Canopy nitrogen distribution is optimized to prevent photoinhibition throughout the canopy during sun flecks

Mitsutoshi Kitao1, Satoshi Kitaoka1,2, Hisanori Harayama1, Hiroyuki Tobita2, Evgenios Agathokleous1 & Hajime Utsugi2

As photoinhibition primarily reduces the photosynthetic light use efficiency at low light, sunfleck-induced photoinhibition might result in a fatal loss of carbon gain in the shade leaves within a canopy with barely positive carbon balance. We hypothesized that shade leaves at the lower canopy might retain a certain amount of leaf nitrogen (N_L) to maintain energy consumption via electron transport, which contributes to circumventing photoinhibition during sunflecks to keep efficient utilization of low light during the rest period of daytime. We investigated excess energy production, a potential measure of susceptibility to photoinhibition, as a function of N_L distribution within a Japanese oak canopy. Optimal N_L distribution, which maximizes canopy carbon gain, may lead to a higher risk of photoinhibition in shade leaves during sunflecks. Conversely, uniform N_L distribution would cause a higher risk of photoinhibition in sun leaves under the direct sunlight. Actual N_L distribution equalized the risk of photoinhibition throughout the canopy indicated by the constant excess energy production at the highest light intensities that the leaves received. Such a homeostatic adjustment as a whole canopy concerning photoinhibition would be a key factor to explain why actual N_L distribution does not maximize canopy carbon gain.

Within a canopy, leaves acclimate to the growth light conditions, where sun-exposed leaves have a higher area-based leaf N pool (N_L) and higher photosynthetic capacity than shaded leaves1–4. Such a gradient of leaf N along the canopy depth is considered as an efficient utilization of the limited N resource to maximize photosynthetic C gain over the canopy of an entire plant in response to the light gradient within a canopy5–7. However, the measured N_L distribution is more uniform than the theoretically optimal N_L distribution to maximize the canopy carbon gain5–10, which means that sun leaves show lower and shade leaves greater N_L than the optimal value6,11. Furthermore, Hikosaka10 reported that the optimal N_L distribution, considering both direct and diffuse light into account, was much steeper than that conventionally estimated under diffuse light only6,7. Several theoretical approaches have been conducted to explain the less steep declines in N_L than the light gradient within canopies accounting other constraints than light, such as leaf morphological plasticity12, photosynthetic capacity at the top of the canopy limited by the maximum nutrient concentration13, and hydraulic conductance14.

Recently, the importance of direct light, i.e., sunflecks within a canopy, was emphasized both in photosynthesis and photoinhibition. Sunflecks can significantly improve C gain in shade leaves of the lower canopy, while shade leaves may suffer from photoinhibition by the high photosynthetic photon flux density (PPFD) of sunflecks15. Photosynthetic efficiency is reduced during the recovery from photoinhibition on the transition from sunflecks to shade, which would result in a considerable reduction in plant productivity16. The degree of photoinhibition has been empirically linked to average or integrated light intensity in photosynthetic plant production models17,18. However, recent studies have reported that plants grown under fluctuating light environments upregulated the photoprotective mechanisms and electron transport compared with those grown under constant light conditions in response to sunflecks, even when the amounts of daily total irradiance are comparable19,20. This suggests that

1Hokkaido Research Center, Forestry and Forest Products Research Institute, Sapporo, 062-8516, Japan.
2Department of Plant Ecology, Forestry and Forest Products Research Institute, Matsumosato 1, Tsukuba, 305-8687, Japan. Correspondence and requests for materials should be addressed to M.K. (email: kitao@ffpri.affrc.go.jp)
leaves may well acclimate to sun flecks (brief and strong irradiance) rather than averaged irradiance, so as to circumvent transient photo-inhibition.

Photo-inhibition primarily reduces the photosynthetic light use efficiency at low PPFD, i.e. a reduction of the initial slope of the photosynthetic light-response curve rather than the light-saturated photosynthetic rate\textsuperscript{17}. In this context, sun-fleck-induced photo-inhibition may result in a fatal loss of carbon gain in the shade leaves with barely positive carbon balance\textsuperscript{18}. To prevent photo-inhibition, electron transport, mainly involved in photosynthesis and photorespiration\textsuperscript{21}, plays an important role in consuming absorbed light energy as well as thermal energy dissipation\textsuperscript{12,22,23}. Excess energy, neither consumed by electron transport nor dissipated as heat, is known to have a close relationship to photoinactivation of PSII under the inhibition of PSII repair\textsuperscript{24,25}.

Leaf N participation for the circumvention of photo-inhibition is specifically involved in electron transport, but not in xanthophyll-related thermal energy dissipation, since xanthophyll pigments, violaxanthin (C\textsubscript{35}H\textsubscript{44}O\textsubscript{3}), anthoxanthin (C\textsubscript{35}H\textsubscript{42}O\textsubscript{3}), and zeaxanthin (C\textsubscript{35}H\textsubscript{46}O\textsubscript{3}), do not contain any N\textsuperscript{26}. As for the fractions of leaf N in Rubisco, and in proteins related to linear electron transport, there can be little differences in the fractions between the upper and lower canopy leaves\textsuperscript{27}. In this context, leaf N gradient within the canopy can closely reflect the gradient of photosynthesis-related proteins. The capacity of electron transport depends on N\textsubscript{L}, which is varied through intra-canopy light gradients\textsuperscript{28,29}. Thus, shade leaves at the lower canopy may retain a certain amount of N\textsubscript{L} to circumvent photo-inhibition during sun-flecks to keep efficient utilization of low PPFD during the rest period of daytime\textsuperscript{16}.

We hypothesized that N\textsubscript{L} should be distributed within a canopy to prevent photo-inhibition both in shade leaves during sun-flecks and sun leaves under the direct sunlight, leading to the least steep N\textsubscript{L} distribution than the typical sun leaves under the direct sunlight, leading to the less steep N\textsubscript{L} distribution than the deep shade leaves and lower ETR in the deep shade leaves and relatively higher ETR in the typical sun leaves were observed with uniform N\textsubscript{L} distribution (Fig. 4b, Table 2).

Results
Total leaf area index (LAI), F\textsubscript{2}, in the forest was estimated to be 5.91 m\textsuperscript{2} m\textsuperscript{-2} based on the stratified clipping method conducted in 2001 and 2003. Based on the LAI cumulated from the canopy top (F), the light extinction coefficient of the canopy (K\textsubscript{c}) was 0.739 in Eq. 6. Based on the relationship between the relative light intensity (I/I\textsubscript{0}) and F (Eq. 6), we derived F of 15 leaves used for the photosynthetic measurements in 2007 by using relative light intensity at each leaf position. The actual distribution of N\textsubscript{L} as a function of F in 2007 was revealed as follows:

\[
N_L = 2.22 \exp(-0.241F) + 0.269\text{(according to Eq. 7 in Methods)}.
\]

Based on the integration of the actual N\textsubscript{L} distribution, total N per unit ground area (N\textsubscript{T}) was calculated as 8.59 g m\textsuperscript{-2}. Optimal N distribution in the canopy was expressed as follows:

\[
N_L = 5.24 \exp(-0.739F) + 0.269\text{(according to Eq. 8 in Methods)}.
\]

N\textsubscript{T} with uniform distribution was estimated to be 1.45 g m\textsuperscript{-2}, estimated as N\textsubscript{T}/F\textsubscript{2}.

Actual and optimal N\textsubscript{L} distributions within the canopy are shown in Fig. 1. Optimal N\textsubscript{L} was higher than the actual value in the case of F < 1.7 m\textsuperscript{2} m\textsuperscript{-2}, whereas it was lower than the actual value in the case of F > 1.7 m\textsuperscript{2} m\textsuperscript{-2}. F of 1.7 m\textsuperscript{2} m\textsuperscript{-2} corresponds to the average integrated daily flux density in June (Q\textsubscript{int}) of 12 mol m\textsuperscript{-2} day\textsuperscript{-1} (Eq. 6).

In the present study, we expediently classified the leaves into four types based on their growth light environments: (1) deep shade leaves (0 < Q\textsubscript{int} < 3 mol m\textsuperscript{-2} day\textsuperscript{-1}, n = 5); (2) moderate shade leaves (3 < Q\textsubscript{int} < 15 mol m\textsuperscript{-2} day\textsuperscript{-1}, n = 4); (3) moderate sun leaves (15 < Q\textsubscript{int} < 25 mol m\textsuperscript{-2} day\textsuperscript{-1}, n = 3); and (4) typical sun leaves (25 < Q\textsubscript{int} < 35 mol m\textsuperscript{-2} day\textsuperscript{-1}, n = 3). Hereafter, we often use "shade leaves" to refer to deep and moderate shade leaves and "sun leaves" to refer to moderate and typical sun leaves. Peak PPFD entering through the canopy (PPFD\textsubscript{max}), i.e. sun-flecks, was considerably high (>700 \textmu mol m\textsuperscript{-2} s\textsuperscript{-1}), even for the deep shade leaves (Fig. 2).

Electron transport rate (ETR) with actual, optimal and uniform N\textsubscript{L} distribution showed similar relationships as a function of N\textsubscript{L} (Fig. 3a). Higher ETR in sun leaves but lower ETR in shade leaves was observed with optimal N\textsubscript{L} distribution when compared with those with actual N\textsubscript{L} distribution (Table 1). To investigate how ETR changed from the actual to the optimal and uniform N\textsubscript{L} distribution, we showed ETR\textsubscript{opt}/ETR\textsubscript{act} and ETR\textsubscript{act}/ETR\textsubscript{act} as a function of Q\textsubscript{int} (Fig. 4a). Higher ETR\textsubscript{opt}/ETR\textsubscript{act} was observed in leaves grown under higher Q\textsubscript{int} compared among leaf types (Fig. 4a, Table 2). When compared with ETR in leaves with actual N\textsubscript{L} distribution for each leaf type, relatively lower ETR in the deep shade leaves and relatively higher ETR in the typical sun leaves were observed with optimal N\textsubscript{L} distribution (Table 2). Conversely, with uniform N\textsubscript{L} distribution, higher ETR\textsubscript{act}/ETR\textsubscript{act} was observed in the deep shade leaves than the other leaves, where relatively higher ETR in the deep shade leaves and lower ETR in sun leaves were observed when compared with those in leaves with actual N\textsubscript{L} distribution (Fig. 4a, Table 2).

Higher thermal dissipation (D) was generally observed in leaves grown under higher Q\textsubscript{int} with any N\textsubscript{L} distribution, whereas the slope become less steep in leaves with optimal N\textsubscript{L} than the actual, and uniform N\textsubscript{L} distribution (Fig. 3b, Table 1). With optimal N\textsubscript{L} distribution, the typical sun leaves showed lower D\textsubscript{opt}/D\textsubscript{act} than the other leaf types, and relatively lower D than that in the typical sun leaves with actual N\textsubscript{L} distribution. Conversely, there was no significant differences in D\textsubscript{opt}/D\textsubscript{act} among leaf types with uniform N\textsubscript{L} distribution, and also there was no difference in D in leaves with uniform N\textsubscript{L} distribution from that in leaves with actual N\textsubscript{L} distribution within each leaf type (Fig. 4b, Table 2).

There was no difference in excess energy production (E) among leaf types with the actual N\textsubscript{L} distribution (Fig. 3c, Table 1). A significantly higher E was observed in shade leaves than in sun leaves with optimal N\textsubscript{L} distribution. Conversely, a significantly higher E was observed in sun leaves than in shade leaves with uniform N\textsubscript{L} distribution (Fig. 3c, Table 1). Similarly, higher E\textsubscript{opt}/E\textsubscript{act} was observed in shade leaves than in sun leaves with...
optimal $N_L$ distribution, whereas higher $E_{\text{uni}}/E_{\text{act}}$ was observed in sun leaves than in shade leaves with uniform $N_L$ distribution (Fig. 4c, Table 2). With optimal $N_L$ distribution, deep shade leaves showed relatively higher $E$, but typical sun leaves showed relatively lower $E$ compared with those in actual $N_L$ distribution (indicated by the asterisks in Table 2). Conversely, with uniform $N_L$ distribution, deep shade leaves showed relatively lower $E$, but sun leaves showed relatively higher $E$ compared with those in actual $N_L$ distribution.

Based on the relationship between variance in $E$ ($s^2$) and the leaf $N$ distribution coefficient ($K_n$), the $K_n$ minimizing $s^2$ was estimated as 0.291 (Fig. 5), which was very close to the $K_n$ in the actual $N_L$ distribution (0.241 in Eq. 7). We also found that the values of $E$ at $\text{PPFD}_{\text{max}}$ with the actual $N_L$ distribution within the canopy of Japanese oak were very similar to those with the $N_L$ distribution to maintain $E$ constant, minimizing the variance in $E$ ($K_n = 0.291$) (Fig. 6).

**Discussion**

We investigated the fate of absorbed light energy (ETR, $D$, and $E$) in leaves with actual, optimal, and uniform $N$ distribution within the canopy of Japanese oak. Excess energy is known to have a close relationship to photoinactivation of PSII under the inhibition of PSII repair [24,25]. There are two mechanisms involved in photo inhibition (defined as photo inactivation of PSII), i.e., an excess energy mechanism and a two-step mechanism [25]. As excess

**Figure 1.** Actual leaf $N$ ($N_L$) distribution in the canopy of Japanese oak as a function of the leaf area index cumulated from the canopy top ($F$). Dashed line indicates optimal $N_L$ distribution. Leaves were classified into four types based on their growth light environments ($Q_{\text{int}}$): (1) deep shade (triangle, $0 < Q_{\text{int}} < 3 \text{ mol m}^{-2} \text{ day}^{-1}$), moderate shade (diamond, $3 < Q_{\text{int}} < 15 \text{ mol m}^{-2} \text{ day}^{-1}$), moderate sun (square, $15 < Q_{\text{int}} < 25 \text{ mol m}^{-2} \text{ day}^{-1}$), and typical sun leaves (circle, $25 < Q_{\text{int}} < 35 \text{ mol m}^{-2} \text{ day}^{-1}$).

**Figure 2.** Maximal photosynthetic photon flux density ($\text{PPFD}_{\text{max}}$) in June at various leaf positions with various leaf $N$ pool ($N_L$) within the canopy of Japanese oak. $\text{PPFD}_{\text{max}}$ was estimated as average daily peak PPFD at each leaf position using the five highest values daily in June. Symbols are the same as in Fig. 1.
energy might be predominantly involved in photoinhibition in the field under visible light especially at the range of relatively high irradiance\textsuperscript{25,30,31}, c.f. 700–1800 μmol m\textsuperscript{-2} s\textsuperscript{-1} during the sunflecks in the present study, we used the chlorophyll fluorescence parameter “excess energy,” as an empirical measure of the sensitivity of photoinhibitory damage based on the former mechanism. It is noteworthy that $E$ during sunflecks for shade leaves or direct sunlight for sun leaves was stable among leaf types with the actual $N_L$ distribution at various canopy depths, whereas a significantly higher $E$ was observed in the shade leaves than sun leaves with the optimal $N_L$ distribution; a significantly higher $E$ in sun leaves than shade leaves with the uniform $N_L$ distribution (Fig. 3c, Table 1).

As photoprotective $D$ was not enhanced in the leaves with decreased $ETR$ (Table 2)\textsuperscript{22}, the decreases in $ETR$ in the shade leaves with an optimal $N_L$ distribution, and in the sun leaves with a uniform $N_L$ distribution both resulted in the increases in $E$. This also suggests that the photoprotective thermal energy dissipation would not respond enough to set off the changes in $ETR$ with different $N_L$ distributions as far as the present model prediction is concerned, partly because of the small variation in $D$ in the fully acclimated leaves to their growth environments (Supplemental Fig. S1) as reported in long-term drought acclimated plants\textsuperscript{32,33}.

In the present study, the optimal $N$ distribution was estimated using the simple Beer’s law with an assumption that leaves received diffuse light only\textsuperscript{67}. Hikosaka\textsuperscript{10} reported that the optimal $N$ distribution under direct and diffuse light, a more realistic condition than diffuse light only\textsuperscript{67}, was steeper than that under diffuse light only.

**Figure 3.** Electron transport rate (ETR) (a), thermal energy dissipation (D) (b), and excess energy (E) (c) at maximal photosynthetic photon flux density (PPFD\textsubscript{max}) as a function of leaf N pool ($N_L$) with actual (closed), optimal distribution (open), and uniform distribution (grey symbols) in the canopy of Japanese oak. Symbols are the same as in Fig. 1.
An enhanced recovery capacity from photoinhibition by bioengineering lead to an increase in biomass of tobacco plants by up to 20% in fluctuating light\(^{16}\). Thus, there is a possibility to reduce the recovery cost from photoinhibition would be negligible for canopy C balance. Rather, reduced photosynthetic ATP production\(^{34}\), and the cost of relaxation of thermal energy dissipation is also reported to be substantial small, about 0.05% of the total photosynthetic electron flow under saturating light\(^{26}\). Therefore, the decrease in photosynthetic efficiency caused by photoinhibition under fluctuating light was not taken into account\(^{16,19,20}\).

We estimated canopy C gain based on light response curves built with different N distribution and incident PPFD with 1-min-intervals in July 2007 (details in Supplementary Information: Estimation of canopy C gain, Figs S2–S5). We used coefficients of light response curves measured at a leaf temperature of \(27 \degree C\), with no correction for the effects of air temperature, and air humidity on photosynthesis. Total canopy C gain was 24.9, 21.3, and 15.8 mol m\(^{-2}\) s\(^{-1}\) on the ground surface basis for optimal, even-E, which makes E constant throughout the canopy with \(K_E = 0.291\) (Fig. 6), and uniform leaf N distribution, respectively. Even-E N distribution, showed a 15% decrease in canopy C gain integrated in July, during a photosynthetically most vigorous period, compared with optimal N distribution. Conversely, uniform N distribution resulted in a 36% decrease in canopy C gain compared with the optimal N distribution. It is noteworthy, not only the sun leaves with higher leaf \(N_L\) with optimal canopy N distribution, but also the shade leaves with lower \(N_L\) compared with those with even-E N distribution contributed to the higher C gain as a consequence of reduced nighttime respiration rate (Supplementary Figure S5).

In this estimation, a decrease in photosynthetic efficiency caused by photoinhibition under fluctuating light was not taken into account\(^{16,19,20}\). An enhanced recovery capacity from photoinhibition by bioengineering lead to an increase in biomass of tobacco plants by up to 20% in fluctuating light\(^{16}\). Thus, there is a possibility to reduce the difference in the canopy C gain between even-E and optimal N distribution, when the photoinhibitory effects on photosynthetic efficiency are accounted. In addition to an acclimation to short-term fluctuating light (sunflecks), as Japanese oak is a gap-dependent species, which needs a gap formation for its regeneration\(^{2,23}\), keeping relatively higher ETR in shade leaves than optimal would be a pre-conditioning for long-term fluctuating light (gap formation) for this species.

The cost of D1 protein turn-over, as a relevant process of PSI repair, is substantially low at most 0.5% of photosynthetic ATP production\(^{14}\), and the cost of relaxation of thermal energy dissipation is also reported to be substantially small, about 0.05% of the total photosynthetic electron flow under saturating light\(^{26}\). Therefore, the recovery cost from photoinhibition would be negligible for canopy C balance. Rather, reduced photosynthetic efficiency during the recovery from photoinhibition on transfer from sun flecks to shade may have a more significant adverse effect on canopy C gain\(^{16}\). In this context, circumvention of photoinhibition via an enhancement of ETR is essential to maintain the canopy C gain by preventing photoinhibition without any decrease in photosynthetic efficiency, in contrary to xanthophyll-related thermal energy dissipation.

Leaf photosynthetic capacity is closely related to leaf morphology such as leaf mass per area (LMA), where leaves with greater LMA generally have greater leaf area-based nitrogen contents and greater photosynthetic capacity\(^{3,35,36}\). During leaf maturation of Japanese oak, LMA reaches its maximum, accompanied with an increase in net photosynthetic rate, several weeks after the leaves are fully expanded and the light environment within a canopy is fixed (Tobita unpublished data\(^{17}\)). Although the sun/shade anatomy of deciduous leaves is mainly determined by the light condition in the previous year\(^{28}\), less extent but some plasticity exists in leaf morphological change in response to current-year light environment\(^{15}\). For Japanese oak, LMA and net photosynthetic rate increased during June, after the leaf expansion had completed at the end of May (Tobita unpublished data). It was for this reason that we chose growth light environments (\(q_{air}, \text{PPFD}_{\text{max}}\)) in June as determining factors for photosynthetic traits. The equalized amount of excessive energy production observed across the various light environments within a canopy (Figs 5 and 6) could be explained if the developments of photosynthesis (involved in ETR) and photoprotection (involved in D) along with the increase in LMA would be continued until the excessive energy production during sunflecks or direct sunlight drops below a certain level both in shade and sun leaves\(^{16,20}\).

### Table 1.

| Parameter   | Leaf type     | Moderate sun | Typical sun | ANOVA |
|-------------|---------------|--------------|-------------|-------|
| Actual \(N_L\) | Deep shade | 38 ± 4\(^{a}\) | 121 ± 10\(^{a}\) | 147 ± 1\(^{a}\) | \(F = 50.0\ P < 0.001\) |
|             | Moderate shade | 80 ± 9\(^{a}\) | 364 ± 7\(^{a}\) | 423 ± 4\(^{a}\) | \(F = 60.8\ P < 0.001\) |
|             | Typical sun   | 143 ± 10     | 162 ± 6     | 163 ± 4    | \(F = 2.2\ ns\) |
| Uniform \(N_L\) | Deep shade | 12 ± 2\(^{a}\) | 149 ± 17\(^{a}\) | 250 ± 13\(^{a}\) | \(F = 95.3\ P < 0.001\) |
|             | Moderate shade | 64 ± 12\(^{a}\) | 358 ± 5\(^{a}\) | 368 ± 4\(^{a}\) | \(F = 44.5\ P < 0.001\) |
|             | Typical sun   | 184 ± 10\(^{a}\) | 133 ± 11\(^{a}\) | 55 ± 14\(^{a}\) | \(F = 32.9\ P < 0.001\) |
| Optimal \(N_L\) | Deep shade | 82 ± 0\(^{a}\) | 82 ± 0\(^{a}\) | 82 ± 0\(^{a}\) | \(F = 19.7\ P < 0.001\) |
|             | Moderate shade | 82 ± 0\(^{a}\) | 82 ± 0\(^{a}\) | 82 ± 0\(^{a}\) | \(F = 19.7\ P < 0.001\) |
|             | Typical sun   | 107 ± 9\(^{a}\) | 207 ± 5\(^{a}\) | 244 ± 3\(^{a}\) | \(F = 54.3\ P < 0.001\) |
The hypothesis of the present study: higher N_L than optimal is needed in the shade leaves to prevent photoinhibition during sunflecks, is partly similar to that proposed by Dewar et al.\textsuperscript{12}, where a lower bound of LMA in the shade leaves exists, in relation to the limitation in leaf morphological plasticity\textsuperscript{23}. Furthermore, the present study also proposes that the upper bound of N_L in sun leaves would be regulated as the minimum required to circumvent photoinhibition under direct sunlight, which might be related to the upper-bound constraint on photosynthetic capacity at the top of the canopy proposed by Lloyd et al.\textsuperscript{13} to explain the less steep N_L decline.

In the present study, water stress during leaf development was considered to be negligible because of a considerable amount of spring snowmelt in the forest (mean maximum snow depth was 114 cm)\textsuperscript{40}. Peltoniemi et al.\textsuperscript{14} proposed that limited hydraulic conductance for the sun leaves may result in a lower NL in sun leaves with actual N_L distribution than the optimal one. In contrast, leaves acclimated to long-term drought often show higher N_L accompanied with higher ETR, than leaves grown without drought stress, to prevent photoinhibition under lower intercellular CO_2 concentration by stomatal closure\textsuperscript{32,33,41,42}. In this context, water stress on sun leaves induced by the limited hydraulic conductance in the upper canopy\textsuperscript{43,44} would not necessarily decrease N_L in sun leaves, but rather increase N_L for preventing photoinhibition. Further investigation is needed on such environmental stresses during leaf development influencing excess energy production to predict leaf N distribution within a canopy.

**Figure 4.** The ratio of ETR (a), D (b) and E (c) with optimal (open) and uniform N_L distribution (grey symbols) (ETR\textsubscript{opt}, D\textsubscript{opt} and E\textsubscript{opt}; ETR\textsubscript{uni}, D\textsubscript{uni} and E\textsubscript{uni}) to those with actual N_L distribution (ETR\textsubscript{act}, D\textsubscript{act} and E\textsubscript{act}, respectively) at PPFD\textsubscript{max} for a given leaf as a function the daily mean of the integrated photon flux density during leaf development (Q\textsubscript{int}). Dashed line indicates the ratios = 1. Symbols are the same as in Fig. 1.
Table 2. The ratio of ETR, D and E with optimal and uniform N_l distribution (ETR_{opt}, D_{opt} and E_{opt}; ETR_{uni}, D_{uni} and E_{uni}) to those with actual N_l distribution (ETR_{act}, D_{act} and E_{act}, respectively) at PPFD_{max} in different leaf types of Q. mongolica. Values are means ± SE for each leaf type. Different letters indicate significant differences among the leaf types at P < 0.05, according to Holm’s pairwise comparisons. ns indicates non-significant effect of leaf type. *Indicates significant differences in ETR, D and E from those of actual N_l distribution in each leaf type at P < 0.05 with t-test.

| Parameter          | Leaf type               | Optimal N_l | ANOVA   |
|--------------------|-------------------------|-------------|---------|
|                    | Deep shade              | Moderate shade | Moderate sun | Typical sun |         |
| ETR_{opt}/ETR_{act} | 0.32 ± 0.04\(^*\)       | 0.79 ± 0.09\(\ast\) | 1.23 ± 0.06\(^c\) | 1.70 ± 0.08\(\ast\) | F = 81.3 P < 0.001 |
| D_{opt}/D_{act}    | 0.96 ± 0.02\(^c\)       | 0.99 ± 0.01\(^c\) | 0.99 ± 0.01\(^c\) | 0.87 ± 0.02\(^c\) | F = 12.2 P < 0.001 |
| E_{opt}/E_{act}    | 1.29 ± 0.04\(^c\)       | 1.18 ± 0.07\(^c\) | 0.82 ± 0.06\(^c\) | 0.34 ± 0.09\(^c\) | F = 47.6 P < 0.001 |
| Uniform N_l        |                         |             |         |            |         |
| ETR_{opt}/ETR_{act} | 2.25 ± 0.26\(^c\)       | 1.07 ± 0.14\(\ast\) | 0.69 ± 0.06\(\ast\) | 0.56 ± 0.09\(\ast\) | F = 17.4 P < 0.001 |
| D_{uni}/D_{act}    | 0.98 ± 0.02             | 1.00 ± 0.01  | 0.99 ± 0.00  | 0.97 ± 0.01  | F = 0.54 ns            |
| E_{uni}/E_{act}    | 0.74 ± 0.03\(^c\)       | 1.04 ± 0.07\(^c\) | 1.28 ± 0.06\(\ast\) | 1.49 ± 0.03\(^c\) | F = 43.4 P < 0.001 |

Figure 5. The relationship between the variance (s^2) in excess energy production (E) at maximal photosynthetic photon flux density (PPFD_{max}) and variable leaf N distribution coefficient (K_n).

Figure 6. Excess energy production (E) at maximal photosynthetic photon flux density (PPFD_{max}) as a function of leaf N pool (N_L) with actual distribution (closed) and the distribution to minimize the variation in E (open symbols) in the canopy of Japanese oak. Symbols are the same as in Fig. 1.
Conclusion

The present study provides a novel insight into the canopy \( N_i \) distribution with respect to circumvention of photoinhibitory damage at the whole-canopy level like a homeostatic adjustment, where plants follow a strategy through which \( N_i \) distribution is not optimized for canopy \( C \) gain but rather regulated to prevent photoinhibition during sunflecks for shade leaves or direct sunlight for sun leaves. This regulation of \( N_i \) distribution contributes to keeping an efficient light utilization capacity during the daytime at the whole-canopy level, by means of minimizing a time lag in the recovery from photoinhibition \(^{46}\), which indicates an important biological plasticity in the response to light environment, and may suggest genetically-driven adjustments for successful acclimation in a changing light environment. Revealing such a physiological coordination of individual leaves for the benefit of the whole canopy will contribute to advancing the understanding of carbon–nitrogen interactions \(^{45,46}\) and energy flow in terrestrial ecosystems. Further studies with different species and at different environmental conditions are needed to demonstrate that the regulation of \( N_i \) distribution found in this study is an unequivocal biological mechanism occurring across species.

Methods

Study site. The present study was conducted in the experimental forest of the Hokkaido Research Center, Forestry and Forest Products Research Institute in Sapporo, Japan (43°N, 141°E; 180 m above sea level). Mean annual precipitation was approximately 900 mm and the mean annual temperature was 7.1 °C. The predominant species were Japanese white birch (Betula platyphylla Sukatchev var. japonica Hara) and Japanese oak (Q. mongolica Fisch. ex Ledeb. var. crispula (Blume) H. Ohashi), which comprised >75% of the plot basal area.

Estimation of the photosynthetic photon flux density (PPFD) at various leaf positions within the canopy. Leaf morphological and physiological traits are determined by light environments under which leaves are developed\(^ {27}\). We considered that light conditions during leaf development in June were important for leaves of Q. mongolica because photosynthesis reached its maximum during this period (Tobita unpublished data). A hemispherical photograph was taken by a digital camera (Coolpix 4500, Nikon, Tokyo, Japan) with a fisheye lens (Fisheye Lens, FC-E8, Nikon) right above each of the leaves that were used for the photosynthetic gas exchange and chlorophyll fluorescence measurements at the summertime in 2007. Based on the photographs, we estimated the incident PPFD for each leaf by using a canopy analysis software (WinScanopy, Regent Instruments Inc., Quebec, Canada)\(^ {47}\) combined with the data of direct and diffuse radiation from the open sky measured by a pyrheliometer (CH1, Kipp & Zonen, Delft, The Netherlands) combined with an automatic solar tracker (ASTX-1, Prede, Tokyo, Japan) and a pyrheliometer (CM21, Kipp & Zonen) with 1-min intervals placed above the canopy in the experimental forest. Instantaneous incident PPFD at each leaf position was estimated as below:

\[
\text{PPFD} = \text{ISF} \times \left[ \text{diffuse radiation from the open sky} \right] + \left[ \text{direct radiation} \right]
\]

where ISF is the indirect (diffuse) site factor estimated by the WinScanopy software. Penetration of the direct radiation is also assessed with the sun track simulated by the software. The average integrated daily quantum flux density at each leaf position in June \((Q_{\text{int}} \text{ mol m}^{-2} \text{ day}^{-1})\) was calculated as a measure of growth light environment based on 1-min-interval PPFD from the 1st to 30th June, 2007. Average daily peak PPFD in June at each leaf position \((\text{PPFD}_{\text{max}})\) was estimated using the daily five highest values during June\(^ {4}\).

Leaf area index (LAI), and light gradients within the canopy. We used the relationship between LAI and light gradients within the canopy of an inventory plot \((50 \times 50 \text{ m})\) of the experimental forest after Utsugi et al.\(^ {48}\). The three predominant canopy tree species, B. platyphylla, Q. mongolica, and Kalopanax septemlobus, were used for the measurements of LAI during the summers of 2001 and 2003. Eight sample trees for each species \((24 \text{ trees in total for each species})\) were felled, and the gradient of LAI was determined by a stratified clipping method with 1-m strata intervals. PPFD within the canopy was also measured with PPFD sensors (Li-190SA, Li-Cor, NE, USA) with 1-m vertical intervals from 1 to 26 m along with permanent steel scaffolding within the canopy of B. platyphylla, Q. mongolica, and K. septemlobus in the area adjacent to the stratified clipping plot. The measurements were carried out under overcast conditions. Relative PPFD for each height was calculated as a mean of 200 replicates, with synchronized measurements of above and below canopy PPFD. The stand, consisting of B. platyphylla, Q. mongolica, and K. septemlobus, was considered to be matured at that time since the age of the stand was about 90 years when the stratified clipping was conducted. We used the relationship between relative PPFD and LAI based on the pooled data for 2001 and 2003\(^ {48}\) to estimate LAI for each leaf measured in 2007 from the relative PPFD. Relative PPFD at each leaf used for the photosynthetic measurement in 2007 was estimated as \((Q_{\text{int}} \text{ at each leaf position})/Q_{\text{max above the canopy}}\), where \(Q_{\text{max above the canopy}}\) was 43.4 \text{ mol m}^{-2} \text{ day}^{-1} measured by the pyrheliometers placed above the canopy.

Measurements of gas exchange and chlorophyll fluorescence. Measurements of gas exchange and chlorophyll fluorescence were conducted at the summertime of 2007 on mature leaves of a Japanese oak (approximately 23 m height and 95-years-old as of 2007), surrounded by the scaffolding described above with a portable photosynthesis measuring system (Li-6400, Li-Cor, Lincoln, NE, USA) combined with a leaf chamber fluorometer (Li-6400-40, Li-Cor). We selected a total of 15 leaves grown at various positions within the canopy \((4 \text{ to } 23 \text{ m in height})\). The net photosynthetic rate \((A_n)\), quantum yield of PSII electron transport \((qP)\), photochemical efficiency of the open PSII \((Fv'/Fm')\) and excitation pressure adjusted for the efficiency of PSII photochemistry \((1 - qQ) Fv'/Fm'\) were measured at a photosynthetic steady state, where \(qP\) is photochemical quenching. The measurements
were conducted under an ambient CO₂ concentration of 360 μmol mol⁻¹ and at various PPFD (0, 50, 100, 200, 300, 600, 1,000, 1,500, and 2,000 μmol m⁻² s⁻¹) provided by a red/blue LED array (Li-6400-40, Li-Cor), with blue light comprising 10% of the total PPFD. Leaf temperature was ≈27 °C during the measurements. After the measurements, the leaves were sampled and used for determination of N_L by the combustion method using an analysis system composed of a N/C determination unit (SUMIGRAPH, NC 800, Sumika Chem. Anal. Service, Osaka, Japan), a gas chromatograph (GC 8A, Shimadzu, Kyoto, Japan), and a data processor (Chromatopac, C.R6A, Shimadzu).

Electron transport rate (ETR) was calculated as ETR = Φ_max × leaf absorbance × light intensity × 0.5²⁴⁻⁹. Leaf absorbance was calculated from a calibration curve between SPAD readings (measured with a SPAD chlorophyll meter, SPAD 502, Minolta, Osaka, Japan) and leaf absorbance.

The responses of ETR to incident irradiance were fitted by the convexity equations²⁸ as below:

\[ 0 = ETR^2 - (\phi PPFD + \phi ETR_{max}) ETR + \phi PPFD \times ETR_{max} = 0 \]  

where \( \phi \) is the initial slope (maximum quantum yield), \( \theta \) the convexity of the curve, and ETR_max the maximum rate of electron transport.

The responses of Fv'/Fm' and (1 - qP) Fv'/Fm' to incident irradiance for each leaf were fitted as follows:

\[ Fv'/Fm' = \gamma_0 + a/(1 + (PPFD/x_0)^b) \]  

\[ (1 - qP) Fv'/Fm' = a(1 - \exp(-bPPFD)) \]  

Analogous to ETR, thermal dissipation (D) and excess energy production (E) were estimated from (1 - Fv'/Fm') × leaf absorbance × light intensity × 0.5 and (1 - qP) Fv'/Fm' × leaf absorbance × light intensity × 0.5, respectively. Each coefficient in Eqs 2–4 was fitted as a function of NF defined as (N_L – N_b) of the leaves used.

Based on the coefficients of light responses in ETR, Fv'/Fm', respectively²²,²⁴. Each coefficient in Eqs 2–4 was fitted as a function of NF defined as (N_L – N_b) of the leaves used. Based on the coefficients of light responses in ETR, Fv'/Fm', where NTF is the total free nitrogen in the canopy and NT and FT are the total leaf N pool and LAI per unit ground area, respectively. N_L is calculated based on the integration of Eq. 2¹¹. A N_L distribution is defined as uniform when all leaves have the same N_L irrespective of leaf position. Therefore, N_L with uniform distribution is determined as:

\[ N_L = N_T / F_T \]  

Based on the estimated N_L with optimal and uniform distribution, we calculated ETR, D, and E under the maximum PPFD during sunflecks for each leaf by using the N_L-dependent photosynthetic coefficients (Supplemental Fig. S1) and Eqs 2–4.

We hypothesized that canopy N might be distributed to maintain E constant throughout the canopy during sunflecks, thus to keep an efficient light utilization capacity by preventing photoinhibition for all leaves across sun and shade environments. Therefore, we estimated the leaf N distribution coefficient (K_n) that minimized the variance in E at PPFD_max across the different leaf types within the canopy, using Eq. 8 with various K_n (0.1 to 1.0 with an interval of 0.1) instead of K_L as follows:

\[ N_L = K_n N_T / (1 - \exp(-K_n F_T)) \exp(-K_n F) + N_h \]  

\[ N_T = N_T / F_T \]  

\[ N_L = N_T / F_T \]  

Actual, optimal and uniform leaf N distribution. We derived the light extinction coefficient of the canopy, K_C³² as follows:

\[ I/I_0 = \exp(-K_L F) \]  

where F is the LAI cumulated from the canopy top, and I and I_0 are PPFD at F and the canopy top, respectively. Actual N_L distribution at depth F in the canopy is also described by an exponential function of F with a photosynthetic leaf N distribution coefficient (K_n):

\[ N_L = (N_0 - N_h) \exp(-K_n F) + N_h \]  

where N_0 is the N_L of the leaves at the canopy top. Optimal N_L distribution was derived after Anten et al.⁶ as follows:

\[ N_L = K_n N_T / (1 - \exp(-K_n F_T)) \exp(-K_n F) + N_h \]  

With

\[ N_T = N_T / F_T \]  

\[ N_T = N_T / F_T \]  

\[ N_T = N_T / F_T \]  

\[ N_T = N_T / F_T \]  

Based on the estimated N_L with optimal and uniform distribution, we calculated ETR, D, and E under the maximum PPFD during sunflecks for each leaf by using the N_L-dependent photosynthetic coefficients (Supplemental Fig. S1) and Eqs 2–4.

We hypothesized that canopy N might be distributed to maintain E constant throughout the canopy during sunflecks, thus to keep an efficient light utilization capacity by preventing photoinhibition for all leaves across sun and shade environments. Therefore, we estimated the leaf N distribution coefficient (K_n) that minimized the variance in E at PPFD_max across the different leaf types within the canopy, using Eq. 8 with various K_n (0.1 to 1.0 with an interval of 0.1) instead of K_L as follows:

\[ N_L = K_n N_T / (1 - \exp(-K_n F_T)) \exp(-K_n F) + N_h \]  

\[ N_T = N_T / F_T \]  

\[ N_T = N_T / F_T \]  

\[ N_T = N_T / F_T \]  

Based on the estimated N_L with optimal and uniform distribution, we calculated ETR, D, and E under the maximum PPFD during sunflecks for each leaf by using the N_L-dependent photosynthetic coefficients (Supplemental Fig. S1) and Eqs 2–4.

We hypothesized that canopy N might be distributed to maintain E constant throughout the canopy during sunflecks, thus to keep an efficient light utilization capacity by preventing photoinhibition for all leaves across sun and shade environments. Therefore, we estimated the leaf N distribution coefficient (K_n) that minimized the variance in E at PPFD_max across the different leaf types within the canopy, using Eq. 8 with various K_n (0.1 to 1.0 with an interval of 0.1) instead of K_L as follows:

\[ N_L = K_n N_T / (1 - \exp(-K_n F_T)) \exp(-K_n F) + N_h \]  

\[ N_T = N_T / F_T \]  

\[ N_T = N_T / F_T \]  

\[ N_T = N_T / F_T \]  

Based on the estimated N_L with optimal and uniform distribution, we calculated ETR, D, and E under the maximum PPFD during sunflecks for each leaf by using the N_L-dependent photosynthetic coefficients (Supplemental Fig. S1) and Eqs 2–4.
Variance ($s^2$) in $E$ was estimated as follows:

$$s^2 = \frac{1}{n} \sum_{i=1}^{n} (E_s - E)^2$$  \hspace{1cm} (12)

Statistical analysis. We expediently classified the leaves into four types based on their growth light environments: (1) deep shade leaves ($0 < Q_{int} < 3 \text{ mol m}^{-2} \text{ day}^{-1}$, $n = 5$); (2) moderate shade leaves ($3 < Q_{int} < 15 \text{ mol m}^{-2} \text{ day}^{-1}$, $n = 4$); (3) moderate sun leaves ($15 < Q_{int} < 25 \text{ mol m}^{-2} \text{ day}^{-1}$, $n = 3$); and (4) typical sun leaves ($25 < Q_{int} < 35 \text{ mol m}^{-2} \text{ day}^{-1}$, $n = 3$). One-way analysis of variance (ANOVA) was used to test the differences among leaf types in ETR, D, and E with actual, optimal, or uniform $N_L$ distribution, and the ratio of ETR, D, and E with optimal or uniform to actual $N_L$ distribution. Furthermore, when the ANOVA returned an overall significant effect of leaf type, the means were further tested using Holm’s pairwise comparisons. The threshold for statistical significance was predefined at an alpha level of 0.05.

Data availability. All data used in this manuscript are present in the manuscript and its supplementary information.

References

1. Björkman, O. Responses to different quantum flux densities. In *Physiological Plant Ecology I: Responses to the Physical Environment* (eds Lange, O. L., Nobel, P. S., Osmond, C. B. & Ziegler, H.) pp. 57–107, http://dx.doi.org/10.1007/978-3-642-68090-8_4 (Springer, 1981).
2. Kitao, M., Lei, T. T., Koike, T., Tobita, H. & Maruyama, Y. Susceptibility to photoinhibition of three deciduous broadleaf tree species with different successional traits raised under various light regimes. *Plant, Cell Environ.* 23, 81–89 (2000).
3. Kitao, M. et al. Leaves of Japanese oak (*Quercus mongolica var. crispula*) mitigate photoinhibition by adjusting electron transport capacities and thermal energy dissipation along the intra-canopy light gradient. *Physiol. Plant.* 146, 192–204 (2012).
4. Peary, R. W. & Sims, D. A. Photosynthetic acclimation to changing light environments: Scaling from the leaf to the whole plant. In *Exploitation of Environmental Heterogeneity by Plants: Ecophysiological processes above- and belowground* (eds Roy, J., Caldwell, M. M. & Pearce, R. P.) 145–174, http://dx.doi.org/10.1007/B978-0-12-155070-7.50010-X (Elsevier, 1994).
5. Field, C. Allocating leaf nitrogen for the maximization of carbon gain: Leaf age as a control on the allocation program. *Oecologia* 56, 341–347 (1983).
6. Anten, N. P. R., Schieving, F. & Werger, M. J. A. Patterns of light and nitrogen distribution in relation to whole canopy carbon gain in C3 and C4 mono- and dicotyledonous species. *Ecologia* 101, 504–513 (1995).
7. Hirose, T. Development of the Monsi-Saeki theory on canopy structure and function. *Ann. Bot.* 95, 483–494 (2005).
8. Anten, N. P. R. Optimal photosynthetic characteristics of individual plants in vegetation stands and implications for species coexistence. *Ann. Bot.* 95, 495–506 (2005).
9. Ninemets, U. & Way, D. Optimization of foliage photosynthetic capacity in tree canopies: Towards identifying missing constraints. *Tree Physiol.* 32, 505–509 (2012).
10. Hikosaka, K. Optimal nitrogen distribution within a leaf canopy under direct and diffuse light. *Plant, Cell Environ.* 37, 2077–2085 (2014).
11. Hirose, T. & Werger, M. J. A. Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. *Oecologia* 72, 520–526 (1987).
12. Dewar, R. C., Tarvainen, L., Parker, K., Wallin, G. & McMurtrie, R. E. Why does leaf nitrogen decline within tree canopies less rapidly than light? An explanation from optimization subject to a lower bound on leaf mass per area. *Tree Physiol.* 32, 520–534 (2012).
13. Lloyd, I. et al. Optimisation of photosynthetic carbon gain and within-canopy gradients of associated foliar traits for Amazon forest trees. *Biogeosciences* 7, 1833–1859 (2010).
14. Peltoniemi, M. S., Duursma, R. A. & Medlyn, B. E. Co-optimal distribution of leaf nitrogen and hydraulic conductance in plant canopies. *Tree Physiol.* 32, 510–512 (2012).
15. Way, D. A. & Peary, R. W. Sunflecks in trees and forests: From photosynthetic physiology to global change biology. *Tree Physiol.* 32, 1066–1081 (2012).
16. Kromdijk, J. et al. Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science* 354, 857–861 (2016).
17. Werner, C., Ryel, R. J., Correia, O. & Beyschlag, W. Effects of photoinhibition on whole-plant carbon gain assessed with a photosynthesis model. *Plant, Cell Environ.* 24, 37–40 (2001).
18. Ninemets, U. & Keenan, T. Photosynthetic responses to stress in Mediterranean evergreens: Mechanisms and models. *Environ. Exp. Bot.* 103, 24–41 (2014).
19. Alter, P., Dreissen, A., Luo, F. L. & Matsubara, S. Acclimatory responses of Arabidopsis to fluctuating light environment: Comparison of different sunfleck regimes and accessions. *Photosynth. Res.* 113, 221–237 (2012).
20. Miyata, K., Ikeda, H., Nakaji, M., Kandel, D. R. & Terashima, I. Rate constants of PSII photoinhibition and its repair, and PSII fluorescence parameters in field plants in relation to their growth light environments. *Plant Cell Physiol.* 56, 1841–1854 (2014).
21. Cornic, G. & Fresneau, C. Photosynthetic carbon reduction and oxygen cycles are the main electron sinks for photosystem II activity during a mild drought. *Ann. Bot.* 89, 887–894 (2002).
22. Demmig-Adams, B. et al. Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. *Physiol. Plant.* 98, 253–264 (2000).
23. Kitao, M., Lei, T. T., Koike, T., Tobita, H. & Maruyama, Y. Tradeoff between shade adaptation and mitigation of photoinhibition in leaves of *Quercus mongolica* and *Acer mon* acclimated to deep shade. *Tree Physiol.* 26, 441–448 (2006).
24. Kato, M. C., Hikosaka, K., Hirotsu, N., Makino, A. & Hirose, T. The excess light energy that is neither utilized in photosynthesis nor dissipated by photoprotective mechanisms determines the rate of photoactivation in photosystem II. *Plant Cell Physiol.* 44, 318–325 (2003).
25. Oguchi, R., Terashima, I., Koji, K. & Chow, W. S. Operation of dual mechanisms that both lead to photoactivation of Photosystem II in leaves by visible light. *Physiol. Plant.* 142, 47–55 (2011).
26. Wilhelm, C. & Selmar, D. Energy dissipation is an essential mechanism to sustain the viability of plants: The physiological limits of improved photosynthesis. *J. Plant Physiol.* 168, 79–87 (2011).
27. Ninemets, U., Kull, O. & Tenhunen, J. D. Within-canopy variation in the rate of development of photosynthetic capacity is proportional to integrated quantum flux density in temperate deciduous trees. *Plant, Cell Environ.* 27, 293–313 (2004).
28. Ninemets, U., Bilger, W., Kull, O. & Tenhunen, J. D. Acclimation to high irradiance in temperate deciduous trees in the field: Changes in xanthophyll cycle pool size and in photosynthetic capacity along a canopy light gradient. *Plant, Cell Environ.* 21, 1205–1218 (1998).
29. Ninemets, U., Bilger, W., Kull, O. & Tenhunen, J. Responses of foliar photosynthetic electron transport, pigment stoichiometry, and stomatal conductance to interacting environmental factors in a mixed species forest canopy. *Tree Physiol.* 19, 839–852 (1999).
30. Korniyeyev, D., Logan, B. A. & Holoday, A. S. Excitation pressure as a measure of the sensitivity of photosystem II to photo-inactivation. Funct. Plant Biol. 37, 943–951 (2010).
31. Vass, I. Role of charge recombination processes in photodamage and photoprotection of the photosystem II complex. Physiol. Plant. 142, 6–16 (2011).
32. Kitao, M. & Lei, T. T. Circumvention of over-excitation of PSII by maintaining electron transport rate in leaves of four cotton genotypes developed under long-term drought. Plant Biol. 9, 69–76 (2007).
33. Kitao, M. et al. Interaction of drought and elevated CO₂ concentration on photosynthetic down-regulation and susceptibility to photoinhibition in Japanese white birch seedlings grown with limited N availability. Tree Physiol. 27, 727–735 (2007).
34. Miyata, K., Noguchi, K. & Terashima, I. Cost and benefit of the repair of photodamaged photosystem II in spinach leaves: Roles of acclimation to growth light. Photosynth. Res. 113, 165–180 (2012).
35. Evans, J. R. Photosynthesis and nitrogen relationship in leaves of C3 plants. Oecologia 78, 9–19 (1989).
36. Keenan, T. F. & Niinemets, Ü. Global leaf trait estimates biased due to plasticity in the shade. Nat. Plants 3, 16201 (2016).
37. Harayama, H., Ishida, A. & Yoshimura, H. Overwintering evergreen oaks reverse typical relationships between leaf traits in a species spectrum. R. Soc. open Sci. 3, 160276 (2016).
38. Eschrich, W., Burchardt, R. & Essiham, S. The induction of sun and shade leaves of the European beech (Fagus sylvatica L.): anatomical studies. Trees 3, 1–10 (1989).
39. Tobita, H. et al. Variation in photoinhibition among Sasa senanensis, Quercus mongolica, and Acer mono in the understory of a deciduous broad-leaved forest exposed to canopy gaps caused by typhoons. Trees 24, 307–319 (2010).
40. Mizoguchi, Y., Yamanouchi, K., Kitamura, K., Nakai, Y. & Suzuki, K. Meteorological observations at the Sapporo forest meteorology research site from 1999 to 2008, Hokkaido, Japan. Bull. For. For. Prod. Res. Inst. 13, 193–206 (2014).
41. Flexas, J., Bota, J., Galmés, J., Medrano, H. & Ribas-Carbó, M. Keeping a positive carbon balance under adverse conditions: Responses of photosynthesis and respiration to water stress. Physiol. Plant. 127, 343–352 (2006).
42. Sperdouli, I. & Moustakas, M. A better energy allocation of absorbed light in photosystem II and less photooxidative damage to chloroplasts. Photosynth. Res. 91, 209–218 (2006).
43. Niinemets, Ü., Sonninen, E. & Tobias, M. Canopy gradients in leaf intercellular CO₂ mole fractions revisited: Interactions between leaf traits in a species spectrum. R. Soc. open Sci. 3, 160276 (2016).
44. Ishii, H. R., Azuma, W., Kuroda, K. & Sillett, S. C. Pushing the limits to tree height: Could foliar water storage compensate for hydraulic constraints in Sequoia sempervirens? Funct. Ecol. 28, 1087–1093 (2014).
45. LeBauer, D. S. & Treseder, K. K. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89, 371–379 (2008).
46. Coskun, D., Britto, D. T. & Kronzucker, H. J. Nutrient constraints on terrestrial carbon fixation: The role of nitrogen. J. Plant Physiol. 203, 93–109 (2016).
47. Chazdon, R. L. & Field, C. B. Photographic estimation of photosynthetically active radiation: evaluation of a computerized technique. Oecologia 73, 525–532 (1987).
48. Utug, H., Tobita, H., Maruyama, Y. & Ishizuka, M. Spatial and seasonal variations in leaf mass per area and their relationship to leaf nitrogen in a secondary northern hardwood forest in Japan. Phyton 45, 245–251 (2005).
49. Genty, B., Briantais, J. M. & Baker, N. R. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta - Gen. Subj. 990, 87–92 (1989).
50. Leiper, J. W. Factors determining the nature of the light response curve of leaves. In Photoinhibition of Photosynthesis: from Molecular Mechanisms to the Field (eds Baker, N. R. & Bowyer, J. R.) 239–254, (BIOS Scientific Publishers, 1994).
51. Hikosaka, K. et al. A meta-analysis of leaf nitrogen distribution within plant canopies. Ann. Bot. 118, 393–47 (2016).
52. Monsi, M. & Saeki, T. Über den Lichteinfall in den Pflanzengesellschaften und seine Bedeutung für die Stoffproduktion. Japanese J. Bot. 14, 22–52 (1953).
53. R Development Core Team. R: A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria. ISBN 3-900051-07-0 (2014).

Acknowledgements
This study was partly supported by the project “Research on response to climate change for agriculture, forestry and fisheries” funded by Agriculture, Forestry and Fisheries Research Council, Japan, and by JSPS KAKENHI Grant Number JP17F17102. Evgenios Agathokleous is a JSPS International Research Fellow (ID No: P17102).

Author Contributions
M.K., H.T. and H.U. conceived the idea; M.K., S.K., H.H., H.T. and H.U. collected the data; S.K. conducted the leaf N analysis; H.H. analyzed hemispheric photographs; M.K. and E.A. led the writing of the manuscript with input from all co-authors; All authors contributed critically to the drafts and gave final approval for publication.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-017-18766-0.

Competing Interests: The authors declare that they have no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.