<table>
<thead>
<tr>
<th>Announcement Type</th>
<th>Announcement Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title</td>
<td>Effective use of high CO₂ efflux at the soil surface in a tropical understory plant.</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Ishida, Atsushi; Nakano, Takashi; Adachi, Minaco; Yoshimura, Kenichi; Osada, Noriyuki; Ladpala, Phanumard; Diloksumpun, Sapit; Puangchit, Ladawan; Yoshimura, Jin</td>
</tr>
<tr>
<td>Citation</td>
<td>Scientific reports (2015), 5</td>
</tr>
<tr>
<td>Issue Date</td>
<td>2015-03-11</td>
</tr>
<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/2433/198826">http://hdl.handle.net/2433/198826</a></td>
</tr>
<tr>
<td>Rights</td>
<td>This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder in order to reproduce the material. To view a copy of this license, visit <a href="http://creativecommons.org/licenses/by/4.0/">http://creativecommons.org/licenses/by/4.0/</a></td>
</tr>
<tr>
<td>Type</td>
<td>Journal Article</td>
</tr>
<tr>
<td>Textversion</td>
<td>publisher</td>
</tr>
</tbody>
</table>

Kyoto University
Effective use of high CO₂ efflux at the soil surface in a tropical understory plant

Atsushi Ishida¹, Takashi Nakano², Minaco Adachi³, Kenichi Yoshimura⁴, Noriyuki Osada⁵, Phanumard Ladpala⁶, Sapit Diloksumpun⁷, Ladawan Puangchit⁷ & Jin Yoshimura⁸,⁹,¹⁰

Many terrestrial plants are C₃ plants that evolved in the Mesozoic Era when atmospheric CO₂ concentrations ([CO₂]) were high. Given current conditions, C₃ plants can no longer benefit from high ambient [CO₂]. *Kaempferia marginata* Carey is a unique understory ginger plant in the tropical dry forests of Thailand. The plant has two large flat leaves that spread on the soil surface. We found a large difference in CO₂ concentrations ([CO₂]) between the partly closed space between the soil surface and the leaves (638 μmol mol⁻¹) and the atmosphere at 20 cm above ground level (412 μmol mol⁻¹). This finding indicates that the plants capture CO₂ efflux from the soil. Almost all of the stomata are located on the abaxial leaf surface. When ambient air [CO₂] was experimentally increased from 400 to 600 μmol mol⁻¹, net photosynthetic rates increased by 45 to 48% under near light-saturated conditions. No significant increase was observed under low light conditions. These data demonstrate that the unique leaf structure enhances carbon gain by trapping soil CO₂ efflux at stomatal sites under relatively high light conditions, suggesting that ambient air [CO₂] can serve as an important selective agent for terrestrial C₃ plants.

The geological record indicates that the C₃ land plants originated during the middle to late Ordovician period (450 to 440 million years ago) when atmospheric CO₂ concentrations ([CO₂]) were still very high (approximately 4% compared with 0.039% at present) and O₂ concentrations ([O₂]) in air were low (approximately 15% compared with 21% at present)²,³. Although the down-regulation of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) under high [CO₂] is a well-known phenomenon⁴, high [CO₂] and low [O₂] in the ancient air would have contributed to an increase in carbon assimilation rates (A) due to the kinetics of Rubisco. A meta-analysis of FACE (free-air CO₂ enrichment) experiments revealed that the average maximum carboxylation rates under doubled [CO₂] were −17% in C₃ crops and −4% in C₃ trees due to dawn-regulation. On average, the increase in light-saturated net photosynthesis under doubled [CO₂] was 13% in C₃ crops and 47% in C₃ trees⁵. This finding may indicate that C₃ plants in the past exhibited increased carbon (C) gain and that more extensive C cycling occurred in forest ecosystems compared with the present era. During the Cenozoic era, atmospheric O₂ concentrations increased and atmospheric [CO₂] became largely depleted, with record minimum [CO₂] during the Oligocene/Miocene epoch (24 million years ago)⁶. Since the advent of the Industrial Revolution, atmospheric [CO₂] has increased rather rapidly due to the modernization of human society and increasing reliance on coal and oil burning. In the photosynthetic CO₂-response curves of C₃ plants, the transition of the limitation from ribulose-1,5-bisphosphate (RuBP) carboxylation limitation to RuBP regeneration limitation is typically observed between ambient and doubled ambient [CO₂]⁷. Thus, C₃ plants are constrained by the carboxylation limit of RuBP in the present-day air [CO₂]. In contrast, photosynthesis in C₄ plants is not limited by low air [CO₂]⁸ because these plants possess the appropriate enzyme (PEP carboxylase) and the specific anatomy in bundle sheath cells required to increase the CO₂ partial pressure around Rubisco sites⁹. C₃ plants have evolved to improve plant carbon and water relations simultaneously during photosynthesis and to cope with declining atmospheric [CO₂] and increasing water demand⁸,⁹. However, C₃ plants have not evolved carbon-concentrating mechanisms in their physiology and anatomy.
Even in present-day ecosystems, sites with high air [CO2], such as forest floors[8] and volcanic vents[9] are observed. The high [CO2] found on forest floors originates from the respiration of soil organisms and plant-root systems. Attention has been focused on the large contributions of sunflecks or sun patches to net C assimilation rates (A) in forest understory plants, indicating strong light limitation[12,13]. However, the potential effects of rising [CO2] on A in understory plants have barely been evaluated. High [CO2] should contribute to the survival of understory plants that experience reduced photosynthetic rates due to water stress[14]. The stable carbon isotope ratios of understory plants indicate that these plants re-fix the efflux C in tropical[15] and cool-temperate forests[16].

High [CO2] that originates from the soil surface dissipated rapidly due to diffusion and mass flow caused by wind. Although wind velocity is reduced near the understory, an extremely gentle breeze is sufficient to diffuse CO2 from the soil surface[17]. Therefore, for understory plants to effectively use this high soil-efflux [CO2], they must trap CO2 near the soil surface. In the present study, we report the discovery of an understory ginger plant, *Kaempferia marginata* Carey (Zingiberaceae), which effectively traps soil-efflux CO2 in the closed space between the soil surface and its leaves. This plant enhances photosynthesis by 45 to 48% under relatively high light conditions. It is a drought-deciduous, perennial herb found in tropical dry forests in Southeast Asia. Based on measurements of ambient air [CO2], photosynthetic capacity, and the stable carbon isotope ratios in the lamina, we demonstrate that this ginger plant makes effective use of high [CO2] on the forest floor.

**Results**

The ginger plant has a unique leaf structure; the individual plant has two flat leaves that spread on the soil surface, and the leaf edges are often curled downward to capture the air under its leaf blades (Fig. 1). The root system is small, indicating that this plant has a poor water uptake capacity. The uppermost height of a single leaf blade is only 24 mm above the ground surface on average and defines a relatively closed space between the leaf blade and the soil surface (Table S1). The stomatal densities were 1.6 mm⁻² and 20.9 mm⁻² on the adaxial and abaxial leaf surfaces, respectively, indicating that approximately all stomata face the soil surface. The distributions of leaf sizes and leaf morphologies indicate that as the leaf size increases with time, the leaf shape gradually becomes rounder (Fig. S1), contributing to an increase in the efficiency of trapping CO2 efflux from the soil surface.

On a sunny day during the rainy season, the average daily [CO2] was 412 μmol mol⁻¹ in the open air at 20 cm above the ground and 638 μmol mol⁻¹ in the space between the leaves and soil surface (Fig. 2). The maximum [CO2] observed in the air space was greater than 1000 μmol mol⁻¹. Nevertheless, [CO2] in the space largely fluctuated with temporal variations in wind velocity. The values (mean ± SD) of the stable carbon isotope ratios ([δ¹³C] in the lamina were −34.9 ± 1.5%o in the ginger plants and −29.1 ± 1.5%o in the upper canopy leaves of woody plants in the dry evergreen forest (our unpublished data on woody plants). The low δ¹³C value in the ginger plants indicates high internal [CO2] in the leaves during the day.

When the ambient-air [CO2] was artificially increased from 400 to 600 μmol mol⁻¹, the A under near-light saturated conditions (800 μmol m⁻² s⁻¹ PPF: photosynthetic photon flux) increased from 5.8 to 8.2 μmol m⁻² s⁻¹, a 45% increase (Fig. 3A). In contrast, under low light conditions (less than 70 μmol m⁻² s⁻¹ PPF), no significant increase was detected in A after elevating [CO2] from 400 to 600 μmol mol⁻¹. We also measured ambient-air CO2 response curves under 500 and 40 μmol m⁻² s⁻¹ PPFs. Both RuBP carboxylation and RuBP regeneration rates were reduced by the low PPF (Fig. 3B). When the ambient-air [CO2] was increased from 400 to 600 μmol mol⁻¹, A increased by 48% under relatively strong sunlight (500 μmol m⁻² s⁻¹ PPF) and by 36% under reduced light (40 μmol m⁻² s⁻¹ PPF) conditions. The data indicate that a significant increase in A in response to elevated [CO2] was more pronounced under sunlit conditions compared with shaded conditions. Sunflecks must thus cooperate with rising [CO2] for enhancing of A[12,13].

**Discussion**

The data presented here indicate that the unique leaf structure of ginger plant enhances C fixation under high light conditions by effectively trapping high [CO2] efflux in the relatively closed space between their leaves and the soil surfaces. In tropical forests, high termite activity at ground level prevents fallen leaves from covering the leaf surface of the ginger plants (Fig. 1A); the leaf litter layer typically remains fairly thin and does not persist for a long period of time. This may be a factor in explaining why the ginger plant has evolved to capture CO2 efflux from soil respiration in tropical forests.

Another unique morphological characteristic of the ginger plant is the small root system (Fig. 1B). Large non-photosynthetic organs are found to have large respiration requirements[16,17]. However, its small root system, the ginger plant has a very low CO2 compensation point at the whole plant level, similar to leafy plants[18]. Because of the small root system, the ginger plant can only grow during the favorable rainy season as an ephemeral plant. Another advantage is the high

![Figure 1](image_url) An understory ginger plant, *Kaempferia marginata* Carey, with a unique leaf structure in a tropical forest in Southeast Asia. (a) field-grown plants, (b) A plant removed from the soil; two large leaves and a poor root system are evident.

![Figure 2](image_url) Diurnal time variations in air CO2 concentration at 20 cm above the ground (open circles) and in the air space between the leaf blade and soil surface (blue circles).
Figure 3 | Photosynthetic responses under high CO2 concentrations and high light conditions. (a) Photosynthetic light response curves (PPF-A curves) at 600 μmol mol⁻¹ CO2 (closed circles) and 400 μmol mol⁻¹ CO2 (open circles), where PPF represents the photosynthetic photon flux at the leaf surface and A is net C assimilation rate. Bars indicate 1 SD, (b) Photosynthetic ambient-air CO2 response curves (Ca-A curves) in a leaf blade at 500 μmol m⁻² s⁻¹ PPF (closed circles) and 40 μmol m⁻² s⁻¹ PPF (open circles), where Ca represents CO2 concentration in outlet gas stream in the LI-6400, i.e., ambient air CO2 concentration.

The pulse-labeling method has been used to determine the time lag from CO2 efflux from soil to leaf C assimilation⁹. The time lag ranges from 12.5 ± 7.5 (mean ± SD) h in grasses to 4 to 5 days in trees. Although the data indicate that interactions between the soil and plants in the C cycles within a single ecosystem exist, most CO2 that originates from the soil will have dissipated from the ecosystem by diffusion during this time period. The low δ¹³C values of ginger plants indicate that they were exposed to high [CO2] and used large amounts of C emitted from the soil. Nevertheless, shady conditions increase internal [CO2] in leaves due to the reduced A, consequently decreasing the δ¹³C values in laminae⁴¹. Therefore, we cannot use δ¹³C values to distinguish between the two potential sources of the effects, shade and high ambient air [CO2]. Overall, we can conclude that root and microbial-derived CO2 are major contributors to carbon assimilation in this ginger plant.

Methods

The study was conducted in July 2008 in a dry evergreen forest in Thailand (14° 29’N, 101° 55’E, 563 m ASL) approximately 180 km northeast of Bangkok during the middle of the rainy season⁴². We selected a population of ginger plants found roadside in a forest with a dense canopy. During three successive days, the diurnal time courses of PPF, ambient air temperatures and relative humidity in air were measured near the center of the plant population (data shown in Fig. S2). On a relatively sunny day, the diurnal time courses of leaf gas exchange and chlorophyll fluorescence were measured from predawn to dusk using an open, portable measurement system (LI-6400, LI-COR, Lincoln, NE) and a chlorophyll fluorescence meter (Mini-PAM, Walz, Effeltrich, Germany), respectively. These measurements were conducted in eight individual plants with relatively large leaves.

While measuring diurnal leaf gas exchange, the diurnal variations in ambient air [CO2] were simultaneously measured with thin-film capacitance CO2 sensors (GM70, Vaisala, Helsinki, Finland) without tube-absorbing air. The CO2 sensors were set at two heights: 1) 20 cm above the ground and 2) in the air space between the leaf blade and the ground surface in an individual plant with a relatively large leaf area. The diameter of the CO2 sensor probe was 18.5 mm, and the leaf diameter was greater than 100 mm. Because of without tube-absorbing and given a large leaf, [CO2] in the air space below the leaf could be directly measured (Fig. S4); it is possible that we did not completely avoid air leaks along the side of the probe, possibly resulting in an underestimation of [CO2].

In the following days, to evaluate the interactive effects of light intensity and [CO2] on A, we measured photosynthetic light responses (PPF-A curve) under different ambient air [CO2] levels and photosynthetic ambient air CO2 responses (Ca-A curve) under different light levels during the daylight hours (Ca refers to ambient air [CO2]).

To evaluate the average internal [CO2] in leaves over a long time period, carbon isotope ratios in the eight laminae were examined with an isotope ratio mass spectrometer (DELTAM USEC), Thermo Fisher Scientific Inc., Cambridge, UK). More detailed information is described in the supplementary information.


soil respiration during rainy seasons. In tropical dry forests in Thailand, where the ginger plant is a native species, the soil respiration rates become double during the rainy seasons²⁰. The mean soil respiration rate is approximately 7.67 μmol m⁻² s⁻¹ in the rainy season and approximately 3.63 μmol m⁻² s⁻¹ in the dry season.

A relatively high irradiance is required to effectively enhance A under elevated [CO2] (Fig. 3B); light levels greater than approximately 6.4% of full sunlight appear to be required to maintain a population of the ginger plant (see Environmental description in Supplementary information). Under sunlit conditions, the risk of photoinhibition increases even in tropical climates, particularly in shaded plants at relatively high temperatures²¹,²². However, in the ginger plant, xanthophyll-cycle dependent non-photochemical quenching (NPQ) appears to prevent chronic photoinhibition (Fig. S3). This unique adaptation to specific microhabitats is reflected by the plant distribution. In the tropical dry forests, the ginger plant is primarily located in the drought-deciduous forests with sparse tree cover and lightly shaded forest floors. In contrast, the ginger plant is primarily located on the trunks of large trees. We hypothesize that the mechanism similar to that of the ginger plant may be identified among the woody plants, the respiration rates per unit stem surface at breast height ranges from 1.2 μmol m⁻² s⁻¹ to 3.5 μmol m⁻² s⁻¹ in the ground but also on the trunks of large trees. We hypothesize that the combination of a closed air space and relatively high sunlight is required to exploit extremely high efflux CO2.

The study was conducted in July 2008 in a dry evergreen forest in Thailand (14° 29’N, 101° 55’E, 563 m ASL) approximately 180 km northeast of Bangkok during the middle of the rainy season⁴². We selected a population of ginger plants found roadside in a forest with a dense canopy. During three successive days, the diurnal time courses of PPF, ambient air temperatures and relative humidity in air were measured near the center of the plant population (data shown in Fig. S2). On a relatively sunny day, the diurnal time courses of leaf gas exchange and chlorophyll fluorescence were measured from predawn to dusk using an open, portable measurement system (LI-6400, LI-COR, Lincoln, NE) and a chlorophyll fluorescence meter (Mini-PAM, Walz, Effeltrich, Germany), respectively. These measurements were conducted in eight individual plants with relatively large leaves.

While measuring diurnal leaf gas exchange, the diurnal variations in ambient air [CO2] were simultaneously measured with thin-film capacitance CO2 sensors (GM70, Vaisala, Helsinki, Finland) without tube-absorbing air. The CO2 sensors were set at two heights: 1) 20 cm above the ground and 2) in the air space between the leaf blade and the ground surface in an individual plant with a relatively large leaf area. The diameter of the CO2 sensor probe was 18.5 mm, and the leaf diameter was greater than 100 mm. Because of without tube-absorbing and given a large leaf, [CO2] in the air space below the leaf could be directly measured (Fig. S4); it is possible that we did not completely avoid air leaks along the side of the probe, possibly resulting in an underestimation of [CO2].

In the following days, to evaluate the interactive effects of light intensity and [CO2] on A, we measured photosynthetic light responses (PPF-A curve) under different ambient air [CO2] levels and photosynthetic ambient air CO2 responses (Ca-A curve) under different light levels during the daylight hours (Ca refers to ambient air [CO2]).

To evaluate the average internal [CO2] in leaves over a long time period, carbon isotope ratios in the eight laminae were examined with an isotope ratio mass spectrometer (DELTAM USEC), Thermo Fisher Scientific Inc., Cambridge, UK). More detailed information is described in the supplementary information.


Acknowledgments

This study was supported by grants-in-aid from the Japan Society for the Promotion of Science (nos. 18255011, 24370009 to A.I., 22255004, 22370010 to J.Y.). We wish to thank the staff of the research station, Dr. T. Artchawakom, and Dr. M. Takahashi for their support.

Author contributions

A.I. and T.N. designed and carried out the major part of the field measurements. M.A., K.Y., N.O. and P.L. carried out the field measurements. S.D. and L.P. designed and prepared the manuscript. A.I. and J.Y. wrote the manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder in order to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/
Supplementary Information

**Title:** Effective use of high CO₂ efflux at soil surface in a tropical understory plant

**Authors:** Atsushi Ishida, Takashi Nakano, Minaco Adachi, Kenichi Yoshimura, Noriyuki Osada, Phanumard Ladpala, Sapit Diloksumpun, Ladawan Puangchit, Jin Yoshimura

**Environmental description and more detailed methods**

*Study site and plant materials*

The study was located in the dry evergreen forest at the Sakaerat Environmental Research Station (14° 29’N, 101° 55’E, 563 m ASL), approximately 180 km northeast of Bangkok in Thailand. Mean annual temperature was 26.2°C, and mean annual rainfall was 1240 mm (Sakurai *et al.* 1998). There is a distinct dry season from November to March (Ishida *et al.* 2006). The soil is of sandstone origin and acidic (around 4.5 in pH), and has relatively poor nutrients with high porosity. *Hovea ferrea* Lanessan (Dipterocarpaceae) is the predominant tree with tall canopies (approximately 25-35 m high) in the evergreen forest. Details in landform and soil characteristics are shown in Pitman (1996) and Murata *et al.* (2009).

The examined ginger plant (*Kaempferia marginata* Carey, Zingiberaceae) is a drought-deciduous perennial herb. The plants are usually found near the roadside in the dry evergreen forests with dense canopies, and inside the drought deciduous forests with sparse canopies in the Sakaerat Environmental Research Station. Thus, the ginger plant seems to favor to a relatively light understory.

*Measurements of leaf size distribution*

We selected a population of the ginger plant found roadside in the dry evergreen forest. To examine the ontogenetic variations of leaf form, we measured the leaf area, leaf length and width, and the top heights of the leaf blades in 150 individual plants with contrasting plants size. The data in leaf shape and the top height are shown in Supplementary Fig. 1 and Supplementary Table 1, respectively.

*Microclimate measurements*

Photosynthetic photon flux (PPF) was measured with quantum sensors (LI-190SB, LI-COR, Lincoln, NE, USA) at an open place and two understory places around the center of a ginger plant population for three consecutive, relatively sunny days from July 15 to July 17 in 2008 in the middle of the wet season. PPF measurement at the open site was conducted on the top of a 45-m high scaffolding tower constructed near the study site, which exceeded the uppermost canopy in the forest. Ambient air temperature ($T_{air}$) and relative humidity (RH) in the understory were simultaneously measured with thermistor and thin-film capacitance sensors, respectively (Model 36355, Hioki-Denki, Nagano, Japan). These sensors were connected with small data loggers (Model 3631 or 3635, Hioki-Denki), and the data were minutely stored. These measurements in the understory were conducted at 20 cm above the ground near the center of the ginger population.
On a sunny day (July 15 in 2008), the diurnal variations in ambient air CO\textsubscript{2} concentrations ([CO\textsubscript{2}]) were directly measured with thin-film capacitance CO\textsubscript{2} sensors (GM70, Vaisala, Helsinki, Finland) without tube-absorbing air. The CO\textsubscript{2} sensors were set at two heights: (1) 20 cm above the ground and (2) in the air space between the leaf blade and the ground surface in a lamina. To measure CO\textsubscript{2} concentrations in the air space, we selected an individual plant with a relatively large leaf area to avoid air leak along the side of the censor probe (Supplementary Figure 4).

Environmental description
Supplementary Figure 2 shows the diurnal time variations in microclimate (PPF, air temperature, and relative humidity in air) in the examined population growing at a roadside in the dry evergreen forest and the diurnal variations in PPF at an open place. The daily total PPF at the study site relative to that of the open site was 6.4%. The understory light levels in tropical evergreen forest are approximately 1% of full sunlight or sometimes less than 1% (e.g., Ashton 1992). Because the ginger plants are not found in deeper-shaded sites in the evergreen forests, the light levels of approximately 6.4% relative to full sunlight appear to be required to maintain a population of the ginger plant.

More detailed methods in the photosynthetic capacity measurements
The leaf photosynthetic capacity in eight individuals was measured with an open, portable measurement system (LI-6400, LI-COR, Lincoln, NE). The measurement was conducted in six healthy, mature leaves. The leaf chamber with 6 cm\textsuperscript{2} was used and the red-blue RED lamp unit was utilized as a light source. Photosynthetic light-response curves were measured on the same eight leaf blades under 600 and 400 µmol mol\textsuperscript{-1} CO\textsubscript{2} in the inlet gas stream with LI-6400. The values of 400 and 600 µmol mol\textsuperscript{-1} CO\textsubscript{2} were approximate and corresponded to daily mean air CO\textsubscript{2} concentrations at the 20 cm above and just below the leaf blades, respectively. PPFs were decreased stepwise from 800, 500, 200, 100, 70, 40, 30, 20, 10, 7, 3, to 0 µmol m\textsuperscript{-2} s\textsuperscript{-1}. Light compensation points and apparent quantum use efficiencies were calculated from linear regressions under very low PPFs (from 0 to 10 µmol m\textsuperscript{-2} s\textsuperscript{-1}). The mean leaf temperature during these measurements was 29.2°C which approximately corresponds to the daytime leaf temperature. Photosynthetic ambient air CO\textsubscript{2}-response curves were measured on the same seven leaf blades under 500 and 40 µmol m\textsuperscript{-2} s\textsuperscript{-1} PPF with LI-6400. In the values that were exposed to sun-flecks and in those without sunflecks during the daytime, the values of 500 and 40 µmol m\textsuperscript{-2} s\textsuperscript{-1} PPF were used, respectively (see Supplementary Fig. 2). The [CO\textsubscript{2}] in the inlet gas stream with LI-6400 increased stepwise from 0, 50, 100, 200, 300, 400, 500, 600, 700, 800, to 1000 µmol mol\textsuperscript{-1}.

More detailed methods in the diurnal variations of leaf gas exchange and chlorophyll fluorescence measurements
The diurnal time change in leaf gas exchange was measured with an open, portable measurement system (LI-6400, LI-COR, Lincoln, NE), from dawn to dusk on 15 July 2008. The measurement was conducted in eight healthy, mature leaves at approximately 30-minute intervals. The leaf chamber with 6 cm\textsuperscript{2} was used and the top part of the chamber was sealed with a clear plastic plate to receive naturally incident PPF. The CO\textsubscript{2} concentration in the inlet gas stream within LI-6400 was adjusted at
600 µmol mol⁻¹, which approximately corresponded to the mean air [CO₂] just below the leaf blades (see Figure 2).

While measuring the leaf gas exchange, the diurnal time variations in chlorophyll a fluorescence were measured with a fluorescence meter (Mini-PAM, Walz, Effeltrich, Germany), according to Bilger et al. (1995). The fiber-optic cable was connected with the clear top-cover of the LI-6400 chamber, while the angle (60°) and the distance between the leaf surface and the fiber-optic cable were manually adjusted. Maximum fluorescence yield (Fₘ) and dark fluorescence yield (Fₒ) in photosystem II (PSII) were determined just before dawn. Just after the measurement of leaf gas exchange, we supplied a saturated-light pulse to the leaf surface. Maximum fluorescence (Fₘ') and steady-state fluorescence (F) in the light-adapted state of PSII were measured during the daytime. Chlorophyll fluorescence parameters were calculated, according to Genty et al. (1989). The potential maximum quantum yield of PSII (Fₘ/Fₘ = (Fₘ-Fₒ)/Fₘ) was calculated from the dark-time measurements made before dawn. For each daytime measurement, the effective quantum yield of PSII (Φₚₛᵦᵢᵦ = (Fₘ'-F)/Fₘ') was calculated. Assuming that photosystem I and II absorb equal amounts of light and the leaf absorbance of lamina is 0.84, the electron transport rate through PSII (ETR) was calculated as, ETR = 0.5 Φₚₛᵦᵢᵦ 0.84 PPF (at the leaf surface). Non-photochemical quenching (NPQ = (Fₘ/Fₘ')-1) was also calculated. Data in chlorophyll fluorescence are showed in Supplementary Figure 3.

Measurements of the nitrogen and stable carbon isotope ratio in lamina and the number and size of stomata

After all measurements, we collected the leaves and then cut leaf discs with a borer. The leaf discs were oven dried (70°C, 72 hr) and weighed to determine leaf dry mass per unit leaf area (LMA). The total nitrogen (N) and carbon (C) contents within the leaf discs were measured with an N-C analyzer (Sumigraph NC-900, Sumitomo-Kagaku, Osaka).

To estimate the averaged internal CO₂ concentrations in leaves for a long time, the stable carbon isotope ratios (δ¹³C) in lamina were determined with an isotope ratio mass spectrometer (DELTA V Plus, Thermo Fisher Scientific Inc., Cambridge, UK). The δ¹³C values were expressed in delta notation relative to a PD Belemnite standard: δ¹³C = (R_sample - R_standard - 1) 1000 (‰), where R_sample is the ¹³C/¹²C ratios of the samples and R_standard is the ¹³C/¹²C ratio of the standard.

The numbers and the pore length of stomata in the adaxial and abaxial leaf surfaces were determined by obtaining replicas of the surface of four healthy leaves with a celluloid plate (Universal Micro-printing, SUMP, Tokyo, Japan).
**Supplementary Table 1: The morphological and physiological characteristics of leaf blades.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>unit</th>
<th>mean</th>
<th>1 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top height of leaf blades above the ground</td>
<td>mm</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td>Area of individual leaf blades</td>
<td>cm²</td>
<td>65.0</td>
<td>36.9</td>
</tr>
<tr>
<td>Length/width ratio in individual leaf blades</td>
<td></td>
<td>1.32</td>
<td>0.30</td>
</tr>
<tr>
<td>Leaf dry mass per leaf area (LMA)</td>
<td>g m⁻²</td>
<td>35.8</td>
<td>4.7</td>
</tr>
<tr>
<td>Nitrogen (N) content per leaf area</td>
<td>mmol N m⁻²</td>
<td>54.8</td>
<td>6.4</td>
</tr>
<tr>
<td>N concentration per leaf dry mass</td>
<td>mmol N g⁻¹</td>
<td>1.54</td>
<td>0.15</td>
</tr>
<tr>
<td>C/N ratio in leaf blades</td>
<td></td>
<td>19.9</td>
<td>2.05</td>
</tr>
<tr>
<td>Stomatal pore length</td>
<td>μm</td>
<td>39.75</td>
<td>6.37</td>
</tr>
<tr>
<td>Stomatal density in adaxial leaf surface</td>
<td>No. mm⁻²</td>
<td>1.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Stomatal density in abaxial leaf surface</td>
<td>No. mm⁻²</td>
<td>21.0</td>
<td>4.3</td>
</tr>
<tr>
<td>Light compensation point</td>
<td>μmol m⁻² s⁻¹</td>
<td>2.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Apparent quantum use efficiency</td>
<td></td>
<td>0.071</td>
<td>0.008</td>
</tr>
<tr>
<td>Area-based dark respiration rates</td>
<td>μmol m⁻² s⁻¹</td>
<td>-0.17</td>
<td>0.08</td>
</tr>
</tbody>
</table>
Supplementary Figure 1: The ontogenetic variations in size and shape of leaf blades in 150 ginger individuals with different plant sizes. (a) The frequency of blade size of single leaves, and (b) the change of leaf shape (the ratio of length to width in each leaf blade) with leaf area.
Supplementary Figure 2: The diurnal time courses in microclimate at the understory during the successive three days from 15 July to 17 July (the mid-rainy season) in 2008. Incident photosynthetic photon flux (PPF) at (a) an open place and (b) the understory (20 cm above the ground), and (c) air temperature ($T_{\text{air}}$) and relative humidity (RH) in air at an understory site.
Supplementary Figure 3: The relationships between photosynthetic capacity and photosynthetic phone flux (PPF) at the leaf surface. Data were obtained from the measurements of diurnal time courses in (a) net photosynthetic rates, (b) PSII quantum yield, (c) electron transport rates through PSII, and (d) Stern-Volmer non-photochemical quenching coefficient.
Supplementary Figure 4: The measurement of CO₂ concentrations in the air space between the leaf blade and the soil surface.

References