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 (ΔP=0), it was found in previous work that lateral CO2 diffusion could affect measurements performed with clamp-on leaf chambers. On the other hand, overpressure (ΔP>0) in leaf chambers has been reported to minimize artefacts possibly caused by leaks in chamber sealing. In the present work, net CO2 exchange rates (NCER) were measured under different ΔP values (0.0–3.0 kPa) on heterobaric and homobaric leaves. In heterobaric leaves which have internal barriers for lateral gas movement, changes in ΔP had no significant effect on NCER. For homobaric leaves, effects of ΔP>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 ΔP on measured NCER was up to 7 μmol CO2 m⁻² s⁻¹ kPa⁻¹. Since many other factors are also involved, neither ΔP=0 nor ΔP>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.

Key words: 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 CO2 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 (Jahneke 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 CO2 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 CO2 concentration was probably an experimental artefact. Such artefacts can be caused either by specific technical problems when CO2 exchange is measured (Jahneke, 2001) or homobaric leaf anatomy (Jahneke 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
movement in homobaric leaves over distances of more than 8 mm (larger than the width of common leaf chamber gaskets) can affect the measurement of respiration in the dark (Jahnke and Krewitt, 2002). The results were obtained when air pressure inside the leaf chamber, \( P_{a,i} \), was the same as outside, \( P_{a} \) (i.e. \( \Delta P=0 \); Fig. 1) indicating that the observed lateral CO₂ transport was 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 Hällgren, 1993; Jahnke, 2001; Pons and Welschen, 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.

**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 (Piersuschka et al., 2005). Growth chambers provided controlled conditions with 14/10 h photoperiod at 400–550 μmol photons m⁻² s⁻¹ (HQL-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 (Tytan FM-360; Millipore, Eschborn, Germany), and kept constant at a range of levels; the differences in CO₂ concentration (\( \Delta c \)) were experimentally altered. For further details of the gas exchange system see Jahnke (2001). \( c_{a} \), atmospheric CO₂ concentration in ambient air; \( c_{a,e} \), atmospheric CO₂ concentration in the incoming (entering) air; \( c_{a,i} \), atmospheric CO₂ concentration in the leaf chamber; \( G \), leaf chamber gaskets; \( GC \), experimental growth chamber; \( L_a \), leaf area underneath the leaf chamber; \( L_1 \), leaf area inside the leaf chamber; \( L_a \), leaf chamber with an inner diameter of 7 cm; \( P_{a} \), air pressure inside the leaf chamber.
between the leaf chamber and the atmosphere was measured by a pressure transducer, Pd (143P05D; 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 (ΔP) 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 (CO₂ 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 CO₂ 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 cm². NCER in the dark were measured at different CO₂ concentrations inside (c_a,i) and outside (c_a) the leaf chamber ranging between 350, 700, and 2000 µl l⁻¹. The resulting gradients in CO₂ concentration between the leaf chamber and ambient air (Δc = c_a,i – c_a) were 350 or 1650 µl l⁻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 Δc=0 (with c_a,i=c_a=350 µl l⁻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 CO₂, gs, over time.

**Calculations and statistical analysis**

Gas exchange data were strictly plotted as net CO₂ 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 CO₂ 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 CO₂ concentration entering the leaf chamber, c_a, was measured under atmospheric pressure while the CO₂ concentration inside the chamber, c_a,i, was calculated considering the chamber pressure, P_a,i (Fig. 1). The impact of changes in ΔP on photosynthesis (ΔA/ΔP; µmol CO₂ 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 CO₂ concentrations, c_i, were calculated according to von Caemmerer and Farquhar (1981) from experiments with whole leaves either under ambient pressure, i.e. ΔP=0, or ΔP=1.0 kPa; V_cmax of 74.6 and 66.3 µmol CO₂ m⁻² s⁻¹, and respiration in the light of 0.7 and 1.0 µmol CO₂ m⁻² s⁻¹ for V. faba and G. max, respectively, were calculated from A/c_i curves (von Caemmerer, 2000) performed under ambient CO₂ 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 CO₂ compensation point in absence of mitochondrial respiration, P_s (37 µbar), were taken from von Caemmerer (2000; Table 2.3).

The impact of air pressure on transpiration under isothermal conditions can easily be calculated. The intercellular air space of leaves is generally considered to be saturated with water vapour pressure, WVPleaf, 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, \( WVP_{\text{air}} \), thus reducing the leaf-to-air water vapour pressure deficit, \( VPD = WVP_{\text{leaf}} - WVP_{\text{air}} \) (Gale, 1972b). This reduction is proportional to the air pressure, and the impact of pressure on transpiration (\( E \)) was calculated as:

\[
E = \frac{g_{\text{leaf}} - \text{VPD}}{P_{\text{a}}} 
\]

with \( P_{\text{a}} \), 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 CO2 concentration and atmospheric pressure were the same in the leaf chamber and ambient air (\( \Delta c = 0 \) and \( \Delta P = 0 \)), the resulting \( \text{NCER}_{\text{ref}} \) or \( E_{\text{ref}} \) were regarded as references and compared with the \( \text{NCER} \) or \( E \) values measured for \( \Delta P \neq 0 \). Pressure-related changes in \( A \) and \( E \) were then calculated as \( \Delta A/\Delta P = (\text{NCER}_{\text{ref}} - \text{NCER})/\Delta P \) 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 CO2 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 \( \mu l \) l\(^{-1} \)) and \( \Delta c = 0 \); Fig. 2A); step 2, \( c_{a,i} \) was increased to 2000 \( \mu l \) l\(^{-1} \) while \( c_a \) remained low (\( \Delta c = 1650 \) \( \mu l \) l\(^{-1} \)); step 3, both \( c_{a,i} \) and \( c_a \) were high at 2000 \( \mu l \) l\(^{-1} \) (\( \Delta c = 0 \)); step 4, \( c_{a,i} \) was lowered while \( c_a \) was kept high (\( \Delta c = -1650 \) \( \mu l \) 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 \( \text{NCER} \) (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 \( \text{NCER} \) indicated an apparent CO2 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 \( \mu l \) l\(^{-1} \); \( \Delta c = 0 \)) (open circles, dotted lines) or only \( c_a \) was raised to 2000 \( \mu l \) l\(^{-1} \) (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 \( c_a \) was increased (2000 \( \mu l \) l\(^{-1} \); \( \Delta c = -1650 \) \( \mu l \) l\(^{-1} \)), measured \( R \) showed significant differences for all examined homobaric species.
Air pressure in clamp-on leaf chambers 2557

Net CO₂ assimilation rates measured under different air pressure on whole leaves

To examine general effects of air pressure on net CO₂ 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 (ΔP=0; Fig. 5A) and the incoming CO₂ concentration (cₐ,i) was set to 357.7±2.0 μmol m⁻² s⁻¹ (Fig. 5B; closed circles; step 1), measured A (positive NCER values) was 10.5±0.04 μmol CO₂ m⁻² s⁻¹ (Fig. 5C); in step 2, A increased significantly to 11.0±0.05 μmol CO₂ m⁻² s⁻¹ when ΔP was raised by 3.0 kPa causing an increase in cₐ,i to a calculated value of 368.6±0.3 μmol m⁻² s⁻¹; in step 3, cₐ,i was reduced to 347.2±0.2 μmol m⁻² s⁻¹ (while ΔP was still 3.0 kPa) causing a drop in cₐ,i and A to values almost identical with those of step 1 when ΔP was zero (Fig. 5B, C). The pressure-related changes in A (Fig. 5C, ΔA/ΔP; μmol CO₂ m⁻² s⁻¹ kPa⁻¹) were measured and compared with calculated values. The mean cₐ,i values applied at ΔP=0 for both heterobaric and homobaric leaves ranged between 350 and 360 μmol m⁻² s⁻¹ and the corresponding leaf internal CO₂ concentrations, cᵢ, were 268.6±13.0 μmol m⁻² s⁻¹ for G. max and 256.1±12.9 μmol m⁻² s⁻¹ for V. faba. These cᵢ 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 μmol CO₂ m⁻² s⁻¹ kPa⁻¹, respectively, for G. max (P=0.819, n=14) and 0.14±0.09 and 0.15 μmol CO₂ m⁻² s⁻¹ kPa⁻¹, respectively, for V. faba (P=0.345, n=15) (Fig. 6).
rate, conductance. The pressure-related change in transpiration was dependent on stomatal resistance gradients, resulting in gas diffusion independent of the absolute CO2 concentration did not affect measured respiration rates were measured in such studies using 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 CO_2 may escape through the stomata. This vertical path, however, is blocked under the gaskets: respired CO_2 must then accumulate within L_g or may laterally escape when there are open channels in the mesophyll. Some fraction of the CO_2 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 CO_2 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 CO_2 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 CO_2 or H_2O 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 CO_2 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 CO₂ (Terasimah et al., 1995) as described in the model of Farquhar et al. (1980). Thus, A was simply dependent on CO₂ 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 ΔP 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 gₗₑᵃᶠ, lateral air flow inside the leaf is proportional to the externally applied pressure gradient (ΔP) when lateral conductivity 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 ΔP are highest, this effect should be larger than for the centre of a clamped leaf area. Consequently, an overpressure can be considered actually to reduce the effective surface area in a clamp-on leaf chamber. When ΔP increases, pressure-driven air flow incorporates additional stomata which would further reduce the effective surface area inside the leaf chamber (Lₑₐᶠ ≪ Lₑ; Fig. 1) and, consequently, apparent A. This is supported by the observation that at high stomatal conductance, even small ΔP 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 measured 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 CO₂ 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, ΔP>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 CO₂ exchange is then mainly by diffusion and, when CO₂ concentrations inside and outside the leaf chamber are different (ΔC≠0), a pressure difference near zero (ΔP=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 ΔP as small as 0.14 kPa (=1.4 mbar), A may be reduced by 1 μmol CO₂ m⁻² s⁻¹ under high gₗₑᵃᶠ as calculated from the data shown in Fig. 7C which would cause an error of 10% when the net photosynthesis is ~10 μmol CO₂ 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 part (the smaller the chamber the bigger potential edge effects); (ii) the difference in CO₂ 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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