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Canopy Carbon- and Water-Use Efficiencies in Response to Temperature and Water Deficit for Wheat

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ABSTRACT

The frequency and intensity of extreme climatic events increase the complexity in assessing climate change impacts on (agro) ecosystem functions and crop production. A better understanding of carbon and water fluxes for crop plants under climate change requires research based on direct canopy-scale measurements. By analysing a canopy gas exchange data set synthesised from 8 years' experimentation under semi-field conditions for the post-anthesis period of five wheat genotypes, we examined canopy carbon and water fluxes as well as carbon use efficiency (CUE) and water use efficiency (WUE) under varying environmental conditions. CUE was variable, and was negatively affected by high temperatures. Moreover, CUE responded differently to daily, daytime, and nighttime temperatures, and was most sensitive to nighttime temperatures. The response of WUE to increasing temperatures was dominated by the response of carbon fluxes, while the relative contribution of water fluxes to WUE responses increased under water deficit. WUE based on gross and net photosynthesis responded differently to environmental variables, primarily due to the differences in CUE. The findings increase our understanding of canopy carbon and water fluxes under various environmental conditions and highlight the necessity for future efforts to improve crop CUE and WUE under climate change.

1 | Introduction

Quantifying impacts of climatic factors, including global warming and the increasing frequency and intensity of extreme weather events (e.g., heat waves and drought spells), on plant physiological processes is challenging, leading to uncertainties in the assessments of carbon and water budgets of crop plants and (agro) ecosystem functions under future climate.

Carbon use efficiency (CUE), defined as the ratio of net canopy photosynthesis (A_{Cnet}) to gross canopy photosynthesis (A_{Cgross}, i.e., the sum of A_{Cnet} and crop respiration), is a key parameter related to carbon balance at canopy scale. The two components of CUE (i.e., photosynthesis and respiration) are sensitive to environmental factors (e.g., temperature, soil moisture, and atmospheric CO_2 concentration; Dusenge et al. 2019; Posch et al. 2019; Fang et al. 2022, 2023). However, whether or not CUE is invariant under environmental changes, particularly long-term changes (weeks to months), is still under debate (Ryan and Asao 2019). Based on experimental observations and theoretical calculations, earlier studies reported that CUE is conserved due to homeostasis and acclimation of carbon fluxes, and, consequently, the proportion of respiration in A_{Cgross} (40%–50%) varies little under a wide range of environmental conditions (Gifford 1995; Waring et al. 1998; Cheng et al. 2000; Amthor 2010; Drake et al. 2019). Hence, some studies suggested that CUE could be empirically assumed to be constant in ecosystem models and crop models for the sake of simplicity

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(Gifford 1995, 2003; Waring et al. 1998). However, this assumption has long been challenged. It has been argued that CUE varies, depending on factors such as environmental variables, stand ages and soil fertility (Collalti and Prentice 2019 and references therein).

Water use efficiency (WUE) is another physiological efficiency trait, even more integral than CUE, as WUE links both carbon and water fluxes and can be defined from leaf to canopy scales (Beer et al. 2009; Niu et al. 2011; Huang et al. 2016). At canopy scale, WUE is defined as the ratio of either A_{Cnet} or A_{Cgross} to evapotranspiration (ET), the sum of plant transpiration and soil evaporation (Hatfield and Dold 2019), with $\mathrm{WUE}_{\mathrm{net}}$ and $\mathrm{WUE}_{\mathrm{gross}}$ being WUE based on A_{Cnet} and A_{Cgross}, respectively. Unlike CUE, WUE has been consistently reported to be variable under changing environmental conditions (Niu et al. 2011; Huang et al. 2016; Hatfield and Dold 2019), as environmental factors affect carbon (i.e., photosynthesis and respiration) and water (i.e., ET) fluxes differently. Therefore, it is necessary to investigate whether the response of WUE (especially at canopy scale) to various environmental variables is primarily regulated by carbon or by water fluxes.

Temperature is one of the most important changing environmental variables under climate change. Previous studies assessing the impact of rising temperature on plant productivity were usually based on annual mean temperature (e.g., Zhao et al. 2017; Tian et al. 2021); however, variation may still be observed to be independent of mean temperature, which could likely be attributed to intra-seasonal thermal variability (Asseng et al. 2015), and even diurnal temperature variability (Lobell et al. 2007; Donat and Alexander 2012; McAusland et al. 2023; Liu et al. 2024). Especially for the diurnal thermal variability, retrospective analysis showed asymmetric nighttime and daytime temperature increases (Liu et al. 2024). McAusland et al. (2023) reported that an increase in nocturnal temperature increased leaf respiration, reduced nocturnal stomatal conductance and crop productivity in wheat, while hardly affecting daytime stomatal conductance and leaf photosynthesis. These results could have important implications for projecting the impact of global warming on plant growth and the productivity of (agro) ecosystems. Zheng et al. (2021) showed that the poor performance of some process-based vegetation models to predict the dynamic relationship between vegetation growth and diurnal temperature might be caused by their reliance on daily mean temperature, instead of daytime and nighttime temperatures separately. Moreover, the impacts of long-term rising temperature and short-term extremely high temperature (heat shock [HS]) on plants can be different probably due to acclimation and adaptation (Smith and Dukes 2013; Fang et al. 2022, 2023). Therefore, it is important to consider these seasonal and diurnal dynamics when assessing the impact of climate warming. Besides thermal variabilities, interactions with other environmental factors (e.g., drought) or non-environmental factors (e.g., genotypic variation) would increase the complexity of climate change impact assessments (Mittler 2006; Eller et al. 2020; Lesk et al. 2022; Webber et al. 2022).

Wheat, one of the major staple food crops in many regions of the world, has the largest area harvested in the world (FAOSTAT: FAO statist 2023). Understanding how variable environmental factors affect carbon allocation and the coupling of carbon and water fluxes in wheat canopies is critical in assessing climate change impacts on global food security. Here, we analysed a data set of canopy gas exchange where five wheat genotypes were grown under natural sunlight for 8 years, during which wheat plant stands were exposed to a broad range of air temperature and drought regimes during the post-anthesis period, a key period for wheat productivity (Triboi and Triboi-Blondel 2002; Yang et al. 2008), when terminal heat and drought prevail (Barnabás et al. 2008; Lobell et al. 2015). This dataset allowed us to systematically assess impacts of air temperature and drought on wheat canopy carbon and water fluxes. Here, the objectives are to answer the following research questions: (i) is CUE invariant? (ii) is the response of WUE to environmental variables primarily driven by carbon or by water fluxes? We hypothesise that: (i) CUE can be affected by environmental changes; (ii) The main drivers of WUE in response to environments depend on environmental factors.

2 | Materials and Methods

2.1 | The Crop Climate Control and Gas Exchange Measurement (C3-GEM) Experimental Platform and Plant Growth Conditions

All experiments were conducted in the Crop Climate Control and Gas Exchange Measurement (C3-GEM) experimental platform located at the INRAE research centre in Clermont-Ferrand, France (Triboï et al. 1996). The C3-GEM platform has four soil-plant-atmosphere-research (SPAR) units which are able to continuously control environmental conditions (e.g., air temperature, relative humidity, and air CO_2 concentration) and measure canopy gas exchange (Supporting Information S1: Figure S1a,b) under semi-field conditions for studying plant stand functions under natural sunlight radiation. The description of the C3-GEM platform, the regulation of the concentration of CO_2 and relative humidity in the SPAR units, the calculation of canopy CO_2 and water exchange rates, and the plant growth conditions are given in the Supporting Information (Supporting Information S1: Methods).

2.2 | Plant Growth Conditions

In all experiments used in this study, plants stands were grown in insulated steel bins ($2 \times 1\,\mathrm{m}^2$ in cross section, 0.5 m deep) filled with a 2:1 (v:v) mixture of soil. Dry seeds were sown in the insulated steel bins at 2.5 cm depth with a density of 578 seeds m^{-2} and an inter-row spacing of 6.25 cm (15 rows), mimicking plant population density and plant competition in the field. Until anthesis, plants were grown outside under natural ambient conditions. In addition to receiving natural precipitation, plants were irrigated as needed to maintain the soil water content above 80% of field capacity. Crops received a total of 15.5–33.0 g N m^{-2} of ammonium nitrate applied in two to three (depending on experiments) splits between tillering and anthesis, to avoid any nutrient deficiency (Supporting

Information S1: Table S1). The plants were sprayed with fungicides and insecticides when needed to control any development of pests or diseases. To minimise the risk of diseases and maintain good soil properties, the insulated bins were managed with a 3–5 years crop rotation. In the experiments reported here wheat was grown after a rapeseed or sunflower crop.

2.3 | Description of the Experiments and Experimental Treatments (Regimes)

Between 1 day before anthesis and 6 days after anthesis (DAA), plant stands were transferred to the SPAR chambers where air temperature and drought treatments were applied, and gas exchange was measured (Figure 1 and Supporting Information S1: Table S1). The plants were irrigated two to three times a week to replace water lost by ET and maintain soil water content above 80% of field capacity.

Gas exchange data were collected during eight independent experiments conducted in the harvest years 1993, 1994, 1996, 1997, 1998, 2000, 2001, and 2002 (hereafter EXP1993, EXP1994, EXP1996, EXP1997, EXP1998, EXP2000, EXP2001, and EXP2002). In total, five winter wheat genotypes were grown, differing from experiment to experiment. The genotype Thésée was grown in EXP1993, EXP1994, EXP1997, and EXP1998, while the genotype Récital was grown in EXP2000, EXP2001, and EXP2002. In EXP2002, the genotypes Arche, Renan, and Tamaro were also grown (Figure 1).

The experiments and treatments (hereafter regimes) are summarised in Figure 1 and Supporting Information S1: Table S1. Experimental regimes are divided into three factor groups, that is, *Temperature*, *Water* and *Genotype* (Supporting Information S1: Table S1), to investigate the effects of short- and long-term temperature changes, the effects of soil water deficit (WD), and the effects of genotypic variation, respectively. See Supporting Information S1: Methods for the description of the classification of regimes according to factors.

2.4 | Plant Sampling and Canopy Nitrogen Content Determination

Plants were sampled every 2–9 days between anthesis and maturity. On each sampling date, plants were collected on $0.2 \times 1.0\,\mathrm{m}^2$ starting from the north side of the plant stands. After each sampling, the semi-occlusive green fabric, adjusted to crop height, was installed around the crops to reduce the edge effect, and the surface area of the canopies was thus corrected in the control software to calculate gas exchange data. The sampled plants were individualised and counted, and three replicates of 20 plants each were analysed separately. The dry mass of leaf laminae was determined after drying to constant mass in a forced air oven at 80°C. Total nitrogen concentration of oven-dried samples was determined by the Kjeldahl digestion method using a Kjeltec 2300 analyser (Foss Tecator AB, Hoeganaes, Sweden). Based on these data, canopy leaf nitrogen mass on a ground area basis ($N_{\mathrm{C.leaf}}$, $\mathrm{g\,N\,m^{-2}}$ ground) was calculated.

2.5 | Calculation of Daily Mean Values of Climatic and Gas Exchange Variables

Climate and canopy gas exchange data during the post-anthesis period were analysed. Daily mean air temperature and gas exchange variables were calculated separately for daytime and nighttime. Daytime and nighttime were defined as between 4:00 and 20:00 h solar time (16 h) and between 20:00 and 4:00 h solar time (8 h), respectively, according to the diurnal course of the photosynthetic photon flux density (PPFD) (Supporting Information S1: Figure S2). During daytime, air temperature, net CO_2 exchange rate, and H_2O exchange rate recorded at 15-min timestep were averaged to calculate the daily mean daytime air temperature (T_{day}, °C), net CO_2 assimilation rate ($A_{\mathrm{Cnet.day}}$, $\mu\mathrm{mol\,m^{-2}\,ground\,s^{-1}}$), and ET rate ($\mathrm{ET_{day}}$, $\mathrm{mmol\,m^{-2}\,ground\,s^{-1}}$), respectively. Correspondingly, daily mean nighttime air temperature (T_{night}, °C), respiration rate (R_{Cnight}, $\mu\mathrm{mol\,m^{-2}\,ground\,s^{-1}}$), and ET rate ($\mathrm{ET_{night}}$, $\mathrm{mmol\,m^{-2}\,ground\,s^{-1}}$) were calculated by averaging the data collected during nighttime. The daily mean air temperature (T_{daily}, °C) was calculated as $(T_{\mathrm{day}} \times 16 + T_{\mathrm{night}} \times 8)/24$. PPFD recorded during daytime were averaged to obtain the daily mean PPFD ($\mathrm{PPFD_{day}}$, $\mu\mathrm{mol\,m^{-2}\,s^{-1}}$).

By applying the Kok method (Kok 1948) to canopy scale, the daily mean daytime crop respiration rate (R_{Cday}, $\mu\mathrm{mol\,m^{-2}\,ground\,s^{-1}}$) was estimated as the intercept of the linear regression between canopy net CO_2 exchange rate and incident PPFD at dawn (between 4:00 and 8:00 h) and dusk (between 16:00 and 20:00 h), that is when $\mathrm{PPFD} \leq 150\,\mu\mathrm{mol\,m^{-2}\,s^{-1}}$. Linear regressions of net CO_2 exchange rate against incident PPFD were performed separately for dawn and for dusk (Supporting Information S1: Figure S3). The R_{Cday} values estimated from data of dawn and dusk periods were comparable (Supporting Information S1: Figure S4a), and R_{Cday} for each day was calculated as the mean of these two periods.

2.6 | Calculation of Daily Integral Values of Climatic and Gas Exchange Variables

The gas exchange variables on daily basis, that is, the daily integral nighttime crop respiration ($R_{\mathrm{Cnight.daily}}$, mol CO_2 $\mathrm{m^{-2}}$ ground $\mathrm{d^{-1}}$), the daily integral daytime crop respiration ($R_{\mathrm{Cday.daily}}$, mol CO_2 $\mathrm{m^{-2}}$ ground $\mathrm{d^{-1}}$), the daily integral daily total crop respiration ($R_{\mathrm{Ctot.daily}}$, mol CO_2 $\mathrm{m^{-2}}$ ground $\mathrm{d^{-1}}$), the daily integral net canopy CO_2 assimilation ($A_{\mathrm{Cnet.daily}}$, mol CO_2 $\mathrm{m^{-2}}$ ground $\mathrm{d^{-1}}$), the daily integral gross canopy CO_2 assimilation ($A_{\mathrm{Cgross.daily}}$, mol CO_2 $\mathrm{m^{-2}}$ ground $\mathrm{d^{-1}}$) and the daily integral ET ($\mathrm{ET_{daily}}$, mm ground $\mathrm{d^{-1}}$) were calculated as follows:

$$R_{\mathrm{Cnight.daily}} = R_{\mathrm{Cnight}} \times 8 \times 10^{-6} \times 3600, \tag{1}$$

$$R_{\mathrm{Cday.daily}} = R_{\mathrm{Cday}} \times 16 \times 10^{-6} \times 3600, \tag{2}$$

$$R_{\mathrm{Ctot.daily}} = R_{\mathrm{Cnight.daily}} + R_{\mathrm{Cday.daily}}, \tag{3}$$

$$A_{\mathrm{Cnet.daily}} = (A_{\mathrm{Cnet.day}} \times 16 - R_{\mathrm{Cnight}} \times 8) \times 10^{-6} \times 3600, \tag{4}$$

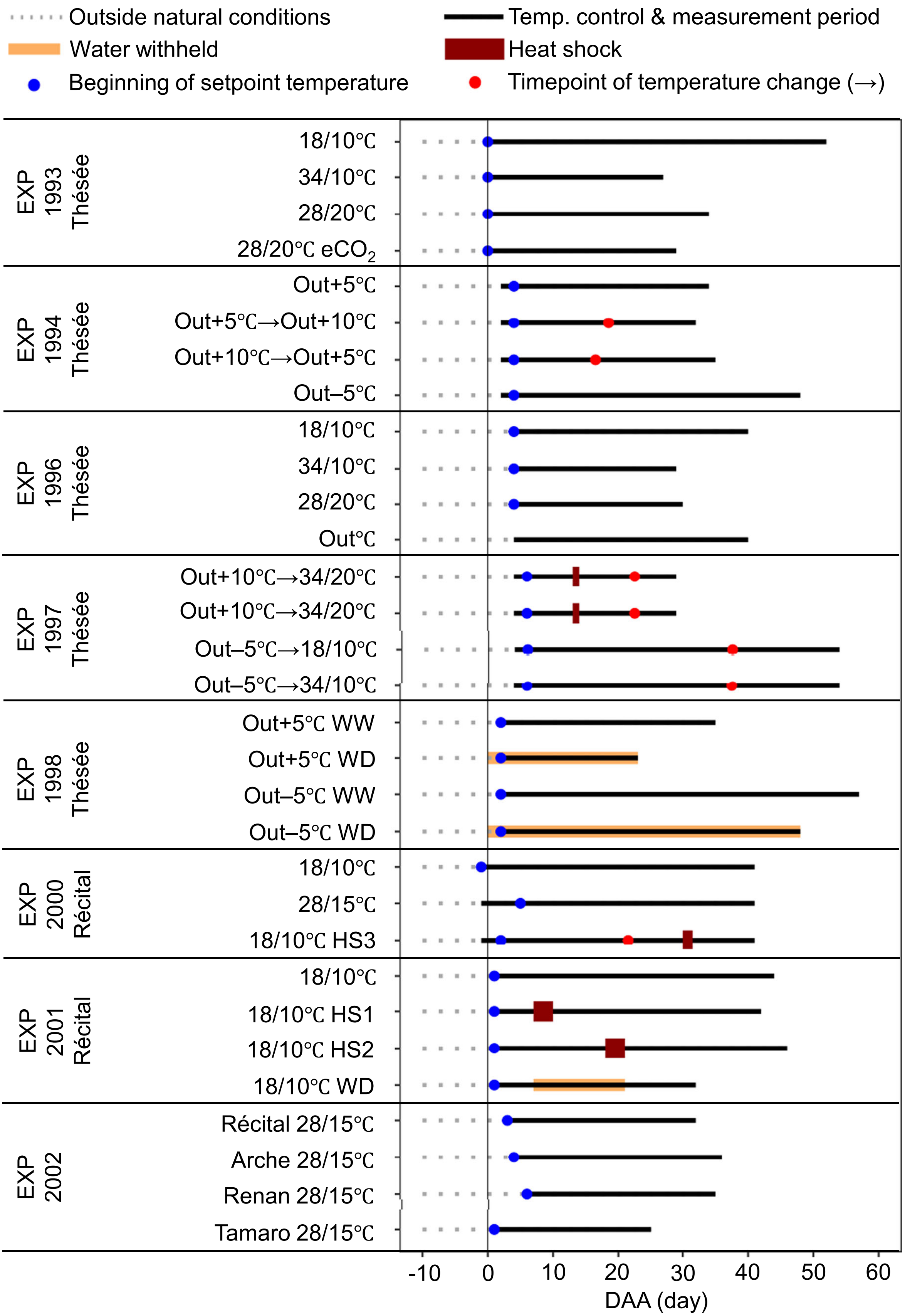

FIGURE 1 | Legend on next page.

$$A_{\text{Cgross.daily}} = A_{\text{Cnet.daily}} + R_{\text{Ctot.daily}}, \tag{5}$$

$$\text{ET}_{\text{daily}} = (\text{ET}_{\text{day}} \times 16 + \text{ET}_{\text{night}} \times 8) \times 10^{-3} \times 3600 \times \frac{18.015}{1000}, \tag{6}$$

where 18.015 (g mol^{-1}) is the molar mass of water, 1000 is the conversion factor from kg to g, and 8 and 16 are the duration in hour of the nighttime and daytime periods, respectively.

2.7 | Calculation of Average and Integral Values of Climatic and Gas Exchange Variables During the Post-Anthesis Period

$N_{\text{C.leaf}}$ reflects both the structure and nitrogen status of the canopy, conceptually representing the product of leaf area index (m^2 leaf area m^{-2} ground) and specific leaf nitrogen (g N m^{-2} leaf area), thereby serving as a good indicator of canopy photosynthetic capacity. In this study, $N_{\text{C.leaf}}$ was directly measured by determining the total nitrogen content of harvested leaves from a known ground area (see Section 2.4), rather than calculated from LAI and specific leaf nitrogen. We used the time course of $N_{\text{C.leaf}}$ (Supporting Information S1: Figure S5 and Table S2) to identify the period of effective canopy photosynthesis during grain filling and the beginning of fast canopy senescence. We described the time course of $N_{\text{c.leaf}}$ during the post-anthesis period using a reversed sigmoid function equation (Yin et al. 2009):

$$N_{\text{C.leaf}} = \begin{cases} (N_{\max} - N_{\text{base}})\left[1 - \left(1 + \frac{t_e - t}{t_e - t_m}\right)\left(\frac{t}{t_e}\right)^{\frac{t_e}{t_e - t_m}}\right] + N_{\text{base}} & \textit{if } t < t_e \\ N_{\text{base}} & \textit{if } t \geq t_e \end{cases}, \tag{7}$$

where, $N_{\max}$ and N_{base} (g N m^{-2} ground) are the maximum and minimum values of $N_{\text{C.leaf}}$, respectively, t (days) is the number of days after anthesis (DAA), t_{m} (days) is the number of days after anthesis when $N_{\text{C.leaf}}$ declines fastest, and t_{e} (days) is the number of days after anthesis when $N_{\text{C.leaf}}$ reaches N_{base} (i.e., when leaves are completely senesced). The period of effective canopy photosynthesis was defined between the start of gas exchange measurement (t_{b}, days; between 1 day before anthesis and 6 DAA depending on the environmental regimes and genotypes; Supporting Information S1: Table S1) and t_{e}. The period before the start of fast canopy senescence was defined as either between t_{b} and the day (t_{80}, days) when ($N_{\max}$–N_{base}) decreased by 20% (named N80%), or between t_{b} and 20 DAA (named DAA20), i.e., when $N_{\text{C.leaf}}$ remained relatively high (Supporting Information S1: Figure S5).

Daily data over the effective period of canopy photosynthesis and before the beginning of fast canopy senescence were either averaged (with subscript 'mean', e.g., $A_{\text{Cnet.mean}}$, mol CO_2 m^{-2} d^{-1}) or integrated (with subscript 'INT', e.g., $A_{\text{Cnet.INT}}$, mol CO_2 m^{-2}) to investigate the effects of long-term growth temperature changes, HS, elevated air CO_2 concentration (eCO_2), and WD on canopy gas exchange during the post-anthesis period. Missing values (2% of total daily data) were linearly interpolated when calculating gas exchange variables over a period of several days.

2.8 | Calculation of Canopy Carbon and Water Use Efficiencies

Canopy CUE and WUE based on A_{Cnet} (WUE_{net}, mol CO_2 m^{-2} ground mm^{-1} H_2O) and A_{Cgross} ($\text{WUE}_{\text{gross}}$, mol CO_2 m^{-2} ground mm^{-1} H_2O) during the period of effective canopy photosynthesis and before the start of fast canopy senescence were calculated as:

$$\text{CUE} = \frac{A_{\text{Cnet.mean}}}{A_{\text{Cgross.mean}}} = \frac{A_{\text{Cnet.INT}}}{A_{\text{Cgross.INT}}}, \tag{8}$$

$$\text{WUE}_{\text{net}} = \frac{A_{\text{Cnet.mean}}}{\text{ET}_{\text{mean}}} = \frac{A_{\text{Cnet.INT}}}{\text{ET}_{\text{INT}}}, \tag{9}$$

$$\text{WUE}_{\text{gross}} = \frac{A_{\text{Cgross.mean}}}{\text{ET}_{\text{mean}}} = \frac{A_{\text{Cgross.INT}}}{\text{ET}_{\text{INT}}}. \tag{10}$$

2.9 | Statistical Data Analyses

Equation (7) was fitted to $N_{\text{C.leaf}}$ data versus the number of days after anthesis using the GAUSS method in PROC NLIN of the statistical software programme SAS (SAS Institute Inc., Cary, NC, USA). We performed multiple linear regression analyses to examine the effects of environmental variables on canopy gas exchange parameters. The models included daytime mean air temperature ($T_{\text{D.mean}}$), nighttime mean air temperature ($T_{\text{N.mean}}$), HS, WD, and eCO_2 as predictors (daily mean air temperature T_{mean} was not included to avoid collinearity). *Genotype* was initially included in the models but was later excluded because of lack of statistical significance. To assess multicollinearity among predictor variables, the variance inflation factor (VIF) was calculated as $1/(1–R^2)$ for each variable, where R^2 is the coefficient of determination from regressing

FIGURE 1 | Schema of the post-anthesis temperature and water regimes evaluated in the experiments analysed in this study. In EXP2000, before temperature regimes changed to 18/10°C, plants were grown at Out–5°C. Arrows (→) and red points indicate a change in the temperature regime; temperatures before and after the slash (/) are day and night temperatures, respectively. The name of the winter wheat genotypes used in each experiment is indicated on the left below the experiment name. Details and codes for experiments and regimes on the left are described in the Supplementary Information Methods section and in Supporting Information S1: Table S1. DAA, days after anthesis; eCO_2, elevated air CO_2 concentration; Out°C, outside ambient temperature; Out+5°C, outside ambient temperature plus 5°C; Out+10°C, outside ambient temperature plus 10°C; Out–5°C, outside ambient temperature minus 5°C; HS, heat shock; HS1, HS2 and HS3 represent heat shocks during the early grain-filling period (7–10 DAA), the mid-grain-filling period (18–21 DAA) and the late grain-filling period (30–31 DAA), respectively; WW, well-watered; WD, water deficit. [Color figure can be viewed at wileyonlinelibrary.com] that variable against all others. All regression models were fitted using the *lm*() function in R (version 4.5.0; R Foundation). VIFs were calculated using the *vif*() function from the car package (Fox and Weisberg 2018).

3 | Results

3.1 | Repeatability of Gas Exchange Measurements in the C3-GEM SAPR Units

Due to the capacity of the C3-GEM facilities (the platform only had four SPAR units), it was not possible to include experimental replications in most experiments. In EXP1997, four chambers were used with two chambers assigned to the temperature treatment 'Out+10°C' and the other two chambers to 'Out−5°C' (Supporting Information S1: Table S1; Figure S6d). Therefore, data from each chamber with the same temperature regime were used to assess the repeatability of gas exchange measurements in the C3-GEM facility. The data showed satisfactory repeatability of the gas exchange measurements based on either directly measured variables (i.e., $N_{\mathrm{C.leaf}}$, T_{daily}, A_{Cnet}, and ET; Panel d in Supporting Information S1: Figures S5, S6, S10, S12, and S13) or indirectly estimated variables (Panel d in Supporting Information S1: Figures S7–S9 and S11).

3.2 | Multiple Regression Analysis for Canopy Gas Exchange Under Various Environments

Given the complexity of treatments in our dataset, we employed multiple regression analysis to investigate their effects on canopy gas exchange. In preliminary multiple regression analyses, the effect of genotype was found to be insignificant; therefore, it was excluded from subsequent analyses. We focused solely on examining the effects of environmental variables, including daytime ($T_{\mathrm{D.mean}}$), nighttime ($T_{\mathrm{N.mean}}$) mean air temperature over the period of effective canopy photosynthesis, and treatments HS, WD, and eCO_2. To assess multicollinearity among predictor variables, VIFs were calculated for each variable. All VIF values for $T_{\mathrm{D.mean}}$, $T_{\mathrm{N.mean}}$, HS, WD, and eCO_2 were < 2.5, indicating low multicollinearity among these predictors (Table 1).

Multiple regression analysis revealed that $T_{\mathrm{D.mean}}$, $T_{\mathrm{N.mean}}$, HS, WD, and eCO_2, differentially affected canopy gas exchange. Overall, among these environmental factors, $T_{\mathrm{D.mean}}$, $T_{\mathrm{N.mean}}$, and WD had the most profound impacts on canopy gas exchange, significantly affecting 11, 9, and 10 out of 16 canopy gas exchange parameters, respectively (Table 1).

$T_{\mathrm{D.mean}}$ had significant negative effects on both respiration ($R_{\mathrm{Cnight.mean}}$, $R_{\mathrm{Ctot.mean}}$, $R_{\mathrm{Cnight.INT}}$, $R_{\mathrm{Cday.INT}}$, $R_{\mathrm{Ctot.INT}}$), and CO_2 assimilation ($A_{\mathrm{Cnet.mean}}$, $A_{\mathrm{Cnet.INT}}$, $A_{\mathrm{Cgross.INT}}$). Notably, to better capture the nonlinear temperature response of CO_2 assimilation, a quadratic term of $T_{\mathrm{D.mean}}$ ($T^2_{\mathrm{D.mean}}$) was introduced into the model (e.g., for $A_{\mathrm{Cnet.mean}}$), which improved the model fit (higher adjusted R^2) and increased the statistical significance of the linear $T_{\mathrm{D.mean}}$ term (Table 1). In addition, $T_{\mathrm{D.mean}}$ negatively affected the effective period of canopy photosynthesis (t_e). In contrast, $T_{\mathrm{D.mean}}$ had a positive effect on $\mathrm{ET}_{\mathrm{mean}}$, but a negative effect on $\mathrm{WUE}_{\mathrm{gross}}$ (Table 1).

$T_{\mathrm{N.mean}}$ showed significant positive effects on respiration parameters ($R_{\mathrm{Cnight.mean}}$, $R_{\mathrm{Cday.mean}}$, $R_{\mathrm{Ctot.mean}}$, $R_{\mathrm{Cnight.INT}}$, $R_{\mathrm{Ctot.INT}}$). In contrast, $T_{\mathrm{N.mean}}$ had significant negative effects on net CO_2 assimilation ($A_{\mathrm{Cnet.mean}}$ and $A_{\mathrm{Cnet.INT}}$) and, as expected, on CUE (Table 1). Additionally, $\mathrm{WUE}_{\mathrm{net}}$ was also negatively affected by $T_{\mathrm{N.mean}}$ (Table 1).

WD had consistently negative effects on all parameters that showed significant associations. In particular, t_e and the integral parameters ($R_{\mathrm{Cday.INT}}$, $R_{\mathrm{Cnight.INT}}$, $R_{\mathrm{Ctot.INT}}$, $A_{\mathrm{Cnet.INT}}$, $A_{\mathrm{Cgross.INT}}$, and $\mathrm{ET}_{\mathrm{INT}}$) were significantly negatively affected by WD. WD also reduced $R_{\mathrm{Cday.mean}}$ and $A_{\mathrm{Cgross.mean}}$ (Table 1).

In contrast to $T_{\mathrm{D.mean}}$, $T_{\mathrm{N.mean}}$, and WD, HS did not significantly affect any of the response variables (Table 1). Additionally, the effects of eCO_2 were relatively limited, with significant impacts observed only on $A_{\mathrm{Cnet.mean}}$ and $A_{\mathrm{Cgross.mean}}$, and on $\mathrm{WUE}_{\mathrm{net}}$ and $\mathrm{WUE}_{\mathrm{gross}}$ (Table 1).

3.3 | Temperature Responses of Canopy Gas Exchange Under Well-Watered Conditions

Given the substantial and statistically significant effects of temperature on canopy gas exchange parameters, we examined their temperature response patterns. All gas exchange parameters calculated over the period of effective gas exchange (Figure 2) and the period before fast canopy senescence (Supporting Information S1: Figures S14 and S15) across all experiments and environmental regimes were plotted against the corresponding daily mean (T_{mean}), daytime mean ($T_{\mathrm{D.mean}}$), and nighttime mean ($T_{\mathrm{N.mean}}$) air temperatures. Given the substantial effects of WD on gas exchange, temperature response analyses were based solely on data for WW conditions.

$R_{\mathrm{Cnight.mean}}$, $R_{\mathrm{Cday.mean}}$, and $R_{\mathrm{Ctot.mean}}$ over the period of effective canopy photosynthesis increased with rising $T_{\mathrm{N.mean}}$, but were not significantly correlated with T_{mean} or $T_{\mathrm{D.mean}}$ (Figure 2a–i). For CO_2 assimilation rates, $A_{\mathrm{Cnet.mean}}$ was negatively correlated with T_{mean}, $T_{\mathrm{D.mean}}$ and $T_{\mathrm{N.mean}}$, but the correlation between $A_{\mathrm{Cnet.mean}}$ and $T_{\mathrm{N.mean}}$ ($R^2 = 0.342$) was stronger than that between $A_{\mathrm{Cnet.mean}}$ and $T_{\mathrm{D.mean}}$ ($R^2 = 0.227$; Figure 2j,k). Compared with $A_{\mathrm{Cnet.mean}}$, $A_{\mathrm{Cgross.mean}}$ over the period of effective canopy photosynthesis showed lower sensitivity to temperature, with a significant correlation observed only with $T_{\mathrm{D.mean}}$ under WW conditions (Figure 2m–o). $\mathrm{ET}_{\mathrm{mean}}$ over the period of effective canopy photosynthesis was positively correlated with T_{mean} and $T_{\mathrm{D.mean}}$, while it was not significantly correlated with $T_{\mathrm{N.mean}}$ (Figure 2p–r).

The average canopy gas exchange rates before the beginning of canopy senescence (N80% and DAA20) were generally higher than that over the period of effective canopy photosynthesis (Supporting Information S1: Figures S14 and S15). Moreover, $R_{\mathrm{Cnight.mean}}$, $R_{\mathrm{Cday.mean}}$, $R_{\mathrm{Ctot.mean}}$, and $\mathrm{ET}_{\mathrm{mean}}$ for both N80% and DAA20 showed better correlations with temperature as compared with that for the period of effective canopy

TABLE 1 | Multiple regression analysis of canopy gas exchange parameters average nighttime ($R_{\mathrm{Cnight.mean}}$), daytime ($R_{\mathrm{Cday.mean}}$), and daily total ($R_{\mathrm{Ctot.mean}}$) respiration rates, average net ($A_{\mathrm{Cnet.mean}}$) and gross ($A_{\mathrm{Cgross.mean}}$) CO_2 assimilation rates, average evapotranspiration rate ($\mathrm{ET}_{\mathrm{mean}}$) over the period of effective canopy photosynthesis, effective duration of active canopy photosynthesis (t_e), integral values of nighttime ($R_{\mathrm{Cnight.INT}}$), daytime ($R_{\mathrm{Cday.INT}}$), and daily total ($R_{\mathrm{Ctot.INT}}$) respiration, net ($A_{\mathrm{Cnet.INT}}$) and gross ($A_{\mathrm{Cgross.INT}}$) CO_2 assimilation, evapotranspiration ($\mathrm{ET}_{\mathrm{INT}}$), and carbon use efficiency (CUE), and water use efficiency based on net CO_2 assimilation ($\mathrm{WUE}_{\mathrm{net}}$) and on gross CO_2 assimilation ($\mathrm{WUE}_{\mathrm{gross}}$) calculated over the period of effective canopy photosynthesis, as a function of environmental variables daytime mean air temperature ($T_{\mathrm{D.mean}}$), nighttime mean air temperature ($T_{\mathrm{N.mean}}$), and heat shock (HS), water deficit (WD), atmospheric CO_2 elevation (eCO_2) treatments, that is, response variable = $b_0 + b_1T_{\mathrm{D.mean}} + b_2T_{\mathrm{N.mean}} + b_3\mathrm{HS} + b_4\mathrm{WD} + b_5eCO_2$[a].

Response parameter	**Intercept** b_0	$T_{\mathrm{D.mean}}$ b_1	$T_{\mathrm{N.mean}}$ b_2	[b]**HS** b_3	[b]**WD** b_4	[b]eCO_2 b_5	[c]$T^2_{\mathrm{D.mean}}$ [c]b_6	R^2_{adj}	n
$R_{\mathrm{Cnight.mean}}$	**0.157****	**−0.007***	**0.010****	−0.016	−0.033	0.001	NA	0.238	30
$R_{\mathrm{Cday.mean}}$	**0.347*****	−0.008	**0.012***	−0.036	**−0.116***	0.167	NA	0.296	30
$R_{\mathrm{Ctot.mean}}$	**0.503*****	**−0.015***	**0.022****	−0.052	−0.149	0.161	NA	0.266	30
$A_{\mathrm{Cnet.mean}}$	**0.757*****	−0.001	**−0.019***	0.148	−0.164	**0.313***	NA	0.384	31
	−1.671*	**−0.005*****	**−0.029*****	0.131	−0.166	0.222	**0.236*****	0.597	31
$A_{\mathrm{Cgross.mean}}$	**1.249*****	−0.014	0.001	0.092	**−0.315***	**0.498***	NA	0.324	30
	0.278	−0.002	−0.003	0.083	**−0.318***	**0.461***	0.081	0.597	30
$\mathrm{ET}_{\mathrm{mean}}$	**3.428****	**0.130***	−0.006	0.849	**−2.206****	−1.479	NA	0.367	31
t_e	**74.361*****	**−1.453*****	−0.221	−3.821	**−10.133*****	−6.695	NA	0.741	31
$R_{\mathrm{Cnight.INT}}$	**11.288*****	**−0.422*****	**0.233***	−1.134	**−3.094****	−0.421	NA	0.465	30
$R_{\mathrm{Cday.INT}}$	**25.544*****	**−0.732*****	0.261	−2.250	**−8.384*****	3.833	NA	0.624	30
$R_{\mathrm{Ctot.INT}}$	**36.779*****	**−1.151*****	**0.494***	−3.370	**−11.468*****	3.206	NA	0.594	30
$A_{\mathrm{Cnet.INT}}$	**42.384*****	**−0.635***	**−0.752***	6.489	**−11.347****	7.403	NA	0.621	31
	−36.304	**−0.168****	**−1.092*****	5.924	**−11.400***	4.446	**7.045***	0.712	31
$A_{\mathrm{Cgross.INT}}$	**78.911*****	**−1.744*****	−0.295	3.011	**−22.887*****	11.3	NA	0.793	30
	49.926	−0.062	−0.427	2.721	**−22.969*****	10.227	1.101	0.793	30
$\mathrm{ET}_{\mathrm{INT}}$	**325.705*****	−3.249	−1.851	34.198	**−139.557*****	−64.547	NA	0.454	31
CUE	**0.616*****	0.009	**−0.023****	0.094	−0.035	0.082	NA	0.333	30
$\mathrm{WUE}_{\mathrm{net}}$	**0.159*****	−0.002	**−0.003***	0.013	−0.0004	**0.079****	NA	0.537	31
$\mathrm{WUE}_{\mathrm{gross}}$	**0.277*****	**−0.006****	0.0003	−0.003	0.012	**0.140*****	NA	0.611	30

Note: Significance level: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. R^2_{adj}: Adjusted R-squared. n: No. of data points. NA: not applicable. Bold values indicate regression coefficients that are statistically significant.

[a] Variance inflation factor (VIF) analysis of the model: VIF values were calculated as $1/(1–R^2)$ for each variable, where R^2 is the coefficient of determination from regressing that variable against all others. VIF values for $T_{\mathrm{D.mean}}$, $T_{\mathrm{N.mean}}$, HS, WD, and eCO_2 were 2.07, 1.96, 1.19, 1.11, and 1.02 ($n = 30$), and 2.11, 2.01, 1.16, 1.10, and 1.02 ($n = 31$), respectively.

[b] Treatments (HS, WD, and eCO_2) as a category variable were quantified by introducing dummy variables. We treated WD as a categorical variable rather than using the amount of irrigation because the irrigation amounts of well-watered treatments were largely determined by t_e. Although the timing of HS application varied from early, to mid, to late grain-filling periods (Supporting Information S1: Table S1), our preliminary analysis indicated that the timing of HS had no significant effect on the response variables; thus, we also treated HS as a binary (dummy) variable indicating the presence or absence of HS.

[c] A quadratic term $T^2_{\mathrm{D.mean}}$ was introduced in the model for CO_2 assimilation variables (response variable = $b_0 + b_1T_{\mathrm{D.mean}} + b_2T_{\mathrm{N.mean}} + b_3\mathrm{HS} + b_4\mathrm{WD} + b_5eCO_2 + b_6T^2_{\mathrm{D.mean}}$) because the response of such parameters to temperature is theoretically assumed nonlinear.

photosynthesis, but this was not the case for $A_{\mathrm{Cnet.mean}}$ (Supporting Information S1: Figures S14 and S15).

t_e was strongly negatively correlations with T_{mean} ($R^2 = 0.754$) and $T_{\mathrm{D.mean}}$ ($R^2 = 0.752$), while it responded less to $T_{\mathrm{N.mean}}$ ($R^2 = 0.422$) (Figure 3a–c). As t_e decreased with rising air temperature, correspondingly, $R_{\mathrm{Cnight.INT}}$, $R_{\mathrm{Cday.INT}}$ and $R_{\mathrm{Ctot.INT}}$ also decreased with increased T_{mean} and $T_{\mathrm{D.mean}}$, but not with $T_{\mathrm{N.mean}}$ (Figure 3d–l). Similarly, $A_{\mathrm{Cnet.INT}}$, $A_{\mathrm{Cgross.INT}}$, and $\mathrm{ET}_{\mathrm{INT}}$ also decreased with increasing air temperature, and they were more correlated with T_{mean} and $T_{\mathrm{D.mean}}$ than with $T_{\mathrm{N.mean}}$ (Figure 3m–u).

The correlation between t_{80} and temperature was weaker than that between t_e and temperature (Supporting Information S1: Figures 3a–c and S16a–c). Consequently, when daily gas exchange variables were integrated before the beginning of canopy senescence, the correlations of $A_{\mathrm{Cnet.INT}}$ and $A_{\mathrm{Cgross.INT}}$ for N80% with temperature was weaker (Supporting Information S1: Figure S16m–r). Moreover, $R_{\mathrm{Cnight.INT}}$, $R_{\mathrm{Cday.INT}}$, $R_{\mathrm{Ctot.INT}}$, and $\mathrm{ET}_{\mathrm{INT}}$ for N80% were insignificantly correlated with temperature (Supporting Information S1: Figures S16d–l,s–u).

CUE was negatively correlated with $T_{\mathrm{N.mean}}$ ($R^2 = 0.404$, $p < 0.001$) and to a less extent with T_{mean} ($R^2 = 0.173$, $p < 0.05$) (Figure 4a–c). Although $\mathrm{WUE}_{\mathrm{net}}$ and $\mathrm{WUE}_{\mathrm{gross}}$ were both negatively correlated with temperature, $\mathrm{WUE}_{\mathrm{gross}}$ was less sensitive to temperature, especially to $T_{\mathrm{N.mean}}$, than $\mathrm{WUE}_{\mathrm{net}}$ (Figure 4d–i).

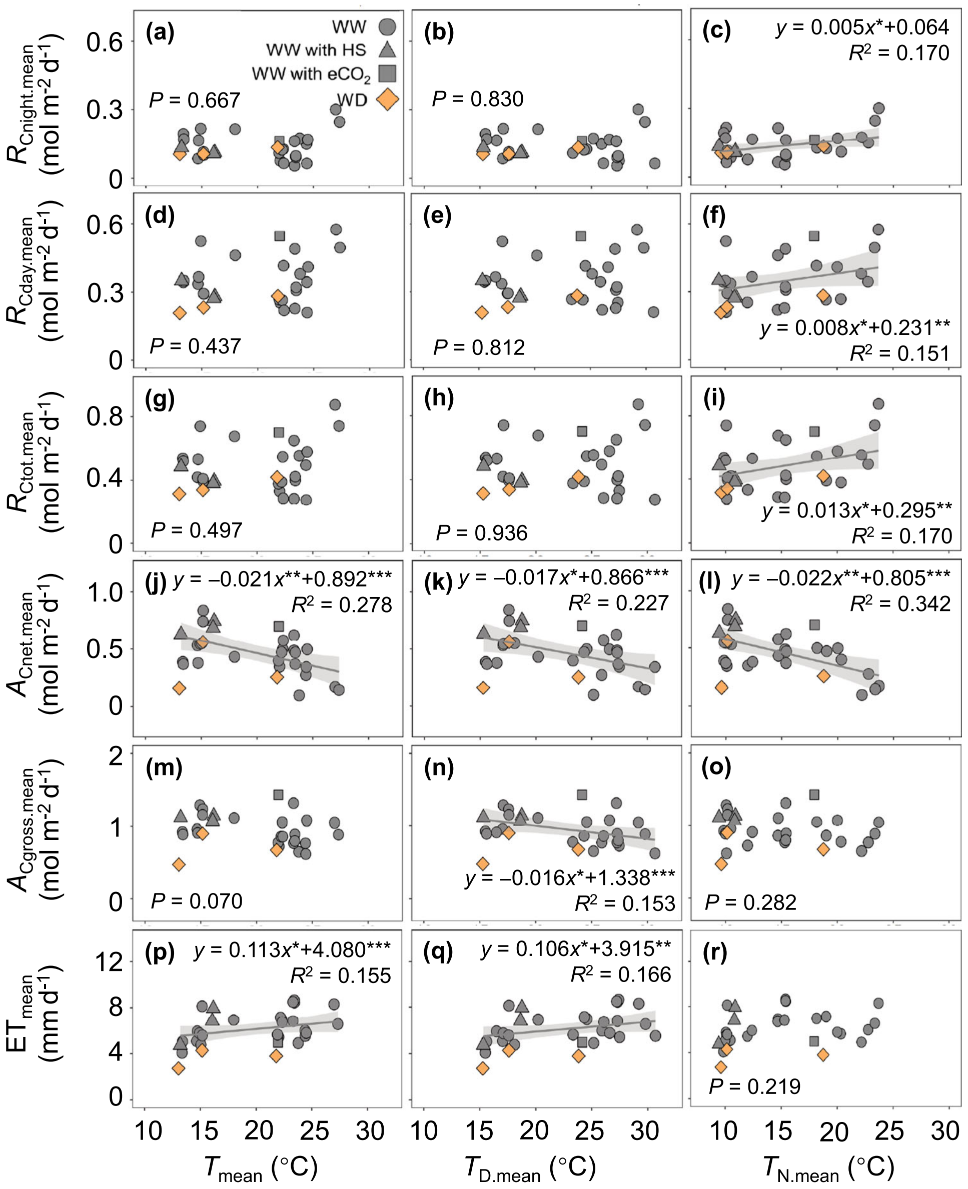

FIGURE 2 | Average canopy gas exchange rates versus average air temperature over the whole post-anthesis measurement period. Nighttime ($R_{\mathrm{Cnight.mean}}$, a–c), daytime ($R_{\mathrm{Cday.mean}}$, d–f), and total respiration ($R_{\mathrm{Ctot.mean}}$, g–i), net ($A_{\mathrm{Cnet.mean}}$, j–l) and gross CO_2 assimilation rates ($A_{\mathrm{Cgross.mean}}$, m–o), and evapotranspiration ($\mathrm{ET}_{\mathrm{mean}}$, p–r) versus daily ($T_{\mathrm{mean}}$; a, d, g, j, m, p), daytime ($T_{\mathrm{D.mean}}$; b, e, h, k, n, q), and nighttime ($T_{\mathrm{N.mean}}$; c, f, i, l, o, r) mean air temperature. Gas exchange data are means over the period of effective canopy photosynthesis. WW, well-watered; WD, water deficit; eCO_2, elevated atmospheric CO_2 concentration; HS, heat shock. Lines are linear regressions fitted to the data for WW conditions and are shown only when significant ($p \leq 0.05$). *, **, and *** indicate statistical significance at $p < 0.05$, < 0.01, and < 0.001, respectively. Shaded area around the regression lines are 95% confidence intervals of the predictions. [Color figure can be viewed at wileyonlinelibrary.com]

4 | Discussion

With a quantitative analysis of canopy gas exchange during the post-anthesis period for wheat grown in multi-year experiments, we systematically investigated to what extent canopy gas exchange can be affected by various environmental factors. We then tried to answer the two research questions: (i) is CUE invariant? (ii) is the response of WUE to environments primarily regulated by carbon fluxes or by water fluxes? Before discussing our main findings and answering these two questions, we first discuss two potential methodological caveats in this study that may affect our conclusions.

4.1 | Can the Kok Method Be Used to Estimate Daytime Canopy Respiration?

Daytime respiration rate (R_{Cday}) is an important component in determining canopy carbon fluxes. Here, we estimated R_{Cday} using the Kok method (Kok 1948), which exploits photosynthetic response to the incident PPFD within the low PPFD range, where the response is expected to be linear. The Kok method tends to underestimate day respiration at leaf scale (Yin et al. 2011; Fang et al. 2022; Yin and Amthor 2024), mainly because it ignores the deviation from linearity of the photosynthetic light response curve with increasing PPFD, as a result of the declining electron transport efficiency. However, the Kok

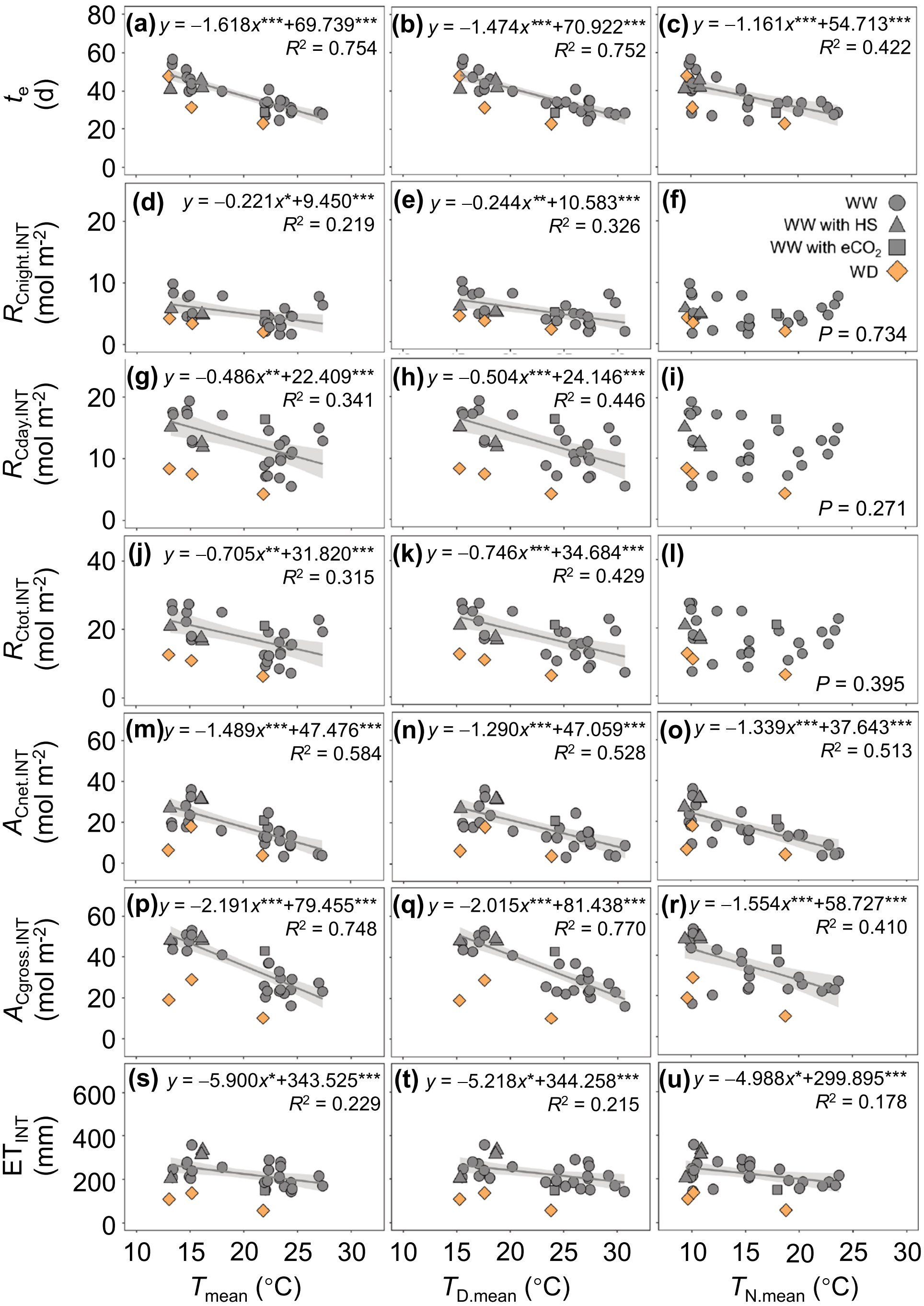

FIGURE 3 | Integral values of canopy gas exchange parameters versus average air temperature over the post-anthesis period of effective canopy photosynthesis. Effective duration of active canopy photosynthesis (t_e, a–c), integral nighttime ($R_{\mathrm{Cnight.INT}}$, d–f), daytime ($R_{\mathrm{Cday.INT}}$, g–i), and daily total respiration ($R_{\mathrm{Ctot.INT}}$, j–l), integral net ($A_{\mathrm{Cnet.INT}}$, m–o) and gross CO_2 assimilation ($A_{\mathrm{Cgross.INT}}$, p–r), and integral evapotranspiration ($\mathrm{ET}_{\mathrm{INT}}$, s–u) versus daily ($T_{\mathrm{mean}}$; a, d, g, j, m, p, s), daytime ($T_{\mathrm{D.mean}}$; b, e, h, k, n, q, t), and nighttime ($T_{\mathrm{N.mean}}$; c, f, i, l, o, r, u) mean air temperature. Data are integral values between the beginning of measurement and the day when canopy was completely senesced. WW, well-watered; WD, water deficit; eCO_2, elevated atmospheric CO_2 concentration; HS, heat shock. Lines are linear regressions fitted to the data for WW conditions and are shown only when significant ($p \leq 0.05$). *, **, and *** indicate statistical significance at $p < 0.05$, < 0.01, and < 0.001, respectively. Shaded area around the regression lines are 95% confidence intervals of the predictions. [Color figure can be viewed at wileyonlinelibrary.com]

method as applied here is expected to be less problematic than when used at leaf scale because the light response curve of canopy photosynthesis is more linear than that of leaf photosynthesis (Supporting Information S1: Figure S3; Sheehy and Mitchell 2015). In addition, while respiration can vary during daytime because of endogenous and environmental rhythmicity (Faber et al. 2022; Fang et al. 2022), daily R_{Cday} values were calculated as the mean of R_{Cday} separately estimated for dawn and dusk hours, to minimise the effect of diurnal variation, which should provide representative estimates of daily R_{Cday}.

It is noteworthy that, unlike many previous findings (e.g., Yin et al. 2011; Gong et al. 2017; Fang et al. 2022), we did not observe light inhibition of respiration, where respiration in the

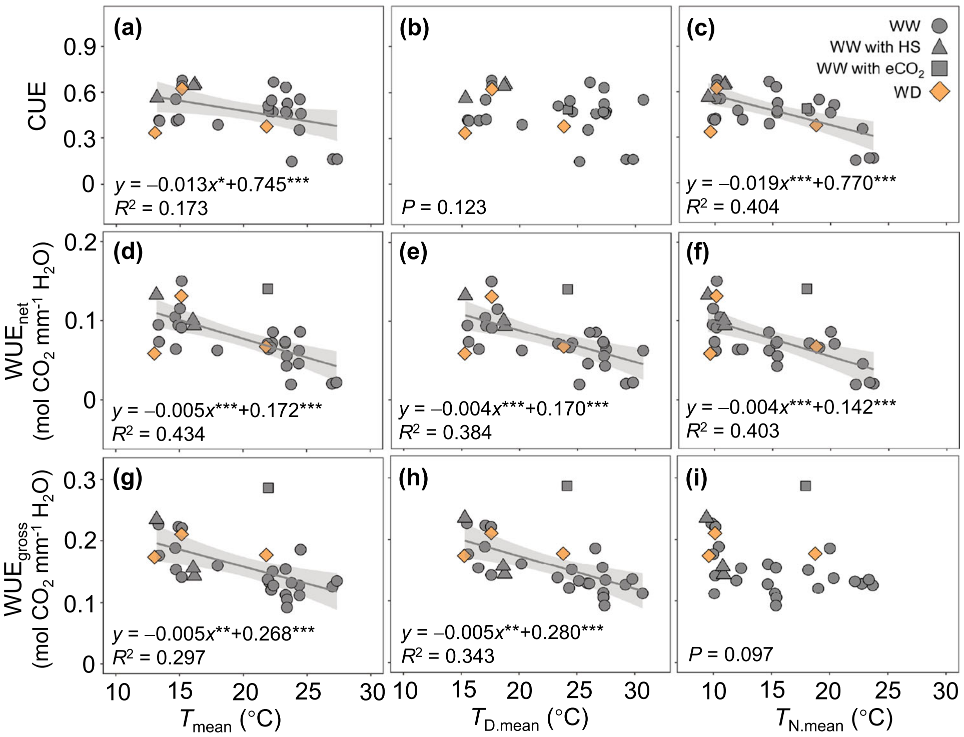

FIGURE 4 | Carbon and water use efficiencies versus air temperature during the period of effective canopy photosynthesis. Carbon use efficiency (CUE, a–c), water use efficiency based on net CO$_2$ assimilation (WUE$_{net}$, d–f) and on gross CO$_2$ assimilation (WUE$_{gross}$, g–i) versus daily (T_{mean}; a, d, g), daytime ($T_{D.mean}$; b, e, h), and nighttime ($T_{N.mean}$; c, f, i) mean air temperature. WW, well-watered; WD, water deficit; eCO$_2$, elevated atmospheric CO$_2$ concentration; HS, heat shock. Lines are linear regressions fitted to the data for WW conditions and are shown only when significant ($p \leq 0.05$). *, **, and *** indicate statistical significance at $p < 0.05$, < 0.01, and < 0.001, respectively. Shaded area around the regression lines are 95% confidence intervals of the predictions. [Color figure can be viewed at wileyonlinelibrary.com]

light is typically lower than respiration in the dark. Instead, we found that R_{Cday} values were generally higher than R_{Cnight} values, which could largely be attributed to the higher air temperatures during the daytime compared to the nighttime (Supporting Information S1: Figure S4c,d). This implies that under natural conditions, temperature-driven stimulation could offset, and even exceed, any inhibition of respiration by light. Nevertheless, there are other CO$_2$-releasing processes that might occur in the day but not in the night (Xu et al. 2024; Yin and Amthor 2024).

4.2 | Do Changes in Canopy Senescence Patterns Under Different Environmental Regimes Affect Estimates of Carbon and Water Use Efficiencies During the Whole Post-Anthesis Period?

Our analyses used the data collected over the period of effective canopy photosynthesis (between the starting day of gas exchange measurements and full canopy senescence). However, including the period after fast canopy senescence in CUE and WUE calculations might lead to inconsistent results as compared with the calculations with data before fast canopy senescence. To verify this, we also calculated the respiration, CO$_2$ assimilation, and ET, and CUE and WUE with data collected before the start of fast senescence, i.e., either N80% or DAA20 (Supporting Information S1: Figures S14–S18). As expected, compared to using data until full canopy senescence, using data before the beginning of fast canopy senescence yielded higher gas exchange rates (Supporting Information S1: Figures S14 and S15), CUE, and WUE (Supporting Information S1: Figure S19), and improved the correlations of CUE and WUE with temperature (Supporting Information S1: Figures S17 and S18). However, in both N80% and DAA20 analyses, some treatments (e.g., HS and intra-seasonal temperature changes) were imposed beyond the selected period; therefore, N80% or DAA20 analyses did not capture the effects of these experimental treatments. Moreover, including the period of canopy senescence in CUE and WUE calculations provides a better estimation of their impact on crop production. Therefore, below we mainly discuss the results of CUE and WUE calculated over the period of effective canopy photosynthesis, i.e., the whole post-anthesis period.

4.3 | Is CUE Invariant?

Whether or not CUE is invariant has long been under debate (Waring et al. 1998; Collalti and Prentice 2019; Ryan and Asao 2019). The common consensus is that CUE is conserved due to the close coupling between photosynthesis and respiration and thus the convergent acclimation of these two physiological processes (Gifford 1995; Cheng et al. 2000; Tjoelker et al. 2008; Smith and Dukes 2013; Aspinwall et al. 2016; Reich et al. 2016; Crous et al. 2017). In line with this consensus, we found a conserved relationship between A_{Cnet} and A_{Cgross} across a wide range of environmental regimes and genotypes regardless of the time scale (daily and several weeks) (Supporting Information S1: Figure S20a,b). Nevertheless, the statistically significant negative intercept of the relationship (Supporting Information S1: Figure S20a,b) suggests that the ratio of A_{Cnet} to A_{Cgross} (i.e., CUE) is variant. The negative intercept could be a result of the respiratory loss when A_{Cgross} tends towards zero.

Our results showed that CUE decreased with increasing T_{mean} (Figure 4a). This was inconsistent with previous studies on wheat (Gifford 1995) and eucalyptus (Drake et al. 2019) showing no effect of warming on CUE at whole plant level. In the study of Gifford (1995), wheat plants were grown under steady-state conditions in growth chambers, and CUE was calculated based on data recorded over a measurement period of only 24 h, with a 12-h photoperiod. However, the homeostasis between photosynthesis and respiration may not be maintained under highly fluctuating environmental conditions during the growing season. In our study, plants were grown outside under natural light environment, and CUE was calculated based on data continuously collected during the whole post-anthesis period. This made it possible to assess CUE over a long-term period and to avoid the potentially uninterpretable and misleading results that could result from spot measurements when other environments (e.g., irradiance and temperature) fluctuate during the measurement period. Moreover, in our study, T_{mean} ranged from 13°C to 27°C, a much wider range than that in Drake et al. (2019) where only an 'Out+3°C' warming treatment was applied. These may contribute to the difference between their results and ours.

While we demonstrated that CUE was negatively correlated with T_{mean}, there was significant scattering around the linear temperature response (Figure 4a). Large variation in the relationship between CUE and annual mean temperature has been observed previously, yet the causes are still unclear (Ryan and Asao 2019). Here, our results may (at least partially) explain the variation in CUE. One explanation is that CUE showed different sensitivities to T_{mean}, $T_{\text{D.mean}}$, and $T_{\text{N.mean}}$ (Figure 4a–c; Table 1), and was most sensitive to $T_{\text{N.mean}}$ that is rarely considered in previous studies on CUE (e.g., Ryan and Asao 2019). Results from both multiple regression and simple linear regression consistently indicated that the sensitivity of CUE to $T_{\text{N.mean}}$ was largely attributable to the positive effect of $T_{\text{N.mean}}$ on respiration rates (Table 1; Figure 2). Recently, at leaf scale, McAusland et al. (2023) reported that the nocturnal heating treatment slightly increased nocturnal respiration, but had little effect on photosynthesis during daytime. Here, at canopy scale, the stronger correlation of CUE with $T_{\text{N.mean}}$ than with $T_{\text{D.mean}}$ might reflect that the homeostasis between photosynthesis and respiration could primarily occur during daytime when both processes occur simultaneously. Note that it is the growth respiration that is probably more associated with photosynthesis, while a direct effect of an increase in temperature is probably on the maintenance component of respiration. Nevertheless, the underlying mechanisms merit further elucidation.

By quantifying the variation in CUE, we observed that intra-seasonal thermal variability, drought stress, and genotypic variation may also lead to large variations in CUE even under close T_{mean} values (Supporting Information S1: Figure S21). By contrast, we observed that HS and eCO_2 had little impact on CUE (Table 1), in agreement with previous studies (Gifford 1995; Cheng et al. 2000). In addition, the fact that the correlation between CUE and temperature was stronger when CUE was calculated during the period before the beginning of fast canopy senescence compared with the period of effective canopy photosynthesis implies that the interaction between environments and canopy senescence may also play a role in determining thermal sensitivity of CUE (Supporting Information S1: Figures S17 and S18). Furthermore, although genotype had no significant effect on CUE in our initial multiple regression analysis (results not shown), the coefficient of variation in CUE among genotypes was 14% (Supporting Information S1: Figure S21a), implying that the effects of genotypic variation should not be neglected (Eller et al. 2020).

4.4 | How Does WUE Respond to Environmental Variables?

Compared with CUE, WUE is more variant (Supporting Information S1: Figures S20c–f and S21), as it integrates carbon and water fluxes, which often respond differently to environmental factors (Niu et al. 2011; Huang et al. 2016; Hatfield and Dold 2019). Here, $A_{\text{Cnet.INT}}$ and $A_{\text{Cgross.INT}}$ decreased by 5% and 4% for each °C of T_{mean} increase, respectively, while ET_{INT} decreased by only 2% per °C increase in T_{mean} (Figure 3m,p,s). Consequently, the negative dependence of WUE_{net} and $\text{WUE}_{\text{gross}}$ on T_{mean} was primarily due to the reductions in $A_{\text{Cnet.INT}}$ and $A_{\text{Cgross.INT}}$ with increasing T_{mean}. This suggests that the responses of WUE_{net} and $\text{WUE}_{\text{gross}}$ to T_{mean} are predominantly regulated by carbon fluxes rather than by water fluxes, which is congruent with the findings of an earlier study in grassland (Niu et al. 2011). However, under WD conditions, the responses of WUE_{net} and $\text{WUE}_{\text{gross}}$ were generally driven by both carbon and water fluxes (Table 1).

The dominance of carbon fluxes for driving WUE variation was further reflected in the differential responses of WUE_{net} and $\text{WUE}_{\text{gross}}$. For instance, $\text{WUE}_{\text{gross}}$ was significantly affected only by $T_{\text{D.mean}}$, whereas, like CUE, WUE_{net} responded solely to $T_{\text{N.mean}}$ (Table 1). Moreover, when CUE varied substantially, WUE_{net} and $\text{WUE}_{\text{gross}}$ showed contrasting response patterns to environmental changes (their coefficients of variation differed much); however, when CUE changed little, the variations in WUE_{net} and $\text{WUE}_{\text{gross}}$ were consistent (their coefficients of variation were similar; Supporting Information S1: Figure S21b). This is probably because compared to $\text{WUE}_{\text{gross}}$, WUE_{net} is additionally affected by CUE (i.e., $\text{WUE}_{\text{net}} = \text{WUE}_{\text{gross}} \times \text{CUE}$).

4.5 | Implications for Climate Change Adaptation

Our findings have significant implications for understanding climate change impacts on (agro) ecosystem functions and productivity. We found that wheat canopy carbon and water fluxes were profoundly influenced by growth temperature, i.e., T_{mean}. Yet, solely focusing on the effect of T_{mean} would overlook some other important information that may induce variation at the same T_{mean}, such as diurnal and intra-seasonal thermal variabilities, as well as interactions between T_{mean} and other (non-)environmental factors (e.g., soil moisture, atmospheric CO_2, and genotype).

CUE determines the carbon budgets of (agro) ecosystems. A simple assumption that respiration accounts for a constant fraction of gross photosynthesis (i.e., a fixed CUE) has been proposed and used in modelling practices (Gifford 1995, 2003; Waring et al. 1998; Van Oijen et al. 2004). This is somewhat understandable, as there are still knowledge gaps about plant carbon budgets due to the difficulty of accurately measuring whole canopy carbon fluxes. Nevertheless, our study demonstrated that CUE is variable, and the variation in CUE could be very high. Therefore, we highlight that assuming a constant CUE in models would forgo opportunities to unravel mechanisms that underlie plant carbon balance under changing environments, in agreement with what Ryan and Asao (2019) advocated.

Our findings further reveal that CUE exhibited different sensitivities to daytime and nighttime temperatures. Retrospective analysis has reported an asymmetric increase in daytime and nighttime temperatures over the past two decades (Liu et al. 2024). Thus, current process-based vegetation/crop models that typically rely on daily mean temperature are expected to produce biased predictions of (agro) ecosystem productivity under global warming (Zheng et al. 2021). To avoid this, modelling studies should thoroughly consider diurnal temperature asymmetry when assessing global warming impacts on (agro) ecosystem productivity. Furthermore, although WUE was primarily regulated by carbon fluxes in warming regimes, the contribution of water fluxes to regulating WUE increased under WD conditions. Thus, it is important to equally account for water fluxes and carbon fluxes for projecting the impacts of future climate on (agro) ecosystems.

5 | Concluding Remarks

In the current study, we analysed a whole-canopy gas exchange data set collected from multi-year experiments for wheat. However, due to constraints in the canopy gas exchange facilities, most treatments were not replicated biologically, and some environmental regimes (e.g., HS and eCO_2) and genotypic comparisons were tested with limited frequency. These limitations may reduce the robustness of some conclusions, particularly those related to HS, eCO_2, and genotypic effects. Nevertheless, our study provides synthesising evidence to answer the two research questions proposed in the Introduction. First, we demonstrated that CUE is altered by long-term high growth temperature. Moreover, CUE shows different sensitivities to T_{mean}, $T_{\mathrm{D.mean}}$, and $T_{\mathrm{N.mean}}$, and is most sensitive to $T_{\mathrm{N.mean}}$. Second, we unravelled that the response of WUE to rising temperature is dominated by the response of carbon fluxes, while the contribution of water fluxes to WUE responses increased under WD. Moreover, the relative importance of carbon fluxes is reflected in the contrasting response patterns of $\mathrm{WUE}_{\mathrm{net}}$ and $\mathrm{WUE}_{\mathrm{gross}}$. Collectively, these findings offer valuable insights into carbon budgets and water fluxes of crop plants at canopy scale and highlight the necessity for considering the variabilities in (non-) environmental factors when modelling and assessing climate change impacts on (agro) ecosystem functions and productivity.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study available at: https://zenodo.org/records/16880088.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Figure S1: Photographs of the Crop Climate Control and Gas Exchange Measurement (C3-GEM) experimental platform. **Figure S2:** Diurnal course of the photosynthetic photon flux density inside chambers across different experiments and regimes. **Figure S3:** Example of the canopy-scale Kok curves. **Figure S4:** Comparisons of crop respiration rates and mean air temperatures between dawn and dusk and between daytime and nighttime. **Figure S5:** Canopy leaf nitrogen mass on a ground area basis versus days after anthesis. **Figure S6:** Daily mean air temperature and daily integral global radiation versus days after anthesis. **Figure S7:** Daily integral night-time respiration versus days after anthesis. **Figure S8:** Daily integral daytime respiration versus days after anthesis. **Figure S9:** Daily integral total respiration versus days after anthesis. **Figure S10:** Daily integral net photosynthesis versus days after anthesis. **Figure S11:** Daily integral gross photosynthesis versus days after anthesis. **Figure S12:** Daily integral evapotranspiration versus days after anthesis. **Figure S13:** Comparisons of measured variables between chamber pairs subjected to the same treatment in EXP1997. **Figure S14:** Post-anthesis canopy gas exchange rates versus average air temperature during the period between the start of gas exchange measurements and when labile canopy N mass per unit ground area had decreased by 20%. **Figure S15:** Post-anthesis canopy gas exchange rates versus average air temperature before 20 days after anthesis. **Figure S16:** Integral values of canopy gas exchange parameters versus average air temperature for the period between the start of gas exchange measurements and when labile canopy N mass per unit ground area had decreased by 20%. **Figure S17:** Carbon and water use efficiencies versus average air temperature for the period between the start of gas exchange measurements and when labile canopy N mass per unit ground area had decreased by 20%. **Figure S18:** Carbon and water use efficiencies versus average air temperature for the period before 20 days after anthesis. **Figure S19:** Comparisons between carbon and water use efficiencies calculated only considering the period before fast senescence and considering over the period of effective canopy photosynthesis. **Figure S20:** Correlations between net CO_2 assimilation and gross CO_2 assimilation, and between canopy CO_2 assimilation and evapotranspiration. **Figure S21:** Coefficients of variation for carbon use efficiency (CUE) and water use efficiency on the basis of net (WUEnet) and gross (WUEgross) CO_2 assimilation. **Figure S22:** Daily volumetric soil water content versus days after anthesis. **Table S1:** Summary of the experiments analysed in this study. **Table S2:** Estimated post-anthesis maximum (N_{max}) and minimum (N_{base}) values of canopy leaf nitrogen mass on a ground area basis ($N_{\mathrm{c.leaf}}$), number of days after anthesis (DAA) when (N_{max} – N_{base}) decreased by 20% ($t80$), number of DAA when the rate of decrease of $N_{\mathrm{c.leaf}}$ was maximum (t_{m}), and number of DAA when $N_{\mathrm{C.leaf}}$ reached N_{base} (t_{e}).