Seasonal fluctuations in growth/decline and single-leaf gas exchange of C₃ turfgrass fields under various light conditions

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Abstract
Seasonal variation in non-assimilatory organ and leaf biomass and necromass, leaf area index (LAI), leaf mass per unit area (LMA), and leaf nitrogen content per unit leaf area of managed C₃ cool-season turfgrass (Kentucky bluegrass, Poa pratensis L.) fields under four light conditions (irradiance levels: 100, 62, 48, 20 ′) were examined in situ over 2 years. Seasonal fluctuations in light and the temperature dependence of the gas exchange of intact leaves grown under three light conditions (irradiance levels: 100, 48, 20 ′) were also examined for 1 year. The magnitude of spring growth and summer decline in non-assimilatory organ and leaf biomass depended on the light conditions. Both non-assimilatory organ and leaf biomass were higher in the sunnier plots, as was the ratio of non-assimilatory organs to whole biomass. A step wise acclimatization to low irradiance reduced the allocation to non-assimilatory organs to maintain leaf biomass, limited LMA to maintain the LAI, and limited the latter to retain leaf nitrogen per unit leaf area, depending on the shade level. Kentucky bluegrass growing under lower light conditions had similar single-leaf photosynthetic abilities until early summer, but the normalized maximum carboxylation rate at 25°C $V_{\text{cmax,25}}$ decreased during the decline of turfgrass in summer, depending on the shade level, which corresponded to the decline in leaf nitrogen content per unit leaf area.

Key words: Biomass, Cool-season turfgrass, Gas exchange parameter, Low irradiance, Seasonal fluctuation.

1. Introduction
The C₃ cool-season turfgrasses often decline under high temperatures. Therefore, to plan and maintain C₃ cool-season turfgrass fields in warm climates and transitional regions, it is essential to evaluate the balance of the carbohydrate metabolism and its relationship to seasonal growth and decline. There have been several previous studies of the decline in root growth and depression of net photosynthesis in C₃ turfgrasses at high temperatures. Xu and Huang (2000 a, b; 2001 a, b) showed that high soil temperature caused a decline in net photosynthesis at the single-leaf and canopy scales, leading to less carbon allocation to the root and a decline in the root growth of creeping bentgrass. Other studies have reported declines in the carbohydrate content of C₃ turfgrasses during the summer (Sweeney et al., 2001; Youngner et al., 1978). Xu and Huang (2003) also showed a decline in turf quality, carbohydrate content and allocation to
roots during the summer. A decline in root growth has been found to precede that of shoots under high temperatures (Carrow, 1996; Xu and Huang, 2000 a, b). Huang and Liu (2003) found that the summer decline in the roots of creeping bentgrass resulted from both a decline in new root production and increased root mortality. These previous studies indicate that the balance of carbohydrate metabolism, carbon allocation, root production, and root mortality are closely related and determine the seasonal fluctuations in turfgrass fields.

Although several studies have reported the effect of low light on the performance of various kinds of turfgrass (e.g. Bunnell et al., 2005; Jiang et al., 2004; Stanford et al., 2005 and Steinke and Stier, 2003), few studies have examined the in situ seasonal growth, decline, in situ single-leaf gas exchange characteristics, and acclimatization of C3 turfgrass under various light conditions. In urban parks and stadiums, turfgrass is sometimes used in shaded areas, although recently, many new international-standard soccer fields have installed natural turf in enclosed and retractable-dome stadiums. Because of the light levels in these stadiums, it has been observed that shading of soccer fields causes the turfgrass to deteriorate due to lack of light. Since the 2002 soccer World Cup, held in Japan and South Korea, many new roofed stadiums have been constructed, making it necessary to develop cultivation techniques to surmount the influence of the roof shading. To plan and maintain such green areas, it is vital to evaluate the influence of light conditions on carbon balance and allocation, and their relationships to seasonal trends in terms of growth and decline.

Our objective was to investigate the growth, decline, and acclimatization of managed C3 cool-season turfgrass (Kentucky bluegrass, Poa pratensis L.) fields under four light conditions. The seasonal variation in non-assimilatory organ and leaf biomass and necromass, the amount of post-mowing leaf cuttings, the leaf area index (LAI), leaf mass per unit area (LMA), and leaf nitrogen content per unit leaf area of managed C3 turfgrass (Kentucky bluegrass) fields under four light conditions were examined in situ for 2 years. We also measured the single-leaf gas exchange characteristics of intact leaves grown under three different light conditions (irradiance levels: Plot A: 100%, Plot C: 48%, Plot D: 20%) for 1 year and derived the major gas exchange parameters to investigate the influence of low irradiance on photosynthesis.

2. Materials and Methods

2.1 Site

The study was conducted on turfgrass fields in a stadium and its surrounding area (34° 39' N, 134° 10' E; <20 m a.s.l.) in Kobe, Hyogo Prefecture, Japan. We established four plots with different light conditions. Plot A was a nursery (600 m²) in an open area, Plot B was another nursery (370 m²) covered by a pole frame, and plots C and D were located in a stadium (9,279 m²) with a retractable roof that was usually open, but surrounded by buildings that screened the sun, depending on the time of day, season, and position in the stadium. The monthly average diurnal variation in photosynthetic photon flux density (PPFD, µmol m⁻² s⁻¹) in the four plots during 2006 is shown in Fig. 1. The plots became more shaded in order of B, C, and D.

![Fig. 1. Monthly average diurnal change in photosynthetic photon flux density (PPFD) in the four plots in 2006. Plot A is the open site, and conditions become more shaded in the order of Plot B, C, and D. All plots were within well-managed C3 turfgrass (Kentucky bluegrass, Poa pratensis L.) fields.](image-url)
D. In Plot B, the pole frame randomly shaded the turf during the day. In Plot C, sunshine was shadowed by the dome of the building in both the early morning and late afternoon. Plot D was mostly shaded except for a brief period in the afternoon. Compared with Plot A, the percentages of cumulative irradiance for 2 years (January 2006 to December 2007) were 62, 48, and 20% in Plots B, C, and D, respectively. Kentucky bluegrass (*Poa pratensis* L., cool-season turfgrass) that had been grown in open fields was laid in plots A and B in April 2004, Plot C in February 2005, and Plot D in September 2005 respectively on a 1:1 blend of decomposed granite (Masa) soil and sand. Observations were carried out from January 2006 to December 2007. In February 2006, the grass in Plot D was renewed with new turf from an open field to examine the decline under the shaded treatment. In these fields, the canopy was homogeneous and completely closed. Adequate water and soil nutrients were supplied (N: P:K=30-40:20:30:35-45 g m⁻²) during the growing season to prevent drought and nutrient stress.

### 2.2 Meteorology, biomass, necromass, LAI, LMA, and leaf nitrogen content

The air temperature was measured at 0.1 m above the soil using Vaisala-type hygro-thermometers (CS500, Campbell Scientific, Inc., Logan, UT, USA), the soil temperature at a depth of 0.01 m, using thermistors (107, Campbell Scientific, Inc.), and PPFD close to the single-leaf gas exchange chamber using PPFD sensors (LI190SB, LI-COR, Lincoln, NE, USA, or IKS-27, Koito Industries Ltd., Kanagawa Prefecture, Japan). Data were collected using data loggers (CR1000, Campbell Scientific, Inc.).

Total vegetation, including soil, was sampled using a hexagonal sampler (area: 210.4 cm²; height: 0.15 m, n=1) in each plot every 2 weeks from January 2006 to December 2007. The dry weights of the leaf biomass, leaf necromass, non-assimilatory organ biomass (roots and stems without chlorophyll), and non-assimilatory organ necromass were measured after washing out the soil and separating the tissue into its component parts. Mowed leaf biomass was sampled using a barrow type lawnmower (area: 2.9 m²; mowing height: approx. 30 mm, n=1) in each plot twice a month from January 2006 to December 2007. Total leaf area measurements were made using an area analysis system (LI-3050C-P, LI-COR), with the leaf mass per unit area (LMA) calculated using the above data. The leaf nitrogen content per unit dry weight was measured using nitrogen and carbon analyzers (Sumigraph NC-22A, Sumika Chemical Analysis Service Ltd., Osaka, Japan, or Flash EA1112, Thermo Fisher Scientific, Waltham, MA, USA).

### 2.3 Single-leaf gas exchange

Gas exchange measurements were made in the controlled chamber of a portable steady-state photosynthesis measurement system (CIRAS-2, PP Systems International Inc., Amesbury, MA, USA). Measurements were made in situ on each intact leaf (n=3 for each plot) at several irradiances: PPFD 400, 800, 1200, 1600, 1000, 500, 250, 120, 60, 30, 15, and 0 µmol m⁻² s⁻¹, supplied by a red LED, at a leaf temperature of approximately 25°C, relative humidity of approximately 50%, and CO₂ concentration of approximately 380 ppm. After the gas exchange measurements at different irradiances, gas exchange rates at leaf temperatures of 5, 10, 15, 20, 25, 30, 35, and 40°C were respectively measured for the same leaves in darkness or at 1200 µmol m⁻² s⁻¹, supplied by a red LED. The range of temperatures used varied seasonally. Our preliminary examination of the gas exchange in the leaves of Kentucky bluegrass showed that gas exchange rates usually peaked at PPFD 1200 µmol m⁻² s⁻¹, and there was neither a sharp increase in leaf temperature of 5-40°C, nor any photosynthesis depression (unpublished results of the authors). Measurements were made in the winter (19 January to 15 February), spring (12 to 25 April), early summer (2 June to 6 July; rainy season), summer (25 to 30 August), and fall (19 October to 10 November) of 2006.

### 2.4 Parameterization procedures

We derived the major gas exchange parameters: normalized dark respiration rate at 25°C, and its temperature dependence \([R_{d25}, \Delta H_r(R_d)]\), normalized maximum carboxylation rate at 25°C, and its temperature dependence \([V_{c25}, \Delta H_c(V_{c25})]\), and the fraction of light used photosynthetically at the chloroplast lamellae \((1-f)\). The analytical model was modified from the Farquhar *et al.* (1980) biochemical model of photosynthesis for C₃ plants (Kosugi *et al.*, 2003). The net CO₂ assimilation rate is described by:

\[
A = V_c \left( 1 - \frac{p(\Gamma_c)}{p(C)} \right) - R_d \tag{1}
\]

\[
p(\Gamma_c) = \frac{p(O)}{2R}
\tag{2}
\]

where \(A\) is the net assimilation rate (µmol m⁻² s⁻¹),
is the rate of carboxylation in the photosynthetic carbon reduction cycle (\(\mu\)mol m\(^{-2}\) s\(^{-1}\)), \(R_0\) is the daytime (non-photorespiratory) dark respiration rate (\(\mu\)mol m\(^{-2}\) s\(^{-1}\)), \(p(I^*)\) is the CO\(_2\) compensation point without non-photorespiratory respiration (Pa), \(\tau\) is the specificity factor of Rubisco, and \(p(C_c)\) (Pa), and \(p(O_2)\) (21,000 Pa) are the partial pressures of CO\(_2\) and O\(_2\), respectively, at the carboxylation and oxygenation sites. The lower value between ribulose-bisphosphate (RuBP) regeneration or the electron-transport limited rate of carboxylation \((W_j; \mu\)mol m\(^{-2}\) s\(^{-1}\)) and the RuBP saturated rate of carboxylation \((W_c; \mu\)mol m\(^{-2}\) s\(^{-1}\)) is \(V_c\).

\[
V_c = \min\{W_j, W_c\}
\]

(3)

\(W_j\) is determined from the potential rate of electron transport at a given PPFD \((J; \mu\)electron m\(^{-2}\) s\(^{-1}\)) and the number of NADPH molecules required to carboxylate one molecule of CO\(_2\) (mol NADPH mol CO\(_2\)) as follows (Farquhar et al., 1980):

\[
W_j = \frac{J}{4 + \frac{8p(I^*)}{p(C_c)}}
\]

(4)

Because the amounts of ATP and NADPH have upper limits, \(J\) is expressed as the smaller root of the following non-rectangular hyperbola representing the relationship with the absorbed PPFD \((\varepsilon Q);\) Farquhar and Wong, 1984),

\[
\theta J^2 - \left[ J_{\text{max}} + \frac{\varepsilon (1-f)}{2} Q \right] J + J_{\text{max}} \frac{\varepsilon (1-f)}{2} Q = 0
\]

(5)

where \(Q\) is the incident PPFD (\(\mu\)mol m\(^{-2}\) s\(^{-1}\)), \(\varepsilon\) is the leaf absorbance of \(Q\), \(f\) is the fraction of PPFD loss not used photosynthetically at the chloroplast lamellae, \(J_{\text{max}}\) is the maximum potential rate of electron transport, and \(\theta\) is a factor defining the convexity in the relationship between the absorbed PPFD and potential electron transport. A value of \(\theta\) equal to 0.9 was approximated using the results of light curve measurements and the rate of electron transport for several plant species, including that investigated (unpublished results of the authors), using a portable chlorophyll fluorometer (MINI-PAM, Heinz Walz GmbH, Effeltrich, Germany). A value of \(\varepsilon\) equal to 0.86 was approximated using measurements of the light penetration of the canopy leaves.

\(W_c\) is written as (Farquhar et al., 1980)

\[
W_c = V_{c_{\text{max}}} \frac{p(C_c)}{p(C_c) + K_c \left(1 + \frac{p(O_2)}{K_o}\right)}
\]

(6)

where \(V_{c_{\text{max}}}\) is the maximum rate of carboxylation (\(\mu\)mol m\(^{-2}\) s\(^{-1}\)), and \(K_c\) and \(K_o\) (Pa) are the Rubisco Michaelis-Menten constants for CO\(_2\) and O\(_2\), respectively. \(V_{c_{\text{max}}}\) was estimated using the one-point method described by Kosugi et al. (2003). Briefly, \(V_{c_{\text{max}}}\) was derived from any point within the low intercellular carbon dioxide concentration (C\(_i\)) range of the A/C\(_i\) relationship after determining \(R_o\). Thus, after excluding the points at which \(W_c\) was not the limiting factor (under field conditions, this usually occurs at low PPFD and ambient CO\(_2\) concentrations), each CO\(_2\) and H\(_2\)O gas exchange data point includes information on \(V_{c_{\text{max}}}\) if we know \(R_o\). This method can reveal more information than the analysis of an A/C\(_i\) curve obtained with a controlled chamber in the field.

Assuming a constant ratio of leaf nitrogen allocation to light-harvesting complex, Rubisco, chlorophyll, and other proteins, \(J_{\text{max}}\) is related to \(V_{c_{\text{max}}}\) as follows:

\[
J_{\text{max}} = K_j J_{c_{\text{max}}}
\]

(7)

A value of \(K_j\) equal to 2.2 was approximated from the results of light-curve measurements of the rate of electron transport for Kentucky bluegrass (unpublished results of the authors) using a portable steady-state photosynthesis measurement system with a chlorophyll fluorometer (LI-6400, LI-COR). The values of \(K_c\), \(K_o\), \(\tau\) and their activation energies used to calculate temperature dependence were taken from Harley et al. (1992) while the Arrhenius function was used to determine the temperature dependencies of parameters \(K_c\), \(K_o\), \(\tau\), and \(R_o\) as:

\[
f(T_{ik}) = f(T_{ic}) \cdot \exp \left[ \frac{\Delta H_c}{R T_{ic}} \right]
\]

(8)

A simplified equation from Sharpe and DeMichele (1977) was used to determine the temperature dependence of \(V_{c_{\text{max}}}\):

\[
f(T_{ik}) = \frac{f(T_{ic}) \cdot \exp \left[ \frac{\Delta H_d}{R T_{ic}} \right]}{1 + \exp \left[ \frac{\Delta S T_{ik} - \Delta H_d}{R T_{ik}} \right]}
\]

(9)
where $f(T_{l})$ is the value of a given parameter at leaf temperature ($T_{l}$), $f(T_{ref})$ is the reference value of that parameter at 25°C ($K_{25}$, $K_{c25}$, $R_{c25}$, $\tau_{c25}$, and $V_{cmax25}$), $\Delta H_{f}$ is the activation energy (J mol$^{-1}$), $\Delta H_{d}$ is the deactivation energy (J mol$^{-1}$), $\Delta S$ is an entropy term (J K$^{-1}$ mol$^{-1}$), and $R$ is the gas constant (8.31447 J K$^{-1}$ mol$^{-1}$). Based on Kosugi et al. (2003), in this study we used the following parameter values:

$K_{25}$ = 27.5 Pa CO$_2$

$\Delta H_{f}(K_{25})$ = 80500 J mol$^{-1}$

$K_{c25}$ = 42000 Pa O$_2$

$\Delta H_{f}(K_{c25})$ = 14500 J mol$^{-1}$

$\tau_{c25}$ = 2321

$\Delta H_{d}(\tau)$ = -29000 J mol$^{-1}$

$\Delta S$ = 650 J K$^{-1}$ mol$^{-1}$

First, we determined $R_{d}$ at the reference temperature and its temperature dependence from Equation (8). Here, $A$ measured in the dark chamber was used to derive $R_{d25}$ and $\Delta H_{f}(R_{d})$ for each plot. $R_{d25}$ and $\Delta H_{d}(R_{d})$ were optimized with a nonlinear least square optimization procedure separately for each plot. Next, we obtained the instantaneous $V_{cmax}$ for each value measured under light-saturated conditions by the one-point method. $V_{cmax}$ can be calculated in reverse using the obtained $R_{d25}$ and $\Delta H_{f}(R_{d})$ and Equations (1), (2), (6), and (8). $V_{cmax25}$ and $\Delta H_{f}(V_{cmax})$ were derived using the obtained $V_{cmax}$ and Equation (9). Finally, we obtained $J$ for each value measured under low PPFD conditions. $J$ can be calculated in reverse using the obtained $R_{d25}$ and $\Delta H_{d}(R_{d})$ and Equations (1), (2), (4), and (8). $1-f$ was derived using the obtained $J$, $V_{cmax25}$ and $\Delta H_{f}(V_{cmax})$, and Equations (5), (7), and (9).

3. Results

3.1 Biomass and necromass

Figures 2a–e show the seasonal changes in non-assimilatory organ and leaf biomass, and their ratio and necromass in the four plots over 2 years. The peak growth season of these turfgrass fields was between April and June (Figs. 2ab). The averages and standard deviations of non-assimilatory organ biomass were 1.33±0.35 (Plot A), 0.81±0.22 (Plot B), 0.45±0.15 (Plot C), and 0.82±0.25 (Plot D) kg m$^{-2}$. The averages and standard deviations of leaf biomass were 0.17±0.05 (Plot A), 0.15±0.04 (Plot B), 0.10±0.04 (Plot C), and 0.08±0.03 (Plot D) kg m$^{-2}$. Among plots A, B, and C, both the average and standard deviation of non-assimilatory organ and leaf biomass were higher in the sunnier plot (Figs. 2ab). The ratio of non-assimilatory organ to whole biomass (non-assimilatory organ biomass and leaf biomass) was also higher in the sunnier plot, except for Plot D, where new turf with a large non-assimilatory organ biomass was planted in February 2006 and then gradually decreased until December 2007 (Fig. 2c). The averages and standard deviations of non-assimilatory organ necromass were 0.26±0.10 (Plot A), 0.20±0.11 (Plot B), 0.26±0.08 (Plot C), and 0.21±0.10 (Plot D) kg m$^{-2}$. The averages and
standard deviations of leaf necromass were 0.10±0.04 (Plot A), 0.08±0.03 (Plot B), 0.07±0.03 (Plot C), and 0.05±0.02 (Plot D) kg m⁻². Leaf necromass was higher in the sunnier plots in summer and accounted for more than half the leaf biomass (Figs. 2be). The necromass of non-assimilatory organs showed no clear differences among the plots, and the ratio of non-assimilatory organ necromass to biomass was low (Figs. 2ad).

3.2 Growth and decline

The yearly cumulative growth and decline of grasses in each plot was evaluated from leaf, non-assimilatory organs, and mowing leaf biomass data (Fig. 3). In the two sunnier plots (A and B), notable spring growth and summer decline in both non-assimilatory organ and leaf biomass was observed, although their magnitude depended on the light conditions. Notable non-assimilatory organ growth was observed in Plot

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Fig. 3. Cumulative growth and decline in leaves and non-assimilatory organs, and the quantity of mowing leaves (gC m⁻²) in the four plots over 2 years, with seasonal changes in air temperature and photosynthetic photon flux density (PPFD) at the open site. The 30-min average PPFD and air temperature values are plotted in the top panel. Plot A is the open site, and conditions become more shaded in the order of Plot B, C, and D. All plots were within well-managed C₃ turfgrass (Kentucky bluegrass, *Poa pratensis* L.) fields. The turf in Plot D was replaced in February 2006 (indicated by an arrow).
A during the warm winter of 2006-2007. In spring, the increase in leaf biomass began in April, whereas that in non-assimilatory organ biomass began earlier in both plots A and B. In Plot C, the amplitude of growth and decline was quite small compared with the two sunnier plots. In Plot D, where new turf with a large non-assimilatory organ biomass was planted in February 2006, a severe decline in non-assimilatory organ biomass was observed in 2006, which continued until the end of 2007.

3.3 Leaf area and leaf nitrogen content

Figures 4abc show the seasonal changes in the leaf area index (LAI), leaf mass per unit area (LMA) and leaf nitrogen content per unit leaf area in the four plots for the 2 years. In the two sunnier plots (A and B), clear bimodal seasonal changes were detected in LAI, with a larger peak between April and July and a smaller peak in the fall. In spring, the increase in LAI started in April, coinciding with the increase in leaf biomass. Since the increase in LAI was quite large compared with that in leaf biomass (Fig. 2b), the LMA and leaf nitrogen content per unit leaf area tended to be small when LAI became large in spring.

The averages and standard deviations of LAI were 3.00±1.23 (Plot A), 3.06±1.10 (Plot B), 2.33±1.00 (Plot C), and 2.27±0.79 (Plot D) m² m⁻². The averages and standard deviations of LMA were 0.059±0.013 (Plot A), 0.050±0.009 (Plot B), 0.046±0.010 (Plot C), and 0.038±0.011 (Plot D) kg m⁻². The leaf areas in the two sunnier plots (A and B) had similar amplitudes and seasonal trends despite differences in leaf biomass, so that the LMA was sometimes larger in the plot with the higher irradiance (A). In the two shadier plots (C and D), the LAI was smaller than in the two sunnier plots (A and B).

The averages and standard deviations of the leaf nitrogen content per unit leaf area were 1.80±0.37 (Plot A), 1.85±0.35 (Plot B), 1.62±0.40 (Plot C), and 1.43±0.41 (Plot D) kg m⁻². The leaf nitrogen content in the two sunnier plots (A and B) was similar, whereas it was smaller in the two shadier plots (C and D) after early summer in Plot D and at late summer in Plot C, and did not recover in the fall in Plot D.

Figure 4d shows the relationship between leaf nitrogen content per unit leaf area and LMA by plot. The leaf nitrogen content per unit leaf area and LMA were significantly related in all four plots, although the r was smaller in the sunnier plots than in the shadier plots. Additionally, the slope of this relationship was smallest in the sunniest plot (Plot A). This can be explained considering that only leaf biomass and LMA in Plot A were sometimes larger than in Plot B; however, this did not apply in the case of LAI and leaf nitrogen content.

It can be summarized from these results that in both LAI and leaf nitrogen content per unit leaf area, the two sunnier plots (A and B) were similar (Figs. 4ac). In contrast, the non-assimilatory organ biomass and its ratio to whole biomass, leaf biomass, and LMA were sometimes higher in Plot A than Plot B (Figs. 2a-c, 4b). A low irradiance (62%, Plot B) did not affect the LAI and leaf nitrogen per unit leaf area of the grass, which is closely related to total vegetation mass and single-leaf photosynthetic ability, and only carbohydrate assimilated with leaf photosynthesis and allocation differed with the light conditions. In Plots
3.4 Single-leaf gas exchange

The light curves of $A$ at 25°C and the temperature dependence of $A$ at high irradiance (PPFD 1200 µmol m$^{-2}$ s$^{-1}$) was compared between plots and seasons (Figs. 5ab). The maximum $A$ in all three plots was obviously small in winter over a wide temperature range, increased in spring, and showed some decrease in early summer (rainy season). There was no clear difference in light curves or the temperature dependence of $A$ between plots in winter, spring, and early summer. In summer, the maximum $A$ of Plot A recovered to the level attained in spring, while decreasing in the two shaded plots (C and D). In the fall, the maximum $A$ recovered in Plot C, but not in Plot D. In terms of temperature dependence, $A$ peaked at leaf temperature values of 25 and 30°C in all plots, except in winter, when the optimal leaf temperature was lower than in other seasons.

$R_d$ values were plotted against simultaneously
Dark respiration rate (R\text{d}), maximum carboxylation rate (V\text{cmax}), and electron transport rate (J) at 25°C and in intact leaves of Kentucky bluegrass (Poa pratensis L.) in relation to leaf temperature or photosynthetic photon flux density (PPFD) for different seasons and plots under different light conditions. The average values from three leaves are plotted. Error bars show standard deviations. Lines show simulated R\text{d} with the Farquhar model, based on the parameter sets in Table 1.

Figure 6. Dark respiration rate (R\text{d}), maximum carboxylation rate (V\text{cmax}), and electron transport rate (J) at 25°C and in intact leaves of Kentucky bluegrass (Poa pratensis L.) in relation to leaf temperature or photosynthetic photon flux density (PPFD) for different seasons and plots under different light conditions. The average values from three leaves are plotted. Error bars show standard deviations. Lines show simulated R\text{d} with the Farquhar model, based on the parameter sets in Table 1.

measured leaf temperature (Fig. 6a), and R\text{d25} and ΔH\text{a}(R\text{d}) and their seasonal fluctuations are shown in Table 1. R\text{d25} was slightly lower in winter than in other seasons for all three plots. In Plot D, ΔH\text{a}(R\text{d}) obviously declined in the summer and fall.

Figure 6b shows the response of V\text{cmax} to leaf temperature by plots and seasons. V\text{cmax25} and ΔH\text{a}(V\text{cmax}), and their seasonal fluctuations are shown in Table 1. Similar to the maximum A, V\text{cmax} values in all three plots were obviously smaller in winter than in other seasons over a wide temperature range, increased in spring and showed some decrease in early summer. There was no clear difference between plots in V\text{cmax25} and ΔH\text{a}(V\text{cmax}) in winter, spring, and early summer, and V\text{cmax25} values had the same seasonal fluctuations as V\text{cmax} values in these seasons. Although V\text{cmax25} recovered in Plot A in the summer, it decreased in Plot C and was low in Plot D. In fall, V\text{cmax25} recovered in Plot C, but further declined in Plot D. Regarding ΔH\text{a}(V\text{cmax}), values for all plots were lower in the winter and increased
in the spring and early summer, with no difference between plots. After the summer, a decline in $\Delta H_a (V_{\text{carmax}})$ was observed in plots C and D. These trends were similar to those of $V_{\text{carmax25}}$.

The light curves of $J$ at 25°C between plots and seasons are shown in Fig. 6c. The values of 1–$f$ and their seasonal fluctuations are given in Table 1. The 1–$f$ of turfgrass grown under low irradiance was larger than in the open site in winter and spring, indicating that the grass acclimatized to the shade, while differences between plots became unclear after early summer.

4. Discussion

4.1 Biomass and necromass

Both non-assimilatory organ and leaf biomass were higher in sunnier rather than shadier plots (Figs. 2ab), as was the ratio of non-assimilatory organs to whole biomass (Fig. 2c). Thus, allocation to the non-assimilatory organs increased with increasing net assimilation under high irradiance.

The large ratio of leaf necromass to biomass and the difference in leaf necromass between plots indicates a large input of leaf litter that depends on the amount of leaf biomass available and suggests the rapid turnover of the grass leaves, whereas a smaller ratio and lack of seasonality of non-assimilatory organ necromass to biomass suggests a longer turnover in the case of non-assimilatory organ biomass (Figs. 2abde).

4.2 Growth and decline

Differences between plots in cumulative growth and decline strongly suggest that both photosynthesis and plant respiration were higher in sunnier rather than shadier sites. These two components depend on light and leaf and soil temperature conditions. Because the sunnier sites had higher leaf and soil temperature affected by light, light conditions eventually governed the growth and decline of the turfgrass field (Fig. 3). Especially in the shadiest plot (Plot D), where new turf with a large non-assimilatory organ biomass was planted at the beginning of the experiment, a severe decline was observed during the latter, suggesting that the grass under this level of shade could not achieve sufficient photosynthesis to match the respiration of the large non-assimilatory organs. However, the ratio of non-assimilatory organs to whole biomass was larger than other plots due to delayed acclimatization, since it was transplanted later than in the other plots.

4.3 Seasonality in LAI, LMA, and leaf nitrogen content

The LAI peaked twice during the year, indicating clear seasonality (Fig. 4a). In early spring, carbohydrate assimilated by leaf photosynthesis was not allocated to the leaves. Seasonal fluctuations in LAI did not simply depend on carbohydrate assimilated with leaf photosynthesis, but rather seemed to be regulated by the phenology of the grass.

<table>
<thead>
<tr>
<th>Year</th>
<th>Season</th>
<th>Date</th>
<th>Plot</th>
<th>$R_{D25}$ ($\mu$mol m$^{-2}$ s$^{-1}$)</th>
<th>$\Delta H_a (R_d)$ (J mol$^{-1}$)</th>
<th>$V_{\text{carmax25}}$ ($\mu$mol m$^{-2}$ s$^{-1}$)</th>
<th>$\Delta H_a (V_{\text{carmax}})$ (J mol$^{-1}$)</th>
<th>1–$f$ (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>Winter</td>
<td>2/15</td>
<td>A</td>
<td>0.91</td>
<td>41461</td>
<td>35.8</td>
<td>39520</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/27</td>
<td>C</td>
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<td>1/19</td>
<td>D</td>
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<td>59138</td>
<td>29.6</td>
<td>35651</td>
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<td>Spring</td>
<td>4/25</td>
<td>A</td>
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<td>70.8</td>
<td>59781</td>
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<td></td>
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<td>4/12</td>
<td>C</td>
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<td>33075</td>
<td>73.1</td>
<td>57847</td>
<td>0.71</td>
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<td></td>
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<td>4/20</td>
<td>D</td>
<td>0.75</td>
<td>47740</td>
<td>67.0</td>
<td>62507</td>
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<td></td>
<td>Early summer</td>
<td>6/2</td>
<td>A</td>
<td>–</td>
<td>–</td>
<td>52.4</td>
<td>63844</td>
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<tr>
<td></td>
<td>(rainy season)</td>
<td>7/6</td>
<td>C</td>
<td>–</td>
<td>–</td>
<td>57.2</td>
<td>77180</td>
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<td></td>
<td></td>
<td>6/28</td>
<td>D</td>
<td>–</td>
<td>–</td>
<td>47.8</td>
<td>73437</td>
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<tr>
<td></td>
<td>Summer</td>
<td>8/20</td>
<td>A</td>
<td>0.95</td>
<td>66606</td>
<td>65.9</td>
<td>59037</td>
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<tr>
<td></td>
<td></td>
<td>8/20</td>
<td>C</td>
<td>1.31</td>
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<td>58153</td>
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<td>8/25</td>
<td>D</td>
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<td>29076</td>
<td>43.1</td>
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<td>11/10</td>
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<td>1.27</td>
<td>32311</td>
<td>73.7</td>
<td>64885</td>
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<td>C</td>
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<td>57.6</td>
<td>48313</td>
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<td>10/20</td>
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<td>0.63</td>
<td>30656</td>
<td>35.0</td>
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Large increases in LAI in the spring also caused a decline in the LMA and leaf nitrogen content per unit leaf area. Single-leaf gas exchange measurements showed that the normalized maximum carboxylation rate at 25°C ($V_{\text{cmax}25}$) peaked in spring (Fig. 6b). This suggests that the decrease in LMA and leaf nitrogen content per unit leaf area in spring accompanying the increase in LAI did not cause any decline in the leaf photosynthetic ability.

### 4.4 Single-leaf gas exchange

In the biochemical photosynthesis model, $R_{\text{a25}}$ and $\Delta H_d(R_d)$ are characteristic of $R_d$, while leaf respiration is affected by the leaf growing conditions and leaf age (Kosugi and Matsuo, 2006). In this study, $R_{\text{a25}}$ was lower in the winter and higher during the growing seasons, when there were many young leaves with vigorous gas exchange.

$V_{\text{cmax}25}$ and $\Delta H_d(V_{\text{cmax}})$ show the potential of Rubisco and thus the single-leaf photosynthetic ability at high irradiance. Low irradiance induced the depression of $V_{\text{cmax}25}$ and $\Delta H_d(V_{\text{cmax}})$ after the summer, depending on the shade level, while leaf and non-assimilatory organ biomass declined in the early summer in all plots (Figs. 2ab). This trend was synchronized with the decline in $V_{\text{cmax}25}$ in early summer (Table 1, Fig. 6b). The leaf nitrogen content per unit leaf area was smaller after the early summer in Plot D and after the summer in Plot C, and did not recover in the fall in Plot D. These seasonal changes in leaf nitrogen content per unit leaf area under different levels of shade were similar to those in $V_{\text{cmax}25}$.

In terms of gross photosynthesis of the ecosystem at the same site, a summer decline in maximum photosynthesis at optimal irradiance was observed in all plots (Kosugi et al., 2010). This trend differed from the single leaf $V_{\text{cmax}}$, which did not show a post-summer decline in Plot A. This was likely because gross photosynthesis of the ecosystem is not only related to $V_{\text{cmax}25}$, which is an index of single-leaf photosynthetic ability, but also to the leaf area index. This relationship should be considered in an analysis using a multi-layer model (e.g. Kosugi et al., 2006) in future.

In low light, $A$ is determined by $1-f$, which is the light use efficiency at low irradiance and influenced by the amount of chlorophyll and electron transfer. $1-f$ is one of the key characteristics for turfgrass in a stadium where adequate light is not available for photosynthesis.

For turfgrass grown in lower light, $1-f$ was higher during the cooler seasons of winter and spring, suggesting that the turfgrass acclimatized to low light conditions and allocated nitrogen to synthesize chloroplast lamellae. In contrast, turfgrass grown in higher light was lower during the cooler seasons, suggesting that the turfgrass avoided the photoinhibition caused by photosynthesis that had been reduced due to the lower air temperature. Under hot conditions the differences between light conditions became accordingly ambiguous after early summer because of the significant photosynthesis in the sunnier plot.

### 4.5 Acclimation to low irradiance

These results suggest that turfgrass fields have positive carbohydrate assimilation with leaf photosynthesis, acclimatization to low irradiance reduces the allocation to non-assimilatory organs to maintain leaf biomass. Furthermore, limited LMA, to retain the LAI, and limited LAI, to maintain the leaf nitrogen per unit leaf area, are carried out step-wise, depending on the shade level. After the summer decline, the leaf nitrogen content per unit leaf area also decreases, although the LAI is maintained at a minimum level during this decline.

The lower leaf nitrogen content per unit leaf area after the early summer in Plot D and in late summer in Plot C compared to the two sunnier plots (A and B) was synchronized with the decline in $V_{\text{cmax}25}$. The leaves of turfgrass grown in shade acclimatized to maintain $V_{\text{cmax}25}$ during the winter and spring, while the 80% shade in Plot D did not affect $V_{\text{cmax}25}$ in the same periods. The decline in $V_{\text{cmax}25}$ caused by low irradiance occurred only at higher temperatures, depending on the shade level.

Thus, light conditions are the critical factor that determine the pattern of growth and decline of turfgrass fields. A stepwise acclimatization of turfgrass to low irradiance occurs depending on the light environment.

### Acknowledgments

This study was conducted under the Industry-Academia-Government Collaboration Program at Kyoto University and was funded by the Obayashi Corporation. We thank Obayashi Road Corporation and Kokusai Ryokka Co. Ltd for help with our field observations at a soccer field. We also thank Dr. Makoto Tani, Mr. Kazuonri Mizuochi, Mr. Kiyoshi Sogo, and Dr. Hiroyuki Chino for their support, and Mr. Shigeru Maruo, Mr. Yuichiro Hayami, and other
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References


