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Differential impact of decreasing relative humidity on photosynthesis under fluctuating light between maize and tomato

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Abstract

Photosynthetic CO_2 assimilation (A_N) under fluctuating light (FL) plays a critical role in plant growth and crop yield. C_4 crops usually reach greater yields than C_3 crops when grown in regions with low relative humidity, which might be partly due to the CO_2 concentration mechanism and higher A_N under FL of C_4 crops. To test this hypothesis, we measured gas exchange and chlorophyll fluorescence under different changing frequencies of FL at contrasting relative air humidity (60% and 15%) for leaves of maize (*Zea mays*, C_4) and tomato (*Solanum lycopersicum*, C_3). In general, a more rapid changing frequency of FL would cause a larger loss in A_N during shade-sun transitions, with 20–38% in maize and 16–52% in tomato. Low relative humidity induced a larger decrease of stomatal conductance (g_s) under FL in tomato than in maize, leading to a much stronger loss in A_N in tomato than in maize. After transition from low to high light, A_N of maize was mainly limited by g_s with negligible non-stomatal limitation, and the stomatal limitation was slightly affected by relative humidity. By comparison, low relative humidity significantly increased the stomatal limitation of A_N in tomato but hardly affected non-stomatal limitation. These results highlight the differential impact of decreasing relative humidity on dynamic photosynthesis and productivity between C_3 and C_4 crops.

1 | INTRODUCTION

Photosynthesis is an important remaining opportunity for further improving the genetic yield potential of our major crops. Measurement, analysis, and improvement of leaf CO_2 assimilation (A_N) have focused largely on photosynthetic capacity under light-saturated steady-state conditions (Scafaro et al. 2011; Driever et al. 2014; Yamori et al. 2016). However, in modern crop canopies of several leaf layers, light is rarely constant, and most of the leaves experience marked light fluctuations throughout the day, owing to cloudy movement, wind-induced change in leaf angle and shading from upper leaves. As a result, most leaves of field crops are rarely under steady-state photosynthesis and dynamic photosynthesis contributes to a significant fraction of carbon gain (Kromdijk et al. 2016a; Slattery et al. 2018; De Souza et al. 2022). Upon transition from sun to shade and back into sunlight, photosynthesis needs several minutes to return to steady-state efficiency, leading to approximately 10–40% loss in potential A_N (Long et al. 2022). Therefore, increasing photosynthetic efficiency under fluctuating light (FL) is a promising strategy for improving plant growth and crop yield (Zhu et al. 2004; Adachi et al. 2019; De Souza et al. 2020; Kimura et al. 2020; Yamori et al. 2020).

Low-light-adapted C_3 and C_4 species showed similar light utilization in FL (Pearcy and Calkin 1983; Pearcy et al. 1985; Krall and Pearcy 1993), but high-light-adapted C_4 plants utilize FL less efficiently than high-light-adapted C_3 plants under low VPD conditions (Kubásek et al. 2013; Li et al. 2021). Therefore, increasing sunfleck utilization efficiency has the potential to improve plant growth and yield of C_4 plants under natural field conditions. Many recent studies investigated the limiting factor of sunfleck utilization efficiency in C_3 crops

(Soleh et al. 2016; Salter et al. 2019; Acevedo-Siaca et al. 2020; De Souza et al. 2020; Li et al. 2020; Sakoda et al. 2022). Unlike immediate CO_2 fixation by Rubisco in the C_3 plant tomato, CO_2 in the C_4 plant maize is first hydrated to HCO_3^- by carbonic anhydrase and assimilated to oxaloacetate by phosphoenolpyruvate carboxylase in mesophyll cells (Yamori et al. 2014). Oxaloacetate is subsequently reduced to malate in mesophyll cells and then transported to bundle sheath cells, in which CO_2 is regenerated from the decarboxylation of malate and fixed by Rubisco. The CO_2 concentration mechanism facilitates relatively high rate of photosynthesis under low g_s conditions. However, little is known about the major limiting factor of sunfleck utilization efficiency in C_4 crops (Li et al. 2021; Wang et al. 2021; Lee et al. 2022). Under the background of global climatic change, uneven rainfall has become increasingly frequent, leading to drought stress or a decrease in relative air humidity. C_4 crops usually reach greater yields than C_3 crops when grown in regions with low precipitation. Many previous studies investigated the effects of drought and dry air on steady-state photosynthesis in C_3 and C_4 plants (Leakey et al. 2006; Ibrahim et al. 2008; Carmo-Silva et al. 2010; Vilagrosa et al. 2012; Zivcak et al. 2013; Sancho-Knapik et al. 2022), but it is unclear whether C_4 crops have higher sunfleck utilization efficiency in dry air than C_3 crops.

Fluctuating light can induce a gradual decrease of stomatal conductance (g_s) owing to the low-light-induced stomatal closure (Kimura et al. 2020; Yamori et al. 2020; Eyland et al. 2021). Upon transition from low to high light, the induction rapidity of g_s is much slower than the activation rate of Rubisco (Sakoda et al. 2021). The absolute g_s at low light and the induction speed of g_s are critical for the induction kinetics of A_N (Kaiser et al. 2020; Kimura et al. 2020; Eyland et al. 2021). Therefore, g_s imposes a significant limitation on A_N under FL in some C_3 plants (Kaiser et al. 2020; Kimura et al. 2020; Eyland et al. 2021; Liu et al. 2022). Under high VPD conditions, g_s usually declines to prevent hydraulic failure, leading to subsequent detrimental effects on steady-state A_N, growth and productivity (Shirke and Pathre 2004; Zhang et al. 2005; Peguero-Pina et al. 2016; Qiu et al. 2017; Merilo et al. 2018; Grossiord et al. 2020; Inoue et al. 2021; Sancho-Knapik et al. 2022). Furthermore, a high VPD slows down the induction rate of g_s in tomato (Kaiser et al. 2017, 2020). Owing to the similar effects of VPD and FL on g_s, dry air might aggravate the decrease of g_s under FL. However, little is known about the impact of high VPD on g_s behavior under FL in C_3 and C_4 plants.

In nature, FL occurs with different frequencies (Valladares et al. 1997; Kimura et al. 2020). Based on the characteristics of g_s under FL (i.e., decrease of g_s at low light and increase of g_s at high light), the duration of low light significantly affects the absolute g_s before transitioning to high light (Yamori et al. 2020; Eyland et al. 2021). Therefore, the changing frequency of FL can significantly influence the photosynthetic efficiency during shade-sun transitions (Arce Cubas et al. 2023). The effects of changing the frequency of FL on g_s and CO_2 assimilation rate have been well clarified in the C_3 model plant *Arabidopsis thaliana* (Kimura et al. 2020; Yamori et al. 2020). Furthermore, the changing frequency of FL influences CO_2 assimilation rate during shade-sun transitions in C_4 species, and C_4 species had stronger and more sustained CO_2 assimilation rate during the low-light phase in FL compared with C_3 species (Arce Cubas et al. 2023). However, it is unclear whether relative humidity interacts with the changing frequency of FL to influence photosynthetic efficiency under FL.

Based on current knowledge about dynamic photosynthesis and its responses to environmental stresses, dry air theoretically aggravates the loss in photosynthetic carbon gain under FL and thus reduces crop production. Therefore, understanding the responses of dynamic photosynthesis to dry air in C_3 and C_4 plants can help us actively solve the global food crisis under future climatic change. In this study, we measured gas exchange and chlorophyll fluorescence under different frequencies of fluctuating light at contrasting relative air humidity for leaves of maize (*Zea mays*, C_4) and tomato (*Solanum lycopersicum*, C_3). The main aims are: 1) to assess whether maize has a higher sunfleck utilization efficiency than tomato in dry air; and 2) to evaluate whether dry air imposes different effects on maize and tomato in terms of g_s and A_N under FL.

2 | MATERIALS AND METHODS

2.1 | Plant materials and growth conditions

Maize (*Zea mays* L. cv. Zhengdan 958) and tomato (*Solanum lycopersicum* Miller cv. Hupishizi) plants were used in this study. Plants were grown in 13-litre plastic pots containing humus soil, with an initial soil N content of 2.1 mg g^{-1}. After seed gemination, pots were placed in an open field at the Kunming Institute of Botany, Chinese Academy of Sciences, China (102°44′31″E, 25°08′24″N, 1950 m of elevation). During the growth period (from July to August 2022), the day/night air temperatures were approximately 30°C/20°C and the maximum light intensity to which the leaves were exposed was approximately 2000 μmol photons m^{-2} s^{-1} (measured by LI-1400, Li-Cor Biosciences) These plants were fertilized with 0.15 g N $plant^{-1}$ every two days using Peters Professional's water solution (N:P:K = 15:4.8:24.1) to avoid any nutrient stress. To prevent any water stress, plants were watered every day with a gradually increasing amount of water to match the plant growth. After normal growth for a month, mature canopy leaves on the top of plants were used for photosynthetic measurements.

2.2 | Gas exchange and chlorophyll fluorescence measurements

The gas exchange and chlorophyll fluorescence were measured simultaneously using a portable photosynthesis system (LI-6400XT; Li-Cor Biosciences) equipped with a leaf chamber fluorometer (Li-Cor Part No. 6400–40, enclosed leaf area: 2 cm^2). This leaf chamber can be full of all individual maize and tomato leaves. The atmospheric CO_2 concentration (400 μmol mol^{-1}), the flow rate of air through the

measuring system (300 mmol min^{-1}), and leaf temperature (30°C) were controlled by an automatic control device in the LI-6400XT. Irradiance was provided by a mixture of red (90%) and blue (10%) LEDs in the fluorometer. Relative air humidity of 60% or 15% was controlled by using a buffer bottle (water was injected in it) and desiccant. Leaves were first illuminated at 1500 µmol photons m^{-2} s^{-1} and 400 µmol mol^{-1} CO_2 concentration for at least 30 min to reach steady-state photosynthesis and stomatal conductance. Afterwards, light intensity was altered between low and high (100 and 1500 µmol photons m^{-2} s^{-1}) every 2, 5, or 10 min for 40 min to simulate different frequencies of FL. Photosynthetic parameters were logged every 1 minute to calculate the kinetics of photosynthesis under FL. After transition from low to high light, the measured cumulative CO_2 assimilation ($CCA_{measured}$) in high-light phase was calculated by integrating

TABLE 1 Steady state photosynthetic parameters of maize and tomato measured at different relative humidity (60% and 15%) conditions. Data are shown as mean ± SE (n = 5–6). Different letters indicate significant differences among these treatments (Tukey's test, $P < 0.05$)

Species and conditions	A_N (µmol m^{-2} s^{-1})	Φ_{PSII}	g_s (mol m^{-2} s^{-1})	T_r (mmol m^{-2} s^{-1})
Maize RH 60%	35.4 ± 1.3^a	0.30 ± 0.01^b	0.22 ± 0.01^c	4.4 ± 0.3^c
Maize RH 15%	34.9 ± 0.7^a	0.30 ± 0.01^b	0.23 ± 0.01^c	9.7 ± 0.5^b
Tomato RH 60%	26.8 ± 0.5^b	0.35 ± 0.01^a	0.51 ± 0.02^a	8.5 ± 0.5^b
Tomato RH 15%	22.9 ± 1.1^c	0.33 ± 0.02^b	0.34 ± 0.04^b	12.6 ± 1.2^a

A_N, net CO_2 assimilation rate; g_s, stomatal conductance; Φ_{PSII}, effective quantum yield of photosystem II photochemistry; T_r, transpiration rate.

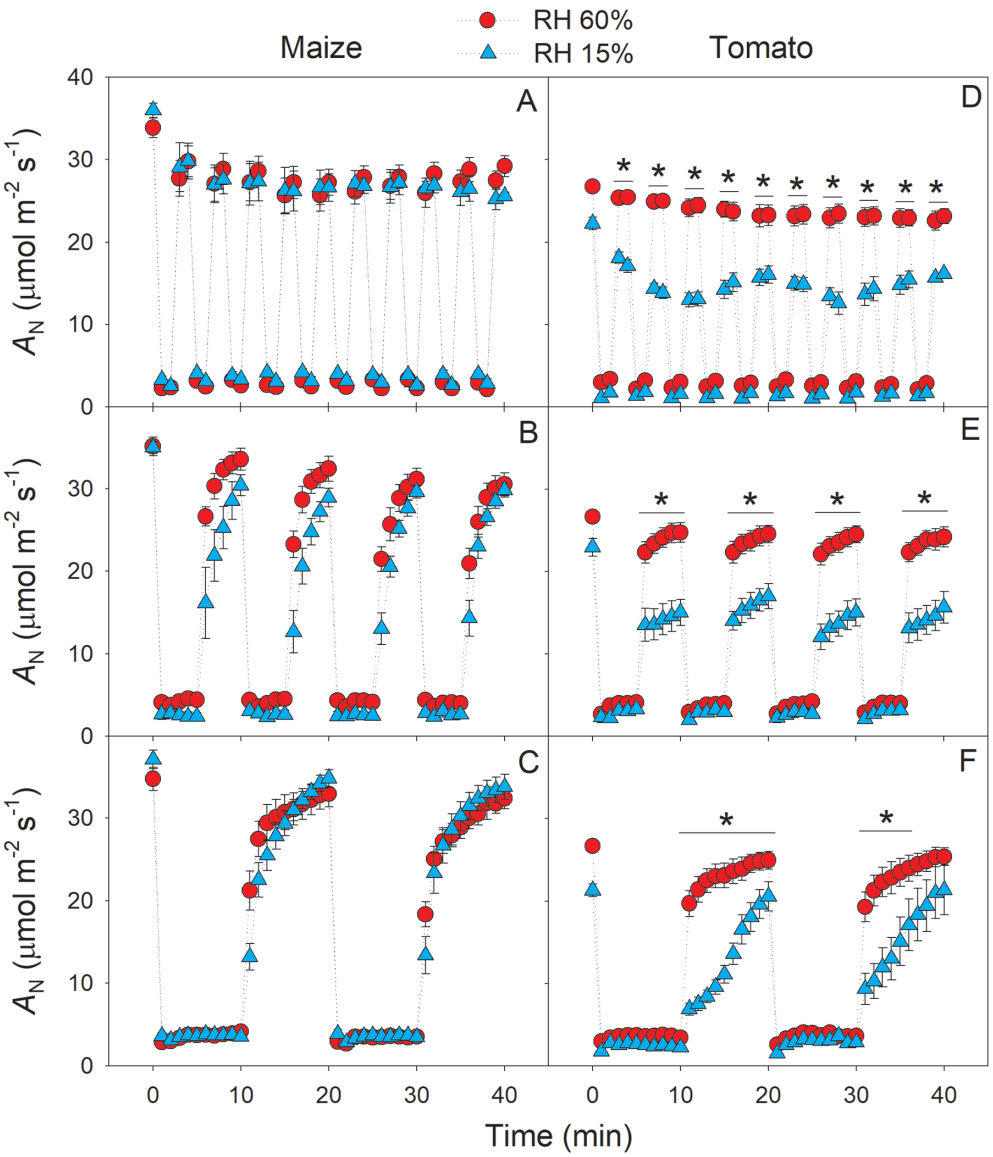

FIGURE 1 Dynamic change of net CO_2 assimilation rate (A_N) under fluctuating light for leaves of maize (A, B, C) and tomato (D, E, F). Fluctuating light alternates between 1500 and 100 µmol m^{-2} s^{-1} every 2 (A, D), 5 (B, E), or 10 min (C, F) at 30°C at different relative humidity (RH 60% and 15%). Asterisk indicates a significant difference between 60% and 15% relative humidity (T-test, $P < 0.05$). Data are means ± SE (n = 5–6 replicates, each being an individual plant).

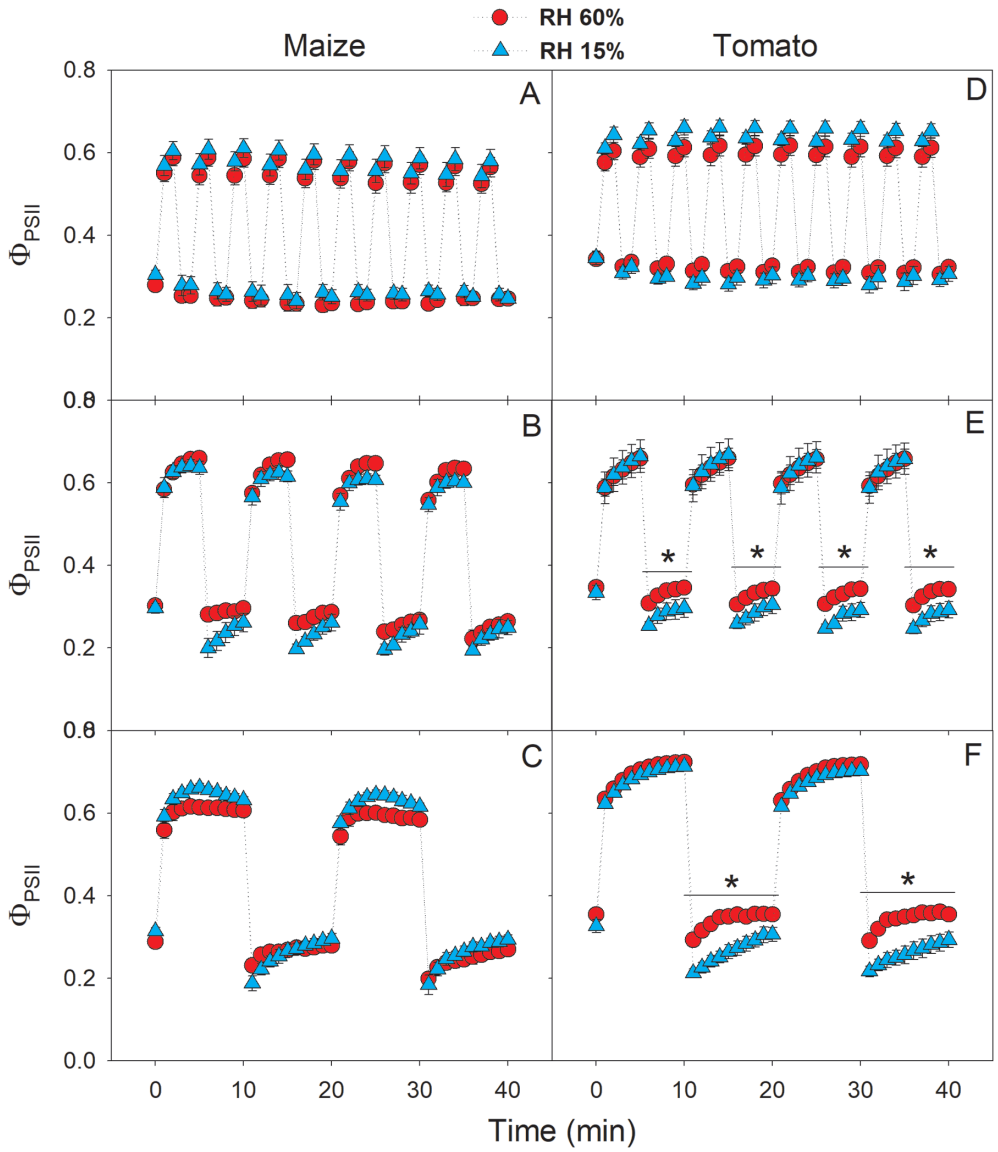

FIGURE 2 Dynamic change of effective quantum yield of photosystem II photochemistry (Φ_{PSII}) under fluctuating light for leaves of maize (A, B, C) and tomato (D, E, F). Fluctuating light alternates between 1500 and 100 μmol m^{-2} s^{-1} every 2 (A, D), 5 (B, E), or 10 min (C, F) at 30°C at different relative humidity (RH 60% and 15%). Asterisk indicates a significant difference between 60% and 15% relative humidity (T-test, $P < 0.05$). Data are means ± SE (n = 5–6).

measured A_N. The maximum potential cumulative CO_2 assimilation ($CCA_{maximum}$) in high-light phase was calculated by integrating the maximum A_N. The maximum A_N indicates the steady-state A_N at 1500 μmol photons m^{-2} s^{-1} and 400 μmol mol^{-1} CO_2 concentration. The percentage of loss in CO_2 fixation after transition from low to high light was calculated by 1 – $CCA_{measured}/CCA_{maximum}$ (Long et al. 2022).

We determined chlorophyll fluorescence parameters using the multi-phase flash (MPF) protocol following recommended procedures (Loriaux et al. 2013). The measuring light intensity and the maximum flash intensity were 1 and 8000 μmol m^{-2} s^{-1}, respectively. The flash intensity decreased by 60% during the second phase of the MPF and the durations of the three flash phases were 0.3 s, 0.7 s, and 0.4 s, respectively. The effective photochemistry quantum yield of photosystem II (Φ_{PSII}) and total electron transport rate through PSII (ETR) were calculated using the following equation (Genty et al. 1989; Krall and Edwards 1992):

$$\Phi_{PSII} = \frac{(F_m' - F_s)}{F_m'}.$$

2.3 | Stomatal and nonstomatal limitations

Stomatal limitation (L_S) and non-stomatal limitation (L_{NS}) imposed on CO_2 assimilation were calculated using the following equations (Farquhar and Sharkey 1982; Bellasio et al. 2018, 2023):

$$L_S = \frac{A_{potCa} - A_{potCiop}}{A_{potCa}}$$

$$L_{NS} = \frac{A_{potCiop} - A_{op}}{A_{potCa}}$$

where A_{potCa} is the potential A that would occur when C_i was equal to ambient [CO_2] at the leaf surface (C_a) if there was no epidermal impediment to CO_2 diffusion into the leaf, as predicted by the A/C_i curve;

$A_{potCiop}$ is the potential A that would occur at the operational C_i, as predicted by the A/C_i curve; A_{op} is the operational A that is measured at the operational C_i. The response of A to C_i was measured at 1500 µmol photons m^{-2} s^{-1} and different C_i conditions, and the A/C_i curve was modelled empirically as a nonrectangular hyperbola (Lee et al. 2022).

2.4 | Statistical analysis

Experimental design was a randomized complete block design with at least five replications from different individual plants. Tukey's test was used to determine whether significant differences ($P < 0.05$) existed among all treatments in maize and tomato. A t-test was used to determine whether significant differences ($P < 0.05$) existed between high and low relative humidity in maize or tomato. The software SigmaPlot 10.0 was used for graphing and fitting.

3 | RESULTS

3.1 | Effects of decreasing relative humidity on steady-state photosynthetic parameters

We first measured the steady-state photosynthetic performance at 1500 µmol photons m^{-2} s^{-1} and contrasting relative humidity (Table 1). At 60% relative humidity, net CO_2 assimilation rate (A_N) in maize was 35.4 ± 1.3 µmol CO_2 m^{-2} s^{-1}, which was significantly higher than that in tomato (26.8 ± 0.5 µmol CO_2 m^{-2} s^{-1}) ($P < 0.0001$). At 15% relative humidity, A_N in maize was 34.9 ± 0.7 µmol CO_2 m^{-2} s^{-1} but significantly decreased to 22.9 ± 1.1 µmol CO_2 m^{-2} s^{-1} in tomato ($P < 0.0001$). The effective quantum yield of photosystem II photochemistry (Φ_{PSII}) at 60% relative humidity was 0.30 ± 0.01 in maize and 0.35 ± 0.01 in tomato. When relative humidity decreased to 15%, values for Φ_{PSII} were 0.30 ± 0.01 and 0.33 ± 0.03 in maize and tomato, respectively. When relative humidity decreased from 60% to 15%, the steady-state g_s was maintained stable at 0.22–0.23 mol m^{-2} s^{-1} in maize, but decreased significantly from 0.51 ± 0.02 to 0.34 ± 0.04 mol m^{-2} s^{-1} in tomato ($P < 0.0001$). Concomitantly, transpiration rate (T_r) significantly increased from 4.4 ± 0.3 to 9.7 ± 0.5 mmol m^{-2} s^{-1} in maize and from 8.5 ± 0.5 to 12.6 ± 1.2 mmol m^{-2} s^{-1} in tomato ($P < 0.0001$).

3.2 | Effects of dry air on temporal kinetics of photosynthetic parameters under FL

Next, we examined photosynthetic performance under FL conditions alternating between 100 and 1500 µmol photons m^{-2} s^{-1} every 2, 5, or 10 min (FL–2 min, FL–5 min, or FL–10 min). When relative humidity decreased from 60% to 15%, A_N in low light phases was almost maintained stable under all FL conditions in maize and tomato (Figure 1A-C). Under FL–2 min condition, sunfleck utilization efficiency of maize was hardly affected by the decrease in relative humidity (Figure 1A). Under FL–5 min and FL–10 min conditions, sunfleck utilization efficiency of maize was slightly suppressed within the first minutes by the low relative humidity (Figure 1B,C). After transfer from low to high light for ten minutes, A_N reached at least 90% of the maximum value in maize at high and low relative humidity. By comparison, low relative humidity largely significantly suppressed sunfleck utilization efficiency in tomato under all FL conditions, especially within the first minutes after transition from low to high light (Figure 1D-F). After transitioning to high light for one minute under FL–5 min and FL–10 min conditions, values for A_N were higher than 24 µmol CO_2 m^{-2} s^{-1} at 60% relative humidity but were lower

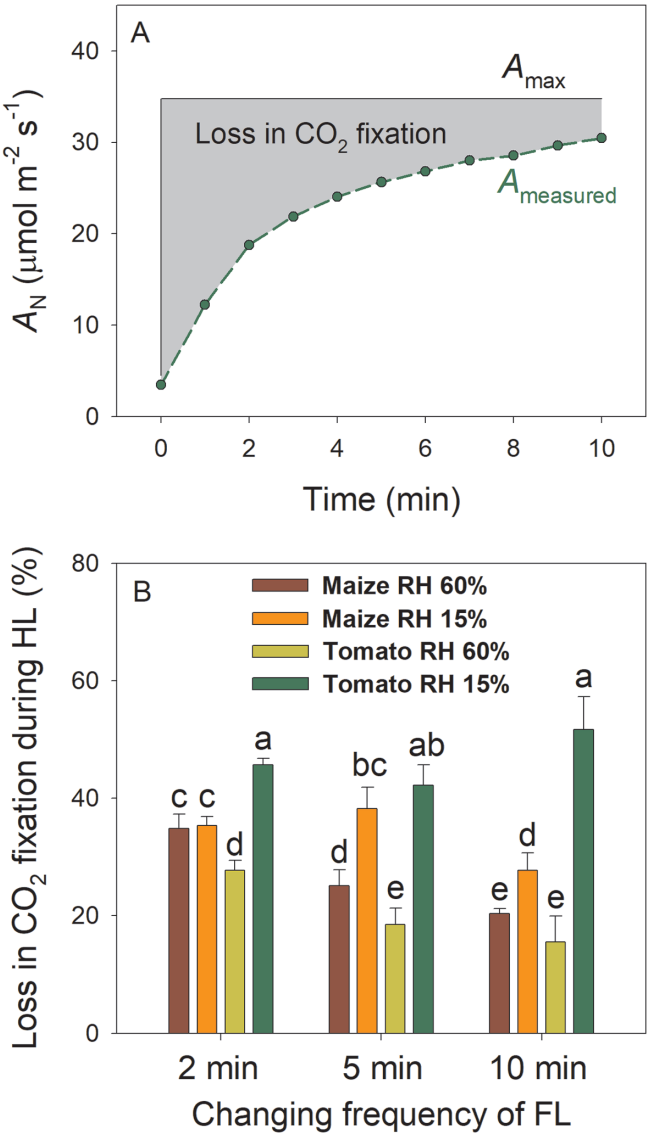

FIGURE 3 The loss in CO_2 fixation after transition from low to high light. (A) A typical model for assessing the loss in CO_2 fixation under fluctuating light. Solid and dashed lines represent the maximum A_N and measured A_N, respectively. (B) The percentage of loss in CO_2 fixation during high-light phases of fluctuating light for maize and tomato measured at 60% and 15% relative humidity. Different letters indicate significant differences among these treatments (Tukey's test, $P < 0.05$). Data are means ± SE (n = 5–6).

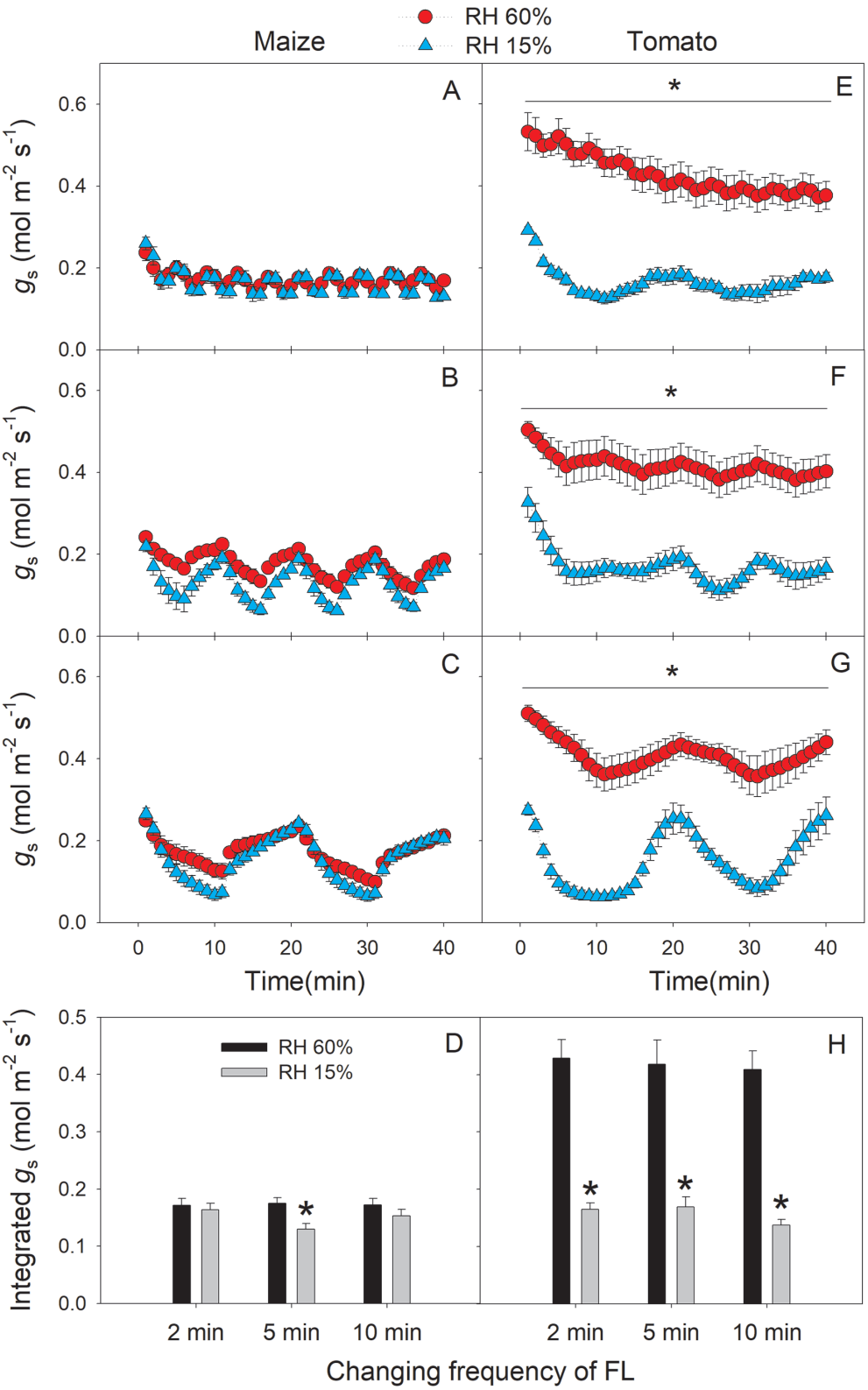

FIGURE 4 Dynamic change of stomatal conductance (g_s) under fluctuating light for leaves of maize (A, B, C) and tomato (E, F, G). Fluctuating light alternates between 1500 and 100 µmol m^{-2} s^{-1} every 2 (A, E), 5 (B, F), or 10 min (C, G) at 30°C and different relative humidity (RH 60% and 15%). Integrated g_s values in maize (D) and tomato (H) were calculated. Asterisk indicates a significant difference between 60% and 15% relative humidity (t-test, $P < 0.05$). Data are means ± SE (n = 5–6).

than 14 µmol CO_2 m^{-2} s^{-1} at 15% relative humidity (Figure 1E,F). Consistent with the performance of A_N, dry air hardly affected Φ_{PSII} in maize leaves under all FL conditions (Figure 2A-C). By comparison, Φ_{PSII} during high-light phases slightly decreased in tomato leaves when exposed to FL–5 min and FL–10 min conditions (Figure 2E-F). After transitioning to high light for five minutes under FL–10 min condition, values for Φ_{PSII} were approximately 0.35 and 0.26 at 60% and 15% relative humidity, respectively.

We further estimated the loss in CO_2 fixation by calculating the difference between potential CO_2 fixation and measured CO_2 fixation

(Figure 3A). At 60% and 15% relative humidity, cumulative CO_2 fixation of maize lost 34.9% ± 0.025 and 35.4% ± 0.015 in FL–2 min, 25.1% ± 0.027 and 38.3% ± 0.036 in FL–5 min, 20.4% ± 0.008 and 27.7% ± 0.03 in FL–10 min, respectively (Figure 3B). In tomato leaves, cumulative CO_2 fixation at 60% and 15% relative humidity lost 27.8% ± 0.017 and 45.7 ± 0.011 in FL–2 min, 18.5% ± 0.028 and 42.2% ± 0.035 in FL–5 min, 15.6% ± 0.044 and 51.7% ± 0.056 in FL–10 min, respectively (Figure 3B). These results indicated that sunfleck utilization efficiency at 60% relative humidity was significantly lower in FL–2 min condition than in FL–5 min and FL–10 min ($P < 0.05$). Furthermore, dry air induced a larger loss in cumulative CO_2 fixation under FL conditions in tomato than in maize.

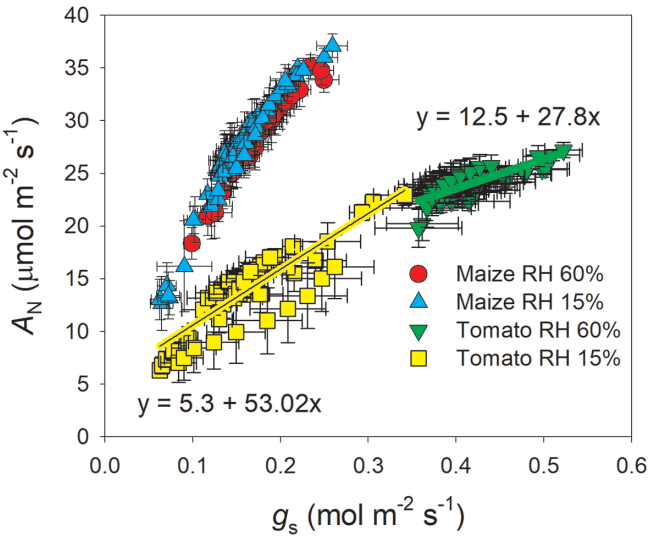

FIGURE 5 Relationship between g_s and A_N during high-light phases of all fluctuating light conditions for maize and tomato measured at 60% and 15% relative humidity. Data are means ± SE (n = 5–6).

Upon transition from high to low light, the decreased g_s rate in maize was aggravated by dry air, leading to a lower g_s before transition to high light in FL–5 min and FL–10 min (Figure 4B,C). When back into high light in FL–5 min and FL–10 min, g_s rapidly increased and the peak of g_s was hardly affected by dry air (Figure 4B,C). Compared with g_s at 60% relative humidity, the integrated g_s of maize leaves at 15% relative humidity decreased by 4%, 26%, and 11% in FL–2 min, FL–5 min and FL–10 min, respectively (Figure 3D). In tomato leaves, g_s under all FL conditions largely decreased at 15% relative humidity when compared with 60% relative humidity (Figure 4E-G). After transition from high to low light, g_s rapidly decreased at 15% relative humidity. Furthermore, within the first five minutes after transitioning to high light, g_s increased very slightly at 15% relative humidity. As a result, when relative humidity decreased from 60% to 15%, the integrated g_s of tomato leaves decreased by 62%, 60%, and 66% in FL–2 min,

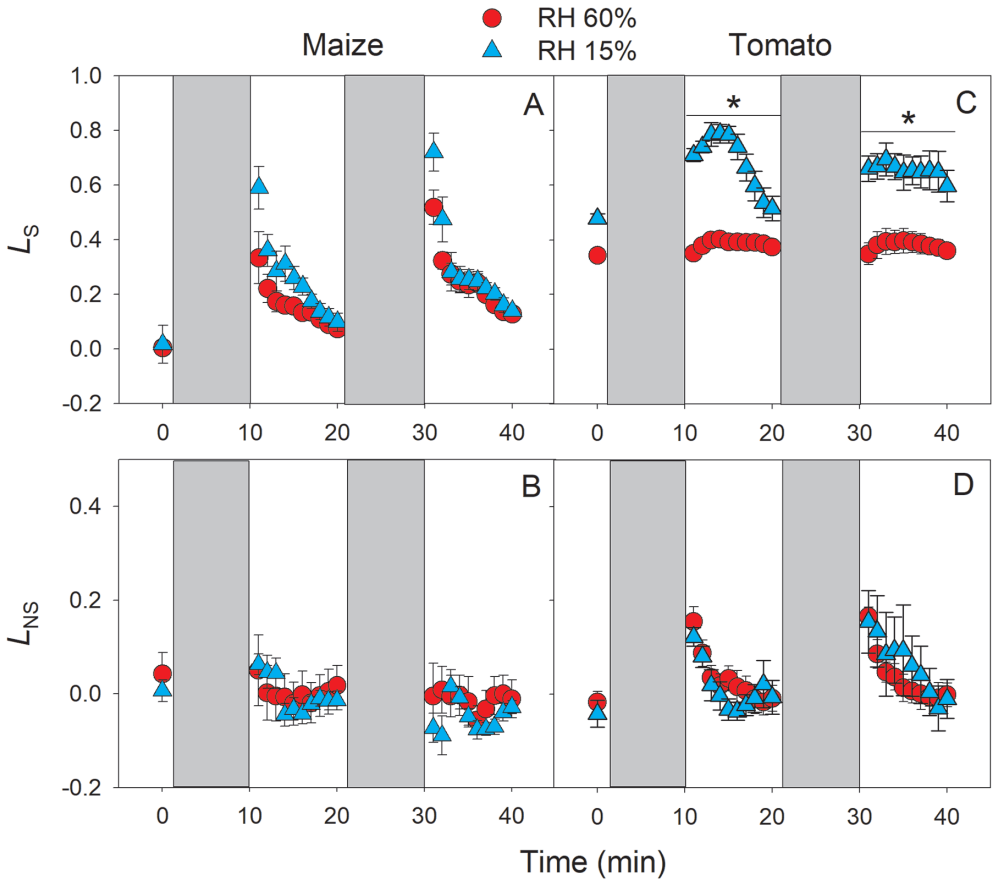

FIGURE 6 Dynamic changes of stomatal and non-stomatal limitations (L_S and L_{NS}) for leaves of maize (A, B) and tomato (C, D) in high-light phases under fluctuating light alternating between 1500 and 100 µmol m^{-2} s^{-1} every 10 min at 30°C and different relative humidity (RH 60% and 15%). Grey bars indicate low-light phases and open bars indicate high-light phases. Asterisk indicates a significant difference between 60% and 15% relative humidity (t-test, $P < 0.05$). Data are means ± SE (n = 5–6).

FL–5 min and FL–10 min, respectively (Figure 4H). Therefore, dry air induced a larger decline of g_s under all FL conditions in tomato than in maize.

3.3 | Photosynthetic limitations under FL

Based on the concurrent changes of g_s and A_N under FL, the relationship between g_s and A_N was analyzed (Figure 5). During high-light phases under FL, g_s was positively correlated to A_N in both species, with a steeper slope in maize. In maize leaves, the slope of relationship between g_s and A_N was not altered by relative humidity. By comparison, in tomato, a steeper slope of relationship at 15% relative humidity than that at 60% relative humidity was found. To further examine the impact of g_s kinetics on A_N, we analyzed stomatal and non-stomatal limitations (L_S and L_{NS}, respectively) during high-light phases. In maize leaves, A_N during shade-sun transitions was significantly limited by g_s with little non-stomatal limitation (Figure 6A,B). Within the first two minutes after transition from low to high light, maize leaves showed high levels of L_S and such temporary L_S was aggravated by dry air. In tomato leaves, L_S and L_{NS} significantly occurred during shade-sun transitions (Figure 6C,D). Furthermore, dry air significantly increased L_S but hardly affected L_{NS} during the whole high-light phases in tomato leaves (Figure 6C,D). Therefore, L_S is more sensitive to dry air in C_3 tomato leaves than in C_4 maize leaves.

4 | DISCUSSION

4.1 | Sunfleck utilization efficiency is affected by the changing frequency of FL

Increasing the ability of plants to adjust their photosynthetic efficiency under FL is an attractive strategy for improving photosynthesis and crop yield (Kromdijk et al. 2016b; Tanaka et al. 2019; Acevedo-Siaca et al. 2020). Here, we determined the photosynthetic performance under FL with different changing frequencies in maize and tomato (Figure 1). The results indicated that the loss in potential photosynthetic carbon gain during sunfleck varied from 20–38% in maize and 16–52% in tomato (Figure 3B). The highest loss in potential photosynthetic carbon gain at 15% relative humidity in tomato (52%) is stronger than the loss in CO_2 fixation under FL as calculated in previous studies (10–40%) (Lee et al. 2022; Long et al. 2022). Furthermore, we examined the loss in photosynthetic carbon gain under FL conditions with different changing frequencies. The results indicated that FL alternating between low and high light every 2 min (FL–2 min) induced a stronger loss in photosynthetic carbon gain than FL alternating between low and high light every 10 min (FL–10 min) (Figure 3B). These results indicated that a quicker changing frequency of FL would cause a lower sunfleck utilization efficiency. Therefore, the changing frequency of FL is an important factor affecting sunfleck utilization efficiency in both maize and tomato. Consistently, a recent study found that photosynthetic efficiency under fluctuating light could be influenced by the fluctuation regime (Arce Cubas et al. 2023).

By transferring plants from low to high light under favorable conditions, photosynthetic induction was reported to be delayed in C_4 plants when compared with C_3 plants (Li et al. 2021; Lee et al. 2022). Consistently, the loss in photosynthetic carbon gain was significantly higher in maize than in tomato when exposed to FL–2 min and FL–5 min at 60% relative humidity (Figure 3B). These results strongly supported that the loss in photosynthetic carbon gain in maize mainly occurred in the first 2 min after transition from low to high light (Lee et al. 2022). However, under condition of FL–10 min at 60% relative humidity, no significant difference in the loss in photosynthetic carbon gain was observed between maize and tomato (Figure 3B), which was consistent with the scheme that photosynthetic efficiency during high-light phase is attributable to species rather than photosynthetic pathway (Arce Cubas et al. 2023). Therefore, the previous conclusion that C_4 plants utilize FL less efficiently than C_3 plants should be revised according to the changing frequency of FL.

4.2 | Photosynthesis under FL in maize is mainly limited by g_s

In previous studies, the difference in sunfleck utilization efficiency between C_4 and C_3 plants was hypothesized to be caused by the slower diffusion of metabolites from mesophyll cells to bundle sheath cells (Kubásek et al. 2013; Lee et al. 2022). However, our present data indicated that such delayed photosynthetic induction in maize was tightly correlated to the response of g_s to light conditions (Figure 5). After transition from high to low light at 60% relative humidity, g_s decreased much faster in maize than in tomato, leading to a much lower initial g_s under low light in maize (Figure 4), which strongly restricted A_N within the first minutes after transition to high light (Figure 6). Therefore, increasing the g_s under low light conditions or accelerating the rate of stomatal opening might provide a new opportunity to improve sunfleck utilization efficiency in maize grown under favorite conditions. Increased g_s or improved stomatal opening has been documented to be efficient to enhance phot osynthetic rate and biomass under FL for C_3 plants such as *Arabidopsis thaliana*, rice and tomato (Kaiser et al. 2020; Kimura et al. 2020; Sakoda et al. 2020; Yamori et al. 2020). Because of the stronger relationship between g_s and A_N during shade-sun transitions in maize than in tomato (Figure 5), manipulating g_s would improve dynamic photosynthesis to a greater extent in C_4 crops than in C_3 crops when cultivated in regions with high precipitation.

4.3 | Maize uses sunfleck more efficiently than tomato in dry air

In nature, FL often occurs with low relative humidity under the background of global climatic change, especially in semi-arid areas and in

dry season. However, little is known about how dry air affects photosynthetic efficiency under FL (Kaiser et al. 2015). We found that the absolute cumulative CO_2 fixation was much higher in maize than tomato (Figure 1). Although dry air generally accelerated the loss in photosynthetic carbon gain under FL in both maize and tomato, the loss extent was significantly lower in maize (Figure 3B). These results strongly indicate that maize utilizes sunfleck more efficiently than tomato in dry air, providing insight into why C_4 crops have higher yield potential than C_3 crops when cultivated in regions with dry air. In the context of adaptive evolution, these important photosynthetic characteristics of C_4 species result in them being more competitive against C_3 species in regions with dry air.

When relative humidity decreased from 60% to 15%, the closing rate of stomata under low light was accelerated in maize (Figure 4), leading to a lower initial g_s and thus restricting sunfleck utilization efficiency within the first minutes (Figure 6). Compared with maize, dry air not only induced a more rapid stomatal closure in low light phases but also inhibited stomatal opening during light induction in tomato (Figure 4), consistent with the impact of VPD on g_s in other C_3 plants (Grossiord et al. 2020). This large decrease of g_s in dry air made g_s the major limitation of photosynthesis during sunfleck in tomato (Figure 6), which was consistent with the significant limitation of g_s during light induction in C_3 plants (De Souza et al. 2020; Liu et al. 2022). Owing to the CO_2 concentration mechanism in maize, a relatively low g_s could be accompanied by a relatively high A_N (Wang et al. 2021; Lee et al. 2022). Therefore, although maize and tomato leaves showed similar g_s under FL conditions in dry air (Figure 4), the absolute A_N during high-light phases was much higher in maize than in tomato (Figures 1 and 5). Therefore, the maintenance of g_s and CO_2 concentration mechanism jointly facilitates the higher sunfleck utilization efficiency in maize than in tomato when exposed to FL conditions in dry air.

5 | CONCLUSIONS

Many previous studies investigated the photosynthetic limitation under FL in C_3 plants, but little is known about the photosynthetic limitation under FL in C_4 plants. We here found that sunfleck utilization efficiency of maize is mainly limited by g_s. Therefore, increasing g_s has the potential to improve dynamic photosynthetic efficiency and biomass for maize grown in high-precipitation regions. When exposed to FL in dry air, g_s decreased only slightly in C_4 maize but largely decreased in C_3 tomato. As a result, dry air induced a smaller loss in photosynthetic carbon gain under FL in maize than in tomato. This result partially explains why C_4 crops usually reach greater yields than C_3 crops when grown in regions with low relative humidity. At high relative humidity, the photosynthetic advantage of C_4 crops compared with C_3 crops is diminished by the relatively high g_s in FL. Taking together, the effect of relative humidity on dynamic photosynthesis should be taken into consideration when modelling crop yield under future climatic change.

AUTHOR CONTRIBUTIONS

WH and YJY designed experiments; QS, XQW and BH performed experiments; QS, XQW and WH analyzed data; and WH wrote the paper, which was edited by other authors.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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