Lateral diffusion of CO_2 from shaded to illuminated leaf parts affects photosynthesis inside homobaric leaves

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Summary

• Gas exchange is generally regarded to occur between the leaf interior and ambient air, i.e. in vertical (anticlinal) directions of leaf blades. However, inside homobaric leaves, gas movement occurs also in lateral directions. The aim of the present study was to ascertain whether lateral CO_2 diffusion affects leaf photosynthesis when illuminated leaves are partially shaded.

• Measurements using gas exchange and chlorophyll fluorescence imaging techniques were performed on homobaric leaves of *Vicia faba* and *Nicotiana tabacum* or on heterobaric leaves of *Glycine max* and *Phaseolus vulgaris*.

• For homobaric leaves, gas exchange inside a clamp-on leaf chamber was affected by shading the leaf outside the chamber. The quantum yield of photosystem II (Φ_{PSII}) was highest directly adjacent to a light/shade border (LSB). Φ_{PSII} decreased in the illuminated leaf parts with distance from the LSB, while the opposite was observed for nonphotochemical quenching. These effects became most pronounced at low stomatal conductance. They were not observed in heterobaric leaves.

• The results suggest that plants with homobaric leaves can benefit from lateral CO_2 flux, in particular when stomata are closed (e.g. under drought stress). This may enhance photosynthetic, instead of nonphotochemical, processes near LSBs in such leaves and reduce the photoinhibitory effects of excess light.

Key words: chlorophyll fluorescence imaging, homobaric leaves, lateral CO_2 flux, light/shade border (LSB), photosynthesis, quantum yield, stomatal conductance, water use efficiency.

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Introduction

Carbon dioxide is trapped mainly by the mesophyll cells of leaves, where it is converted into organic compounds by photosynthesis. This creates the driving force for the subsequent delivery of CO_2 into leaves from the ambient air (Nobel, 1991). The gradient in CO_2 concentration determines both the direction and the rate of net CO_2 transport. Consequently, gas exchange is generally studied in vertical (anticlinal) directions of leaves, including boundary layers, stomata, intercellular air spaces, cell walls, membranes and liquid phases of mesophyll cells and, finally, the envelope and matrix of chloroplasts (Parkhurst, 1994).

In addition to this vertical transport, CO_2 can also move in lateral (paradermal) directions through the intercellular air spaces of leaves. Lateral gradients in CO_2 concentration are needed, as well as the absence of barriers, to enable internal lateral gas movement. This is a trait of homobaric leaves in which intercellular air spaces are laterally interconnected. Heterobaric leaves, on the other hand, have bundle sheath extensions that span the gap between the upper and the lower epidermis, forming physical barriers for lateral gaseous diffusion (Neger, 1918). Jahnke & Krewitt (2002) demonstrated that the lateral diffusion of CO_2 inside homobaric leaves is effective over distances of at least 8 mm (the width of leaf chamber gaskets) and can cause artefacts in gas exchange

measurements performed using clamp-on leaf chambers. Literature on lateral gas diffusion inside leaves is still rather scarce and, in general, restricted to short diffusion distances. The uneven distribution of closed stomata over a leaf blade may result in different intercellular CO_2 concentrations (c_i) in different mesophyll compartments of heterobaric leaves, causing nonuniformly distributed photosynthesis; this is unlikely to occur in homobaric leaves because of internal lateral CO_2 diffusion (Terashima, 1992). It was found, using high-resolution fluorescence imaging, that the photosynthetic activity of guard cells was influenced by the lateral diffusion of CO_2 when only a small area of the leaf was illuminated; respiration in the surrounding (shaded) area overrode any influence of changes in atmospheric CO_2 concentration (c_a) (Lawson *et al.*, 2002). In addition, recent findings confirm that conductivities of homobaric leaves can be even larger in the lateral than in the vertical (anticlinal) directions (Pieruschka *et al.*, 2005).

In the present work, we posed the question of whether lateral CO_2 exchange inside leaves could play a role in partially shaded leaves. Generally, even under sunny conditions, only the outermost leaves of a canopy are fully exposed to light, while the others are at least partly or temporarily shaded. Only the upper five 'layers' of a canopy are above light compensation while, in lower layers, respiration may exceed assimilation (Nobel *et al.*, 1993). In particular, understory plants demonstrate an extreme example where light fluctuations over time contribute largely to the carbon gain of the plants (Pearcy & Pfitsch, 1994; Pearcy *et al.*, 1996). However, not only temporal fluctuations, but also spatial heterogeneities in the distribution of light, may contribute to net carbon gain; when some parts of a leaf are illuminated and others are shaded, internal lateral gradients in CO_2 concentration can develop across light/shade borders (LSB). Shaded leaf sections may then act as an internal CO_2 source, whereas illuminated areas are sinks for CO_2 owing to photosynthetic activity. Shading or illuminating a leaf outside a clamp-on leaf chamber might then influence the leaf internal CO_2 concentration gradients in lateral directions and alter the net CO_2 exchange rates (NCER) of the leaf part enclosed in the leaf chamber. Furthermore, the leaf chamber gaskets of a leaf chamber artificially seal the stomata, and (respiratory) CO_2 released underneath has to escape laterally. Such artificial closure of stomata, together with partial shading of a leaf, was simulated here by fixing a nontranslucent and gas-impermeable tape on both sides of a leaf. The effect of laterally diffusing CO_2 was then visualized in the adjacent illuminated leaf areas by using chlorophyll fluorescence imaging, which is widely applied to measure heterogeneities in leaf photosynthesis (Oxborough, 2004).

Photosynthesis is progressively diminished under drought stress, while the exact mechanisms of this reduction are still under debate (Pankovic *et al.*, 1999; Medrano *et al.*, 2002a; Parry *et al.*, 2002; Tezara *et al.*, 2002; Kitao *et al.*, 2003). However, it has been found recently that stomatal conductance represents an integrative basis for the overall effects of drought, and photosynthetic responses are understood to be a direct adjustment of photosynthetic metabolism to CO_2 availability (Flexas *et al.*, 2002; Medrano *et al.*, 2002b; Bota *et al.*, 2004). Low intercellular CO_2 concentrations occurring under stomatal closure may cause light stress, even at low light intensities (Long *et al.*, 1994; Ort & Baker, 2002). To avoid excess light, plants have developed different mechanisms. For example, changes in leaf orientation, relative to direct solar irradiance, affect the amount of light absorbed by a leaf and, consequently, photosynthetic activity, transpiration rate and temperature (Cornic & Massacci, 1996). In order to protect the photosynthetic apparatus from photoinhibitory damage, excess light energy is consumed by photorespiration in C_3 plants (Osmond *et al.*, 1997; Wingler *et al.*, 1999; Ort, 2001; Cornic & Fresneau, 2002; Medrano *et al.*, 2002a; Ort & Baker, 2002) and thermal energy dissipation of absorbed light is associated with the light-induced formation of zeaxanthin (Demmig-Adams & Adams III, 1992; Horton *et al.*, 1996). Heat dissipation may also provide tolerance to rapidly fluctuating excitation pressure (Külheim *et al.*, 2002). When stomatal conductance decreases at an advanced stage of drought stress, down-regulation of photosystem II activity was observed, resulting in reduced electron transport rates and an increase in thermal energy dissipation (Flexas *et al.*, 2002; Medrano *et al.*, 2002b; Omasa & Takayama, 2003; Souza *et al.*, 2004), which may be mediated by cycling electron transport (Cornic *et al.*, 2000; Golding & Johnson, 2003).

We hypothesize that lateral CO_2 fluxes inside homobaric leaves have beneficial effects under conditions when parts of the leaves are under high light while others are shaded: partial shading of a leaf may cause lateral CO_2 diffusion to adjacent illuminated leaf areas, thereby enhancing overall leaf photosynthesis. This must be especially important in plants under drought stress where stomata are largely closed and CO_2 uptake from ambient air is hindered. Refixation of respiratory CO_2, supplied from shaded (remote) parts inside a leaf, can then help to increase photosynthetic efficiency in illuminated areas and attenuate the effects of drought stress by reducing potential damage of the photosynthetic apparatus arising from overexcitation. The goal of the present work was to test whether the hypothesized effect of lateral CO_2 diffusion on photosynthesis across LSBs does exist and to evaluate whether it provides an appreciable contribution to leaf photosynthesis.

Materials and Methods

Plant material

Plants of *Glycine max* (L.) Merr. cv. Williams and *Phaseolus vulgaris* L. cv. Saxa, both with heterobaric leaves (Terashima, 1992; Jahnke, 2001), and plants of *Vicia faba* L. cv. Hangdown Grünkernig and *Nicotiana tabacum* L. var. Samsun, both having homobaric leaves (Terashima *et al.*, 1988; Terashima, 1992;

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Jahnke & Krewitt, 2002) were grown from seeds in soil (Einheitserde Typ P; Balster Einheitserdewerk GmbH, Fröndenberg, Germany) mixed with perlite (4 : 1, v/v) in 1 l pots. The plants were watered periodically with nutrient solution and the growing conditions were as previously described (Jahnke, 2001; Pieruschka *et al.*, 2005). Plants were used for the experiments 6–8 wk after sowing.

Gas exchange measurements

Gas exchange was measured by an open gas exchange system, LI-6400 (LI-COR Bioscience, Lincoln, NE, USA), on leaves of plants under different drought stress, by stopping irrigation for 1–4 d. The experiments were performed in an experimental cabinet with controlled CO_2 concentration (350 ± 10 μl l^{-1}), temperature (28 ± 0.3°C) and air humidity [relative humidity (rh) = 50 ± 5%; vapour pressure deficit (VPD) = 1.9 kPa]. NCER was measured at an atmospheric CO_2 concentration of 350 μl l^{-1}. The leaf part inside the clamp-on leaf chamber was exposed to photosynthetic photon flux density (PPFD) of 500 μmol (photons) m^{-2} s^{-1}, whereas the leaf area outside the chamber was either shaded or illuminated by a light unit (FL-460; Walz GmbH, Effeltrich, Germany), providing a PPFD of approx. 450–500 μmol (photons) m^{-2} s^{-1}. When the leaf area outside the leaf chamber was shaded, it still received light of approx. 1–5 μmol (photons) m^{-2} s^{-1}. The light intensities were measured using an LI-185B sensor (LI-COR Inc.). Statistical analysis was performed by analysis of variance (ANOVA) using the software SigmaStat (SPSS GmbH Software, München, Germany).

Measurement of chlorophyll fluorescence

The experiments with well-watered plants were performed under laboratory conditions (approx. 25°C and 50% rh). Experiments in which drought stress was applied were performed in an experimental cabinet at air temperatures of 28 ± 0.5°C; air humidity was 50 ± 5% rh, equivalent to a VPD of 1.9 kPa. Plants exposed to drought stress were not irrigated for *c.* 48 h before starting an experiment. At that time, the first symptoms of wilting were already visible on some leaves of *V. faba* and *N. tabacum* plants, while the leaves of *G. max* and *Ph. vulgaris* showed no visible impairments.

Chlorophyll fluorescence was measured using a pulse-modulated fluorometer with spatial resolution (Imaging-PAM Chlorophyll Fluorometer; Walz GmbH). A leaf area of *c.* 20 × 14 mm (camera resolution 640 × 480 pixels) was measured, which is within the maximum sample area of the instrument (Walz, 2003). Homogeneity of actinic light, provided by the light unit of the system, was tested as follows. The camera of the Imaging-PAM was replaced with a commercial camcorder (DLR-TRV8E PAL; Sony Deutschland GmbH, Köln, Germany) and the actinic light was recorded on white filter paper at different light intensities. The images thus obtained were then transferred from the camcorder to a computer via firewire cable and a frame grabber (DVBK-2000E; Sony). The resulting images (739 × 568 pixels) were gamma-corrected (gamma = 2.0) by the computer program Scion Image (Scion Corporation; www.scioncorp.de). It was found that pixel luminousness was highest in the middle of the illuminated area but did not vary by more than 5% from the average value of all pixels within the illuminated area of 2.9 cm^2 at all light intensities tested. The kinetics of maximal chlorophyll fluorescence (F_m) was tested using a Teaching-PAM (Walz GmbH) and it was assured that the F_m value reached a plateau within the time of the saturation pulse of the Imaging-PAM (800 ms) for all plants investigated.

After plants were kept in the dark for *c.* 1 h, the leaves were clamped in the fluorometer, and minimum (F_o) and maximum (F_m) fluorescence values were recorded. When actinic light was switched on, maximum fluorescence in the light (F'_m) and steady-state fluorescence before the flash (F_t) were measured (cf. Walz, 2003), while saturated light flashes were applied at intervals of 20 or 30 s. This allowed calculation of the effective quantum yield of photosystem II (Φ_{PSII}) (cf. Genty *et al.*, 1989), and nonphotochemical quenching (NPQ) was calculated as $NPQ = [(F_m - F'_m)/F'_m]$ (cf. Maxwell & Johnson, 2000).

In a first set of experiments, leaves were partially shaded by templates made from black gas-tight adhesive tapes, which were fixed on both the upper and lower surfaces of the leaves. Chlorophyll fluorescence was then measured inside the illuminated area of *c.* 1 × 1 cm. In a second set of experiments, shading was performed by simply putting templates of black paper on the upper side of the leaves. In both sets of experiments, leaves were adapted to the dark before being clamped in the imaging fluorometer and F_o and F_m measured. Thereafter, actinic light was switched on, providing a PPFD of 290 μmol m^{-2} s^{-1} to the illuminated leaf area where chlorophyll fluorescence was measured. PPFD values below the templates of adhesive tape or black paper were *c.* 0 and 1–3 μmol m^{-2} s^{-1}, respectively. Statistical analysis was performed using ANOVA.

Results

Gas exchange rates when leaves were shaded or illuminated outside the leaf chamber

Leaf areas outside the clamp-on leaf chamber were either illuminated or shaded, while inside the leaf chamber constant light conditions were maintained (Fig. 1a). For homobaric leaves of *V. faba*, the NCER changed significantly between the treatments (Fig. 1b), whereas for heterobaric leaves of *G. max*, shading or illuminating had no effect (Fig. 1c). In homobaric leaves, the difference between the NCER obtained when the leaf area outside the chamber was illuminated ($NCER_{light}$) or shaded ($NCER_{shade}$), decreased with increasing stomatal conductance (g_{leaf}) (Fig. 2a); the values of $NCER_{light}$ and $NCER_{shade}$ differed significantly, except for very large values of g_{leaf}. The shading effect, however, was underestimated

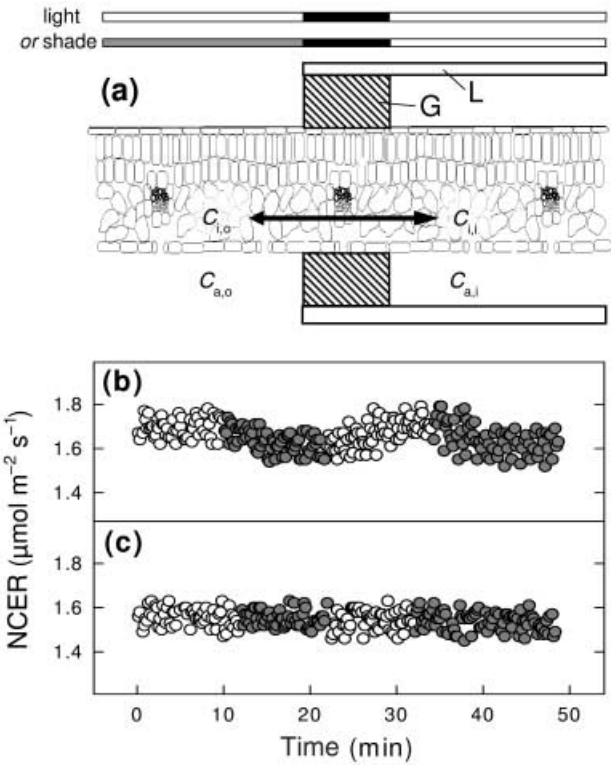

Fig. 1 Measurements of net CO_2 exchange rates (NCER) of leaf areas enclosed in a clamp-on leaf chamber when leaf parts outside the chamber were either illuminated or in shade. (a) Schematic cross-section of a leaf partially enclosed in a leaf chamber. The horizontal bars at the top indicate the experimental procedure: leaves were alternately illuminated (white bar) or shaded (grey bar) outside the leaf chamber, while the clamped section was permanently illuminated. As a result of the clamp, leaves were inevitably shaded under the gaskets (indicated by the black bars above the washers) in both the light and shade treatment. There was no change in light intensity at the clamped part of the leaves. G, leaf chamber gaskets; L, chamber lids; $c_{i,i}$, leaf internal CO_2 concentration inside the leaf chamber; $c_{i,o}$, leaf internal CO_2 concentration outside the leaf chamber. The atmospheric CO_2 concentration outside and inside the leaf chamber ($c_{a,o}$ and $c_{a,i}$, respectively) was 350 μl l^{-1}. NCER are shown (b) for a homobaric leaf of *Vicia faba* and (c) for a heterobaric leaf of *Glycine max*. The circles represent NCER when the leaf parts outside the clamp-on leaf chamber were either shaded (grey) or illuminated (white).

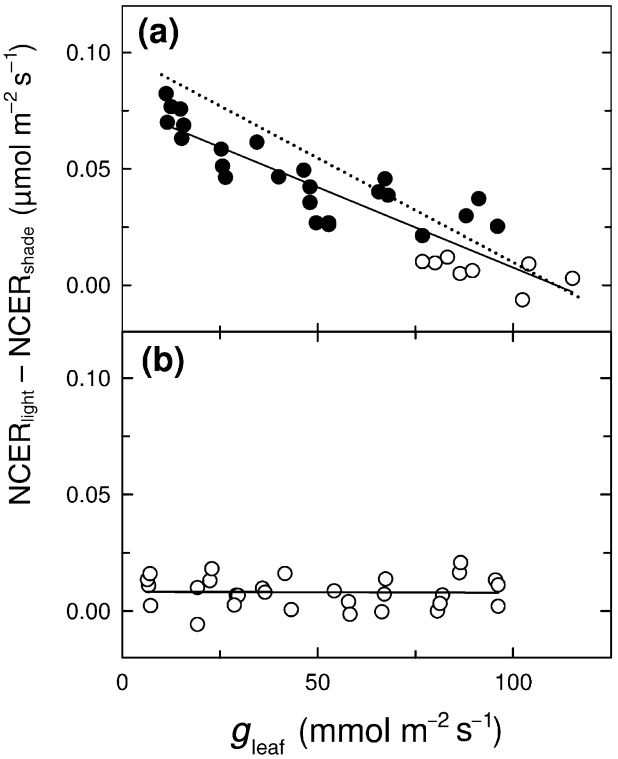

Fig. 2 Differences between net CO_2 exchange rates (NCER), when the leaf parts outside the leaf chamber were either illuminated ($NCER_{light}$) or shaded ($NCER_{shade}$), are plotted vs. stomatal conductance (g_{leaf}). Measurements are shown (a) for *Vicia faba* (homobaric) and (b) for *Glycine max* (heterobaric). Black circles represent statistically significant and white circles nonsignificant differences between $NCER_{light}$ and $NCER_{shade}$, performed by analysis of variance (ANOVA) ($P < 0.05$). Unbroken lines denote linear regression through all presented points; the dotted line in (a) is plotted to indicate that the measured differences between $NCER_{light}$ and $NCER_{shade}$ were presumably underestimated by at least 30%. This is because approx. 30% of the leaf area outside the measured (clamped) leaf part was continuously shaded by the handle of the leaf chamber (i.e. only the remaining 70% could be either shaded or illuminated).

by approx. 30%, as indicated by the dotted line in Fig. 2(a): the leaf area outside the rectangular chamber was accessible to light only from three sides, whereas the forth (long) side was continuously shaded by the handle of the clamp-on leaf chamber of the LI-6400. In heterobaric leaves, differences between $NCER_{light}$ and $NCER_{shade}$ were not significant and were independent of g_{leaf} (Fig. 2b).

Chlorophyll fluorescence imaging experiments in which leaf chamber sealing was simulated

Leaves of well-irrigated *V. faba* and *Ph. vulgaris* plants were shaded by nontranslucent gas-tight adhesive tapes forming rectangular frames that mimicked a leaf chamber sealing tightly fixed to both (adaxial and abaxial) sides of the leaves. Stomata were effectively sealed by the tapes, and respiratory CO_2 released in the masked areas eventually had to move laterally. Distinct differences in quantum yield (Φ_{PSII}; Fig. 3a–d) were observed for the leaves of *V. faba*. The images of Φ_{PSII} in Fig. 3(a–c) were obtained 10, 25 and 45 min after the light was switched on. We arbitrarily defined five regions of interest (ROI), each 1 mm wide, to pool data with a given distance to the shade (ROI 1–5; Fig. 3c). For each ROI, averaged Φ_{PSII} values were calculated, and temporal changes in Φ_{PSII} after illumination are shown in Fig. 3(d). Quantum yield was higher close to the shade (ROI 1) than in the centre of the illuminated leaf segment (ROI 5), forming a Φ_{PSII} gradient across the different ROIs. The differences in Φ_{PSII} were highest *c.* 10 min after the light was switched on but were still present after 50 min (Fig. 3d).

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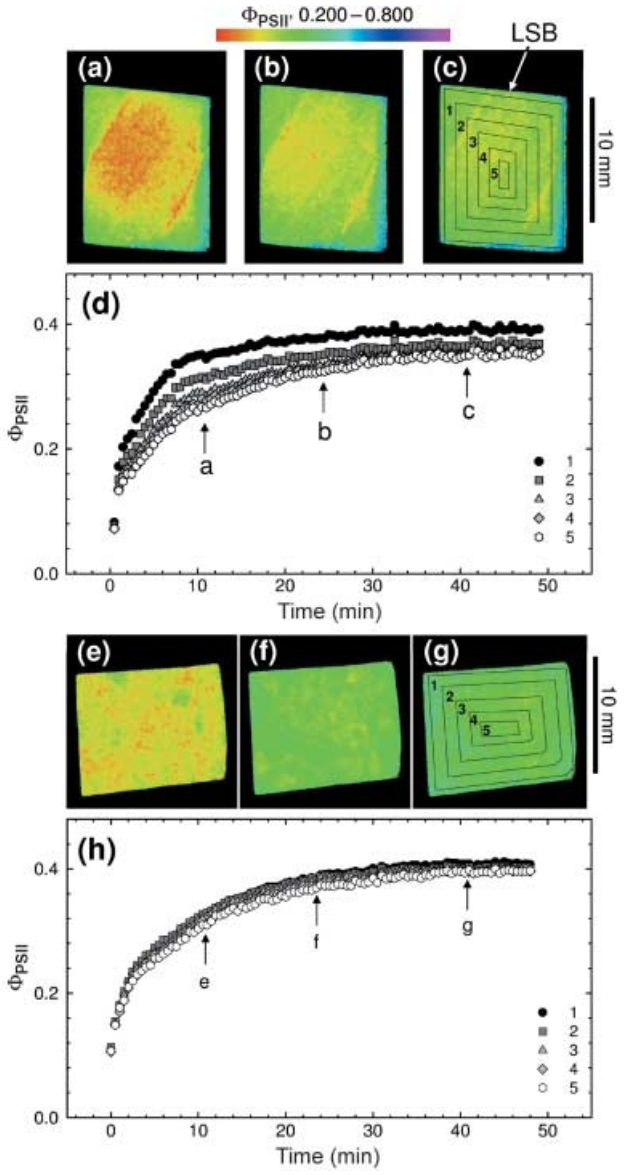

Fig. 3 Quantum yield of photosystem II (Φ_{PSII}) of rectangular leaf areas exposed to actinic light [290 µmol (photons) m^{-2} s^{-1}] and shaded outside by nontransparent, gas-tight adhesive tapes (black areas in a–c and e–g) on both the adaxial and abaxial side of the leaves to simulate sealing by leaf chamber gaskets. Measurements are shown for (a–d) *Vicia faba* (homobaric) and (e–h) *Phaseolus vulgaris* (heterobaric). The experiments were performed on well-watered plants. Data were pooled in five regions of interest, each 1 mm wide [regions of interest (ROI) 1–5; for clarity drawn only in c and g] and at a given distance from the light/shade border (LSB). The experiments started at time 0 when the dark-adapted leaves were illuminated with actinic light. The time-points when the Φ_{PSII} images of (a–c) and (e–g) were taken are indicated by arrows in (d) and (h) where the temporal changes in Φ_{PSII} after illumination are shown.

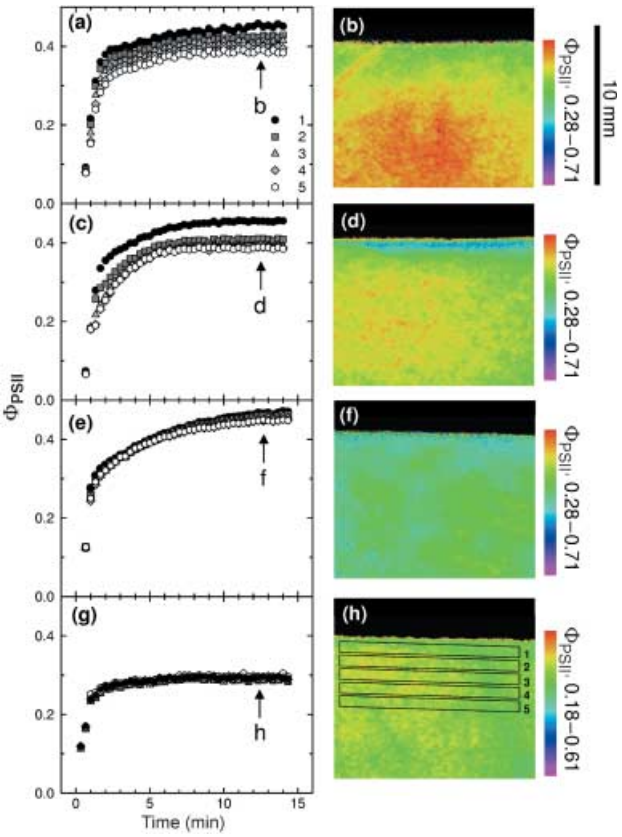

Fig. 4 Quantum yield of photosystem II (Φ_{PSII}) measured in leaves of different plant species under drought stress. Parts of the leaves were partially shaded (black areas in b, d, f and h), while others were exposed to actinic light of 290 µmol (photons) m^{-2} s^{-1}. Results were obtained either on homobaric leaves of *Vicia faba* (a, b) and *Nicotiana tabacum* (c, d), or on heterobaric leaves of *Phaseolus vulgaris* (e, f) and *Glycine max* (g, h). Data were pooled in five regions of interest, each 1 mm wide [regions of interest (ROI) 1–5; shown only in h] and with a given distance from the light/shade border (LSB). The experiments started at time 0 when the dark-adapted leaves were illuminated with actinic light. Temporal changes of Φ_{PSII} are presented in (a), (c), (e) and (g); the arrows indicate the time-points when the images shown in (b), (d), (f) or (h) were taken.

When heterobaric leaves of *Ph. vulgaris* were treated in the same way, the quantum yield was rather homogeneously distributed over the illuminated leaf area, which did not change between 10, 25 and 35 min after light was switched on (Fig. 3e–g). Only minor effects on Φ_{PSII}, with respect to the distance from the shade, were observed after illumination (ROI 1–5, Fig. 3h) which, as analysed for seven replicates, were not significant (as shown later on in Fig. 5c).

Chlorophyll fluorescence imaging of leaves when the plants were under drought stress

In a second series of experiments, the leaves of plants were partially shaded with black paper, a treatment by which stomata in the shaded region were not sealed as in the previous experiments. However, to enhance our hypothesized effect of lateral CO_2 diffusion from shaded to illuminated leaf areas, plants were put under drought stress in order to decrease stomatal conductance and thus vertical CO_2 diffusion. As

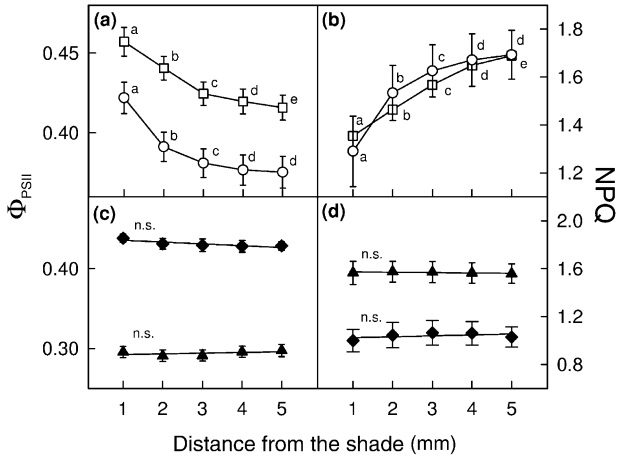

Fig. 5 Quantum yield of photosystem II (Φ_{PSII}) and nonphotochemical quenching (NPQ) of illuminated leaf areas, with respect to distance from the shade, were measured on plants under drought stress. In (a), Φ_{PSII} values, and in (b), NPQ values of homobaric leaves of *Vicia faba* (open squares) or *Nicotiana tabacum* (open circles) are shown. In (c), Φ_{PSII} values, and in (d), NPQ values of heterobaric leaves of *Phaseolus vulgaris* (closed diamonds) or *Glycine max* (closed triangles) are presented. Values represent the arithmetic means ± standard error of the mean (SEM) (n = 7). For the homobaric leaves, statistically significant differences between values at consecutive distances from the shade are denoted by different letters ($P < 0.05$). For the heterobaric leaves, differences between the values at various distances from the shade were not significant (n.s.).

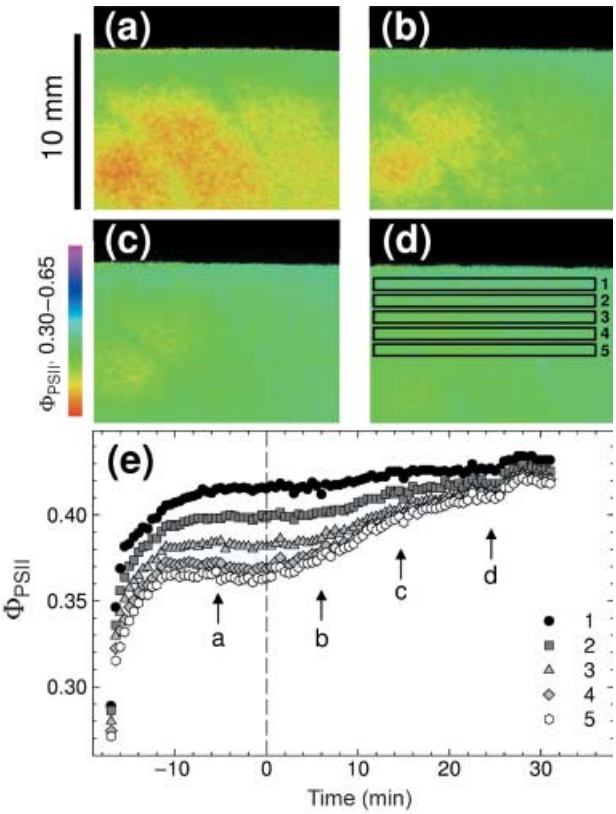

Fig. 6 Quantum yield of photosystem II (Φ_{PSII}) of a homobaric leaf of *Vicia faba* when part of the leaf was shaded (black areas in a–d). In (a), the Φ_{PSII} image was taken while the plant was under drought stress, and the Φ_{PSII} images shown in (b–d) were obtained after the plant was rewatered. Data were pooled in five regions of interest, each 1 mm wide [regions of interest (ROI) 1–5; drawn only in d], and with a given distance from the LSB. In (e), temporal changes in Φ_{PSII}, before and after the plants were rewatered at time 0, are presented. The arrows indicate the time-points when the Φ_{PSII} images of (a)–(d) were made.

before, we defined five regions of interest, each 1 mm wide, to pool data with a given distance to the LSB (cf. Fig. 4h). Temporal changes in Φ_{PSII}, averaged over the five ROIs, are shown in Fig. 4. For the homobaric leaves of *V. faba* (Fig. 4a,b) and *N. tabacum* (Fig. 4c,d), Φ_{PSII} was substantially larger at ROIs close to the LSB. In contrast, no influence of shade was observed for the heterobaric leaves of *Ph. vulgaris* (Fig. 4e,f) and *G. max* (Fig. 4g,h).

To further analyse the effects of lateral diffusion, we performed seven replicates of the above experiments to determine Φ_{PSII} and NPQ at steady-state and at various distances from the LSB. For the homobaric leaves of *V. faba* and *N. tabacum*, changes in Φ_{PSII} and NPQ with distance from the shade were significant (Fig. 5a,b). The highest values of Φ_{PSII} were located close to the LSB at ROI 1, decreasing with distance from the shade (Fig. 5a); inversely to Φ_{PSII}, the values of NPQ were lowest at ROI 1 and increased with distance from the LSB (Fig. 5b). Taking the centre of the illuminated area (ROI 5), where the influence of the shade was least as a reference, the Φ_{PSII} at ROI 1 was 13.0% larger for *V. faba* and 12.6% for *N. tabacum*; correspondingly, the NPQ values decreased by 19.6% and 24.8%, which is almost twice as large as for Φ_{PSII}. This distance dependence was not present in well-watered plants of *V. faba* (data not shown), which correlates with the gas exchange experiments where no significant impact of shading was observed at high g_{leaf} values (cf. Fig. 2a). In contrast to homobaric leaves, the heterobaric leaves of *Ph. vulgaris* and *G. max* showed no such dependence on distance from shade, even under drought stress (Fig. 5c,d).

Chlorophyll fluorescence imaging of leaves when drought stressed plants were rewatered

To study the impact of stomata opening on the observed effects on Φ_{PSII} values along an LSB, drought-stressed *V. faba* plants were rewatered in the course of the experiments (Fig. 6). As in Fig. 4(a), a gradient in Φ_{PSII} developed between ROI 1–5, with the highest values near the LSB while the plant was under drought stress (Fig. 6a; before time 0 in e). However, when the plant was rewatered, the Φ_{PSII} became homogeneously distributed with time across the illuminated leaf area (Fig. 6b–d) and the gradient in Φ_{PSII} disappeared after *c.* 20–25 min (Fig. 6e). Such effects were never found when heterobaric leaves of *G. max* or *Ph. vulgaris* were treated in the same way (data not shown).

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Discussion

In the standard setup of gas exchange measurements with clamp-on leaf chambers, only the leaf part enclosed in the chamber is considered. The leaf parts outside a chamber are not taken into account, and their potential contribution to processes inside the chamber is ignored. Different CO_2 concentrations inside and outside a leaf chamber, however, may substantially affect measurements of respiration in the dark when homobaric leaves are investigated (Jahnke & Krewitt, 2002). These observations led us to question whether changing the light intensities on leaf areas outside a chamber may cause similar artefacts in measurements of photosynthesis. In principle, such a treatment should have no effect at all. However, when a homobaric leaf of *V. faba* was shaded outside the chamber, the apparent NCER became smaller (cf. Fig. 1). This response can be explained by higher c_i values in the (shaded) leaf part outside the leaf chamber as a result of CO_2 released by respiration leading to a net lateral CO_2 flux towards the illuminated clamped leaf part.

When gas exchange measurements are performed on fully illuminated leaves, the very use of clamp-on leaf chambers leads to shading by both the gaskets and the handle. Respiratory CO_2, originating from the leaf areas below the gaskets, can only escape laterally, thereby affecting gas exchange measurements performed inside the leaf chamber. This reasoning is supported by the chlorophyll fluorescence experiments in which leaf chamber gaskets were simulated with black adhesive tape (see Fig. 3a–d). The stomata in the shaded area were effectively sealed by this treatment, and an increase in quantum yield within the illuminated leaf area adjacent to the shade was observed for homobaric *V. faba* leaves. We interpret this result by an increase in c_i within the shaded leaf area, which causes lateral CO_2 transport and, as a consequence, higher photosynthetic rates in the illuminated area along the LSB. The gas exchange measurements, on the other hand, indicated lower apparent photosynthetic NCER inside the leaf chamber when leaves were shaded outside (cf. Fig. 1b). The results obtained by gas exchange measurements and chlorophyll fluorescence imaging therefore appear to be in conflict. However, lateral fluxes of CO_2 across the LSBs can easily explain this apparent contradiction. On the one hand, additional CO_2 is available for photosynthesis in the illuminated areas, which is supported by the chlorophyll fluorescence data presented above (higher Φ_{PSII} values close to the LSBs). On the other hand, an increase in c_i lowers the CO_2 gradient, and thus CO_2 fluxes, between ambient air and the leaf mesophyll, resulting in a decrease in the measured NCER. This, however, is an experimental artefact: gas exchange measurements only detect changes in leaf external CO_2 and cannot reflect true NCER when there is an internal supply of CO_2 into a clamped leaf region.

Stomatal conductance is the main mechanism by which plants control gas exchange and leaf temperature (Farquhar & Sharkey, 1982). Stomatal conductance decreases under mild or moderate drought stress, which leads to a reduction in c_i, thereby affecting photosynthesis (Lawlor, 2002). The data presented here show that the potential effect of lateral diffusion on NCER is also dependent on stomatal conductance (Fig. 2a). Lateral CO_2 fluxes depend on the CO_2 supply from ambient air (i.e. in a vertical direction) determined by stomatal opening: the impact of lateral diffusion on photosynthetic NCER in illuminated leaf areas near LSBs was large when the g_{leaf} was low and declined with higher g_{leaf} values (cf. Fig. 2a). The contribution of stomatal conductance became apparent also in the chlorophyll fluorescence imaging experiments. When stomata were artificially sealed by adhesive tape, Φ_{PSII} gradients near the LSBs were large at the beginning because the experiment started with dark-adapted leaves in which the stomatal conductance was low (Fig. 3d); the gradients in Φ_{PSII} declined with time as a result of the gradual reopening of stomata after illumination (cf. Pearcy *et al.*, 1996). The interplay between vertical and lateral fluxes becomes even clearer when a single leaf of *V. faba* is observed under a changing water supply. During drought stress, respiratory CO_2 released in the shaded leaf part augmented CO_2 availability in the illuminated leaf area near the LSB (indicated by the Φ_{PSII} gradient across the different ROIs in Fig. 6). Rewatering caused two major changes as a consequence of stomatal reopening: the Φ_{PSII} gradient decreased, indicating a diminishing impact of lateral CO_2 transport, while (absolute) Φ_{PSII} values increased as a result of the re-establishment of CO_2 supply from ambient air. We therefore conclude that lateral CO_2 supply across LSBs may contribute to photosynthesis of homobaric leaves under drought stress, but cannot fully substitute 'normal' CO_2 supply via the stomata.

When stomatal conductance decreases, photoinhibitory damage through excess light poses a threat to the photosynthetic apparatus of leaves. In C_3 plants, the photosynthetic apparatus is protected from photoinhibition by various mechanisms, such as photorespiration (Osmond *et al.*, 1997; Ort, 2001; Cornic & Fresneau, 2002; Medrano *et al.*, 2002a; Ort & Baker, 2002) and heat dissipation through light-induced formation of zeaxanthin (Demmig-Adams & Adams III, 1992). At natural stands, most leaves are only partially illuminated owing to self-shading inside a canopy and, at LSBs, have to cope with high (or even extreme) differences in light intensity. The increased quantum yield near LSBs, observed in the present study, may then provide an additional, yet-unknown mechanism to reduce light stress in homobaric leaves. The increase in c_i owing to lateral CO_2 fluxes from shaded to illuminated leaf parts causes higher quantum yield and Φ_{PSII}, and can also explain the observed decrease in NPQ (cf. Fig. 5). Interestingly, the relative decrease in NPQ was larger than the increase in photosynthetic efficiency. We interpret this as a first indication that protection from overexcitation through lateral CO_2 fluxes across LSB could be even more beneficial for leaves under drought stress than the higher yield in CO_2

assimilation. Plants that can withstand drought stress are more effective in conserving tissue hydration than drought-susceptible plants (Grzesiak *et al.*, 1999). They reduce water loss by stomatal closure but then have to cope with a diminished supply of CO_2. Considerable gas transport and, consequently, refixation of respiratory CO_2 from remote parts of the leaves, however, are only possible if intercellular space is open for gas transport in the lateral directions. This is a characteristic trait of homobaric leaves for which lateral gas conductivities have been reported to be even larger than gas conductivities in vertical directions (Pieruschka *et al.*, 2005). When stomatal conductance decreases and lateral conductance remains constant in such leaves, more respiratory CO_2 is internally available for refixation, and the effects of lateral CO_2 diffusion on photosynthesis and energy dissipation become pronounced (cf. Fig. 5). With rising temperatures, which cause an increase in VPD and force stomatal closure to prevent water loss (Mott & Parkhurst, 1991), the effects described might become even larger.

These observations may lead to the hypothesis that the homobaric leaf anatomy is an adaptation to the specific environmental conditions in which the evaporative demand of plants is high. Refixation of respiratory CO_2 released from (remote) shaded leaf parts could result in higher water use efficiency (WUE). These processes cannot occur in plants that have heterobaric leaves. Plants with high WUE generally grow in relatively dry habitats (Larcher, 2003) and one may then speculate whether homobaric leaf anatomy may prevail in plant species native to such areas. Wylie (1952) presented a survey on 348 plant species with respect to the occurrence of bundle sheath extensions, which are the main barriers for lateral gas movement. Plants with homobaric leaves (*c.* 40% of the species) were from warmer regions, whereas those with heterobaric leaves were mostly from northern (temperate) areas. In particular, the investigated woody species featuring homobaric leaves favoured evergreen habitats and many showed xeromorphic leaf modifications (Wylie, 1952). In warmer habitats, plants may at least temporarily face low relative humidity (i.e. high VPD), which is one of the key factors mediating changes in stomatal sensitivity to CO_2 (Monteith, 1995; Talbott *et al.*, 2003). Thus, lateral gas conductance and internal CO_2 refixation may also favour the efficient use of water under unfavourable conditions.

The proposed hypothesis, that the potential to use CO_2 from remote leaf areas is beneficial for plants with homobaric leaves, has yet to be evaluated under natural conditions.

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Note added in proof

Morison *et al.* (2005) have recently published similar phenomena on homobaric *Commelina communis* leaves. The results obtained were quantitatively different from the results presented in this work. Although we have not yet examined *C. communis* leaves in detail, we believe that these differences are largely due to different degrees in homobaric leaf anatomy.

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