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Hysteresis response of photosynthetic parameters in different plants to external CO₂ concentration

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Abstract: Global warming caused by increasing CO₂ concentration has caused deviations in the original patterns of plant photosynthesis and transpiration in response to CO₂. Additionally, it has exacerbated agricultural water shortages, making the requirement to improve water use efficiency (WUE) more urgent than ever. To solve this problem, this study used a rectangular hyperbolic-modified model with two successive periods in reverse order to analyze the hysteresis responses of the net photosynthetic rate (P_N), transpiration rate (E) and WUE of *Triticum aestivum*, *Rumex crispus*, and *Taraxacum officinale* to CO₂ concentration. The results show that different plant species follow obvious hysteresis laws: If hysteresis occurs between periods of different plants, P_N and E exhibit hysteresis with a positive main effect, and

WUE shows hysteresis with a negative main effect. During the same period of P_N and E , hysteresis occurring in the forward and negative periods shows a positive effect; However, for WUE, the hysteresis effect is opposite to that of P_N and E . This result provides deeper insights into the response of plant photosynthesis, transpiration, and WUE values to changes in CO_2 concentration. It helps enhance our understanding of the relationship between plant growth and climate change, offering scientific evidence to support maintaining agricultural production and ensuring food security.

Keywords: CO_2 response; Hysteresis; Net photosynthetic rate; Period; Water-use efficiency.

1.Introduction

In recent years, the global CO_2 concentration has increased from the pre-industrial level [$280 \mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$] to $400 \mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$, and is expected to reach $800 \mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$ by the end of this century ^[1]. Meanwhile, the global warming caused by rising CO_2 levels has led to deviations in the original response patterns of photosynthesis and transpiration in plants. Additionally, the trend of water resource shortages induced by global warming will severely impact industrial and agricultural production and the lives of residents. Previous research by Serôdio et al. showed that photosynthetic organisms exhibit significant differences in fluorescence parameters due to the cumulative effects of light when responding to increases or decreases in light intensity ^[2]. This phenomenon is referred to as hysteresis. Studying hysteresis may be beneficial for adjusting plant photosynthetic responses to CO_2 , helping to address issues such as reduced available water resources and abnormal photosynthetic responses in plants. Therefore, to address the global climate problems caused by the continuously increasing CO_2 concentration, it has become an urgent scientific task to fully understand the responses of photosynthetic parameters, such as crop water use efficiency, to changes in CO_2 concentration, as well as the hysteresis phenomenon that occurs during this process.

WUE is an important parameter to characterize the relationship between plant

59 water consumption and CO₂ assimilation capacity ^[3]. WUE reflects the water use
60 characteristics of plant leaves at various levels, including individual leaves, plants,
61 and populations ^[4], demonstrates the plant's sensitivity to climate change ^[5], and plays
62 a key role in the land-atmosphere water carbon cycling ^[6]. Therefore, WUE is widely
63 recognized as an important determinant of the yield of plants under stress ^[7]. The
64 factors that affect net photosynthetic rate (P_N) and transpiration rate (E) also affect
65 WUE, with CO₂ being particularly important ^[8]. Changes in its concentration directly
66 affect the plant photosynthesis and transpiration by altering the responses of stomatal
67 conductance (g_s) ^[9], which in turn affects the WUE of plants ^[10].

68 Ihnken et al. proposed that the response measured under certain light levels not
69 only depends on the instantaneous light conditions but also on the light history ^[11].
70 Building on this, in order to account for the impact of exposure time on plant
71 responses to light, Serôdio et al. suggested using hysteresis light curves (HLC) for
72 measurement ^[2]. Hysteresis light curves are an experimental approach focused on the
73 cumulative effects of light, providing information about the time-dependent nature of
74 photosynthetic light responses. By comparing the light increase phase with the light
75 decrease phase, hysteresis caused by high light exposure can be quantitatively
76 analyzed. The magnitude and direction of the hysteresis can reveal activation and
77 subsequent relaxation processes in photosynthesis induced by high light. These
78 studies offer a new perspective on understanding the response of photosynthetic
79 parameters, such as water use efficiency, to CO₂ under global warming conditions and
80 provide potential strategies to address abnormal plant responses to CO₂ under climate
81 change.

82 The Free-Air CO₂ Enrichment (FACE) system allows plants to be continuously
83 exposed to high CO₂ concentrations while maintaining natural environmental factors
84 to the greatest extent. However, Allen et al. found that the CO₂ fluctuation range in
85 the FACE system is more than 10 times greater than that under natural conditions ^[12].
86 Research by Bunce et al. suggests that the drastic CO₂ fluctuations lead to an
87 underestimation of plant photosynthesis and long-term growth under high CO₂
88 conditions ^[13]. This can be partially explained by the nonlinear response of

photosynthesis to CO₂ concentrations, as well as the reduction in the photochemical efficiency of Photosystem II and stomatal conductance due to CO₂ fluctuations, though other unknown mechanisms may also be involved. Studying the physiological mechanisms of plant responses to fluctuating CO₂ is essential for better understanding plant photosynthetic parameters in response to CO₂ concentrations and providing a theoretical foundation for related research.

Physiological status and photosynthetic characteristics, including CO₂ saturation point, CO₂ compensation point, and carboxylation efficiency, can be obtained by analyzing photosynthetic CO₂ response curves ^[14]. Existing studies on photosynthetic CO₂ response curves mostly focus on the effects of monotonic changes in CO₂ concentration on various photosynthetic parameters. Inspired by hysteresis light curves and CO₂ fluctuation, this study aims to determine the photosynthetic CO₂ response curves using a closed-loop CO₂ concentration period in reverse order.

Therefore, considering the existence of kidney-shaped and dumbbell-shaped guard cells in plants, this study used monocotyledonous and dicotyledonous materials to quantitatively evaluate the response characteristics of plant P_N , E and WUE to periodic CO₂ changes using a model method, and revealed the differences in response modes among plant species to CO₂. Furthermore, it provided theoretical support for the research and practices for optimizing water use efficiency in the context of global climate change.

2.Results

2.1 Hysteretic response to periodic changes in CO₂

In the photosynthetic CO₂ response curves obtained based on the forward and negative periods, different plants exhibited varying hysteresis responses. Taking *Triticum aestivum* as an example, in the response of P_N of *T. aestivum* to CO₂ (Fig.1a), the positivity and negativity of $H_{(CO_2)}$ shifted two times at 0 μmol(CO₂) mol⁻¹ (Corresponding to measurement sequence 6), and 300 μmol(CO₂) mol⁻¹ (Corresponding to measurement sequence 10). At the highest CO₂ concentration (800 μmol(CO₂) mol⁻¹), the P_N of the negative period was slightly lower than the

corresponding value of the forward period with $H_{(\text{CO}_2)}$ of $0.908 \mu\text{mol m}^{-2} \text{s}^{-1}$, whereas at the beginning of the forward period, the forward and negative periods showed the most significant difference with $H_{(\text{CO}_2)}$ reaching $-2.621 \mu\text{mol m}^{-2} \text{s}^{-1}$. Similarly, the *Rumex crispus*, and *Taraxacum officinale* also showed significant differences in both the forward and negative periods, and exhibited a hysteresis effect (Fig.1b,c).

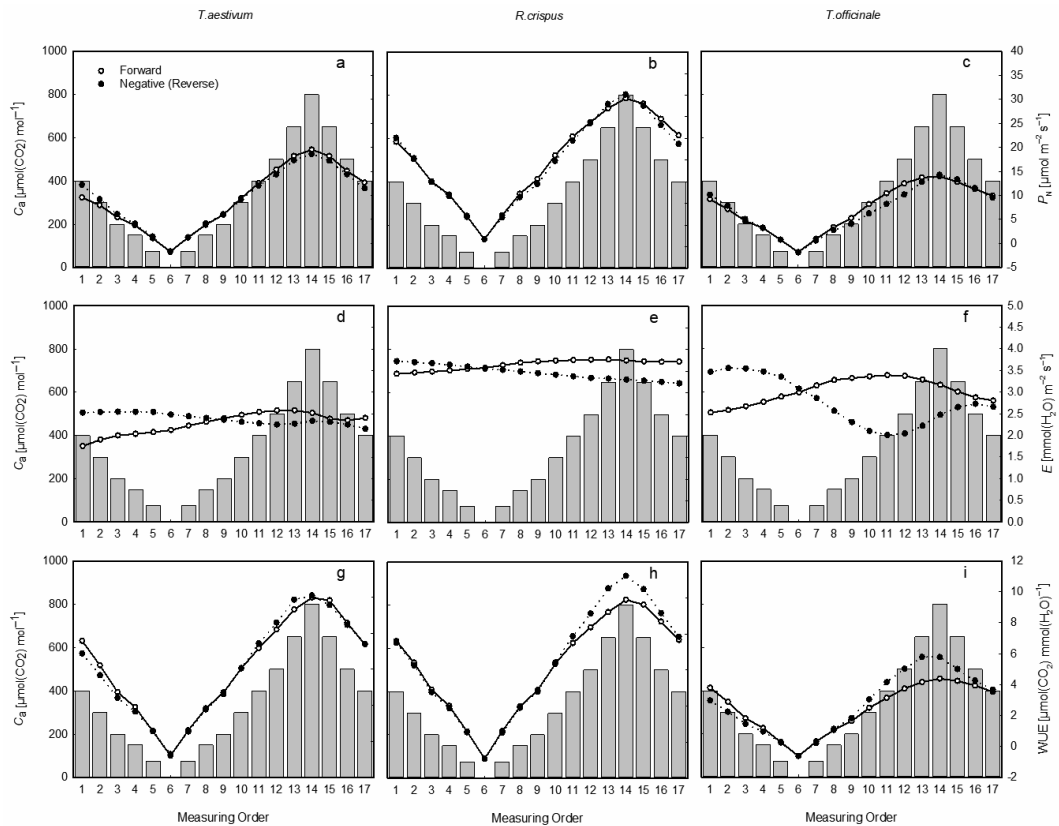


Fig.1 Responses of P_N (a-c), E (d-f) and WUE (g-i) to changes in CO_2 concentration in *T. aestivum*, *R. crispus*, and *T. officinale*. The gray bars represent CO_2 concentration, with the solid line indicating the forward period, measured in the order of 1 to 17; the dashed line is the negative period, measured in the opposite order of the forward period, i.e., 17 to 1. Each photosynthetic parameter was averaged from five biological replicates. To clearly demonstrate the hysteresis, the vertical error bars are omitted, with specific values provided in Supplementary Table 1-9. C_a —Atmospheric CO_2 concentration; P_N —Net photosynthetic rate; E —Transpiration rate; WUE—Water-use efficiency.

Although the response of E of *T. aestivum* to CO_2 was relatively smooth and did not fluctuate as significantly as P_N , there was a significant difference between the positive and negative periodic responses to CO_2 concentration changes. The $H_{(\text{CO}_2)}$ of

E shifted from negative to positive when the CO_2 concentration was $200 \mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ (Fig.1d, corresponding to measurement sequence 9). When the CO_2 concentration exceeded the natural range ($400\text{--}800 \mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$), the E hysteresis response was different from in the $0\text{--}400 \mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ range, but the magnitude of the E response remained consistently small throughout. Similarly, these also occurred in *R. crispus* and *T. officinale* (Fig.1e,f).

In the response of the WUE of the *T. aestivum* to CO_2 (Fig.1g), the positivity and negativity of $H_{(\text{CO}_2)}$ shifted five times at $75 \mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ (Corresponding to measurement sequence 5), $75 \mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ (Corresponding to measurement sequence 7), $300 \mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ (Corresponding to measurement sequence 10), $650 \mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ (Corresponding to measurement sequence 15), and $400 \mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ (Corresponding to measurement sequence 17). However, despite the frequent fluctuations in the $H_{(\text{CO}_2)}$ values of *T. aestivum*, the differences between the values of the forward and negative periods within the measurement range are not significant. In contrast, for *R. crispus* and *T. officinale*, the $H_{(\text{CO}_2)}$ values clearly turned negative at $300 \mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ and $150 \mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$, respectively. This indicates that at moderate to high CO_2 levels, the negative period of WUE for *R. crispus* and *T. officinale* is significantly higher than during the forward period. Similar results were obtained by measuring the WUE in the forward and negative periods during the morning and afternoon, respectively, to exclude the effect of the continuous measurement (Supplementary Fig.1), thus minimizing any influence of the end of the forward period on the beginning of the negative period.

2.2 The response differences of different species within period

The values of P_N and E at the end of the period were greater than those at the beginning, regardless of whether they were the forward or negative periods. In contrast, WUE decreased at the end of the period in all plants except *R. crispus*, suggesting that periodic changes in CO_2 may reduce water use efficiency. The same pattern of within-period hysteresis was observed across plants (Table 1): If there was significant hysteresis of P_N , E and WUE within a single period for different plants

($H_w > 0.1$), both P_N and E exhibited positive hysteresis in both the forward and negative periods, while WUE showed negative hysteresis.

2.3 Hysteresis effects of P_N in different plant species within period

No significant hysteresis was found in the P_N response within CO₂ periods, except for a positive hysteresis in the forward period for *T. aestivum* (Fig.1a-c). The H_w values for *T. aestivum* were 0.244 in the forward and 0.058 in the negative period. For *R. crispus*, the H_w values were 0.074 in the forward and 0.068 in the negative period. For *T. officinale*, the H_w values were 0.072 in the forward and 0.062 in the negative period.

2.4 Hysteresis effects of E in different plant species within period

Differences in the E response were observed between monocots and dicots within a single CO₂ period (Fig.1d-f). The H_w values for *T. aestivum* in the forward and negative periods were 0.271 and 0.148, respectively; for *R. crispus*, the H_w values were 0.075 and 0.137; and for *T. officinale*, the H_w values were 0.099 and 0.233. These results suggest that all plants exhibited positive hysteresis in the negative period. However, while both dicots showed no significant hysteresis in the forward period, *T. aestivum*, a monocot, exhibited negative hysteresis in the forward period.

2.5 Hysteresis effects of WUE in different plant species within period

In the WUE responses of different plants within the period (Fig.1g-i), the H_w values for *T. aestivum* in the forward and negative periods were -0.034 and -0.098, respectively; for *R. crispus*, the H_w values were 0.011 and -0.057; and for *T. officinale*, the H_w values were -0.069 and -0.189. Notably, only *T. officinale* exhibited negative hysteresis in the negative period.

2.6 Hysteresis effects of g_s in different plant species within period

Differences in the g_s response were also observed between monocots and dicots within a single CO₂ period (Fig.2a,b,c). The H_w values for *T. aestivum* in the forward

and negative periods were 0.149 and -0.158, respectively; for *R. crispus*, the H_w values were -0.026 and -0.079; and for *T. officinale*, the H_w values were -0.020 and -0.207. These results indicate that g_s of monocots exhibits significant hysteresis in response to periodic CO_2 changes. Moreover, according to the Table 1 and Fig.2, the response trend of g_s to CO_2 is highly consistent with that of E to CO_2 .

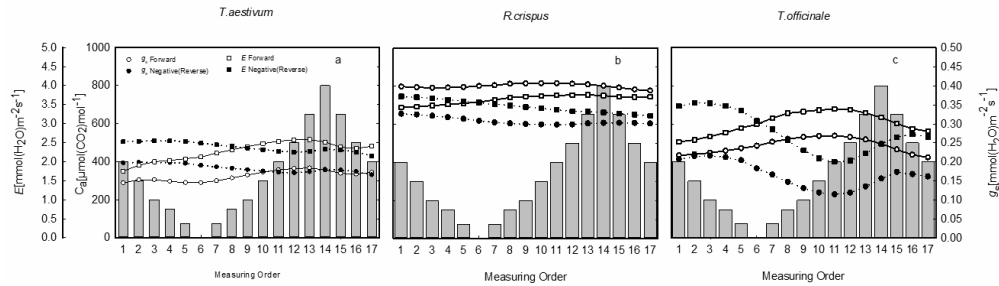


Fig.2 The response of g_s and E of *T. aestivum* (a), *R. crispus* (b) and *T. officinale* (c) to forward and negative periodic changes in CO_2 concentration. The gray bars represent the CO_2 levels, with the solid line indicating the forward period and the dashed line representing the negative period. g_s is denoted by circles, and E is represented by squares. Both g_s and E were obtained from the average of five biological replicates. C_a —Atmospheric CO_2 concentration; g_s —Stomatal conductance; E —Transpiration rate.

2.7 Hysteresis effects in the range of monotonic CO_2 changes (The hysteresis between periods)

In Fig.3, the R^2 of the established model exceeded 0.98, $P < 0.001$, indicating a highly significant fit. For P_N of different plants (Fig.3a-c), only *T. officinale* exhibited an increase followed by a decrease in response to rising CO_2 concentration during the forward period, whereas P_N in all other plants increased consistently across the range. E of all plants reached its extreme value within the range (Fig.3d-f). The response of WUE varied depending on the differing patterns of P_N and E , but the WUE values of different plants all exhibited an overall increase across different periods (Fig.3g-i). There were similar hysteresis response patterns in P_N , E and WUE of different plants: In cases where obvious hysteresis was observed in the positive and negative $H_{(CO_2)}$ ranges of P_N , E and WUE of different plants ($H_b > 0.1$), P_N and E exhibited positive hysteresis or a mixed negative-positive hysteresis response with positive hysteresis as

the main effect, while WUE showed a mixed hysteresis response of positive followed by negative hysteresis, with negative hysteresis being the main effect.

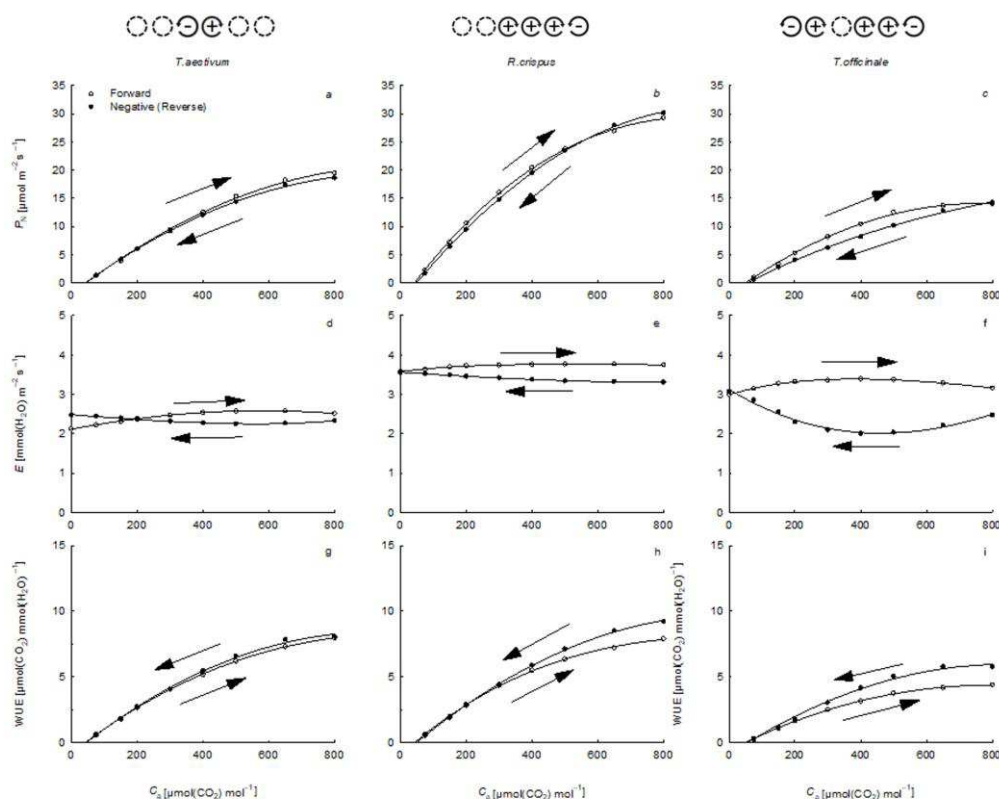


Fig.3 Response of photosynthetic parameters to forward and negative periods in *T. aestivum*, *R. crispus*, and *T. officinale*. The curve modelling range covers all CO₂ levels, and the CO₂ level increases monotonically from left to right within the range. The arrow direction indicates the order of CO₂ level measurement, and the positive (clockwise) and negative (counterclockwise) symbols at the top indicate the hysteresis direction. *a-c*, *d-f* and *g-i* show the responses of different plant species for P_N , E and WUE, respectively. Each photosynthetic parameter was averaged from five biological replicates. C_a –Atmospheric CO₂ concentration; P_N –Net photosynthetic rate; E –Transpiration rate; WUE–Water-use efficiency.

2.8 Hysteresis effects of P_N of different plants in the range of monotonic CO₂ changes

There are significant differences in the hysteresis effects of P_N of different plants within the range of monotonic CO₂ changes (Fig.3*a-c*). In the response of P_N of different plants, the $H_{(CO_2)}$ of *T. aestivum* increased continuously. It was negative in

the range of 0–187 $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ and became positive at 187 $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$. However, the negative and positive hysteresis indexes H_b were only -0.094 and 0.036 , respectively, indicating no significant hysteresis. The $H_{(\text{CO}_2)}$ of *R. crispus* first increased and then decreased, reaching positive and negative extremes at 231 and 800 $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$, respectively, with H_b values of 0.077 and -0.046 , showing no significant hysteresis. The $H_{(\text{CO}_2)}$ of *T. officinale* reached its negative extreme at 0 $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ ($H_b = -0.109$) and increased with CO_2 concentration from 0 to 431 $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$. The $H_{(\text{CO}_2)}$ became positive at 25 $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$, peaked at 431 $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ ($H_b = 0.192$), and then gradually decreased, becoming negative at 778 $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$. However, H_b was only 0.019 in the range of 778–800 $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$. Finally, a mixed negative-positive hysteresis response was observed across this range.

2.9 Hysteresis effects of E of different plants in the range of monotonic CO_2 changes

In the response of E in different plants, all the negative extremes of $H_{(\text{CO}_2)}$ occurred at 0 $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ (Fig.3d-f). For *T. aestivum*, $H_{(\text{CO}_2)}$ increased, then decreased, shifted to positive at 154 $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$, and reached a maximum at 593 $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$, with H_b values of -0.148 and 0.177 , respectively, forming a negative-positive hysteresis response. In contrast, *R. crispus* showed no negative hysteresis, with $H_{(\text{CO}_2)}$ increasing and then decreasing, peaking at 718 $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ ($H_b = 0.115$), indicating a positive hysteresis. For *T. officinale*, $H_{(\text{CO}_2)}$ shifted to positive at 18 $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$, peaked at 512 $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ with an H_b of 0.525 , showing clear positive hysteresis. However, its H_b in the negative range was only -0.045 , which can be considered as showing no significant negative hysteresis response.

2.10 Hysteresis effects of WUE of different plants in the range of monotonic CO_2 changes

In the response of WUE in different plants (Fig.3g-i), *T. aestivum* showed a

decrease followed by an increase in $H_{(\text{CO}_2)}$, shifting to negative at 312 $\mu\text{mol}(\text{CO}_2)$ mol^{-1} , and reaching positive and negative extremes at 0 and 609 $\mu\text{mol}(\text{CO}_2)$ mol^{-1} , respectively. However, the H_b values were only 0.097 and -0.011, indicating no significant hysteresis. For *R. crispus*, $H_{(\text{CO}_2)}$ increased and then decreased, peaking at 57 $\mu\text{mol}(\text{CO}_2)$ mol^{-1} , shifting to negative at 172 $\mu\text{mol}(\text{CO}_2)$ mol^{-1} , with the most pronounced negative hysteresis effect at the highest CO_2 concentration ($H_b = -0.171$), forming a mixed positive-negative hysteresis. *T. officinale* exhibited a similar pattern to *R. crispus*, with a strong positive hysteresis at the lowest CO_2 concentration ($H_b = 0.288$). $H_{(\text{CO}_2)}$ shifted to negative at 87 $\mu\text{mol}(\text{CO}_2)$ mol^{-1} and reached a negative extreme at the highest CO_2 concentration ($H_b = -0.252$), forming a mixed positive-negative hysteresis.

3. Discussion

The results of this study indicate that hysteresis occurred in the response of P_N , E and WUE to periodic changes in ambient CO_2 across various plant species, including both monocots and dicots. Significant differences in the hysteresis responses were observed between species and parameters.

Overall, when hysteresis occurred between periods, the E of monocot *T. aestivum* exhibited a mixed negative-positive hysteresis response, with positive hysteresis being the dominant effect. In contrast, E of dicots showed a consistent positive hysteresis response. For P_N , no significant hysteresis was observed within periods, except in *T. aestivum*, where a positive hysteresis was seen in the forward period. Additionally, E in different plant species showed positive hysteresis in the negative period, although this response was less pronounced in the dicots in the forward period. To obtain more accurate conclusions, it may be necessary to use a greater variety of plant species as subjects for further research.

For WUE, the negative hysteresis peaked at the highest CO_2 concentration for both dicots between periods. Both showed a positive-negative mixed hysteresis response, with the negative hysteresis as the main effect. In contrast, the monocot *T. aestivum* exhibited no significant hysteresis, although $H_{(\text{CO}_2)}$ also showed a positive

-to-negative trend. Additionally, by comparing the 400-800-400 $\mu\text{mol (CO}_2\text{) mol}^{-1}$ segment of the forward and negative periods (Fig.1g-i), it can be observed that the WUE response to CO_2 changes is more pronounced in the negative period, especially in *R. crispus* and *T. officinale*. This is because the forward period first reduces the CO_2 concentration, leading to an increase in stomatal conductance, which results in a higher transpiration rate within the corresponding CO_2 range of the forward period, thus causing a weaker WUE response to CO_2 changes in the forward period. Within a single period, significant hysteresis was only observed in *T. officinale*, which exhibited negative hysteresis during the negative period. No significant hysteresis was found in the forward period of *T. officinale* or in any period for the other plants. The absence of significant hysteresis in most cases suggests that plants can rapidly respond to up- or down-regulation of CO_2 concentration during activation and adaptation, leading to minimal loss in photosynthetic yield during the CO_2 response.

In this study, *T. officinale* had lower P_N compared to the other plants, which may explain its significant hysteresis. When we compared monocots like *T. aestivum* and dicots like *R. crispus*, it was found that dicotyledons generally exhibited a greater hysteresis response. This difference may be attributed to potential of monocots to adapt to environmental changes by increasing stomatal density ^[16], which allow them to respond more quickly and efficiently to environmental changes in open habitats.

The change in VPD at the end of the forward and negative periods was less than 10% from the initial value, and the response patterns of E and g_s were similar (Fig.2), which aligns with $g_s = E / \text{VPD}$ ^[17]. This suggests that the observed changes in E were primarily driven by stomatal factors. Existing research indicates that although leaf WUE shows a linear relationship with sub-stomatal CO_2 concentration, there is no significant correlation between leaf WUE and mesophyll conductance ^[18,19]. This suggests that the variation in WUE may have a weak correlation with E and is mainly driven by P_N .

Furthermore, this study found that the response of g_s to CO_2 in the plant leaves was not limited to a nonlinear decrease, but also exhibited other patterns, which is consistent with the findings of Li et al. and Batke et al. ^[20,21]. For the forward period,

g_s initially increased and then decreased with increasing CO_2 concentration. This response has proven difficult to predict through modeling ^[22], and the underlying mechanisms remain unclear. When we compared the response of E across different plants, it was found that E of both dicots exhibited positive hysteresis, whereas E of *T. aestivum* showed a significant negative-positive mixed hysteresis response, with positive hysteresis predominating. Additionally, the E of dicots was higher than that of monocots, which contrasts with the conclusion of Hasegawa et al. ^[23]. Whether the higher P_N in monocots is achieved at the cost of great water consumption requires further investigation.

When we compared the WUE responses of different plants within periods, it was found that all dicots exhibited negative hysteresis, peaking at the highest CO_2 concentration. In contrast, the hysteresis response in monocots was either not significant or decreased with increasing CO_2 concentration, suggesting that monocot WUE is more adaptive to CO_2 changes. This finding supports the conclusions of Hu et al. ^[16], which highlight that grassland monocots enhance their ability to adapt to environmental changes through abundant stomatal morphological diversity. The response patterns of P_N and WUE in different plants were extremely similar, primarily because the magnitude of the E response to CO_2 concentration was relatively small within a single CO_2 period, even though E exhibited significant hysteresis characteristics. This is similar to the findings of Li et al. ^[24]. Their research showed that elevated CO_2 had little effect on the stomatal conductance and transpiration rate of wild-type barley, but it increased the photosynthetic rate, leading to higher WUE at the stomatal, leaf, and whole plant levels. Overall, the response of transpiration to CO_2 shows significant differences compared to photosynthesis. This is because CO_2 is a key raw material for photosynthesis, so when CO_2 concentration increases, the rate of photosynthesis also increases. However, stomatal opening and closing are influenced by multiple factors such as water availability, light intensity, and air humidity. Although an increase in CO_2 concentration may lead to a greater degree of stomatal closure to reduce water loss, if water supply is sufficient, the closure of stomata may not be enough to significantly affect the transpiration rate. Furthermore,

the results of this study indicate that the changes in E are primarily driven by stomatal factors, and the correlation between leaf WUE and stomatal conductance is weak. Therefore, changes in WUE were mainly driven by P_N , while E had a lesser impact. Additionally, the magnitude of WUE hysteresis was mainly determined by E , given that P_N hysteresis had a minimal effect. This suggests that efforts to improve water conservation should focus more on improving the photosynthetic rate rather than solely on reducing the transpiration rate.

The results of this study show that elevated CO_2 concentration can enhance WUE, which is consistent with the findings of many previous studies [24-27]. Li et al. found that this is mainly due to the increase in photosynthetic rate and the reduction in stomatal conductance and transpiration rate caused by elevated atmospheric CO_2 , leading to higher WUE, with the underlying mechanism related to ABA levels in plants [25]. Chai et al. found that between 1982 and 2015, every 100 ppm increase in atmospheric CO_2 led to a $5.65 \pm 1.14\%$ increase in global WUE [28]. This suggests that agricultural production could achieve a higher WUE by increasing CO_2 concentration in the field.

It is worth noting that the response of WUE to CO_2 is also influenced by factors such as soil water content, research scale, plant growth rate, and plant genotype. Li et al. found that the increase in WUE induced by elevated CO_2 is attributed to the combined effects of P_N and E , whereas the increase in WUE due to water deficit is associated with a decrease in E [24]. Water deficit reduces the impact of CO_2 elevation on plants. Research by Knauer et al. shows that as the scale increases, the sensitivity of WUE to CO_2 decreases, with the WUE trend at larger scales being much smaller than the estimates from individual forest ecosystems [29]. Li et al. found that slower-growing species generally have lower WUE than faster-growing species, but the increase in WUE due to elevated CO_2 is greater in the former [26]. Soba et al. found that low WUE genotypes, due to their higher stomatal conductance, have a larger response to increased CO_2 in terms of leaf daily photosynthetic carbon gain, while high WUE genotypes compensate for this difference by increasing leaf area [30]. These factors will influence the practical application of maintaining high WUE

through elevated CO₂ in agricultural production. Therefore, how to establish appropriate plant cultivation conditions remains an area for further research.

Notably, all dicots displayed a stable negative hysteresis in WUE, indicating that dicots can effectively limit water loss while maintaining a high CO₂ assimilation rate, which is an important physiological trait for avoiding excessive drought stress.

To date, there is limited research on the effects of photosynthetic hysteresis in plants, with most studies focusing on the asymmetric response of plant photosynthesis to light. This emphasis on light response complicates the analysis, as it often overlooks the daily light hysteresis caused by fluctuations in natural light intensity, which can influence the magnitude of the observed hysteresis. Furthermore, no studies have yet examined the differences in photosynthetic hysteresis across plant systems influenced by CO₂. This study provides a novel perspective by exploring the variations in CO₂-induced hysteresis responses between monocots and dicots, offering new directions for future research.

In this study, the monotonic range comparison model of Serôdio et al. [2] was referred to when comparing the positive and negative hysteresis within the period of photosynthetic parameters, but their *H* index was not utilized. The monotonic range comparison model was used because it applies a symmetric approach using the same CO₂ concentrations for both the increasing and decreasing phases, allowing for a direct comparison of parameter measurements at identical CO₂ levels. This model is particularly suited for observing hysteresis effects. On the other hand, using their *H* index would pose challenges, such as its inability to express the direction of mixed hysteresis and the risk of misrepresenting the degree of hysteresis. In cases of mixed hysteresis, the *H* index could yield a small value, even for a response exhibiting significant hysteresis, due to the large range integrals involved. In this study, CO₂ levels ranged from 0 to 800 μmol(CO₂) mol⁻¹, with each level applied for 120 s, allowing plants to reach high or even supersaturated CO₂ levels in sufficient time to highlight the main response differences of photosynthetic parameters. However, the degree of change in *g_s* is closely related to the duration of continuous CO₂ exposure. The activation, adaptation, and inhibition of photosynthetic CO₂ responses, which are

linked to stomatal changes and internal enzyme activities, further complicate this relationship. Therefore, the effects of different CO₂ levels on photosynthetic parameters are inherently tied to their application time (CO₂ history) and concentration gradient. To better optimize the response of photosynthetic parameters, further research is needed to adjust CO₂ concentrations and exposure durations effectively.

The various photosynthetic parameters of different plants fall within distinct ranges (e.g., P_N ranges from approximately 0–35 $\mu\text{mol m}^{-2} \text{s}^{-1}$, E ranges from approximately 0–5 $\text{mmol}(\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}$, and WUE ranges from approximately 0–15 $\mu\text{mol}(\text{CO}_2) \text{mmol}(\text{H}_2\text{O})^{-1}$ in the plants selected in this study). As a result, the unnormalized H_b and H_w indexes may not adequately capture the differences between these parameters. Additionally, normalization methods, such as the H index defined by Serôdio et al. [2], may overcompensate, leading to hysteresis indexes that do not reflect the true direction or magnitude of the effect. Despite efforts to refine the H index, it remains insufficient to fully characterize aspects such as the curve shape or the dynamic process of hysteresis with CO₂ concentration. Therefore, further experiments and studies are needed to more accurately and comprehensively characterize the hysteresis in different photosynthetic parameters. This would help clarify certain features of the hysteresis phenomenon not captured by classical response curves, elucidate the underlying mechanisms, and establish a more detailed response model.

This study demonstrated significant hysteresis effects in the photosynthetic parameters P_N , E and WUE across different plant species. Notably, dicot species such as *R. crispus* and *T. officinale* exhibited stronger hysteresis responses compared to monocots like *T. aestivum*. Despite these differences, several consistent patterns emerged:

(1) Between periods, when hysteresis occurred, for both P_N and E , hysteresis was generally positive or showed a mixed negative-positive response, with positive hysteresis being the dominant effect. WUE in dicots displayed a mixed positive-negative hysteresis response, with negative hysteresis peaking at the highest

CO₂ concentration. In contrast, monocots like *T. aestivum* showed no significant hysteresis in WUE.

(2) Within the same period, no significant hysteresis was observed in the different periods of P_N , WUE and the forward period of E in dicots. When hysteresis was present, it was positive for both P_N and E in both forward and negative periods across different plants, while WUE exhibited the opposite hysteresis pattern (negative) compared to P_N and E .

The investigation and quantification of hysteresis in photosynthetic parameters like P_N , E and WUE in monocots and dicots under varying CO₂ levels provide valuable insights into how different plants adapt to increasing global CO₂ concentrations and periodic CO₂ fluctuations. This knowledge is crucial for understanding plant responses to environmental changes, improving photosynthetic efficiency in agricultural practices, and addressing challenges related to water scarcity and enhanced plant growth and development under changing climate conditions.

4.Methods

4.1 Plant material and growth conditions

The test materials were *Triticum aestivum* (Monocots, Gramineae, *Triticum* genus) and *Rumex crispus* (Dicots, Polygonaceae, *Rumex* genus), *Taraxacum officinale* (Dicots, Compositae, *Taraxacum* genus), grown at the North Campus of Northwest A&F University (Yangling District, Xianyang City, Shaanxi Province, 34°16' 56.24" N, 108°4' 27.95" E). The average temperature ranged from 9°C to 20°C, and the light intensity was 1,200 $\mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$, and the total annual rainfall is 496.1 mm, with rainfall concentrated between July and October. All selected plants showed good growth and high representativeness.

The plant collection was conducted as part of Northwest A&F University's academic research activities. The samples were obtained from university property, which is not a protected area, and involved common species that are routinely managed by the grounds staff. To the best of our knowledge, no specific permits were required for this type of academic collection on institutional property, in accordance

with standard practices at our institution. We confirm that the collection complied with all relevant regulations and had no adverse impact on the plant community.

4.2 Determination of parameters

To avoid the impact of water stress on the measurements, the CO₂ response curve and parameters such as P_N , g_s , intercellular CO₂ concentration (C_i), E , Vapor Pressure Deficit (VPD) and leaf temperature (T_{leaf}) were measured using a portable photosynthetic instrument (*LI-6400*, *LI-COR Inc.*, USA) on sunny days after rain from 9:00 to 11:30 and in the afternoon from 14:00 to 16:00. In this study, 17 CO₂ concentrations (Forward period, 400, 300, 200, 150, 75, 0, 75, 150, 200, 300, 400, 500, 650, 800, 650, 500, and 400 $\mu\text{mol (CO}_2\text{) mol}^{-1}$) were set, each maintained for 120 s, illuminated by a built-in red and blue light source with an intensity of approximately 1,200 $\mu\text{mol (photon) m}^{-2} \text{ s}^{-1}$, simulating natural light. The measurement order during the negative period was reversed compared to the forward period.

Five independent plants with similar growth status and spaced more than 400 meters apart were selected as biological replicates for each species, and for each plant, the widest part of the largest intact leaf was measured during both the forward and negative periods, with the average of five plants representing the response of photosynthetic parameters to CO₂ in that species.

4.3 Model building and Data processing

4.3.1 A fitting model for the response curve of various photosynthetic parameters to CO₂

As the rectangular hyperbola-modified model provides a better fit than other models ^[15], this model was selected for the response of P_N , E , WUE and other plant photosynthetic parameters to ambient CO₂ concentration:

$$P_N = a \frac{1 - bC_a}{1 + cC_a} C_a - d \quad (1)$$

where a represents the initial carboxylation efficiency of the CO₂ response curve, C_a is the atmospheric CO₂ concentration, b , c and d are coefficients (the units of b and c are $\text{mol } \mu\text{mol}^{-1}$, and the unit of d is $\mu\text{mol m}^{-2} \text{ s}^{-1}$). Similarly, the model fitting method

for E and WUE follows that in equation (1).

4.3.2 Water use efficiency in the leaf level

$$WUE = \frac{P_N}{E} \quad (2)$$

4.3.3 Quantitative description of the hysteresis effect between periods

In this study, a non-coincidence phenomenon in response to forward and negative periodic changes in CO_2 concentration was observed in the main parameters such as P_N , E and WUE. Following Serôdio et al. [2], this is referred to as the hysteresis effect in response to CO_2 concentration. To evaluate this effect quantitatively, the hysteresis function $H_{(CO_2)}$ was obtained by subtracting the forward and negative periodic fitting model equations of the same photosynthetic parameter:

$$H_{(CO_2)} = P_N^+ - P_N^- \quad (3)$$

where "+" represents the forward period, and "-" represents the negative period. The calculation method of $H_{(CO_2)}$ for E and WUE is the same as in equation (3). Information on the cumulative effect of CO_2 concentration on plants can be obtained from the magnitude and direction of $H_{(CO_2)}$. If $H_{(CO_2)} > 0$, it means that the value of the photosynthetic parameter in the forward period is higher than in the negative period, and vice versa. When hysteresis occurs, $H_{(CO_2)} > 0$ is considered to be a positive hysteresis; otherwise, it is negative hysteresis.

To determine whether hysteresis occurs and to characterize the hysteresis response of different plants, the maximum absolute value of $H_{(CO_2)}$ within the positive and negative hysteresis ranges of different photosynthetic parameters is obtained. This value is then divided by the maximum absolute value of the corresponding parameter at the same CO_2 level in the response curve to calculate the hysteresis index between periods, H_b :

$$|H_b| = \frac{|H_{(CO_2)}|_{\max}}{|P_N|_{\max}}, \begin{cases} H_{(CO_2)} > 0, H_b > 0 \\ H_{(CO_2)} < 0, H_b < 0 \end{cases} \quad (4)$$

H_b can be used to compare the hysteresis between the forward and negative periods in different plants, with its positivity and negativity remaining consistent with $H_{(CO_2)}$. If

the absolute value of H_b is less than 0.1, there is considered to be no hysteresis effect. The calculation of H_b for E and WUE follows the same method as in equation (4).

4.3.4 Quantitative description of the hysteresis effect within the period

Similarly to H_b , the initial-state values of plant photosynthetic parameters such as P_N , E , and WUE are subtracted from the end-state values, then divided by the maximum response value at the corresponding CO_2 level for normalization. The normalized result is defined as the hysteresis index within the period (H_w):

$$H_w = \frac{P_{N(C_t)} - P_{N(C_0)}}{P_{N_{max}}} \quad (5)$$

where C_t and C_0 are the final and initial CO_2 concentration, respectively. Equation (5) is also used to calculate H_w for E and WUE. H_w enables the comparison of the hysteresis across different plants following periodic CO_2 changes, where $H_w > 0$ presents positive hysteresis and $H_w < 0$ presents negative hysteresis. When the absolute value of H_w is less than 0.1, no significant hysteresis is considered.

4.4 Data statistics and analysis

Wolframalpha was used for data analysis and Sigmaplot 14.0 for graphing, while IBM SPSS Statistics 25 was used for significance difference analysis, with the Waller-Duncan method applied for the significance analysis.

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Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Abbreviations:

C_a	Atmospheric CO ₂ concentration
C_i	Intercellular CO ₂ concentration
C_t	Final CO ₂ concentration
C_0	Initial CO ₂ concentration
E	Transpiration rate
g_s	Stomatal conductance
H_b	The hysteresis index between periods
$H_{(CO_2)}$	The function of hysteresis
H_w	The hysteresis index within the period
P_N	Net photosynthetic rate
T_{leaf}	leaf temperature
VPD	Vapor pressure deficit
WUE	Water-use efficiency

Highlights:

- P_N , E and WUE of different plants exhibit hysteresis responses to CO₂ periodic changes.
- The difference of response patterns between monocot and dicot plants is large.
- Monocot or dicot plants followed a similar hysteresis pattern, respectively.

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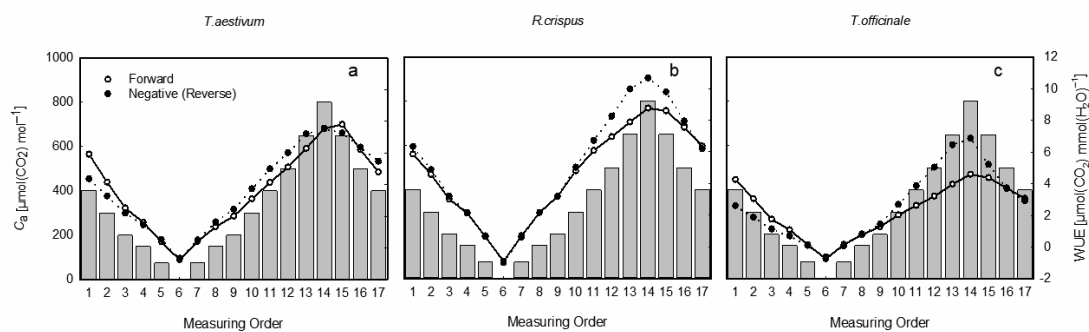
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Species	Parameters	H_b index		H_w index	
		Positive	Negative	Forward	Negative
<i>T. aestivum</i>	P_N	0.036 ⁽²⁾	-0.094 ⁽¹⁾	0.244	0.058
	E	0.177 ⁽²⁾	-0.148 ⁽¹⁾	0.271	0.148
	WUE	0.097 ⁽¹⁾	-0.011 ⁽²⁾	-0.034	-0.098
	g_s	0.061 ⁽²⁾	-0.238 ⁽¹⁾	0.149	-0.158
<i>R. crispus</i>	P_N	0.077 ⁽¹⁾	-0.046 ⁽²⁾	0.074	0.068
	E	0.115	-	0.075	0.137
	WUE	0.319 ⁽¹⁾	-0.171 ⁽²⁾	0.011	-0.057
	g_s	0.266	-	-0.026	-0.079
<i>T. officinale</i>	P_N	0.192 ⁽²⁾	-0.109 ⁽¹⁾	0.072	0.062
	E	0.525 ⁽²⁾	-0.045 ⁽¹⁾	0.099	0.233
	WUE	0.288 ⁽¹⁾	-0.252 ⁽²⁾	-0.069	-0.189
	g_s	0.573	-	-0.020	-0.207

H_b —The hysteresis index between periods; H_w —The hysteresis index within the period; P_N —Net photosynthetic rate; E —Transpiration rate; WUE—Water-use efficiency; g_s —Stomatal conductance. For the H_b index⁽ⁱ⁾, positive, negative refer to the positivity and negativity of H_b , respectively, and i refers to the order of occurrence of H_b with the corresponding positivity or negativity in the curve. For the H_w index, forward, negative refer to the measuring period as forward and negative period, respectively.



Supplementary Fig.1 Response of WUE to forward and negative periodic variation of CO_2 concentration in *T. aestivum*, *R. crispus*, and *T. officinale*. The gray bars represent the applied CO_2 levels. The forward period (solid line) was measured in the morning, while the negative period (dashed line) was measured in the afternoon and in reverse order to the forward period. C_a —Atmospheric CO_2 concentration; WUE—Water-use efficiency.

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Supplementary Table 1 Response of P_N of *T. aestivum* to forward and negative periodic changes in CO₂ concentration

Measuring Order	$P_N[\mu\text{mol m}^{-2} \text{s}^{-1}]$		
	Forward	Negative (Reverse)	$H_{(\text{CO}_2)}$
1	$9.579 \pm 10^{-4\text{de}}$	$12.200 \pm 1.539^{\text{bc}}$	$-2.621 \pm 1.437^{\text{c}}$
2	$7.966 \pm 0.687^{\text{ef}}$	$9.209 \pm 0.838^{\text{d}}$	$-1.243 \pm 0.995^{\text{bc}}$
3	$5.448 \pm 0.482^{\text{fg}}$	$6.088 \pm 0.434^{\text{e}}$	$-0.640 \pm 0.622^{\text{abc}}$
4	$3.799 \pm 0.265^{\text{gh}}$	$4.122 \pm 0.203^{\text{e}}$	$-0.323 \pm 0.332^{\text{ab}}$
5	$1.094 \pm 0.029^{\text{hi}}$	$1.431 \pm 0.030^{\text{f}}$	$-0.337 \pm 0.140^{\text{ab}}$
6	$-1.678 \pm 0.076^{\text{i}}$	$-1.757 \pm 0.032^{\text{g}}$	$0.079 \pm 0.206^{\text{ab}}$
7	$1.228 \pm 0.043^{\text{hi}}$	$1.282 \pm 0.037^{\text{f}}$	$-0.053 \pm 0.399^{\text{ab}}$
8	$3.892 \pm 0.420^{\text{gh}}$	$4.156 \pm 0.243^{\text{e}}$	$-0.264 \pm 0.894^{\text{ab}}$
9	$5.988 \pm 0.901^{\text{fg}}$	$6.033 \pm 0.505^{\text{e}}$	$-0.045 \pm 1.111^{\text{ab}}$
10	$9.411 \pm 2.203^{\text{de}}$	$9.165 \pm 1.021^{\text{d}}$	$0.246 \pm 1.497^{\text{ab}}$
11	$12.538 \pm 4.220^{\text{cd}}$	$12.037 \pm 1.652^{\text{bc}}$	$0.501 \pm 1.927^{\text{ab}}$
12	$15.349 \pm 6.020^{\text{bc}}$	$14.383 \pm 2.115^{\text{b}}$	$0.965 \pm 1.926^{\text{a}}$
13	$18.192 \pm 7.896^{\text{ab}}$	$17.330 \pm 2.529^{\text{a}}$	$0.862 \pm 1.764^{\text{a}}$
14	$19.526 \pm 8.845^{\text{a}}$	$18.618 \pm 2.336^{\text{a}}$	$0.908 \pm 2.210^{\text{a}}$
15	$18.192 \pm 7.698^{\text{ab}}$	$17.253 \pm 1.833^{\text{a}}$	$0.940 \pm 1.547^{\text{a}}$
16	$15.098 \pm 4.915^{\text{bc}}$	$14.369 \pm 0.949^{\text{b}}$	$0.729 \pm 1.142^{\text{ab}}$
17	$12.679 \pm 3.259^{\text{cd}}$	$11.491 \pm 10^{-4\text{cd}}$	$1.188 \pm 1.005^{\text{a}}$

$H_{(\text{CO}_2)}$ —The function of hysteresis; P_N —Net photosynthetic rate; In the forward and negative periods, the SD of P_N are generated by dividing by the corresponding values at the CO₂ level of 400 $\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$ to normalize to eliminate individual differences and multiply them with the average value; The calculation of $H_{(\text{CO}_2)}$ has not undergone additional normalization.

Supplementary Table 2 Response of P_N of *R. crispus* to forward and negative periodic changes in CO₂ concentration

Measuring Order	$P_N[\mu\text{mol m}^{-2} \text{s}^{-1}]$		
	Forward	Negative (Reverse)	$H_{(\text{CO}_2)}$
1	19.211±10 ^{-4ef}	20.100±1.409 ^{bcd}	-0.889±1.519 ^{de}
2	15.220±0.205 ^f	15.407±0.639 ^{ef}	-0.187±1.094 ^{cde}
3	10.054±0.183 ^g	9.911±0.209 ^g	0.142±0.707 ^{bcd}
4	6.994±0.141 ^g	6.634±0.104 ^g	0.360±0.443 ^{bcd}
5	2.067±0.030 ^h	1.880±0.033 ^h	0.187±0.250 ^{bcd}
6	-3.312±0.033 ⁱ	-3.298±0.102 ⁱ	-0.014±0.211 ^{bcd}
7	2.210±0.029 ^h	1.741±0.035 ^h	0.469±0.268 ^{bcd}
8	7.222±0.184 ^g	6.448±0.074 ^g	0.774±0.295 ^{abc}
9	10.609±0.375 ^g	9.460±0.129 ^g	1.149±0.385 ^{abc}
10	16.048±0.736 ^f	14.787±0.354 ^f	1.261±0.512 ^{ab}
11	20.441±1.176 ^d	19.502±0.827 ^{cd}	0.938±0.713 ^{abc}
12	23.750±1.290 ^{cd}	23.489±1.490 ^b	0.261±1.055 ^{bcd}
13	26.943±1.566 ^{abc}	27.971±2.952 ^a	-1.028±1.315 ^e
14	29.270±2.335 ^a	30.149±3.809 ^a	-0.878±1.211 ^{de}
15	28.032±2.361 ^{ab}	27.569±2.647 ^a	0.463±1.097 ^{bcd}
16	24.482±1.701 ^{bcd}	23.095±0.886 ^{bc}	1.387±1.197 ^{ab}
17	20.751±1.078 ^{de}	18.724±10 ^{-4de}	2.027±1.314 ^a

$H_{(\text{CO}_2)}$ —The function of hysteresis; P_N —Net photosynthetic rate; In the forward and negative periods, the SD of P_N are generated by dividing by the corresponding values at the CO₂ level of 400 $\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$ to normalize to eliminate individual differences and multiply them with the average value; The calculation of $H_{(\text{CO}_2)}$ has not undergone additional normalization.

Supplementary Table 3 Response of P_N of *T. officinale* to forward and negative periodic changes in CO₂ concentration

Measuring Order	$P_N[\mu\text{mol m}^{-2} \text{s}^{-1}]$		
	Forward	Negative (Reverse)	$H_{(\text{CO}_2)}$
1	$9.194 \pm 10^{-4\text{abc}}$	$10.106 \pm 0.611^{\text{ab}}$	$-0.913 \pm 1.552^{\text{d}}$
2	$7.118 \pm 0.147^{\text{abcde}}$	$7.803 \pm 0.444^{\text{abc}}$	$-0.685 \pm 1.224^{\text{d}}$
3	$4.647 \pm 0.165^{\text{cdef}}$	$5.017 \pm 0.245^{\text{bcd}}$	$-0.370 \pm 0.820^{\text{cd}}$
4	$3.179 \pm 0.073^{\text{cdef}}$	$3.229 \pm 0.153^{\text{cde}}$	$-0.050 \pm 0.581^{\text{cd}}$
5	$0.752 \pm 0.021^{\text{ef}}$	$0.762 \pm 0.022^{\text{de}}$	$-0.011 \pm 0.353^{\text{cd}}$
6	$-1.847 \pm 0.084^{\text{f}}$	$-1.918 \pm 0.043^{\text{e}}$	$0.070 \pm 0.250^{\text{bcd}}$
7	$0.923 \pm 0.020^{\text{def}}$	$0.565 \pm 0.014^{\text{de}}$	$0.358 \pm 0.314^{\text{abcd}}$
8	$3.348 \pm 0.034^{\text{cdef}}$	$2.781 \pm 0.111^{\text{cde}}$	$0.568 \pm 0.478^{\text{abcd}}$
9	$5.253 \pm 0.284^{\text{bcdef}}$	$4.043 \pm 0.241^{\text{bcde}}$	$1.210 \pm 0.624^{\text{abc}}$
10	$8.148 \pm 1.061^{\text{abcd}}$	$6.197 \pm 0.568^{\text{abcd}}$	$1.951 \pm 0.807^{\text{ab}}$
11	$10.417 \pm 2.175^{\text{abc}}$	$8.175 \pm 1.087^{\text{abc}}$	$2.242 \pm 1.283^{\text{a}}$
12	$11.157 \pm 3.205^{\text{ab}}$	$10.148 \pm 1.492^{\text{ab}}$	$2.337 \pm 1.679^{\text{ab}}$
13	$12.747 \pm 5.338^{\text{a}}$	$12.755 \pm 1.584^{\text{a}}$	$0.931 \pm 2.208^{\text{abcd}}$
14	$14.386 \pm 6.654^{\text{abc}}$	$14.240 \pm 0.867^{\text{abc}}$	$-0.272 \pm 1.419^{\text{cd}}$
15	$13.645 \pm 4.565^{\text{abcde}}$	$13.295 \pm 0.532^{\text{abcd}}$	$-0.510 \pm 0.899^{\text{abcd}}$
16	$11.686 \pm 2.459^{\text{bcde}}$	$11.499 \pm 0.239^{\text{bcd}}$	$-0.189 \pm 1.059^{\text{abcd}}$
17	$9.865 \pm 1.340^{\text{bcdef}}$	$9.477 \pm 10^{-4\text{bcde}}$	$0.430 \pm 1.408^{\text{abcd}}$

$H_{(\text{CO}_2)}$ —The function of hysteresis; P_N —Net photosynthetic rate; In the forward and negative periods, the SD of P_N are generated by dividing by the corresponding values at the CO₂ level of 400 $\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$ to normalize to eliminate individual differences and multiply them with the average value; The calculation of $H_{(\text{CO}_2)}$ has not undergone additional normalization.

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Supplementary Table 4 Response of *E* of *T. aestivum* to forward and negative periodic changes in CO₂ concentration

Measuring Order	<i>E</i> [$\mu\text{mol m}^{-2} \text{s}^{-1}$]		
	Forward	Negative (Reverse)	<i>H</i> (CO ₂)
1	1.754 ± 10^{-4a}	2.525 ± 0.597^a	-0.772 ± 0.583^f
2	1.902 ± 0.224^a	2.538 ± 0.574^a	-0.636 ± 0.503^{ef}
3	2.000 ± 0.482^a	2.546 ± 0.554^a	-0.546 ± 0.379^{def}
4	2.034 ± 0.653^a	2.544 ± 0.539^a	-0.510 ± 0.274^{cdef}
5	2.077 ± 0.833^a	2.538 ± 0.510^a	-0.461 ± 0.281^{bdef}
6	2.122 ± 0.993^a	2.481 ± 0.492^a	$-0.358 \pm 0.380^{abcdef}$
7	2.227 ± 1.241^a	2.443 ± 0.485^a	$-0.216 \pm 0.527^{abcdef}$
8	2.314 ± 1.357^a	2.401 ± 0.484^a	$-0.087 \pm 0.581^{abcdef}$
9	2.395 ± 1.413^a	2.360 ± 0.481^a	0.036 ± 0.582^{abcde}
10	2.477 ± 1.453^a	2.317 ± 0.483^a	0.160 ± 0.561^{abcd}
11	2.540 ± 1.460^a	2.278 ± 0.483^a	0.262 ± 0.540^a
12	2.576 ± 1.456^a	2.252 ± 0.471^a	0.323 ± 0.533^a
13	2.580 ± 1.418^a	2.270 ± 0.426^a	0.310 ± 0.501^a
14	2.518 ± 1.288^a	2.333 ± 0.306^a	0.185 ± 0.398^{abc}
15	2.384 ± 1.082^a	2.310 ± 0.256^a	0.074 ± 0.325^{abcde}
16	2.351 ± 0.971^a	2.252 ± 0.173^a	0.098 ± 0.266^{abcd}
17	2.405 ± 0.960^a	2.151 ± 10^{-4a}	0.253 ± 0.322^{ab}

E—Transpiration rate; *H*(CO₂)—The function of hysteresis. In the forward and negative periods, the SD of *E* are generated by dividing by the corresponding values at the CO₂ level of 400 $\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$ to normalize to eliminate individual differences and multiply them with the average value; The calculation of *H*(CO₂) has not undergone additional normalization.

Supplementary Table 5 Response of *E* of *R. crispus* to forward and negative periodic changes in CO₂ concentration

Measuring Order	<i>E</i> [mmol(H ₂ O) m ⁻² s ⁻¹]		
	Forward	Negative (Reverse)	<i>H</i> _(CO₂)
1	3.441±10 ^{-4a}	3.730±0.430 ^a	-0.289±0.551 ^e
2	3.468±0.033 ^a	3.709±0.423 ^a	-0.241±0.532 ^{de}
3	3.489±0.064 ^a	3.683±0.415 ^a	-0.194±0.504 ^{cde}
4	3.518±0.095 ^a	3.647±0.407 ^a	-0.130±0.471 ^{bcd}
5	3.549±0.110 ^a	3.604±0.413 ^a	-0.054±0.447 ^{abcde}
6	3.580±0.127 ^a	3.560±0.411 ^a	0.019±0.412 ^{abcde}
7	3.640±0.168 ^a	3.530±0.390 ^a	0.109±0.367 ^{abcde}
8	3.697±0.172 ^a	3.493±0.364 ^a	0.205±0.325 ^{abcde}
9	3.728±0.175 ^a	3.455±0.328 ^a	0.273±0.283 ^{abcde}
10	3.741±0.175 ^a	3.419±0.303 ^a	0.321±0.265 ^{abcd}
11	3.758±0.191 ^a	3.380±0.267 ^a	0.378±0.219 ^{abc}
12	3.766±0.216 ^a	3.343±0.218 ^a	0.423±0.184 ^{ab}
13	3.771±0.250 ^a	3.328±0.173 ^a	0.443±0.178 ^a
14	3.747±0.298 ^a	3.307±0.125 ^a	0.440±0.194 ^{ab}
15	3.725±0.339 ^a	3.283±0.085 ^a	0.422±0.233 ^{ab}
16	3.717±0.394 ^a	3.253±0.047 ^a	0.464±0.288 ^a
17	3.721±0.440 ^a	3.219±10 ^{-4a}	0.502±0.356 ^a

E—Transpiration rate; *H*_(CO₂)—The function of hysteresis. In the forward and negative periods, the SD of *E* are generated by dividing by the corresponding values at the CO₂ level of 400 μmol(CO₂) mol⁻¹ to normalize to eliminate individual differences and multiply them with the average value; The calculation of *H*_(CO₂) has not undergone additional normalization.

Supplementary Table 6 Response of *E* of *T. officinale* to forward and negative periodic changes in CO₂ concentration

Measuring Order	<i>E</i> [μmol m ⁻² s ⁻¹]		
	Forward	Negative (Reverse)	<i>H</i> (CO ₂)
1	2.528 ± 10 ^{-4a}	3.467 ± 1.097 ^a	-0.939 ± 1.313 ^c
2	2.583 ± 0.089 ^a	3.553 ± 1.096 ^a	-0.970 ± 1.307 ^c
3	2.670 ± 0.199 ^a	3.544 ± 1.045 ^a	-0.875 ± 1.296 ^c
4	2.772 ± 0.297 ^a	3.472 ± 0.981 ^a	-0.700 ± 1.243 ^c
5	2.893 ± 0.475 ^a	3.355 ± 0.937 ^{ab}	-0.462 ± 1.197 ^{bc}
6	2.991 ± 0.665 ^a	3.077 ± 0.922 ^{abc}	-0.086 ± 1.173 ^{bc}
7	3.149 ± 1.094 ^a	2.859 ± 0.836 ^{abc}	0.291 ± 1.054 ^{abc}
8	3.281 ± 1.472 ^a	2.565 ± 0.742 ^{abc}	0.716 ± 0.963 ^{abc}
9	3.324 ± 1.764 ^a	2.304 ± 0.663 ^{abc}	1.019 ± 0.919 ^{ab}
10	3.361 ± 1.951 ^a	2.099 ± 0.585 ^{abc}	1.262 ± 0.921 ^a
11	3.391 ± 2.055 ^a	2.006 ± 0.522 ^{abc}	1.385 ± 0.888 ^a
12	3.376 ± 2.006 ^a	2.040 ± 0.506 ^{abc}	1.335 ± 0.793 ^a
13	3.286 ± 1.852 ^a	2.222 ± 0.543 ^{abc}	1.064 ± 0.728 ^{ab}
14	3.166 ± 1.679 ^a	2.475 ± 0.570 ^c	0.691 ± 0.574 ^{abc}
15	3.010 ± 1.449 ^a	2.652 ± 0.442 ^{ab}	0.358 ± 0.239 ^{abc}
16	2.874 ± 1.303 ^a	2.727 ± 0.169 ^c	0.147 ± 0.239 ^{abc}
17	2.805 ± 1.285 ^a	2.658 ± 10 ^{-4abc}	0.146 ± 0.416 ^{abc}

E—Transpiration rate; *H*(CO₂)—The function of hysteresis. In the forward and negative periods, the SD of *E* are generated by dividing by the corresponding values at the CO₂ level of 400 μmol(CO₂) mol⁻¹ to normalize to eliminate individual differences and multiply them with the average value; The calculation of *H*(CO₂) has not undergone additional normalization.

Supplementary Table 7 Response of WUE of *T. aestivum* to forward and negative periodic changes in CO₂ concentration

Measuring Order	WUE[$\mu\text{mol m}^{-2} \text{s}^{-1}$]		
	Forward	Negative (Reverse)	$H_{(\text{CO}_2)}$
1	$5.570 \pm 10^{-4\text{de}}$	$4.866 \pm 0.314^{\text{ef}}$	$0.705 \pm 0.921^{\text{a}}$
2	$4.218 \pm 0.286^{\text{fg}}$	$3.657 \pm 0.194^{\text{g}}$	$0.561 \pm 0.460^{\text{a}}$
3	$2.728 \pm 0.272^{\text{hi}}$	$2.414 \pm 0.105^{\text{h}}$	$0.314 \pm 0.152^{\text{a}}$
4	$1.888 \pm 0.187^{\text{ij}}$	$1.642 \pm 0.065^{\text{h}}$	$0.246 \pm 0.093^{\text{a}}$
5	$0.533 \pm 0.028^{\text{k}}$	$0.576 \pm 0.010^{\text{i}}$	$-0.023 \pm 0.097^{\text{a}}$
6	$-0.805 \pm 0.028^{\text{l}}$	$-0.718 \pm 0.006^{\text{j}}$	$-0.087 \pm 0.093^{\text{a}}$
7	$0.605 \pm 0.034^{\text{ik}}$	$0.540 \pm 0.014^{\text{i}}$	$0.065 \pm 0.089^{\text{a}}$
8	$1.829 \pm 0.283^{\text{ijk}}$	$1.770 \pm 0.072^{\text{h}}$	$0.059 \pm 0.322^{\text{a}}$
9	$2.707 \pm 0.616^{\text{i}}$	$2.618 \pm 0.135^{\text{h}}$	$0.089 \pm 0.525^{\text{a}}$
10	$4.046 \pm 1.206^{\text{gh}}$	$4.059 \pm 0.276^{\text{fg}}$	$-0.013 \pm 0.701^{\text{a}}$
11	$5.161 \pm 1.571^{\text{efg}}$	$5.438 \pm 0.462^{\text{de}}$	$-0.277 \pm 0.743^{\text{a}}$
12	$6.207 \pm 2.242^{\text{cde}}$	$6.586 \pm 0.752^{\text{bc}}$	$-0.379 \pm 1.089^{\text{a}}$
13	$7.309 \pm 3.072^{\text{abc}}$	$7.851 \pm 1.179^{\text{a}}$	$-0.542 \pm 1.626^{\text{a}}$
14	$7.966 \pm 3.258^{\text{a}}$	$8.086 \pm 0.765^{\text{a}}$	$-0.119 \pm 1.372^{\text{a}}$
15	$7.820 \pm 2.754^{\text{ab}}$	$7.565 \pm 0.443^{\text{ab}}$	$0.255 \pm 0.947^{\text{a}}$
16	$6.573 \pm 2.055^{\text{bcd}}$	$6.460 \pm 0.177^{\text{cd}}$	$0.112 \pm 0.600^{\text{a}}$
17	$5.382 \pm 1.425^{\text{def}}$	$5.394 \pm 0.001^{\text{e}}$	$-0.012 \pm 0.607^{\text{a}}$

$H_{(\text{CO}_2)}$ —The function of hysteresis; WUE—Water-use efficiency. In the forward and negative periods, the SD of WUE are generated by dividing by the corresponding values at the CO₂ level of 400 $\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$ to normalize to eliminate individual differences and multiply them with the average value; The calculation of $H_{(\text{CO}_2)}$ has not undergone additional normalization.

Supplementary Table 8 Response of WUE of *R. crispus* to forward and negative periodic changes in CO₂ concentration

Measuring Order	WUE[$\mu\text{mol}(\text{CO}_2) \text{ mmol}(\text{H}_2\text{O})^{-1}$]		
	Forward	Negative (Reverse)	$H_{(\text{CO}_2)}$
1	5.601 $\pm$ 10 ^{-4e}	5.503 $\pm$ 0.354 ^{cd}	0.098 $\pm$ 0.941 ^a
2	4.401 $\pm$ 0.063 ^f	4.249 $\pm$ 0.246 ^e	0.152 $\pm$ 0.713 ^a
3	2.891 $\pm$ 0.051 ^g	2.758 $\pm$ 0.130 ^f	0.132 $\pm$ 0.478 ^a
4	1.998 $\pm$ 0.039 ^h	1.867 $\pm$ 0.067 ^f	0.131 $\pm$ 0.311 ^a
5	0.585 $\pm$ 0.008 ⁱ	0.542 $\pm$ 0.012 ^g	0.043 $\pm$ 0.109 ^a
6	-0.929 $\pm$ 0.010 ^j	-0.933 $\pm$ 0.018 ^h	0.004 $\pm$ 0.086 ^a
7	0.612 $\pm$ 0.008 ⁱ	0.513 $\pm$ 0.013 ^g	0.099 $\pm$ 0.130 ^a
8	1.972 $\pm$ 0.063 ^h	1.892 $\pm$ 0.065 ^f	0.080 $\pm$ 0.240 ^a
9	2.876 $\pm$ 0.136 ^g	2.795 $\pm$ 0.113 ^f	0.081 $\pm$ 0.311 ^a
10	4.333 $\pm$ 0.271 ^f	4.407 $\pm$ 0.239 ^{de}	-0.075 $\pm$ 0.457 ^{ab}
11	5.492 $\pm$ 0.447 ^e	5.869 $\pm$ 0.383 ^c	-0.038 $\pm$ 0.533 ^{abc}
12	6.359 $\pm$ 0.560 ^d	7.133 $\pm$ 0.561 ^b	-0.773 $\pm$ 0.604 ^{bcd}
13	7.209 $\pm$ 0.734 ^{bc}	8.520 $\pm$ 0.919 ^a	-1.311 $\pm$ 0.619 ^d
14	7.894 $\pm$ 1.040 ^a	9.216 $\pm$ 1.056 ^a	-1.321 $\pm$ 0.475 ^d
15	7.621 $\pm$ 1.157 ^{ab}	8.477 $\pm$ 0.750 ^a	-0.856 $\pm$ 0.380 ^{cd}
16	6.680 $\pm$ 0.995 ^{cd}	7.147 $\pm$ 0.269 ^b	-0.467 $\pm$ 0.336 ^{abc}
17	5.660 $\pm$ 0.767 ^e	5.834 $\pm$ 10 ^{-4c}	-0.174 $\pm$ 0.347 ^{abc}

$H_{(\text{CO}_2)}$ —The function of hysteresis; WUE—Water-use efficiency. In the forward and negative periods, the SD of WUE are generated by dividing by the corresponding values at the CO₂ level of 400 $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ to normalize to eliminate individual differences and multiply them with the average value; The calculation of $H_{(\text{CO}_2)}$ has not undergone additional normalization.

Supplementary Table 9 Response of WUE of *T. officinale* to forward and negative periodic changes in CO₂ concentration

Measuring Order	WUE[$\mu\text{mol m}^{-2} \text{s}^{-1}$]		
	Forward	Negative (Reverse)	$H_{(\text{CO}_2)}$
1	3.792 ± 10^{-4ab}	2.965 ± 0.394^{bcde}	0.827 ± 1.602^a
2	2.877 ± 0.108^{abc}	2.235 ± 0.230^{cdef}	0.642 ± 1.232^{ab}
3	1.814 ± 0.108^{bcd}	1.447 ± 0.106^{defg}	0.366 ± 0.810^{abc}
4	1.189 ± 0.058^{cde}	0.957 ± 0.051^{efg}	0.232 ± 0.556^{abc}
5	0.272 ± 0.009^{de}	0.241 ± 0.007^{fg}	0.030 ± 0.212^{abcd}
6	-0.631 ± 0.011^e	-0.630 ± 0.024^g	-0.001 ± 0.197^{abcd}
7	0.308 ± 0.010^{de}	0.214 ± 0.006^{fg}	0.094 ± 0.200^{abc}
8	1.060 ± 0.080^{cde}	1.128 ± 0.075^{defg}	-0.068 ± 0.540^{abcd}
9	1.635 ± 0.173^{bcde}	1.816 ± 0.175^{cdefg}	-0.181 ± 0.767^{abcd}
10	2.492 ± 0.386^{abcd}	3.039 ± 0.464^{bcde}	-0.547 ± 1.045^{abcd}
11	3.136 ± 0.578^{abc}	4.148 ± 0.805^{abc}	-1.012 ± 1.157^{bcd}
12	3.740 ± 0.754^{ab}	5.022 ± 1.291^{ab}	-1.283 ± 1.257^{cd}
13	4.166 ± 0.942^a	5.783 ± 2.296^a	-1.617 ± 0.946^d
14	4.383 ± 1.325^{abc}	5.777 ± 2.791^{abcd}	-1.393 ± 0.649^{cd}
15	4.236 ± 1.597^{abcd}	5.001 ± 1.315^{bcdef}	-0.765 ± 0.360^{abc}
16	3.933 ± 1.368^{abcd}	4.261 ± 0.294^{cdef}	-0.328 ± 0.301^{abc}
17	3.528 ± 1.073^{abcd}	3.657 ± 10^{-4cdef}	-0.129 ± 0.208^{ab}

$H_{(\text{CO}_2)}$ —The function of hysteresis; WUE—Water-use efficiency. In the forward and negative periods, the SD of WUE are generated by dividing by the corresponding values at the CO₂ level of 400 $\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$ to normalize to eliminate individual differences and multiply them with the average value; The calculation of $H_{(\text{CO}_2)}$ has not undergone additional normalization.

Supplementary Table 10 Fitting equation of CO₂ response curve within the monotonic variation range of CO₂ concentration

Species	Periods	Equations	R^2
<i>T. aestivum</i>	Forward	$P_N = 0.045(1 - 4.996 \times 10^{-4}x)/(1 + 9.725 \times 10^{-15}x) - 2.033$	0.999
	Negative	$P_N = 0.044(1 - 5.143 \times 10^{-4}x)/(1 + 5.354 \times 10^{-15}x) - 1.841$	1.000
	Forward	$E = 0.002(1 - 8.766 \times 10^{-4}x)/(1 - 6.582 \times 10^{-5}x) + 2.119$	0.998
	Negative	$E = -6.905 \times 10^{-4}(1 - 0.001x)/(1 - 5.962 \times 10^{-4}x) + 2.488$	0.995
	Forward	$WUE = 0.020(1 - 4.717 \times 10^{-4}x)/(1 + 1.385 \times 10^{-4}x) - 0.802$	1.000
	Negative	$WUE = 0.020(1 - 5.408 \times 10^{-4}x)/(1 + 1.300 \times 10^{-14}x) - 0.888$	0.998
<i>R. crispus</i>	Forward	$P_N = 0.082(1 - 4.882 \times 10^{-4}x)/(1 + 2.857 \times 10^{-4}x) - 3.467$	1.000
	Negative	$P_N = 0.073(1 - 5.231 \times 10^{-4}x)/(1 + 3.819 \times 10^{-15}x) - 3.473$	1.000
	Forward	$E = 0.001(1 - 5.910 \times 10^{-4}x)/(1 + 0.002x) + 3.577$	0.991
	Negative	$E = -5.719 \times 10^{-4}(1 - 7.091 \times 10^{-4}x)/(1 - 0.0003x) + 3.566$	0.996
	Forward	$WUE = 0.023(1 - 4.209 \times 10^{-4}x)/(1 + 4.728 \times 10^{-4}x) - 0.965$	1.000
	Negative	$WUE = 0.022(1 - 5.012 \times 10^{-4}x)/(1 + 5.574 \times 10^{-15}x) - 1.029$	1.000
<i>T. officinale</i>	Forward	$P_N = 0.042(1 - 6.567 \times 10^{-4}x)/(1 + 9.077 \times 10^{-15}x) - 2.072$	0.999
	Negative	$P_N = 0.033(1 - 1.128 \times 10^{-4}x)/(1 + 6.257 \times 10^{-4}x) - 1.846$	0.999
	Forward	$E = 0.003(1 - 0.001x)/(1 + 0.001x) + 2.990$	0.996
	Negative	$E = -0.005(1 - 0.001x)/(1 - 3.002 \times 10^{-4}x) + 3.132$	0.981
	Forward	$WUE = 0.013(1 - 5.946 \times 10^{-4}x)/(1 + 1.153 \times 10^{-4}x) - 0.635$	1.000
	Negative	$WUE = 0.016(1 - 5.939 \times 10^{-4}x)/(1 + 1.599 \times 10^{-14}x) - 0.892$	0.995

E —Transpiration rate; P_N —Net photosynthetic rate; WUE—Water-use efficiency. In this table, x is the intensity of PAR [$\mu\text{mol m}^{-2} \text{s}^{-1}$].