Daytime warming during early grain filling offsets the CO$_2$ fertilization effect in rice

Guoyou Zhang$^{1,6}$, Kazuhiro Ujiie$^{2,8}$, Mayumi Yoshimoto, Hidemitsu Sakai$^{1,7}$, Takeshi Tokida$^*$, Yasuhiro Usui$^*$, Hitomi Wakatsuki$^*$, Miwa Arai$^*$, Hiroki Ikawa$^*$, Hirofumi Nakamura$^3$ and Toshihiro Hasegawa$^*$

1 Institute for Agro-Environmental Sciences, National Agriculture and Food Research Organization (NARO), Tsukuba, Ibaraki 305-8604, Japan
2 Institute of Agricultural and Life Sciences, Academic Assembly, Shimane University, Matsue, Shimane 690-8504, Japan
3 Central Region Agricultural Research Center, NARO, Tsukuba, Ibaraki 305-8666, Japan
4 Hokkaido Agricultural Research Center, NARO, Sapporo, Hokkaido 062-8555, Japan
5 Taiyo Keiki Co. Ltd, 3-2-5, Kawagishi, Toda, Saitama 335-0015, Japan
6 Key Laboratory of Agrometeorology of Jiangsu Province, School of Applied Meteorology, Nanjing University of Information Science & Technology, Nanjing 210044, People's Republic of China
7 Present address; Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki 305-8686, Japan.
8 Authors made the same contribution.
* Authors to whom any correspondence should be addressed.
E-mail: hsakai@affrc.go.jp and thase@affrc.go.jp

Keywords: chalky grains, climate change, heat stress, free-air CO$_2$ enrichment (FACE), Oryza sativa, spikelet sterility, temperature-free-air controlled enhancement (T-FACE)

Abstract
Increasing concentrations of atmospheric CO$_2$ are projected to have positive effects on crop photosynthesis and yield (CO$_2$ fertilization effect, CFE). High-temperature events, such as heatwaves, during sensitive periods can have significant negative impacts on crop yield and quality; however, the combined effects of elevated CO$_2$ (EC) and short-period elevated temperature (ET) have not been determined in the open field. Here, we show a strong negative interaction between EC and ET obtained from a temperature-free-air controlled enhancement treatment embedded in a season-long free-air CO$_2$ enrichment (FACE) experiment on a japonica rice cultivar, Koshihikari, over three seasons at the Tsukuba FACE facility in Ibaraki, Japan. CFE was 15% at ambient temperature, but it was reduced to 3% by ET, where canopy surface temperature (Tc) was elevated by ~1.6 °C for 20 d after flowering. Reductions in CFE mainly arose from poor grain setting at Tc above ~30 °C. High Tc also increased the percentage of chalky grains and substantially decreased the grain appearance quality, although the threshold temperature varied between the seasons. Simultaneous increases in atmospheric CO$_2$ concentration and air temperature are expected to increase daytime canopy temperatures more than air warming alone, thereby affecting grain yield and quality. Crop models without these processes are likely to underestimate the negative impacts of climate change on crop yield and quality. The development of adaptation measures against heat stress, particularly during reproductive and grain-filling periods, needs to be enhanced and accelerated.

1. Introduction
Climate change, including global warming and increases in frequency and intensity of extremes, is projected to negatively impact the yield of the world’s major staple crops [1]. A recent systematic review of crop simulation studies of four major crops (maize, rice, soybean, and wheat) has shown an overall negative impact on crop yield without adaptation, with median effects of per °C increase in global temperature ranging from −8% to −2% depending on crop species [2]. The effects differ greatly among locations
and are heavily dependent on current temperature levels; adverse effects will appear larger and sooner in current warmer regions, likely because they will be exposed to extreme heat sooner. However, many models do not simulate the short-period extreme climate events well [3, 4], suggesting that yield losses due to extreme weather events may be underestimated.

Increases in atmospheric CO₂ concentrations are expected to counter the adverse effects of increasing temperatures by enhancing photosynthesis and grain yield (GY), particularly in C₃ crop species [5]. However, the scale of the CO₂ fertilization effect (CFE) remains variable and is thus an important source of uncertainty in response to climate change [5]. In early studies, crop responses to elevated CO₂ (EC) were generally examined using environmentally controlled chambers; however, in the late 1980s, free-air CO₂ enrichment (FACE) technology was developed, and applied to various plant species to examine open-field plant responses to EC. FACE studies provide valuable datasets for testing crop simulation models under open field conditions; they have been used to compare multiple crop models to determine the uncertainty of yield projections in wheat, rice, and maize [6–8], contributing to crop model improvements [9]. One of the shortcomings of FACE studies is that experiments have been conducted at a limited number of sites and that interactions with other environmental factors have not been rigorously tested. Recent analyses of long-term FACE experiments suggest that unfavorable weather conditions, such as heat, cold, and drought, may reduce CFE [10], and that analysis of the effects of season-long climatic conditions may overlook important in-season variations that may affect yield and CFE [11].

Rice is the most important food crop in Asia, and is widely grown in different climate zones. Reproductive growth processes are sensitive to extreme temperatures, posing a significant threat to stable production under climate change [12]. Earlier studies using growth chambers have revealed negative interactions between EC and heat [13, 14]. The results of FACE studies on rice conducted over 13 years in Japan also showed that the CFE on yield (CFEyield) decreased linearly with an increase in temperature for 30 d after heading [15], possibly caused by reductions in sink capacity, grain setting, and grain filling. Under climate change, unseasonal weather and short-period heat extremes are projected to occur more frequently [16]. The effects of short-period elevated temperatures (ET) during a sensitive stage on CFE need to be better understood to predict the effects of climate change on crops more accurately and to develop appropriate adaptations.

Temperature-free-air controlled enhancement (T-FACE) technologies have been developed to test crop and vegetation responses to ET by heating crop canopy downward using an array of infra-red heaters [17]. By combining T-FACE with FACE, which elevates the atmospheric CO₂ concentration in an octagonal or circular area by fumigating CO₂ from peripheries, a direct examination of the combined effects of temperature and CO₂ in open fields is possible. A limited number of FACE × T-FACE studies on soybean, rice, and wheat have shown that season-long ETs depress C₃ plant productivity and crop yield, which cannot be compensated for by EC, indicating a loss of CFE due to seasonal warming [18, 19]. However, the effects of short-period ET on CFE are not well understood.

Grain setting and active grain growth occur for a few weeks right after heading and are highly sensitive to temperatures [20, 21]. The previous findings from more than 10 years of rice FACE studies also suggest that temperatures during the early grain-filling period could modify CFE [15]. We, therefore, conducted a FACE × T-FACE experiment to examine the effect of short-period warming after the heading stage on CFE in rice. The warming treatment was imposed during the daytime only for the following two reasons: (a) grain-setting is highly sensitive to temperature at the time of flowering, which generally occurs around mid-day [22], and (b) we anticipated that reduced transpiration by EC would modify the canopy temperature in the presence of solar radiation [23], possibly affecting CO₂ and temperature effects on GY and quality. The experiments were conducted at the Tsukuba FACE site, located in Ibaraki Prefecture, Japan, for 3 years under well-fertilized and fully-irrigated conditions, eliminating the influence of factors other than temperature that could potentially affect CFE [24] (see section 2). The CO₂ treatment was imposed over the entire growing season from late May to mid-September [25]. Daytime canopy warming was initiated at the onset of flowering. Although the same heating system was used for both CO₂ treatments, increases in canopy temperature were greater in EC than in ambient CO₂ (AC): 1.6 °C in EC and 0.6 °C in AC (table 1), as was anticipated owing to a decrease in stomatal conductance in EC, reducing transpirational cooling [26].

2. Method

2.1. Site for the field experiment

The Tsukuba FACE site (www.naro.affrc.go.jp/archive/niaes/outline/face/english/) is located in Tsukubamirai City, Ibaraki Prefecture, Japan (35°58′N, 139°60′E, 10 m above sea level) [25]. The soil at the site has a bulk density of 0.87 Mg m⁻³ and comprises 36% sand, 40% silt, and 23% clay [27]. FACE: an octagonal area of approximately 240 m² (internal diameter of 17 m) was used for each CO₂ treatment. In the EC plots, pure CO₂ was released.
Table 1. Daytime mean canopy temperature and weather conditions during the T-FACE × FACE experiments.

| Year | Treatment | Daytime canopy temperature (°C) | Mean air temperature (°C) | Solar radiation (MJ m⁻² d⁻¹) | Precipitation (mm) |
|------|-----------|---------------------------------|--------------------------|------------------------------|-------------------|
| 2015 | CTRL      | 29.8                            | 27.5                     | 19.1                         | 50                |
|      | EC        | 30.4                            |                          |                              |                   |
|      | ET        | 30.2                            |                          |                              |                   |
|      | EC + ET   | 31.3                            |                          |                              |                   |
| 2016 | CTRL      | 28.9                            | 26.8                     | 18.7                         | 142               |
|      | EC        | 29.8                            |                          |                              |                   |
|      | ET        | 29.5                            |                          |                              |                   |
|      | EC + ET   | 30.8                            |                          |                              |                   |
| 2017 | CTRL      | 27.2                            | 25.5                     | 12.9                         | 115               |
|      | EC        | 27.6                            |                          |                              |                   |
|      | ET        | 27.9                            |                          |                              |                   |
|      | EC + ET   | 28.6                            |                          |                              |                   |

CTRL stands for ambient CO₂ concentration and ambient canopy temperature as control; ET, EC, and EC + ET stand for elevated canopy temperature, elevated CO₂ concentrations alone, and the combination of EC and ET. See daily changes in daytime canopy temperatures in supplementary figure S2.

from the windward sides to maintain the CO₂ concentration measured at the central point approximately 200 μmol mol⁻¹ above AC, which is projected to reach in the mid-century under high emission scenarios [28]. This target concentration has been most commonly used in many other large-scale FACE studies [29], facilitating comparison with other studies. We started the CO₂ treatments on 31 May for all 3 years and terminated at maturity on 11, 17, and 19 September in 2015, 2016, and 2017, respectively. The season-long daytime average CO₂ concentration in AC and EC, respectively, were 383 ± 12.7 and 579 ± 20.2 μmol mol⁻¹ in 2015, 391 ± 11.6 and 586 ± 21.7 μmol mol⁻¹ in 2016, and 391 ± 12.8 and 585 ± 16.3 μmol mol⁻¹ in 2017. T-FACE: canopy warming treatments (ET) were designed as subplots (4 m² in 2015 and 5.1 m² in 2016 and 2017) in the AC and EC plots, with two replicates in 2015 and three replicates in 2016 and 2017. We installed four (2015) or six (2016 and 2017) infrared heaters (1000 W, FTE-1000, MOR Electric Heating Assoc. Cosmstock Park, MI, USA) 1 m above the canopy surface to increase the canopy temperature (figure 1). We aimed to raise canopy temperature in ET by 2 °C relative to the non-heated reference in each CO₂ treatment, using a proportional-integral-derivative controller, but the voltage was not large enough to reach the target temperature, resulting in almost continuous heating. The ET treatment was imposed from 09:00 to 15:00 for 3 weeks (2015 and 2016) or 4 weeks (2017) from the onset of flowering, from 29 July to 18 August, from 1 to 21 August, and from 3 to 23 August, respectively, for EC and AC in 2016, and from 22 July to 18 August 2017. A combination of AC and ambient (reference) temperature (AT) was designated as control (CTRL). We grew a temperate japonica cultivar, Koshihikari, the most widely planted cultivar in Japan for more than three decades [30], under fully-irrigated and well-fertilized conditions. See supplementary materials for crop management practices.

2.2. Weather observations
General weather components, including air temperature, relative humidity, and solar radiation, were measured at the FACE site. Air temperature and relative humidity were measured within a radiation shield with forced ventilation (MP45D; Vaisala, Helsinki, Finland). The canopy temperature in each subplot was monitored and recorded using infrared radiometers (SI-III; Apogee Instruments, Inc., Logan, UT, USA). Monthly records of weather variables are provided in supplementary table S1.

2.3. Field survey and sample analysis
Heading date: We recorded panicle emergence for eight hills in each plot every 2–3 d, and determined the heading date as the date on which 50% of the productive tillers reached panicle emergence.

Yield and yield component analysis: Yield determination and analysis were performed as previously reported [27]. In general, we collected aboveground plant parts from 18 hills (equivalent to 0.81 m²) in September (supplementary table S1). The harvested plants were air-dried in a rain shelter. We then threshed each rough sample was then split into three sub-samples to determine the aboveground biomass, panicle number, spikelet weight, brown rice yield, and moisture content (grain moisture: grain moisture tester, Riceter f, Kett Electric Laboratory, Tokyo, Japan; straw moisture: gravimetric method after oven-drying at 80 °C). Harvest index was derived as GY divided by aboveground biomass.

Sterility and grain filling inhibition analysis: We collected 15 panicles in each subplot at maturity and then stored in 80% ethanol solution. The number of
sterile and fertile spikelets per panicle was determined through visual inspection. The sterility % was expressed as the percentage of sterile spikelets relative to the total number of spikelets. The proportion of ripened spikelets (RS) and the classification of unfilled spikelets were analyzed for the sample collected yield using solutions of different specific gravity (SG; 80% ethanol solution with SG = 0.86; water, SG = 1.0; ammonium sulfate solution, SG = 1.06) [31]. RS was classified as spikelets that sank in an ammonium sulfate solution (SG > 1.06). The lightest-filled spikelets were classified as those that floated in the ethanol solution (SG < 0.86), but were not classified as sterile.

Grain appearance quality analysis: Unsieved brown rice samples taken from the subsamples (SG > 1.0) used for yield determination were analyzed for grain appearance quality. The grain appearance quality was measured using a grain quality inspector (RGQI20A; Satake Corp., Hiroshima, Japan) covering the following traits: type of chalky kernel (supplementary figure S1), grain width, grain length, and grain thickness [32].

Statistical analysis: Analysis of variance was conducted using a split-split-plot design, in which year was treated as the main factor, CO\textsubscript{2} as the split-plot factor, and temperature as the split-split-plot factor. The mixed-model-REML procedure of the SAS package (SAS Add-In 7.13 for Microsoft Office, SAS Institute, Tokyo, Japan) was used to test the statistical significance of each factor, using a fixed effect (year, CO\textsubscript{2}, and temperature) and a random effect (block), and Type III sums of squares were computed using restricted maximum likelihood estimates for F-tests. The numerator and denominator degrees of freedom (df), F ratios, and P values for each test are shown. All data were checked for normality using the distribution analysis procedure in the SAS package, and then an appropriate transformation was employed to ensure constant variance. We also calculated percentage changes relative to CTRL to represent the effects of EC, ET, and EC + ET.

The effects of Tc on aboveground biomass, harvest index, and spikelet sterility were examined by comparing simple linear and segmented regression models. First, a simple linear regression was fitted to these relationships using the ordinary least square method. If the residuals of the regression suggested non-linearity, a piecewise linear function was fitted using the Levenberg–Marquardt algorithm in the nonlinear curve fit procedure of OriginPro 2017 J software (OriginLab Corporation, Northampton, MA, USA). We used piecewise regression to determine the breakpoint temperatures. Linear and piecewise regression models were compared using adjusted $R^2$, F-statistics for the additional sum of squares, and Akaike’s Information Criteria. For the response of chalky grain (CG) % to Tc, regression lines were
compared among different years using the lm function in R, version 3.6.0 software (R Core Team, Vienna, Austria).

Data availability: All datasets necessary to replicate the conclusions of this study are available in the manuscript or the supplementary material.

3. Results

3.1. Climatic conditions

The T-FACE treatment period was warmest in 2015 with the highest solar radiation and was coolest in 2017 with the lowest solar radiation (table 1). The difference between the two seasons was 2.0 °C in air temperature and 6.2 MJ m⁻² d⁻¹ in solar radiation. Daytime canopy temperature (Tc) in CTRL had even a greater year-to-year difference than air temperature, ranging from 27.2 °C to 29.6 °C and ranked as 2015 > 2016 > 2017. However, the Tc differences between the treatments were consistent across seasons. Tc was higher in EC than AC by 0.4 °C–0.9 °C without warming. The ET treatment increased Tc to a greater extent in EC by 0.9 °C–1.0 °C than in AC by 0.4 °C–0.7 °C. As a result, Tc in EC + ET was higher than that in CTRL by 1.4 °C–1.6 °C during the canopy warming treatment in all 3 years (supplementary figure S2).

3.2. Yield and yield components

Across the 3 years, EC significantly increased GY (total weight of husked rice) (F₁,₅ = 6.797, P = 0.0478; figure 2(a), supplementary table S2), whereas ET significantly reduced it (F₁,₁₀ = 17.940, P = 0.0017). The interactive effects of EC + ET on GY were also significant (F₁,₁₀ = 7.412, P = 0.0215); CFEyield averaged 15% at ambient temperature (AT), but was reduced to 3% by ET. The effect of EC + ET on GY varied significantly among years (F₂,₁₀ = 11.030, P = 0.0030), being −4%, 3%, and 9% in 2015, 2016, and 2017, respectively.

Aboveground biomass was consistently increased by EC in both AT and ET in all three seasons (F₁,₅ = 7.908, P = 0.0375; figure 2(b), supplementary table S2), whereas no effect was observed with the ET treatment. In contrast, the harvest index was decreased in ET alone by 1% (F₁,₁₀ = 9.769, P = 0.0108; figure 2(c)), and decreased by 3% in ET + EC compared to that in CTRL (F₁,₁₀ = 6.969, P = 0.0247; figure 2(c)).

EC increased the percentage of RS (F₁,₅ = 10.143, P = 0.0244, figure 2(d), supplementary table S2) by 10 points (15% relative to CTRL), averaged across the three seasons, whereas the effect of ET on RS was significantly negative (F₁,₁₀ = 8.918, P = 0.0137, figure 2(d)) and varied greatly depending on the year and CO₂ concentration (F₂,₁₀ = 5.351, P = 0.0264, figure 2(d)). Single filled-grain weight (SGW) slightly, albeit non-significantly, increased with EC or ET alone, but when EC and ET were combined, SGW did not increase, resulting in a negative interaction between CO₂ and temperature (F₁,₁₀ = 20.919, P = 0.0010; figure 2(e), supplementary table S2).

The classification of unfilled spikelets showed that spikelets that failed to pollinate (sterile spikelets) and stopped growing during the early grain-filling stage (early growth cessation) togethe accounted for 54%–80% of unfilled spikelets (supplementary table S3). In particular, ET and ET + EC significantly increased spikelet sterility (F₁,₁₀ = 35.6, P = 0.0001 for temperature (T); F₁,₁₀ = 10.8, P = 0.0081 for CO₂ × T; supplementary table S3), but the effects varied among seasons (F₂,₅ = 8.48, P = 0.0247 for year (Y) × CO₂; F₂,₁₀ = 17.41, P = 0.0006 for Y × T), suggesting that spikelet sterility was largely responsible for the variations in RS and GY.

3.3. Grain appearance quality

EC degraded the grain appearance quality by reducing the percentage of undamaged grains (UDG, CG, figure 2(f), supplementary table S4) by as much as 16 points (F₁,₅ = 28.122, P = 0.0032; supplementary table S4). Although ET had no effect, UDG was 23% lower in EC + ET than in CTRL. The percentage of CGs (figure 2(g)), especially those with a chalky area in the basal part of the grains (F₁,₁₀ = 144.344, P = 0.0001, figure 2(h)), was significantly increased by EC and EC + ET relative to CTRL.

3.4. Tc effects on yield components and quality

Tc averaged for the treatment period had no significant effects on aboveground biomass (figure 3(a)), but harvest index decreased with increasing Tc (figure 3(b)). A piecewise regression model fit significantly better than a simple linear model, with a breakpoint temperature (Tc,b) of 30.5 ± 0.6 °C (supplementary table S5). Tc was also a major factor for spikelet sterility, accounting for the variations observed between treatments and between seasons (figure 3(c)). A clear breakpoint existed for the spikelet sterility and Tc relationship, where spikelet sterility increased almost linearly with Tc above 29.9 °C + 0.3 °C and remained unchanged in the lower temperature range (figure 3(c), supplementary table S6). CG increased with Tc each year, but the relationship was different in 2017 (figure 3(d)); CG at the same Tc was much greater in 2017.

4. Discussion

Three-year open-field experiments in the paddy field demonstrated that 1.4 °C–1.6 °C canopy warming imposed at the critical grain growth phase could offset the positive effects of EC on GY, CFEyield, depending on the reference temperature levels. These results have implications for rice yield projections under climate change, where the frequency and severity of heat-waves are projected to increase [16]. Currently, most
Grain yield (a), aboveground biomass (b), harvest index (c), % of ripened spikelets (d), single grain weight (e), and grain appearance quality traits (f–h) of Koshihikari harvested from FACE × T-FACE experiments. Grain yield (GY, the weight of husked grain or brown rice) is expressed on a 15% moisture content basis. Aboveground biomass is expressed on 0% moisture content. Harvest index (HI) is unhusked grain yield divided by aboveground biomass, both on a 0% moisture content basis. % of ripened spikelets refers to spikelets that have a specific gravity >1.06; single grain weight refers to mean grain weight of grains of width >1.7 mm. Values for the percentage of undamaged grains, chalky grains, and white-base grains can be found in the methods and supplementary materials. CTRL stands for ambient CO$_2$ concentration and ambient canopy temperature as control; ET, EC, and EC + ET stand for elevated canopy temperature, elevated CO$_2$ concentrations alone, and the combination of EC and ET. Vertical bars attached to the rectangles of the histogram are the standard error for the mean (s.e.m.) for $n = 2$ in 2015 and $n = 3$ in 2016 and 2017. Triangles represent relative values to CTRL (refer to the right axis). The statistical significance for CO$_2$, temperature (T), and their interaction is shown in each panel. ***, **, and *, indicating the significant effect at $P < 0.001$, 0.01, and 0.05, respectively. ns stands for not significant. Data and ANOVA results for all sources of variance, including year and its interaction with CO$_2$ and temperature, are presented in supplementary table S2.

Figure 2.

Rice simulation models generally do not account for the negative interaction between EC and ET [7], and thus potentially provide optimistic yield projections.

Previous open-field studies on a few crops have also shown that ET limits CFE$_{yield}$ [18, 19]. The treatments were season-long, cumulatively affecting various processes, including the life cycle, photosynthesis, nutrient uptake, and grain growth. In this study, however, we imposed the canopy warming treatment only in a short window from the onset of flowering to mid-grain filling, retaining the positive effects of EC on biomass production under both temperature regimes. This was supported by the non-significant relationship between TC and aboveground biomass (figure 3(a)). On the other hand, the effects of EC on harvest index differed depending on temperature treatment and years (figure 2(c)); EC alone increased harvest index by 3%, but EC + ET decreased it by
Figure 3. Relationships between daytime canopy temperature (Tc) and aboveground biomass (a), harvest index (b), percentage of sterile spikelets (c), or percentage of chalky grains (d). Tc was a mean for 6 h (09:00–15:00) during the canopy warming treatment period. Open symbols, ambient CO₂ concentration and ambient canopy temperature; closed blue, EC; closed red, ET; closed gray: EC + ET. The best fit model is presented. See model comparison and parameters in supplementary table S5.

3% compared with that in CTRL (supplementary table S5). Differences between years and treatments were mainly reflected in Tc, reducing harvest index, particularly in the high Tc range above 30.5 °C (figure 3(c), supplementary table S5), suggesting that biomass allocation to the grains was responsible for the reduced CFE yield in ET.

Grain setting and filling are the major processes that affect biomass allocation to grains and are characterized by RS and/or SGW. Previous studies have found inconsistent results regarding the combined effects of ET and EC on RS, depending on the experimental methods, rice cultivars, and temperature conditions [19, 27, 32]. We also found that the effect of EC + ET on RS varied among seasons; changes relative to CTRL ranged from negative (8%) to positive (7%) (figure 2(d)), corresponding to its effects on GY. In contrast, the effects of EC and ET on SGW were consistent across the three seasons (figure 2(e)), suggesting that the year-to-year difference in the effects of canopy warming on CFE yield was mainly induced by RS (supplementary table S2)[14].

Further analysis of unfilled grains revealed that the reduction in RS was mainly caused by Tc-related spikelet sterility (supplementary table S3, figure 3(c)). In our study, multiple factors affected Tc during the treatment period. First, EC alone raised Tc by as much as 0.9 °C. Second, irradiance from the infrared heaters increased Tc in ET but more greatly in EC than in AC. The treatment effects of EC and EC + ET on Tc were likely mediated by reduced stomatal conductance by EC, limiting transpirational cooling [23]. Third, background ATs and solar radiation varied greatly among seasons, creating >2 °C Tc differences between years (table 1). Regardless of the mechanisms or factors, Tc alone explained most of the variation in spikelet sterility (figure 3(c)), indicating that spikelet sterility observed in this study was induced by heat.

There is ample evidence that excessive heat during the flowering stage induces spikelet sterility in rice, as confirmed by observations in controlled environments [22] and open fields in various rice-growing regions [33]. In this study, we showed that the negative effects appeared only above
the threshold temperature, daytime Tc of approximately 30 °C (figure 3(c)). Temperature thresholds for heat-induced sterility have been variously reported, but a comprehensive data analysis by [34] showed that 33 °C is the critical threshold air temperature above which sterility increases. Direct comparison with the current study is difficult because canopy and air temperatures are different [33, 35, 36], but the threshold Tc of 30 °C is low enough to induce heat-induced sterility even under current temperature conditions. When combined with rising CO2, Tc can increase faster than the rate of global warming. These observations indicate the need to use Tc or organ temperatures for spikelet sterility projections, suggesting the need to incorporate Tc for projecting heat stress under climate change.

Early cessation of grain growth was significantly reduced by EC in both AT and ET (supplementary table S3). The mechanisms for early growth cessation are unclear, but there is some evidence that insufficient assimilate supply can induce early grain growth cessation [37]. It is possible that EC enhances photosynthesis and provides more assimilate supply, thereby reducing early growth cessation. This could be another possible mechanism for CFEyield, but the negative effects of Tc on spikelet sterility overrode the positive effects of EC on grain growth, offsetting CFEyield.

In rice, EC and ET are known to decrease the grain appearance quality [32, 38, 39], and our results confirmed that EC degrades the grain appearance quality by increasing the percentage of CGs (figure 3(b)). Any increase in CG reduces the rice grade, price, and milling quality, reducing head rice yield and thereby farmers’ income [21]. In particular, grains with a chalky area in the basal part of the grains increased in EC + ET, in agreement with previous reports examining the EC effect alone [32]. Similar to spikelet sterility, Tc explained the variation in CG, except for the 2017 season (figure 3(d)), when solar radiation was ~30% lower than in the other 2 years (table 1), suggesting that lower solar radiation exacerbates grain quality.

Although this study was conducted at one site using one cultivar, some quantitative findings can provide useful insights into the potential improvement of crop model predictions. In our study, a Tc range observed in this study (treatment average from 27.1 °C to 31.3 °C) was caused by various factors, including background air temperatures (seasons), infrared heating, and CO2 concentrations. Nevertheless, Tc alone accounted for most of the variation in spikelet sterility and harvest index, indicating that an accurate estimate of Tc is key to reducing the yield projection uncertainty. Other environmental factors, including humidity, solar radiation, and wind speed, can also be involved in Tc. In addition, soil and nutrient limitations can raise Tc by reducing leaf stomatal conductance and transpirational cooling, thereby exacerbating heat stress. The importance of canopy or organ temperatures in assessing heat stress has been recognized for rice [35, 36] and other field crops [40, 41]; the present study has revealed that it is also important for estimating CFEyield via grain setting and growth processes.

The cultivar used in this study was a standard temperate japonica cultivar with medium tolerance to heat-induced sterility [42, 43] and to grain appearance quality degradation [30]. The CO2 response of Koshihikari is also considered medium among various cultivars, including high-yielding indica cultivars [27, 44, 45]. Increasing heat tolerance for heat sterility and grain quality and/or enhancing CFE is a key strategy to raise the upper threshold temperature for heat stress. Breeding for improving CFEyield is an attractive strategy for major C3 crops, and consideration for potential tradeoffs is necessary for abiotic stresses exacerbated by EC, as carefully reviewed by Dingkuhn et al [46].

5. Conclusion

This study provides direct evidence that even a slight canopy warming (1.4 °C–1.6 °C) during the critical growth phase can offset the CFE. Reductions in CFE mainly arose from poor grain setting at Tc above ~30 °C. The grain appearance quality also decreased substantially through an increase in CG with increasing Tc, but the threshold temperature varied between seasons. Simultaneous increases in atmospheric CO2 concentration and air temperature are expected to increase daytime canopy temperatures more than air warming alone, thereby affecting GY and quality. Crop models without these processes are likely to underestimate the negative impacts of climate change on yield and quality. Data on the critical threshold canopy or organ temperatures are still limited because most other studies have used air temperatures. Canopy micrometeorological observations and reproductive physiological measurements of rice must be conducted in diverse environments. Our study demonstrated that the adverse effects of EC and ET were largely due to heat stress. Therefore, the development of adaptation measures against heat stress, particularly during reproductive and grain-filling periods, needs to be enhanced and accelerated.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

Acknowledgments

This work was supported in part by the MAFF through ‘Development of Technologies for Mitigation and Adaptation to Climate Change in
Agriculture, Forestry, and Fisheries,’ Grant-in-Aid for Scientific Research on Innovative Areas (JP262520004) by the Japan Society for the Promotion of Science (JSPS), and the Environment Research and Technology Development Fund (JPMERF20S11820) of the Environmental Restoration and Conservation Agency of Japan. We thank Dr Bruce Kimball and Dr Carl Bernacchi for their technical advice on T-FACE implementation. We also thank the team members of the Tsukuba FACE at the Institute for Agro-Environmental Sciences, NARO, for their help with field and laboratory measurements. Guoyou Zhang was a JSPS Fellow (No. 16F16096) and is now working at the Nanjing University of Information Science and Technology (NUIST) under the support of the National Natural Science Foundation of China (42077209) and the startup foundation for introducing talent of NU IST (003035).

Conflict of interest

The authors declare no competing interests.

Author contributions

All authors participated in this research from experimental design, field work, sample collection, and analysis until the completion of this manuscript; particularly, their contributions with different focuses are shown as follows: Guoyou Zhang conducted the experiments, analyzed the data and wrote the manuscript. Kazuhiro Ujiie conducted the experiments and collected and analyzed the samples. Mayumi Yoshimoto designed the study, maintained the FACE and T-FACE experiments, conducted the experiments and collected the micrometeorological data. Hidemitsu Sakai led the group, managed the Tsukuba FACE project, designed the research, maintained the FACE and T-FACE experiments, conducted the FACE project, designed the research, maintained the FACE and T-FACE experiments, conducted the experiments, collected and analyzed the samples, and wrote the manuscript. Hiroki Ikawa conducted field experiments. Miwa Arai conducted field experiments. Hitomi Wakatsuki conducted the statistical analyses. Yasuhiro Usui conducted field experiments, conducted the experiments, and wrote the manuscript. Takeshi Tokida maintained the FACE and T-FACE experiments, conducted the experiments, and analyzed the samples. Toshihiro Hasegawa led the group, managed the Tsukuba FACE project, designed the research, maintained the FACE and T-FACE experiments, conducted the experiments, managed the Tsukuba FACE project, designed the research, maintained the FACE and T-FACE experiments, collected and analyzed the samples, and wrote the manuscript. All authors have checked and approved the manuscript.

ORCID iDs

Guoyou Zhang https://orcid.org/0000-0001-8825-2621
Kazuhiro Ujiie https://orcid.org/0000-0002-074-9029
Hidemitsu Sakai https://orcid.org/0000-0003-4790-1933
Takeshi Tokida https://orcid.org/0000-0001-7245-2952
Yasuhiro Usui https://orcid.org/0000-0003-3239-0907
Hitomi Wakatsuki https://orcid.org/0000-0002-9861-5921
Miwa Arai https://orcid.org/0000-0002-5377-7820
Hiroki Ikawa https://orcid.org/0000-0002-4984-8067
Toshihiro Hasegawa https://orcid.org/0000-0001-8501-5612

References

[1] Bezner Kerr R, Hasegawa T and Lasco R 2022 Food, fibre, and other ecosystem products Climate Change 2022: Impact, Adaptation, and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change ed H O Pörtner (Cambridge: Cambridge University Press) pp 673–816
[2] Hasegawa T, Wakatsuki H, Ju H, Vyas S, Nelson G C, Farrell A, Deryng D, Meza F and Makowski D 2022 A global dataset for the projected impacts of climate change on four major crops Sci. Data 9 58
[3] Sun T, Hasegawa T, Liu B, Tang L, Liu L, Cao W and Zhu Y 2021 Current rice models underestimate yield losses from short-term heat stresses Glob. Change Biol. 27 402–16
[4] Heinricke S, Frieler K, Jägermeyr J and Mengel M 2022 Global gridded crop models underestimate yield responses to droughts and heatwaves Environ. Res. Lett. 17 044026
[5] Rosenzweig C et al 2014 Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison Proc. Natl Acad. Sci. USA 111 3268–73
[6] O’Leary G J et al 2015 Response of wheat growth, grain yield and water use to elevated CO2 under a free-air CO2 enrichment (FACE) experiment and modelling in a semi-arid environment Glob. Change Biol. 21 2670–86
[7] Hasegawa T et al 2017 Causes of variation among rice models in yield response to CO2 examined with free-air CO2 enrichment and growth chamber experiments Sci. Rep. 7 14858
[8] Durand J-L et al 2018 How accurately do maize crop models simulate the interactions of atmospheric CO2 concentration levels with limited water supply on water use and yield? Eur. J. Agron. 100 67–75
[9] Toreti A et al 2020 Narrowing uncertainties in the effects of elevated CO2 on crops Nat. Food. 1 775–82
[10] Obermeier W A et al 2017 Reduced CO2 fertilization effect in temperate C3 grasslands under more extreme weather conditions Nat. Clim. Change 7 137
[11] Bishop K A, Leakey A D B and Ainsworth E A 2014 How seasonal temperature or water inputs affect the relative response of C3 crops to elevated [CO2] a global analysis of open top chamber and free air CO2 enrichment studies Food Energy Secur. 3 33–45
Wassmann R, Jagadish S V K, Sumfleth K, Puthak H, Howell G, Isaila A, Serraj R, Redona E, Singh R K and Heuer S 2009 Regional vulnerability of climate change impacts on Asian rice production and scope for adaptation Advances in Agronomy vol 102 (Amsterdam: Elsevier Inc.) pp 91–133

Matsui T, Namuco O S, Ziska L H and Horie T 1997 Effects of high temperature and CO$_2$ concentration on spikelet sterility in indica rice Field Crop Res. 51 213–9

Chaturvedi A K, Bahuguna R N, Shah D, Pal M and Jagadish S V K 2017 High temperature stress during flowering and grain filling offsets beneficial impact of elevated CO$_2$ on assimilate partitioning and sink-strength in rice Sci. Rep. 7 8227

Hasegawa T, Sakai H, Tokida T, Usui Y, Yoshimoto M, Fukuoka M, Nakamura H, Shimono H and Okada M 2016 Rice free-air carbon dioxide enrichment studies to improve assessment of climate change effects on rice agriculture Adv. Agr. Syst. Model 7 45–68

IPCC 2021 Summary for Policymakers Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change ed V Masson-Delmotte et al (Cambridge: Cambridge University Press) pp 3–32

Kimball B A, Conley M M, Wang S, Lin X, Luo C, Morgan J and Smith D 2008 Infrared heater arrays for warming ecosystem field plots Glob. Change Biol. 14 309–20

Ruiz-Vera U M, Siebers M H, Drag D W, Ort D R and Bernacchi C J 2015 Canopy warming caused photosynthetic acclimation and reduced seed yield in maize grown at ambient and elevated [CO$_2$] Glob. Change Biol. 21 4237–49

Cai C et al 2016 Responses of wheat and rice to factorial combinations of ambient and elevated CO$_2$ and temperature in FACE experiments Glob. Change Biol. 22 836–74

Eyshi Rezaei E, Webber H, Gaiser T, Naab J and Ewert F 2015 Heat stress in cereals: mechanisms and modelling Eur. J. Agron. 46 98–113

Morita S, Wada H and Matsue Y 2016 Countermeasures for heat damage in rice grain quality under climate change Plant Prod. Sci. 19 1–11

Satake T and Yoshida S 1978 High temperature-induced sterility in indica rice Jpn. J. Crop Sci. 47 6–10

Yoshimoto M, Oue H and Kobayashi K 2005 Energy balance and water use efficiency of rice canopies under free-air CO$_2$ enrichment Agric. For. Meteorol. 133 226–46

Kimball B A 2016 Crop responses to elevated CO$_2$ and interactions with H$_2$O, N, and temperature Curr. Opin. Plant Biol. 31 36–43

Nakamura H, Tokida T, Yoshimoto M, Sakai H, Fukuoka M and Hasegawa T 2012 Performance of the enlarged rice-FACE system using pure CO$_2$ installed in Tsukuba, Japan J. Agric. Meteorol. 68 15–23

Yoshimoto M, Oue H, Takahashi N and Kobayashi K 2005 The Effects of FACE (free-air CO$_2$ enrichment) on temperatures and transpiration of rice panicles at flowering stage J. Agric. Meteorol. 60 597–600

Hasegawa T et al 2013 Rice cultivar responses to elevated CO$_2$ at two free-air CO$_2$ enrichment (FACE) sites in Japan Funct. Plant Biol. 40 148–59

Meinshausen M et al 2010 The shared socio-economic pathway (SSP) greenhouse gas concentrations and their extensions to 2500 Geosci. Model Dev. 13 3571–605

Ainsworth E A and Long S P 2021 30 years of free-air carbon dioxide enrichment (FACE): what have we learned about future crop productivity and its potential for adaptation Glob. Change Biol. 27 27–49

Kobayashi A, Hori K, Yamamoto T and Yano M 2018 Koshihikari: a premium short-grain rice cultivar—it’s expansion and breeding in Japan Rice 11 15

Kobata T, Akiyama Y and Kawaoaka T 2010 Convenient estimation of unfertilized grains in rice Plant Prod. Sci. 13 289–96

Usui Y, Sakai H, Tokida T, Nakamura H, Nakagawa H and Hasegawa T 2016 Rice grain yield and quality responses to free-air CO$_2$ enrichment combined with soil and water warming Glob. Change Biol. 22 1256–70

Yoshimoto M et al 2022 Monitoring canopy micrometeorology in diverse climates to improve the prediction of heat-induced spikelet sterility in rice under climate change Agric. For. Meteorol. 316 108660

Bhoermanalhali R, Sathishraj R, Tack J, Naibey L L, Muthurajan R and Jagadish K S V 2016 Temperature thresholds for spikelet sterility and associated warming impacts for sub-tropical rice Agric. For. Meteorol. 221 122–30

Matsui T, Kobayasi K, Nakagawa H, Yoshimoto M, Hasegawa T, Reinke R and Angus J 2014 Lower-than-expected floret sterility of rice under extremely hot conditions in a flood-irrigated field in New South Wales, Australia Plant Prod. Sci. 17 245–52

Julia C and Dingkuhn M 2012 Variation in time of day of anthesis in rice in different climatic environments Eur. J. Agron. 43 166–74

Kobata T, Yoshida H, Masiko U and Honda T 2013 Spikelet sterility is associated with a lack of assimilate in high-spikelet-number rice Agron. J. 105 1821–31

Krishnan P, Ramakrishnan B, Reddy K R and Reddy V R 2011 High-temperature effects on rice growth, yield, and grain quality Advances in Agronomy vol 111 ed D L Sparks (Amsterdam: Elsevier Inc.) pp 87–206

Myers S S et al 2014 Increasing CO$_2$ threatens human nutrition Nature 510 139–42

Siebert S, Ewert F, Eyshi Rezaei E, Kage H and Grafl R 2014 Impact of heat stress on crop yield—on the importance of considering canopy temperature Environ. Res. Lett. 9 044012

Webber H et al 2017 Canopy temperature for simulation of heat stress in irrigated wheat in a semi-arid environment: a multi-model comparison Field Crop Res. 202 21–35

Matsui T, Kobayasi K, Kataga H and Horie T 2005 Correlation between viability of pollenization and length of basal dehiscence of the theca in rice under a hot-and-humid condition Plant Prod. Sci. 8 109–14

Maruyama A, Weerakoon W M W, Wakiyama Y and Ohba K 2013 Effects of increasing temperatures on spikelet fertility in different rice cultivars based on temperature gradient chamber experiments J. Agron. Crop Sci. 199 416–23

Sakai H, Tokida T, Usui Y, Nakamura H and Hasegawa T 2019 Yield responses to elevated CO$_2$ concentration among Japanese rice cultivars released since 1882 Plant Prod. Sci. 22 352–66

Yoshinaga S et al 2020 Analysis of factors related to varietal differences in the yield of rice (Oryza sativa L.) under free-air CO$_2$ enrichment (FACE) conditions Plant Prod. Sci. 23 19–27

Dingkuhn M, Luquet D, Fabre D, Muller B, Yin X and Paul M J 2020 The case for improving crop carbon sink strength or plasticity for a CO$_2$-rich future Curr. Opin. Plant Biol. 56 259–72