Effects of Vertical Gradient of Leaf Nitrogen Content on Canopy Photosynthesis in Tall and Dwarf Cultivars of Sorghum

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Abstract: The vertical profile of leaf nitrogen (N) content per unit leaf area (NLA) is important for increasing crop productivity via optimizing N use for canopy photosynthesis. To investigate the effects of plant height on the optimality, we analyzed the NLA profiles with respect to light gradient twice during vegetative growth in canopies of tall and dwarf cultivars of sorghum. The gradients of the NLA profiles relative to the light gradients were similar in the two cultivars although the vertical light gradient was steeper in the dwarf cultivar with doubled leaf area density (LAD). This suggests that light attenuation is more influential on the NLA profile than is plant height or LAD. The advantage of the observed NLA profile for the canopy CO2 uptake as compared to the uniform NLA profile was similar in the cultivars except when the N allocation rate to the canopy decreased relative to the leaf area expansion in the dwarf cultivar. These results suggested that the optimality of the advantage of the NLA gradient may not be directly influenced by the plant height, but by the balance between N allocation and leaf area expansion. The balance may be altered by the difference in the biomass allocation in the shoot between the tall and dwarf sorghum. These factors are to be taken into consideration in breeding programs that target stature in order to potentially increase production in sorghum.

Key words: C₄, Canopy structure, Height, Leaf area density, Sorghum.

Canopy photosynthesis, integrated photosynthesis of individual leaves composing the canopy, represents the gross primary productivity of plant community. Light is the ultimate source of energy for photosynthesis, and the canopy architecture is considered important; especially with respect to light interception for canopy photosynthesis and crop growth / yield (Nobel et al., 1993; Monsi and Saeki, 2005). In addition, vertical nitrogen (N) distribution throughout the canopy can also be an important factor since N content per unit leaf area (N_LA) affects photosynthetic performance of a leaf (Sage and Pearcy, 1987). Canopies of various crops (Sadras et al., 1993; Shiraiwa and Sinclair, 1993; Dreccer et al., 2000; van Oosterom et al., 2010) as well as natural herbaceous vegetation (Field, 1983; Hirose and Werger, 1987a) show vertical N_LA gradients. The N_LA gradient has been regarded as a plastic response to light environment within the canopy, which optimizes N use with respect to canopy photosynthesis through N reallocation from the lower to the upper leaves.

The relative advantages of different N_LA profiles can be assessed by calculating daily canopy photosynthesis (P_d) for a particular light distribution pattern based on N-dependent leaf photosynthetic rates (Hirose and Werger, 1987b). The calculated gains in P_d for an actual N_LA profile against a uniform one, ranges from 6 to 41% (see Hirose, 2005). These findings also emphasize that the relationships between canopy structure and N_LA profile rather than canopy structure per se is to be considered for assessing canopy photosynthesis and its efficiency.

Sorghum is one of the most widely grown C₄ crops in the world (Brown, 1999). In sorghum, modifying height has been one of the breeding targets for improving potential grain yield mainly through increasing shoot biomass production (George-Jaeggli et al., 2013). Dwarf genotypes show shorter height through shortening of internode lengths while leaf area and maturity are generally unchanged (George-Jaeggli et al., 2011). Thus, plant height may alter the light distribution within the canopy by changing the leaf area per unit volume of space or leaf area density (LAD). In addition, light distributed more uniformly within sorghum canopy as the number of dwarfing genes increased, probably in association with more

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Abbreviations: N, nitrogen; N_L, leaf N content per unit leaf area; N_0, N_LA at the uppermost canopy; K_n, Coefficient of nitrogen distribution; P_c, canopy photosynthesis rate; P_d, daily canopy photosynthesis.
erect leaves (George-Jaeggli et al., 2013). If the N reallocation is influenced by plant height or LAD, the canopy photosynthesis may be affected by the altered $N_{LA}$—light relationship. Van Oosterom et al. (2010) compared the $N_{LA}$ gradient on the basis of canopy depth between the dwarf and the tall sorghum genotypes. They suggested that the steeper $N_{LA}$ gradient might compensate the less total canopy N content for the canopy photosynthesis of the tall sorghum, but whether or not the plant height affected the $N_{LA}$ profile and the canopy photosynthesis remained unclear because the light gradients and the photosynthetic performance were not measured in their studies. Therefore, by applying methods from the optimization studies mentioned above, we evaluated the canopy photosynthesis and the optimality of the $N_{LA}$ profile in canopies of tall and dwarf, cultivars of sorghum during the vegetative growth.

### Materials and Methods

1. **Canopy photosynthesis**

   We calculated canopy photosynthesis following Anten et al. (1998), with some minor modifications. Photon flux density (PFD) was assumed to be attenuated within the leaf canopy (1998), with some minor modifications. Photon flux density ($F_N$) at a given depth ($F_D$) can be given by differentiating $I$ with respect to $F$:

   $$I = I_0 \exp (-K_{I2})$$  \hspace{1cm} (1)

   where $F$ is the cumulative LAI from the top of the canopy and $I_0$ and $I$ are the PFD on a horizontal level above the canopy and at $F$, respectively. $K_{I2}$ is the coefficient of light extinction. The PFD on a leaf surface ($K_L$) at a certain depth ($F$) can be given by differentiating $I$ with respect to $F$:

   $$K_L = K_{I2} I$$  \hspace{1cm} (2)

   A nonrectangular hyperbola was used to formulate the light response curve of individual leaf net photosynthesis ($P_n$):

   $$P_n = [(P_{max} + \phi I_0) - [(P_{max} + \phi I_0)^2 - 4P_{max} \phi \theta I_0]^{1/2}] / 2 \theta - R_d$$  \hspace{1cm} (3)

   where $P_n$ is the net leaf photosynthetic rate; $P_{max}$ is light-saturated photosynthesis; $R_d$ is dark respiration; and $\theta$ and $\phi$ are the curvature factor and quantum yield, respectively (Hirose and Werger, 1987b). Leaf photosynthesis was related to $N_{LA}$ by assuming that $P_{max}$ and $R_d$ increased linearly as $N_{LA}$ increased (Hirose and Werger, 1987b):

   $$P_{max} = a_d N_{LA} + b_d$$

   $$R_d = a_d N_{LA} + b_d$$  \hspace{1cm} (4)

   where $a_d$ and $b_d$ are the slope and y-intercept of the $P_n$ vs. $N_{LA}$ relationship, whereas $a_d$ and $b_d$ are the slope and y-intercept of the $R_d$ vs. $N_{LA}$ relationship. The curvature factor ($\theta$) and the quantum yield ($\phi$) were assumed to be independent of $N_{LA}$.

   The $N_{LA}$ at a certain depth ($F$) in the canopy was described with an exponential function of $F$:

   $$N_{LA} = N_0 \exp (-K_{N}F)$$  \hspace{1cm} (5)

   where $K_N$ is the coefficient of leaf N allocation and $N_0$ is the $N_{LA}$ value at the top of the canopy. $K_N$ increased with an increasing gradient of the $N_{LA}$ profile in which the upper leaves in the canopy exhibit higher N concentrations than lower leaves. By eliminating $F$ from Equations (1) and (5), the relationship between $N_{LA}$ and PFD was formulated as follows:

   $$N_{LA} = N_0 (I / I_0)^{KN/2}$$  \hspace{1cm} (6)

   or $N_{LA} / N_0 = (I / I_0)^{KN/2}$  \hspace{1cm} (7)

   While eq. (6) describes the absolute value of $N_{LA}$ profile with respect to the relative PFD within canopy, eq. (7) describes the $N_{LA}$ profile relative to the top of the canopy.

   The canopy photosynthesis ($P_C$) is an integration of the instantaneous leaf photosynthesis ($P_L$) throughout the canopy at a given $I$. The daily total $P_C$ ($P_D$) is an integration of the $P_C$ with respect to the time of day where $I$ is assumed to follow a squared sine curve with a maximum of 2000 $\mu$mol m$^{-2}$ s$^{-1}$ and a 13 h day length, the approximate average day length during the entire growing season. We calculated $P_w$, $P_C$, and $P_D$ for the two different $N_{LA}$ profiles, namely uniform and actual profiles, with fixed total amount of N allocated to the canopy (i.e. total canopy N content). When the uniform $N_{LA}$ profile was assumed, the $N_{LA}$ was synonymous to the average canopy N content (i.e. total canopy N content divided by the canopy leaf area). The total canopy N content was measured at each sampling (see below). The $P_w$, $P_C$, and $P_D$ values were expressed as per unit of land area.

2. **Growth of stands**

   The model was applied to stands of two different cultivars of sorghum, *Sorghum bicolor* (L.) Moench cv. X8361A (Takii & Co.,Ltd) and Kazetachi (Nagano Animal Exp. Stn.). X8361A is a tall variety with late maturity whereas Kazetachi is a dwarf variety with very late maturity (Anas and Yoshida, 2004). A field experiment was carried out at the University of the Ryukyus in Okinawa, Japan (26°15’N, 127°45’E; altitude 127 m). Seeds of both cultivars were sown on May 9, 2011 at a density of 5.6 m$^{-2}$ (spacing of 0.6 x 0.3 m). Prior to seeding, the soil was fertilized with N (200 kg N ha$^{-1}$ as (NH$_4$)$_2$SO$_4$), phosphorus (110 kg P ha$^{-1}$ as P$_2$O$_5$) and potassium (110 kg K ha$^{-1}$ as KCl). The plot size was 6 x 24 m for each cultivar. Rainfall was supplemented by irrigation as necessary for 2 weeks after sowing so that the germination and the growth of seedlings were kept from drought stress. Standard meteorological measurements were obtained from a meteorological tower set in the field. The crop received 587 mm of rainfall throughout the entire growing season, and no significant water limitation occurred. The total incident solar radiation on the crop throughout entire growing season was about 1400 MJ m$^{-2}$ with the average of 16.7 ± 6.7 MJ m$^{-2}$ d$^{-1}$. The average of maximum and minimum temperatures were 29.3 ± 2.8 and 24.3 ± 2.7°C, respectively, at this site.

3. **Canopy structure**

   Plant sampling was conducted twice during the growing
period before flowering occurred. The first set of samples was collected on 13 and 14 July 2011 (T1), and the second set on 29 July and 1 Aug 2011 (T2) for X8361A and Kazetachi stands, respectively. Applying the stratified clipping method (Monsi and Saeki, 2005), we determined the canopy structure and light distribution. The PFD (400—700 nm) was measured at height increments of 40 cm from the ground using three PAR light sensor bars (#s366816, Spectrum Technologies, Inc.) inserted between three rows within the canopy; another PAR light sensor bar was used concurrently to measure the PFD above the canopy. The PFD data were read simultaneously within and above the canopy with a data logger (Datum-Y, Yokogawa) under overcast skies. Immediately after measurement of the light climate, four adjacent plants between the rows (two plants per row) were cut at ground level and transported to the laboratory. In the laboratory, the plants were clipped every 40 cm along their length starting from the base and divided into stems, leaves and dead leaves. At each sampling, we replicated this procedure three times per cultivar (i.e. 3 plots in a field). Leaf area was measured with a leaf area meter (LI3100, Li-Cor). Dry weights were determined after oven drying at 70°C for at least 3 days. The dried leaves from each layer of the sample in a plot were mixed together and cut into pieces. Then, subsamples of the pieces were ground in a vibrating mill (TI-100, CMT Co., Ltd.), and their N content was determined with a NC-analyzer (NC-80, Shimadzu).

4. Gas exchange measurements
These measurements were conducted on 9–12 and 26–29 July in X8361A and Kazetachi stands, respectively. Photosynthesis and dark respiration were measured with a portable open gas exchange system (LCpro+, ADC Bioscientific, Ltd.) with a broad leaf chamber window area of 0.25 cm². The source of light was an LCpro+ red and blue LED lamp placed on top of the leaf clippings, while outside light was shut out using a black jacket covering the chamber. Measurements were performed under environmental humidity and CO₂ conditions. An assessment of the infrared gas analyzer was performed every night during the measurement period using a compressed air cylinder with a known CO₂ concentration (400 ppm; Sumitomo Seika Chemicals Co., Ltd.), and the machine was recalibrated if necessary. Photosynthesis measurements were conducted on attached leaves (27 per cultivar) from different, random heights within the canopy. Photosynthetic light response curves were constructed by reducing the PFD in six steps from 1600 μmol m⁻² s⁻¹. The average leaf temperature was 31.5 ± 1.8°C. Following the gas exchange measurements, leaf segments were sampled at 10 cm increments from the measured point, and the leaf area, dry weight and N content were determined using the procedure described above.

5. Statistical analysis
Analysis of variance (ANOVA) was used to test for the main effects of the growing period and cultivars. Analysis of covariance (ANCOVA) was employed to test for differences in the light gradient vs the cumulative LAI and the NLA gradient vs the light gradient between the cultivars and growing periods. Results were assumed to be significant at P < 0.05.

Results
1. Parameters of leaf gas exchange
The Pₘₐₓ ranged widely from 19.7 to 61.5 μmol m⁻² s⁻¹ for X8361A and from 12.6 to 53.9 μmol m⁻² s⁻¹ in Kazetachi. The Pₘₐₓ increased linearly with the increase in Nₐ in X8361A with the slope values of 43.1 and 49.9 μmol g⁻¹ N s⁻¹ in X8361A and Kazetachi, respectively (Fig. 1). There was also a positive correlation between R₉ and Nₐ though the relationship was more scattered than that between Pₘₐₓ and Nₐ (Fig. 1). ANCOVA suggested no significant difference in either slope values (a₉ or a₈) between the two cultivars. The R₉ tended to be higher in Kazetachi than X8361A at a comparable Nₐ. The average measured values of the quantum yield (φ) were 0.0642 and 0.0630 whereas the average curvature factors (θ) were 0.879 and 0.909 in X8361A and Kazetachi, respectively. When the Pₘₐₓ and the R₉ were plotted against measured leaf height, a positive correlation was found in both relationships (Fig. 1). As the measurement height
decreased, the $P_{\text{max}}$ decreased more sharply in Kazetachi than X8361A because of the steeper $N_{\text{LA}}$ gradient.

2. Canopy structure and light and $N_{\text{LA}}$ profiles

The shoot mass was significantly larger in X8361A than in Kazetachi at $T_2$ due to an increase in stem mass (Fig. 2). In contrast, the two cultivars were similar in the leaf mass at each sampling period. At $T_2$, the leaf mass represented 20 and 31% of the total shoot mass in X8361A and Kazetachi, respectively. The LAI was similar in the two cultivars, and the difference in plant height resulted in a difference in the LAD (Table 1). With the shorter plant height, the mean LAD in Kazetachi significantly exceeded that of X8361A by 1.5 and 2.0 times at the $T_1$ and $T_2$, respectively. Even though the plant height increased from $T_1$ to $T_2$, the LAD was conserved during the two periods due to the increase in LAI (Table 1).

Vertically, leaves were allocated in upper canopy layers in X8361A whereas leaves were concentrated in the middle layer in Kazetachi. These allocation patterns of leaves were maintained from $T_1$ to $T_2$ in both cultivars (Fig. 3). The $N_{\text{LA}}$ decreased with decreasing height in both cultivars, and the vertical $N_{\text{LA}}$ gradient tended to be steeper in Kazetachi than X8361A (Fig. 3). The canopy light extinction coefficients ($K_L$) for different growing periods in each cultivar ranged from 0.48 to 0.58 with the coefficient of determination ranging from 0.845 to 0.974 (Fig. 4). ANCOVA suggested that there were no significant differences in $K_L$ either between the two cultivars or the two sampling periods.

The $N_{\text{LA}}$ at the uppermost layer of the canopy varied among plots at each sampling. Consequently, $N_0$ obtained using eq. (6) in each plot varied largely, i.e., at most $N_0$ ranged from 1.02 to 1.29 g m$^{-2}$ for Kazetachi at $T_2$. The mean $N_0$ value was calculated as 1.36 g m$^{-2}$ ($T_1$) and 1.28 g m$^{-2}$ ($T_2$).

![Fig. 2. Dry mass per unit ground area of stem, leaves and dead leaves.](image)

![Fig. 3. Vertical distribution of leaf area density (LAD, left) and leaf $N$ content per unit leaf area ($N_{\text{LA}}$, right). Values are means ± SE across 3 measurements.](image)

| Cultivars  | Growing period | Plant height (m) | LAI (m$^2$ m$^{-2}$) | LAD (m$^2$ m$^{-3}$) |
|------------|----------------|------------------|----------------------|----------------------|
| X8361A     | $T_1$          | 2.14 (0.03)      | 3.35 (0.13)          | 1.54 (0.05)          |
|            | $T_2$          | 3.60 (0.11)      | 4.39 (0.23)          | 1.24 (0.04)          |
| Kazetachi  | $T_1$          | 1.42 (0.07)      | 3.24 (0.17)          | 2.29 (0.04)          |
|            | $T_2$          | 2.03 (0.06)      | 4.96 (0.04)          | 2.46 (0.05)          |

Table 1. Plant height, leaf area index (LAI) and leaf area density (LAD) in two different sorghum cultivars. The numbers in parentheses represent SEM.

### Significance
- Cultivars (C) ** ns ** **
- Growing period (G) ** ns **
- $C \times G$ ** ns **

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m$^{-2}$ (T$_1$) for X8361A, and 1.18 g m$^{-2}$ (T$_2$) and 0.92 g m$^{-2}$ (T$_3$) for Kazetachi, respectively. We obtained $K_N$ / $K_L$, the gradient of the $N_{LA}$ profile in relation to local light environment ($I / I_0$), using eq. (7) instead of eq. (6) in order to avoid the variation in the $N_0$ (Fig. 5). In fact, the higher values for coefficient of determination (0.64 – 0.84) were observed using eq. (7) as compared to those (0.50 – 0.78) obtained using eq. (6). The two cultivars showed similar $K_N$ / $K_L$ values at each sampling period (Fig. 5). The $N_{LA}$ gradient became slightly shallower from T$_1$ to T$_2$ as indicated by the decrease in the $K_N$ / $K_L$ without significant difference. The $N_{LA}$ at the lowermost layer reached down to approximately 50 – 60% of the $N_0$.

3. Canopy photosynthesis with respect to $N_{LA}$ profile

The estimated daily canopy photosynthesis ($P_D$) together with the parameters of the $N_{LA}$ profile for estimating $P_D$ under the actual and the uniform $N_{LA}$ profiles are shown in Table 2. X8361A had larger average canopy N content (indicated as uniform $N_0$) because it had a greater total canopy N content than Kazetachi. Then, the actual $N_0$ was also larger in X8361A than in Kazetachi. The estimated advantage of the actual $N_{LA}$ profile in each cultivar over the uniform profile was 4 – 9% (Table 2). The advantage remained constant at 4 – 5% from T$_1$ to T$_2$ in X8361A whereas the advantage increased from 5 to 9% in Kazetachi. The increase in the advantage was due to the decrease in the $P_D$ under the uniform $N_{LA}$ profile rather than the increase in the actual $P_D$.

In each sampling period, the actual $P_D$ was higher in X8361A than in Kazetachi by 11 – 12%. The daily canopy dark respiration (RD), integration of the canopy dark respiration under the dark period, was 0.22 mol m$^{-2}$ (T$_1$) and 0.29 mol m$^{-2}$ (T$_2$) in X8361A, and 0.28 mol m$^{-2}$ (T$_1$) and 0.35 mol m$^{-2}$ (T$_2$) in Kazetachi. These constituted 13, 19, 17 and 23% of the $P_D$ respectively. Accordingly, the value of $P_D$ on the daytime basis (i.e. $P_{RD}$) was 2.12 mol m$^{-2}$ (T$_1$), and 2.26 mol m$^{-2}$ (T$_2$) in X8361A, and 2.09 mol m$^{-2}$ (T$_1$) and 2.21 mol m$^{-2}$ (T$_2$) in Kazetachi, respectively. The difference in $P_D$ between the two cultivars was smaller.

![Fig. 4. Relative PFD ($I / I_0$) as a function of cumulative LAI from the top of the canopy (F). For different symbols see figure 1. Solid and broken lines respectively indicate fitted curves for X8361A and Kazetachi: X8361A: $I / I_0 = 0.91exp^{-0.06F}$, $R^2 = 0.971$ ($P < 0.01$), Kazetachi: $I / I_0 = 0.85exp^{-0.08F}$, $R^2 = 0.974$ ($P < 0.01$) at T$_1$, and X8361A: $I / I_0 = 1.0exp^{-0.13F}$, $R^2 = 0.845$ ($P < 0.01$) Kazetachi: $I / I_0 = 0.82exp^{-0.08F}$, $R^2 = 0.974$ ($P < 0.01$) at T$_2$.](image)

![Fig. 5. Relative leaf N ($N_{LA} / N_0$) as a function of relative PFD ($I / I_0$). For different symbols see figure 1. The lines fitted are: X8361A: $N_{LA} / N_0 = 0.99(I / I_0)^{0.271}$, $R^2 = 0.708$ ($P < 0.01$), Kazetachi: $N_{LA} / N_0 = 1.01(I / I_0)^{0.250}$, $R^2 = 0.845$ ($P < 0.01$) at T$_1$, and X8361A: $N_{LA} / N_0 = 0.98(I / I_0)^{0.251}$, $R^2 = 0.636$ ($P < 0.01$), Kazetachi: $N_{LA} / N_0 = 1.00(I / I_0)^{0.231}$, $R^2 = 0.732$ ($P < 0.01$) at T$_2$.](image)

| $N_{LA}$ profile | X8361A | Kazetachi |
|------------------|---------|-----------|
|                  |         | T$_1$     | T$_2$     | T$_1$     | T$_2$     |
| $N_0$ (g m$^{-2}$)         | uniform | 1.05      | 1.02      | 0.94      | 0.70      |
| $K_N$ | actual | 1.36      | 1.28      | 1.18      | 0.92      |
|         | uniform | 0         | 0         | 0         | 0         |
| $P_D$ (mol CO$_2$ m$^{-2}$ s$^{-1}$) | actual | 1.59      | 1.62      | 1.44      | 1.39      |

Table 2. Factors determining the $N_{LA}$ profile ($N_{LA}$ at the top of the canopy, $K_N$: $N_{LA}$ as a function of the cumulative LAI) and simulated daily canopy photosynthesis ($P_D$) under the uniform and the actual $N_{LA}$ profiles. The numbers in parentheses represent the percentage of the $P_D$ under the uniform $N_{LA}$ profile. Note that the $N_0$ under the uniform $N_{LA}$ profile is the same as the average canopy N content (total canopy N content divided by the canopy leaf area).
on the daytime basis than that on the daily basis as shown in Table 2.

The advantage of the actual $N_{la}$ profile over the uniform one can be attributed to the difference between leaf and canopy photosynthesis in their light responses (Fig. 6). Unlike the light response curve at a leaf level, there was no clear light saturation point in the canopy light response curve. The canopy dark respiration rate, y-intercept of the canopy light response curve, was higher in Kazetachi at each sampling period and increased from T1 to T2 in both cultivars. The differences in the canopy light curve between the actual and the uniform $N_{la}$ profile was obvious at the PFD above the canopy ($I_0$) of above 1000 μmol m$^{-2}$ s$^{-1}$ (Fig. 6).

In order to see how the $N_{la}$ gradient affected canopy photosynthesis via vertically modifying photosynthetic capacity of individual leaves, vertical profiles of $P_{max}$ and $P_{n}$ were illustrated in Fig. 7. At T1, the average canopy N content was similar in the two cultivars (Table 2) leading to the similar $P_{max}$ under the uniform $N_{la}$ profiles (Fig. 7 left). The uniform $P_{max}$ decreased from T1 to T2 in Kazetachi due to the decrease in the average canopy N content (Table 2), whereas the uniform $P_{max}$ in X8361A changed little as the average canopy N content was maintained. With the observed $N_{la}$ gradient, $P_{max}$ decreased from the top to the bottom of the canopy by about 20 μmol m$^{-2}$ s$^{-1}$ in both cultivars, and the difference from the uniform $P_{max}$ was about ±10 μmol m$^{-2}$ s$^{-1}$. The actual $P_{max}$ exceeded uniform ones above the middle of the canopy. The vertical profiles of $P_{n}$ (Fig. 7 right) was like a vertical LAD profile (Fig. 3), showing their peak at a similar height. PFD decreased to less than 50% of the $I_0$ level at around the peak, and, below the peak, $P_{n}$ showed sharper decline than the LAD profile, suggesting the high dependence of canopy photosynthesis on upper layers where light is abundant. When $I_0$ is 1000 μmol m$^{-2}$ s$^{-1}$ at which light availability predominantly limited the canopy photosynthetic rate (Fig. 6), the $P_{n}$ profiles were not affected by the $N_{la}$ profile (not shown) because light does not saturate leaf photosynthesis at any height of the canopy. On the other hand, when $I_0$ is 2000 μmol m$^{-2}$ s$^{-1}$ at which the $N_{la}$ gradient influences the canopy photosynthetic rate (Fig. 6), light at the higher layer of the canopy appeared to saturate leaf photosynthesis. As a consequence, the increase in $P_{max}$ at the higher layer of the canopy resulted in the increase in canopy photosynthesis, whereas the decreased $P_{max}$ at the lower layer did not have a negative impact (Fig. 7).

**Discussion**

1. **Leaf gas exchange characteristics in relation to $N_{la}$ in sorghum**

As many studies have shown (e.g., Sato and Tsuno, 1975; Sage and Pearcy, 1987; Anten et al., 1995; Anten et al., 1998; Dreccer et al., 2000), close linear relationships were observed between $P_{max}$ and $N_{la}$ across the wide range of $P_{max}$ in our study (Fig. 1). Sato and Tsuno (1975) observed...
different $P_{\text{sat}}$ in a single leaf of sorghum divided into top, middle and base parts, and found that the difference can be explained by $N_{\text{LAI}}$. Accordingly, the observed $P_{\text{sat}}$ was appropriately expressed as a function of the $N_{\text{LAI}}$. The slopes of the $P_{\text{sat}}$-$N_{\text{LAI}}$ regression ($a_b$) were greater than the values observed in C$_4$ species (Hirose and Werger, 1987b; Sage and Pearcy, 1987; Anten et al., 1995; Dreccer et al., 2000) but were close to those found in other C$_3$ plants; for example, 37.1 in *Amaranthus retroflexus* (Sage and Pearcy, 1987), 35.5 for *Paspalum fasciculatum* and 40.5 for *Hyparrhenia rufa* (Anten et al., 1998). Because the $a_b$ indicates photosynthetic carbon gain at a given N investment in leaf, sorghum has a higher leaf photosynthetic nitrogen use efficiency (PNUE) than other species. In addition, light saturation point increases as $P_{\text{sat}}$ increases. Then, the vertical $N_{\text{LAI}}$ gradient creates the gradient of light saturation point as well as of $P_{\text{sat}}$.

$R_b$ was an important factor for the $P_{\text{sat}}$ estimation since $R_b$ constituted a large proportion of the $P_{\text{sat}}$ more than 20% at most in this study and partly the reason for the lower $P_{\text{sat}}$ in Kazetachi than in X8361A. In the model we applied here, $R_b$ was simply expressed as the sum of the $R_b$ for whole leaves (i.e. LAI) regardless of the $N_{\text{LAI}}$ profile during the dark period. Then, $R_b$ should be positively related to $a_b$, $b_b$ and total canopy N content because of the linear relationship between $R_b$ and $N_{\text{LAI}}$. Since the total canopy N content was larger in X8361A (3.5 and 4.5 g m$^{-2}$ at T$_1$ and T$_2$) than in Kazetachi (3.1 and 3.5 g m$^{-2}$ at T$_1$ and T$_2$), the higher $R_b$ in Kazetachi was derived from the estimated larger $a_b$ and $b_b$ values. The increase in $R_b$ from T$_1$ to T$_2$ was, on the other hand, due to the increase in total canopy N content. Thus, the sensitivity of the leaf dark respiration to the $N_{\text{LAI}}$ (i.e. $a_b$ and $b_b$) along with the total canopy N content could have large influence on $P_{\text{sat}}$.

2. Effects of canopy height on canopy photosynthesis in sorghum

In the present study, the vertical density of the canopy in X8361A (tall) and Kazetachi (dwarf) cultivars was different. On the other hand, $K_s$ in the two cultivars was not different meaning that the light availability was similar at a certain canopy depth expressed as the cumulative LAI (Fig. 3). Hence, the light was vertically attenuated more sharply in the dwarf cultivar with a higher LAD. Nevertheless, the $N_{\text{LAI}}$ relative to the light availability within the canopy ($I_0/I_s$) was similar in the two cultivars since the vertical $N_{\text{LAI}}$ gradient was also steeper in the dwarf cultivar (Figs. 1 and 3). In other words, the dwarf cultivar has adjusted the $N_{\text{LAI}}$ profile in response to the local light environment like the tall cultivar. The $K_s$ / $K_r$ value of 0.24 in 20X densely populated sorghum stands (Anten et al., 1995) is within the range of values (0.20 – 0.27) found in this study. The small effect of LAI (varied with different planting densities) on the $N_{\text{LAI}}$ gradient relative to the canopy depth (i.e. $K_s$) were observed in *Helianthus annuus* (Sadras et al., 1993) and *Glycine max* (Shiraïwa and Sinclair, 1993). These findings may support the conclusion that light attenuation is a major factor controlling the $N_{\text{LAI}}$ gradient. In contrast, van Oosterom et al. (2010) obtained a $K_s$ of 0.09 and 0.14 for dwarf and tall sorghum genotypes, respectively. Because the dwarf types likely have less steeper light gradients than the tall types (George-Jaeggli et al., 2013), although this was not the case in our study, $N_{\text{LAI}}$ might have distributed more uniformly (i.e. low $K_s$) following the light gradient, in their study.

3. Effects of canopy height on advantage of $N_{\text{LAI}}$ gradient and productivity in sorghum

The advantage of the $N_{\text{LAI}}$ gradient over the uniform profile was small compared to the reported values for other plant species (Hirose, 2005). Furthermore, the advantage was only significant at a high PFD. By contrast, Schieving et al. (1992) found that the advantage of the actual $N_{\text{LAI}}$ gradient was still as high as 20% even when noon maximum $I_0$ was assumed to be only 500 μmol m$^{-2}$ s$^{-1}$ in C$_4$ tall herbs with the smaller $a_b$ of 12.5 CO$_2$ g$^{-1}$ N s$^{-1}$ (Hirose and Werger, 1987a). In the case of sorghum having larger PNUE, photosynthetic capacity of individual leaves is not saturated at this low PFD (Fig. 6). Even when the higher amount of light is available, the advantage was small because of the large photosynthetic capacity with the observed average $N_{\text{LAI}}$.

On the other hand, the increase in the advantage found in the dwarf cultivar was associated with the decrease in the average $N_{\text{LAI}}$. When the average $N_{\text{LAI}}$ declined at T$_2$, so did the $P_{\text{sat}}$ at the upper canopy layer. The canopy photosynthesis became light-limited, and the $N_{\text{LAI}}$ gradient brought a greater advantage, as has been predicted with the similar canopy photosynthesis model (Anten et al., 1995; Leuning et al., 1995). The decrease in the average $N_{\text{LAI}}$ indicates the decreased N allocation rate to the canopy relative to the leaf expansion. At T$_2$, total canopy N content as well as total stem N content was significantly lower in Kazetachi than in X8361A, whereas the average stem N content (i.e. total N content divided by stem dry mass) was similar (not shown). Probably, the pronounced dilution of the $N_{\text{LAI}}$ in the dwarf cultivar is associated with the smaller stem N capacity in the dwarf than in the tall cultivar (Fig. 2). On the other hand, higher average $N_{\text{LAI}}$ was observed throughout the growing period in the shorter sorghum with the lower stem mass (van Oosterom et al., 2010), suggesting that the decreased N allocation relative to canopy development is not a general feature in dwarf sorghum.

In accordance with other studies (van Oosterom et al., 2010; George-Jaeggli et al., 2011, 2013), the dwarf cultivar produced less stem mass and shoot mass than the tall cultivar. The smaller fraction of stem dry mass in the dwarf cultivar may reduce the stem respiration, and thus the bulk shoot respiration. Despite the adjustment of the $N_{\text{LAI}}$
gradient to the steeper light profile in the canopy, the dwarf cultivar had a smaller $P_d$ than the tall cultivar due to the larger respiratory carbon loss. Thus, the larger canopy respiration (associated with the higher respiration rates on N basis in the leaves) counteracted the potentially smaller stem respiration in the dwarf cultivar. In addition, higher stem respiration losses per unit dry weight have been observed in dwarf plants compared to tall plants in wheat (Rawson and Evans 1971; Gent and Kiyomoto 1997). The reduced stem growth may have caused carbohydrate accumulation in either stem or leaves, and subsequently increased the respiration rate of these organs (Azcón-Bieto et al., 1983; Azcón-Bieto and Osmond 1983). The restriction in stem elongation also may direct excess assimilates to root growth, resulting in smaller shoot mass (George-Jaeggl et al., 2013). The dwarf cultivar had lower N content in leaves at a later vegetative stage, which reduced the canopy photosynthesis despite the greater advantage of leaf N gradient in canopy photosynthesis. Therefore, N uptake and allocation as well as biomass production in each organ (i.e. stem, leaf, and root) should interact with the $N_{IA}$ profile and are important factors for the resource use efficiency. These factors are to be taken into consideration if breeding programs want to target stature in order to potentially increase production in sorghum.

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