,c}}$ as calculated from the data shown in Fig. 7C which would cause an error of 10% when the net photosynthesis is $\sim$10 μmol CO$_2$ m$^{-2}$ s$^{-1}$. This rough calculation may even be an underestimate since the chamber gaskets covered only a small apical segment of the enclosed leaf (Fig. 1). When small leaf chambers are completely filled, the edge-to-area ratios are much bigger (Long and Bernacchi, 2003) and pressure-related effects must be larger. The impact of lateral gas flux (diffusive and/or pressure driven) on gas exchange is rather complex with regard to the highly variable magnitude of the potential effects on measured gas exchange rates. Factors that may contribute to the impact of lateral gas movement are (i) the size of the clamp-on leaf chamber defining the edge-to-area ratio of the enclosed leaf (the smaller the chamber the bigger potential edge effects); (ii) the difference in CO$_2$ concentration between the leaf chamber and the ambient air; (iii) the difference in air pressure between the leaf chamber and the ambient air; (iv) the stomatal conductance; and (v) the lateral gas conductivity of the leaf mesophyll clamped underneath the gaskets.

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