

Photosynthesis of rice leaves with a parallel venation is highly tolerant to vein severing

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Abstract

Vein severing in plants caused by leaf damage is common in fields where crops are cultivated. It is hypothesized that leaves with complex reticulate venation can withstand hydraulic disturbances caused by vein severing, thereby preserving leaf carbon assimilation. However, limited research focuses on vein damage of leaves with parallel venation. We studied how vein-severing affected the photosynthetic traits of rice (*Oryza sativa*) leaves in seconds, minutes and days, under varying water-demand conditions and differing extents of water supply disruption. Rice leaves completely lost their photosynthetic capacity within 2.5 minutes after excision. Severing the midrib resulted in reduced light-saturated photosynthetic rate (A), stomatal conductance (g_{sw}) and transpiration rate (E) by 2.6, 6.8 and 5.9%, respectively, already after thirty minutes. We further investigated the photosynthetic trait responses to various extents of leaf width severing, while keeping the midrib functional. Surprisingly, A , g_{sw} and E in the downstream area of the severed leaves largely remained stable, showing minimal variation across different leaf width severing ratios. These traits declined only slightly even under increased ambient light intensity and leaf-to-air vapor pressure deficit. This sustained photosynthesis post-severing is attributed to the efficient lateral water transport. Long-term leaf damage slightly but not significantly, impacted the downstream photosynthetic traits within five days post-severing. However, a more pronounced reduction in gas exchange during leaf senescence was observed nine days after severing. These findings suggested that rice leaves can tolerate hydraulic disturbances from vein severing and maintain functionality under various conditions, which is crucial for crop yield stability. However, long-term consequences require further investigation.

KEYWORDS

hydraulic, lateral water transport, parallel venation, photosynthesis, rice, vein severing

1 | INTRODUCTION

As vascular plants exchange water and CO₂ with the ambient atmosphere through stomatal pores on leaf surfaces, a strong functional linkage between the water transport (i.e., hydraulic capacity) and photosynthetic performance in plants must exist (Brodrribb and Feild, 2010; Brodrribb

et al., 2007; Buckley, 2019; Xiong and Nadal, 2020). Leaf vein density is the primary factor influencing the ability to supply water for opening stomata (Boyce et al., 2009; Brodrribb et al., 2005). High vein density increases the amount of specialized xylem tissues responsible for water transport closer to evaporation sites within the leaf (McKown et al., 2010; Rockwell and Holbrook, 2017; Zwieniecki and Boyce, 2014).

This high water transport capacity not only enhances leaf photosynthetic capability through supplying a high stomatal conductance (Brodribb et al., 2007; Scoffoni et al., 2016) but also improves its ability to stay cool under high air temperatures (Buckley et al., 2017). By examining data across a broad spectrum of plant species, it has been widely confirmed that the vein traits are dependable predictors of leaf photosynthetic capacity (Boyce and Zwieniecki, 2012; Brodribb et al., 2007), and this relationship is mediated by hydraulic conductance (Scoffoni et al., 2016).

The cohesion–tension theory posits that water is transported in xylem conduits under tension (Dixon and Joly, 1895). If the water column is interrupted, the xylem conduit is left with a near vacuum filled only with water vapor, termed xylem embolism (Guan et al., 2021; Tyree and Sperry, 1989; Urli et al., 2013). Studies over the past few decades have shown that xylem embolism is a major determinant of plant mortality during drought and heat events (Anderegg et al., 2016; Brodribb et al., 2021; Choat et al., 2018; McDowell et al., 2008; Tonet et al., 2023). Similarly, hydraulic conduction is also lost through damage to xylem vessels by herbivores, weather events, or disease (Delaney and Higley, 2006; Duarte et al., 2023; Oleksyn et al., 1998; Sack et al., 2008).

Plants suffering from insect pests, diseases and meteorological disasters are quite common under natural conditions (Figure S1; Ristaino et al., 2021; van der Meijden, 2015). For instance, herbivory is still prevalent worldwide in spite of pest control (van der Meijden, 2015), and its intensity and frequency will only increase with global warming (Deutsch et al., 2018; Raffa et al., 2013), leading to more events of leaf and hence vein damage. It is widely believed that the water flow networks of plants, such as the venation system in leaves, evolved to prevent the propagation of xylem damage during long-term evolution (Brodribb et al., 2016a; Roth-Nebelsick et al., 2001). In fact, architectures with many hierarchically nested loops are often employed in artificially complex networks to maintain optimal functionality in the presence of load fluctuations or damage (Corson, 2010; Katifori et al., 2010; Price and Weitz, 2014). Several previous studies have investigated the hydraulic tolerance to xylem damage in highly reticulated venation leaves and demonstrated the strong tolerance of damage to veins (Nardini and Salleo, 2003; Sack et al., 2008; Salleo et al., 2003). The alternative water paths provided by the nearby veins branching from undamaged veins were thus suggested to be the key to buffering the hydraulic disturbance (Nardini et al., 2001; Sack et al., 2008). Therefore, leaves with fewer vein loops, as they have fewer alternative water paths if the xylem is damaged, may have less hydraulic robustness.

Grasses exhibit a distinct vein pattern in which the veins run parallel, in contrast to reticulate venation, lacking apparent anastomosis, suggesting limited lateral water transport capacity and a potential vulnerability to vein damage. Microscopic examination reveals that these parallel veins in grasses are interconnected by a few commissural veins, forming a grid-like vein system (Kuo et al., 1972; Sakaguchi and Fukuda, 2008; Scarpella et al., 2003). Yet, anatomical studies show that a commissural vein usually contains a single xylem conduit with a diameter of less than 10 μm (Altus et al., 1985) and the frequency of these commissural veins between two nearly longitudinal veins is

relatively low compared with minor veins (Ueno et al., 2006). This indicates a limited transport efficiency of the commissural veins, posing challenges for damaged leaves in replenishing water loss, especially under high water demand conditions like intense light, elevated VPD, or high temperatures.

Grass leaves with vein damage may survive due to commissural veins and other xylem-independent water transport paths, but they may experience reduced stomatal conductance and photosynthetic rate. If the leaf gas exchange rate decreases immediately after damage and persists for days even after healing, leaf damage will lead to decreased biomass accumulation, which can ultimately affect grain yield in cereal crops (Lawlor, 2002; Zhu et al., 2010). Since the grass family includes all major cereals which feed the world population (Shiferaw et al., 2011), such as rice, maize, and wheat, it is crucial to understand the photosynthetic responses of grass leaves to vein damage in order to manage crops and assess yield losses after herbivory and/or other events. We hypothesized that the gas exchange rate of parallel-veined leaves is affected by any harm to the leaf veins, and the extent of this damage would significantly dictate the reduction in the gas exchange rate. To investigate the hypothesis, rice, a model grass species, was selected as the representative species. Firstly, we analyzed the alterations in leaf gas exchange rates due to various degrees of damage to the upstream veins. Secondly, by elevating both the surrounding light intensity and the VPD, we intensified the water requirements to assess the impact of vein damage on gas exchange under heightened water demand scenarios. Beyond short-term impacts, we also examined the long-term effects of leaf damage on its gas exchange.

2 | METHODS AND MATERIALS

2.1 | Plant materials and environment

Huanghuazhan (HHZ), a widely used conventional rice (*Oryza sativa*) cultivar in China, was selected in the current study. Seeds were germinated and grown in seedling trays. Seedlings at the three-leaf stage were transplanted to 11.0 L pots containing 10.0 kg dry soil with a density of three hills per pot. Plants were grown outdoors at the Huazhong Agricultural University experimental station before measurements were conducted. Compound fertilizer (N: P_2O_5 : K_2O = 16%: 16%: 16%) was mixed into the soil at 5.0 g per pot before transplanting. The visible water layer was maintained throughout the whole growth period to avoid soil water stress. Pesticides and fungicides were applied regularly to prevent insect pests and diseases, respectively, and weeds were removed manually.

All gas exchange measurements were carried out between 30 to 60 days after transplanting, during the tillering stage. To minimize environmental disturbances during the measurements, potted rice plants, previously grown outdoors, were relocated into a walk-in growth chamber (GR48, Conviron) one day before the measurements. The conditions within the growth chamber were maintained at a 28/24°C day/night temperature, 65% relative humidity, a CO_2

concentration of 400 ppm, and the light intensity on the leaf surface was $400 \mu\text{mol m}^{-2} \text{s}^{-1}$. Gas exchange was measured using a portable photosynthetic system Li-cor 6800 (Li-COR Inc.). Throughout all measurements, the leaf cuvette environment was set to $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photo flux density (PPFD), a leaf temperature of 30°C and a CO_2 concentration of 400 ppm in the reference cuvette. Gas exchange measurements were performed on the upper one-third point of the new fully expanded leaf.

2.2 | Rapid response of gas exchange to leaf severing

To assess the tolerance to various extents of hydraulic disturbance, we severed the rice leaves at varying width percentages using a razor blade to make a long, clean cut along the longitudinal middle. Due to the close proximity of the veins, damaging some mesophyll tissue was inevitable during the severing process. The leaf width severed percentage is defined as the ratio of the cut length to the leaf's width. We included an extreme case, leaf excision (LE, 100% of leaf width severed), as a reference to estimate the time that leaf function could persist after water supply was completely blocked. Considering the crucial role of the midrib in water transport, we specifically examined its impact on leaf function. This was done by contrasting two treatments: midrib severing (MS), where only the midrib was cut while keeping other veins intact, and leaf partial severing (LPS), involving the cutting of leaf width to varying degrees while preserving the midrib. The severing percentages in LPS treatments ranged from 11.0 to 91.0%. On average, the midrib width constitutes approximately $11.9 \pm 1.3\%$ of the total width of rice leaves across various samples.

To investigate the dynamic response of gas exchange to vein damage, gas exchange parameters were continuously recorded with a time interval of 10 s. Before data recording, leaves were acclimatized in the cuvette (6800-01A; 2 cm^2 area) for at least 30 min and the variations of both A and g_{sw} were less than 5% within 16 minutes in order to avoid confounding of stomatal oscillation. Leaf severing treatments of LE, MS and LPS were performed after 5 minutes of gas exchange data recording. Thereafter, gas exchange parameters were automatically logged for another 30 minutes. The impacts of leaf severing treatments on gas exchange parameters were calculated by dividing the difference between gas exchange parameters 30 minutes after the leaf was severed and the average values before the leaf was severed by the latter. The leaf-to-air vapor pressure deficit (VPD) inside the leaf cuvette was kept at 1.5 kPa, other environmental factors were set as described above. There were eight and five replicates for LE and MS, respectively. LPS was performed on 20 newly expanded and healthy leaves.

As the ambient light intensity in the growth chamber was lower than that inside the gas exchange cuvette, the leaf parts outside the cuvette may serve as an additional water pool influencing the short-term responses of gas exchange to leaf severing. We therefore increased the ambient light intensity by adding a cold white LED array light source (Weichuang Electronic Technology Limit) to investigate

the effects of ambient light intensity. During the measurements, the leaves were kept horizontal with fish lines, the light intensity on the leaf surface outside the leaf cuvette uniformly reached $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ (low ambient light intensity: LAL) or $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ (high ambient light intensity: HAL). Except ambient light intensity, other environmental factors were kept still as described above. For MS, five leaves were measured under LAL, and another 12 leaves were measured under HAL. For LPS, 20 leaves were measured under LAL, and another 26 leaves were measured under HAL. Measurements were conducted on different plants in the same status under the two light conditions.

To further increase the water demand downstream of the damaged leaf sites under the high ambient light intensity condition ($1000 \mu\text{mol m}^{-2} \text{s}^{-1}$), gas exchange responses to a step-by-step increase of leaf-to-air VPD were investigated in leaves with leaf severing treatments of MS and LPS and without severing (CK). The VPD in the leaf cuvette was regulated by changing relative humidity and keeping the leaf temperature at 30°C . A VPD response curve was measured by changing VPD from 1.5 to 3.5 kPa with an interval of 0.5 kPa. Except the VPD in the leaf cuvette, other environmental factors were the same as mentioned above. There were nine, twelve and eleven replicates for CK, MS and LPS, respectively.

2.3 | Gas exchange adaptation to leaf severing

The long-term effects of leaf severing on gas exchange parameters were investigated. Gas exchange parameters were measured every four days on leaves with half of the leaf width cut at the middle longitudinally and uncut leaves (control) on other tillers of the same plants. Measurements were performed four times in total on the same leaves marked before, until the A of the control group showed a significant reduction compared with the initial measurement. The measurements were performed under $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ ambient light intensity and 1.5 kPa VPD in the leaf cuvette. Other environmental conditions both inside and outside the leaf cuvette were the same as mentioned above. Seven leaves were measured for each treatment.

2.4 | Measurement of lateral water transport

To trace water transport after vein damage, we employed red foundation pen ink to stain the excised leaf. Beyond HHZ leaves, we also used leaves of white-leaf rice, which is an inbred variety with partial white mesophyll cells and showing white strips in leaves, grown with the same protocol as HHZ as mentioned above, to detect the water transport pathway (Figure S2). Rice tillers were sampled underwater, encapsulated in double black bags, and transported to the laboratory, ensuring the cut-surface remained submerged (see Du et al., 2020). Once in the lab, we recut the end underwater and placed it into a 50 mL conical flask filled with distilled water. After removing non-target leaves from the stem, we kept the newly expanded leaves in darkness for 30 minutes. Following this, we severed the leaves in the

middle, analogous to the LPS leaf severing treatment with the severing percent of 50%), using a razor blade and introduced 2 mL of red ink into the conical flask. These prepared leaves were then exposed to a customized light source to induce leaf transpiration, with light intensity at the leaf level of approximately $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$. We observed the details of staining under a dissecting microscope once the leaf noticeably turned red, which took about 20 minutes. Concurrently, we froze and embedded part of the downstream area tissues with Tissue-Tek® O.C.T. Compound (Sakura Finetek). We viewed these frozen cross-sections under a microscope located in a -20°C walk-in freezer.

To investigate the role of lateral water transport in maintaining leaf function after longitudinal vein damage, we used the portable photosynthetic system Li-Cor 6800 equipped with a $3 \times 3 \text{ cm}^2$ Clear-top cuvette (6800-12A) to measure the leaf gas exchange parameters of leaves subjected to varying degrees of lateral connection separation. The larger leaf cuvette was chosen for the convenience of leaf severing treatments. In detail, longitudinal veins were blocked by two transverse cuts at the upper one-third of a leaf and the lateral connection in the area between the two transverse cuts was partly blocked by a longitudinal cut just alongside the midrib (Figure 5A). The percent of the longitudinal cut or the damaged percent of the lateral connection refers to the ratio between the length of the longitudinal cut and the interval distance of two transverse cuts, ranging from 0–100%. Before gas exchange measurements, the newly expanded leaves were kept horizontal with fish lines and adapted to $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity for 30 minutes. Gas exchange parameters were measured at 1.5 kPa VPD, 30°C leaf temperature and 400 ppm CO_2 concentration. Gas exchange parameters were recorded 5 minutes before the leaf was severed and 30 minutes after the leaf was severed. In total 27 leaves were measured.

2.5 | Data analysis

All data were cleared up in Microsoft Excel and analyzed using *tidyverse*, *rstatix* and *agricolae* packages in R 4.0.2 (R Core Team, 2022). To test whether leaf severing treatments had influenced leaf functions, we compared the effect size of each leaf severing treatment on gas exchange parameters with zero using the student's t-test. The difference in effect size among leaf severing treatments was analyzed using ANOVA and LSD. The impacts of VPD or light intensity on gas exchange parameters and the effect size of leaf severing treatments were also analyzed using ANOVA and LSD.

3 | RESULTS

The light-saturated gas exchange was highly sensitive to the leaf water supply. Photosynthetic rate (A), transpiration rate (E) and stomatal conductance (g_{sw}) slightly increased in the first few seconds and then cliff dropped after LE was conducted (Figure 1). The average decline rates were $4.9 \pm 1.1 \mu\text{mol m}^{-2} \text{s}^{-1} \text{min}^{-1}$, $3.2 \pm 1.0 \text{mmol m}^{-2} \text{s}^{-1} \text{min}^{-1}$, $0.2 \pm 0.07 \text{mol m}^{-2} \text{s}^{-1} \text{min}^{-1}$ in the

first two minutes for A , E and g_{sw} under LE treatment, respectively (Table 1). Stomatal conductance was reduced by $89.1 \pm 15.4\%$ in 2 minutes and stomata were completely closed (lower than $0.05 \text{mol m}^{-2} \text{s}^{-1}$) within 2.5 ± 0.2 minutes ($n = 8$) after the LE was conducted. The photosynthetic rate declined slower than g_{sw} and reduced to lower than $5 \mu\text{mol m}^{-2} \text{s}^{-1}$ within 3 minutes after leaf excision. We then investigated the downstream area gas exchange responses to varying water supply capacity with different proportions of leaf width severed. When the midrib, which accounts for an average 11.9% of the rice leaf width, was severed (MS treatment), values of A , E and g_{sw} in the downstream area were slightly decreased with the average rates of $10.4 \pm 36.4 \times 10^{-3} \mu\text{mol m}^{-2} \text{s}^{-1} \text{min}^{-1}$, $28.4 \pm 11.6 \times 10^{-3} \text{mmol m}^{-2} \text{s}^{-1} \text{min}^{-1}$, $2.1 \pm 1.1 \times 10^{-3} \text{mol m}^{-2} \text{s}^{-1} \text{min}^{-1}$, respectively, in the first two minutes (Table 1). Although the reductions were statistically significant, after thirty minutes of MS conducted, the declines in A , g_{sw} and E were only 3.98%, 9.75%, and 7.99%, respectively (Figure 1, Table 1). Then the midrib was kept, but the other part of the leaf was severed in different width proportions (LPS treatment), and, surprisingly, A and g_{sw} in the downstream area were largely maintained (Figure 1). As shown in Figure 2, gas exchange parameters were weakly correlated with leaf width severed proportions, indicated by the low correlation coefficient and slope values of the regression lines.

Although the PAR inside the gas exchange cuvette was photosynthetically saturated, only a part of the downstream area was covered by the cuvette, and another part of the leaf area was exposed to the relatively low ambient light condition (LAL; about $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the growth chamber). We then increased ambient PAR to $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ (HAL) to confirm whether leaf severing affects downstream area gas exchange under high transpiration demands. The stable g_{sw} measured under the HAL conditions were significantly higher than under the LAL condition (Figure 3). Saturated gas exchange parameters measured under HAL conditions responded similarly to leaf severing as observed in the LAL condition (Figure 3). Even under field conditions, A and g_{sw} of severed leaves did not largely drop (Figure S5).

Under HAL conditions, the responses of light-saturated gas exchange parameters to VPD changes were investigated on leaves with different leaf width severed ratios. We found that undamaged leaves and partly severed leaves showed different E and g_{sw} responses to VPD changes (Figure 4). The E of undamaged leaves increased significantly as VPD increased from 1.5 kPa to 2.5 kPa, and then E was insensitive to further increase in VPD. Midrib-severed leaves showed a significant increase in E when VPD was increased from 1.5 kPa to 3 kPa, and E decreased sharply when VPD continued to increase from 3 kPa to 3.5 kPa. The g_{sw} of leaves with severed midribs was significantly lower than that of undamaged leaves when VPD exceeded 2.5 kPa. Similarly, leaves in LPS treatment showed relatively low g_{sw} compared with undamaged leaves. However, a significant decrease in g_{sw} was observed only at a VPD of 2.5 kPa. The A of leaves in both LPS and MS treatments showed a similar response to VPD changes, except midrib-severed leaves at a VPD of 3.5 kPa.

Given that the continuous water supply was the prerequisite to maintaining the gas exchange rate under high ambient light intensity and high VPD, the water supply perpendicular to the direction of the veins was examined. The red ink tracing test showed that after the veins were cut, water could be transported laterally from the undamaged veins to the severed veins through the intervein tissues (Figure 5F-H). The efficiency of lateral water transport was further investigated by undertaking gas exchange experiments that changed the lateral water supply by longitudinally severing the lateral water path in different proportions. It turned out that the A , g_{sw} and E of the downstream area significantly declined with the reduction of transverse connection (Figure 5B-D). For every 10% reduction of transverse connection, the values of A , g_{sw} and E reduced by 3.7, 3.2 and 3.2%, respectively.

In this study, we further investigated the long-term effects of vein damage on leaf functions. The gas exchange parameters showed a

significant reduction with leaf age (Figure 6). On the first and the fifth day after half of the leaf width was severed, the gas exchange parameters of undamaged leaves and damaged leaves showed no difference. Nine days after leaf-severing, gas exchange parameters significantly declined compared to the values on the first day of treatment, and the declines were greater in severed leaves compared with the undamaged leaves. On the first nine days after leaf severing, the A of undamaged leaves declined from 32.6 ± 0.9 to $27.8 \pm 1.5 \mu\text{mol m}^{-2} \text{s}^{-1}$, and g_{sw} reduced from 0.64 ± 0.03 to $0.46 \pm 0.05 \text{ mol m}^{-2} \text{s}^{-1}$. In the same period, the A reduced from 33.0 ± 1.4 to $25.4 \pm 0.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ and g_{sw} reduced from 0.63 ± 0.06 to $0.36 \pm 0.02 \text{ mol m}^{-2} \text{s}^{-1}$ in severed leaves. The gas exchange parameters of severed leaves were significantly lower than the values of undamaged leaves on the ninth and thirteenth days after leaf severing.

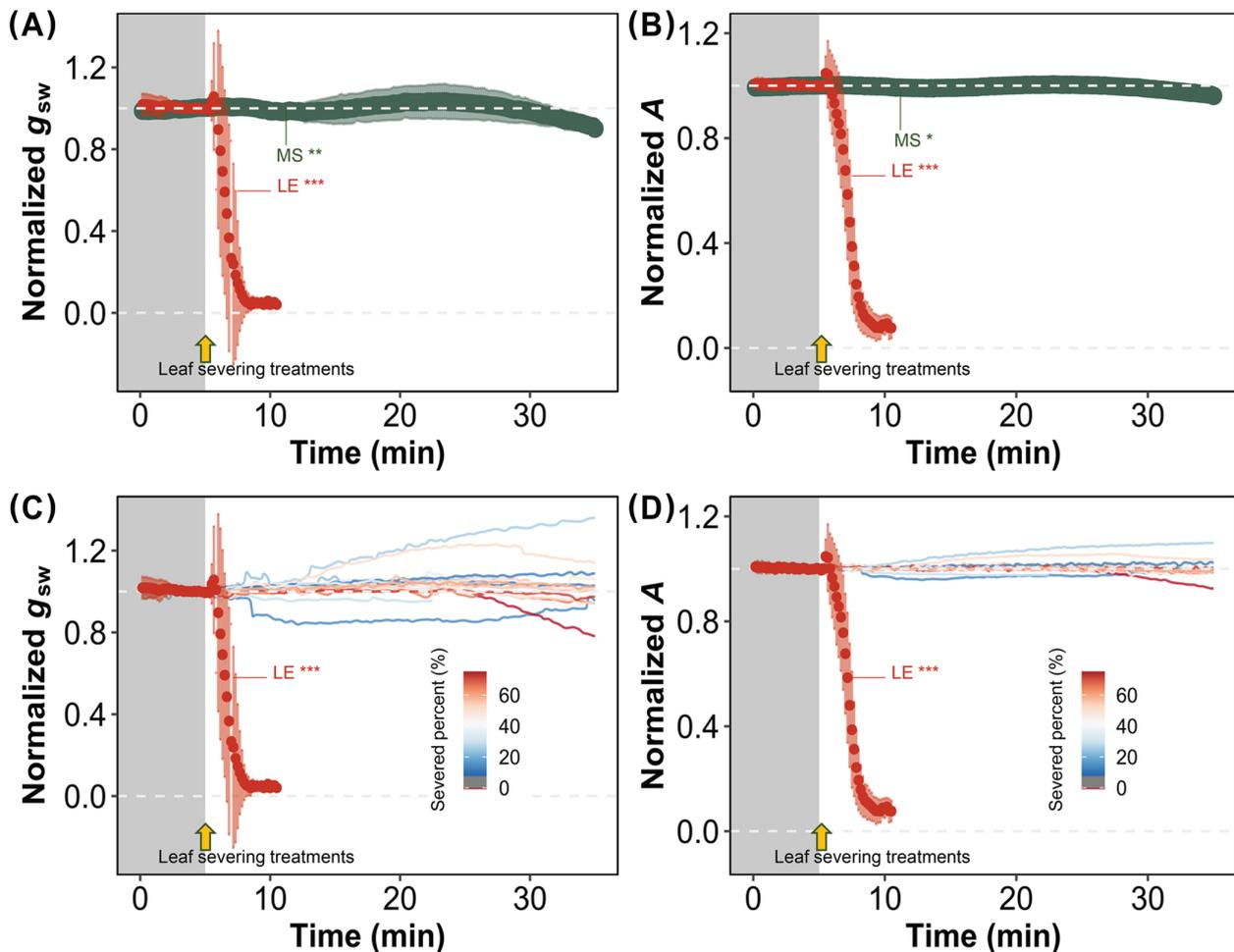


FIGURE 1 Dynamic changes of normalized gas exchange parameters following leaf severing. (A, C) Stomatal conductance (g_{sw}) and (B, D) Light-saturated photosynthetic rate (A) were monitored. Leaf severing treatments, including leaf partial severing (LPS), midrib severing (MS) and complete leaf excising (LE), were performed fifth minutes after achieving steady-state gas exchange, as indicated by the yellow arrow. The gas exchange parameters (g_{sw} , A) for the upper one-third of the leaves were measured under specific conditions ($400 \mu\text{mol m}^{-2} \text{s}^{-1}$ ambient light intensity and 1.5 kPa VPD) in the leaf cuvette. The gas exchange parameters were normalized by dividing each instant by the mean value prior to severance. Mean values and standard deviations are reported for LE ($n = 8$) and MS ($n = 5$), while individual LPS measurements are shown in different colors ($n = 20$). The effects of leaf severance on g_{sw} , and A 30 minutes after the leaves were severed were evaluated using the student's t -test. The significance levels were denoted as follows: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns = not significant at $p < 0.05$.

TABLE 1 The instant rates, difference between instant rate and initial rate (Delta), average response speed and response percent of stomatal conductance (g_{sw}), transpiration rate (E) and light-saturated photosynthetic rate (A) after the leaf was partly severed (LPS), the midrib was severed (MS) or the leaf was excised (LE), the midrib was severed (MS) or the leaf was excised (LE). Mean value \pm standard errors are shown. Statistical analyses were based on LSD analysis. Same letters among the three leaf severing treatments at the same time indicate no significant difference (two-way ANOVA, LSD test, $P < 0.05$)

Time (minutes)	Leaf severing treatments	Instant value			Delta			Response speed			Response in percent					
		A	E	g_{sw}	A	E	g_{sw}	A	E	g_{sw}	A	E	g_{sw}			
		($\mu\text{mol m}^{-2} \text{s}^{-1}$)	($\text{mmol m}^{-2} \text{s}^{-1}$)	($\text{mol m}^{-2} \text{s}^{-1}$)	($10^{-3} \mu\text{mol m}^{-2} \text{s}^{-1}$)	($10^{-3} \text{mmol m}^{-2} \text{s}^{-1}$)	($10^{-3} \text{mol m}^{-2} \text{s}^{-1}$)	($10^{-3} \mu\text{mol m}^{-2} \text{s}^{-1} \text{min}^{-1}$)	($10^{-3} \text{mmol m}^{-2} \text{s}^{-1} \text{min}^{-1}$)	($10^{-3} \text{mol m}^{-2} \text{s}^{-1} \text{min}^{-1}$)	(%)	(%)	(%)			
0	LPS	28.2 \pm 0.6 a	7.8 \pm 0.3 a	0.55 \pm 0.02 a	-	-	-	-	-	-	-	-	-	-	-	-
	MS	30.2 \pm 1.1 a	8.7 \pm 0.7 a	0.62 \pm 0.05 a	-	-	-	-	-	-	-	-	-	-	-	-
	LE	28.7 \pm 1.7 a	7.7 \pm 0.6 a	0.52 \pm 0.06 a	-	-	-	-	-	-	-	-	-	-	-	-
0.5	LPS	28.2 \pm 0.6 a	7.9 \pm 0.3 a	0.56 \pm 0.02 a	-1.6 \pm 31 b	25 \pm 10 a	0.7 \pm 1 a	-32.2 \pm 61.5 b	49.8 \pm 20.7 a	1.4 \pm 1.9 a	-0.13 \pm 0.12 b	0.29 \pm 0.14 a	0.11 \pm 0.18 b			
	MS	30.2 \pm 1.1 a	8.7 \pm 0.7 a	0.62 \pm 0.05 a	-1 \pm 18 b	15 \pm 4 a	0.1 \pm 0.1 a	-2.0 \pm 36.3 b	31.0 \pm 7.1 a	0.2 \pm 0.2 a	0 \pm 0.06 b	0.17 \pm 0.03 a	0.01 \pm 0.02 b			
	LE	29.8 \pm 1.2 a	7.8 \pm 0.5 a	0.53 \pm 0.05 a	1091 \pm 526 a	128 \pm 131 b	12.3 \pm 13 a	2182.8 \pm 1052.8 a	255.7 \pm 261.9 a	24.6 \pm 26.0 a	4.66 \pm 2.22 a	2.51 \pm 2.07 a	4.34 \pm 3.33 a			
1	LPS	28.2 \pm 0.6 a	7.9 \pm 0.3 ab	0.55 \pm 0.02 a	21 \pm 41 a	30 \pm 18 a	1.1 \pm 1.4 a	21.1 \pm 40.8 a	29.5 \pm 17.9 a	1.1 \pm 1.4 a	0.07 \pm 0.14 a	0.26 \pm 0.21 a	0.21 \pm 0.27 a			
	MS	30.2 \pm 1.1 a	8.7 \pm 0.7 a	0.62 \pm 0.05 a	55 \pm 36 a	57 \pm 10 a	2.6 \pm 1.6 a	55.5 \pm 36.1 a	57.1 \pm 9.7 a	2.6 \pm 1.6 a	0.19 \pm 0.12 a	0.48 \pm 0.19 a	0.43 \pm 0.28 a			
	LE	28.5 \pm 0.8 a	7.0 \pm 0.4 b	0.43 \pm 0.03 b	-2405 \pm 865 a	-1700 \pm 558 b	-150.2 \pm 47.4 b	-2405.4 \pm 864.6 a	-1699.8 \pm 558.2 b	-150.2 \pm 47.4 b	-7.87 \pm 3.12 b	-19.76 \pm 7.33 b	-24.94 \pm 9.02 b			
1.5	LPS	28.2 \pm 0.6 a	7.7 \pm 0.3 a	0.55 \pm 0.02 a	48 \pm 37 a	11 \pm 27 a	-0.1 \pm 2.4 a	31.9 \pm 24.6 a	7.4 \pm 18.2 a	-0.1 \pm 1.6 a	0.18 \pm 0.13 a	0.20 \pm 0.35 a	0.07 \pm 0.44 a			
	MS	30.2 \pm 1.1 a	8.8 \pm 0.7 a	0.62 \pm 0.05 a	62 \pm 57 a	62 \pm 9 a	2.9 \pm 1.6 a	41.5 \pm 38.1 a	41.5 \pm 6.3 a	1.9 \pm 1.1 a	0.04 \pm 0.12 a	0.71 \pm 0.11 a	0.49 \pm 0.28 a			
	LE	24.2 \pm 1.6 b	4.4 \pm 0.7 b	0.26 \pm 0.05 b	-5878 \pm 1275 b	-3342 \pm 1193 b	-258.9 \pm 90.6 b	-3918.8 \pm 850.2 b	-2228.1 \pm 795.2 b	-172.6 \pm 60.4 b	-19.87 \pm 4.48 b	-49.81 \pm 9.39 b	-40.95 \pm 17.32 b			
2	LPS	28.2 \pm 0.6 a	7.7 \pm 0.3 a	0.55 \pm 0.02 a	16 \pm 52 a	35 \pm 21 a	1.4 \pm 2 a	8.2 \pm 25.8 a	17.4 \pm 10.5 a	0.7 \pm 1.0 a	0.06 \pm 0.18 a	0.52 \pm 0.28 a	0.51 \pm 0.35 a			
	MS	30.2 \pm 1.1 a	8.2 \pm 0.4 a	0.58 \pm 0.03 a	21 \pm 73 a	57 \pm 23 a	4.1 \pm 2.2 a	10.4 \pm 36.4 a	28.4 \pm 11.6 a	2.1 \pm 1.1 a	0.08 \pm 0.24 a	0.69 \pm 0.32 a	0.31 \pm 0.24 a			
	LE	19.0 \pm 1.8 b	1.4 \pm 1.6 b	0.08 \pm 0.09 b	-9717 \pm 2277 b	-6358 \pm 2079 b	-442.5 \pm 134.9 b	-4858.3 \pm 1138.3 b	-3179.1 \pm 1039.3 b	-221.3 \pm 67.4 b	-32.46 \pm 7.93 b	72.89 \pm 22.09 b	-89.05 \pm 15.38 b			
2.5	LPS	28.2 \pm 0.6 a	7.9 \pm 0.3 a	0.56 \pm 0.02 a	-35 \pm 46 a	36 \pm 23 a	2.2 \pm 1.9 a	-13.8 \pm 18.6 a	14.3 \pm 9.3 a	0.9 \pm 0.8 a	-0.12 \pm 0.16 a	0.46 \pm 0.30 a	0.57 \pm 0.39 a			
	MS	30.2 \pm 1.1 a	8.2 \pm 0.4 a	0.58 \pm 0.03 a	-8 \pm 58 a	49 \pm 26 a	-1.5 \pm 2.7 a	-3.3 \pm 23.3 a	19.6 \pm 10.6 a	-0.6 \pm 1.1 a	-0.02 \pm 0.20 a	0.26 \pm 0.18 a	-0.23 \pm 0.41 a			
	LE	10.5 \pm 1.7 b	0.9 \pm 0.8 b	0.04 \pm 0.04 b	-18198 \pm 2739 b	-6807 \pm 1337 b	-476.8 \pm 92.3 b	-7279.3 \pm 1095.5 b	-2722.8 \pm 534.9 b	-190.7 \pm 36.9 b	-61.42 \pm 7.72 b	-92.17 \pm 9.17 b	-94.40 \pm 7.04 b			
3	LPS	28.2 \pm 0.6 a	7.9 \pm 0.3 a	0.55 \pm 0.02 a	48 \pm 46 a	10 \pm 17 a	1.8 \pm 0.8 a	16.0 \pm 15.5 a	3.2 \pm 5.5 a	0.6 \pm 0.3 a	0.16 \pm 0.16 a	0.25 \pm 0.25 a	0.44 \pm 0.14 a			
	MS	30.1 \pm 1.1 a	8.7 \pm 0.7 a	0.62 \pm 0.05 a	-51 \pm 28 a	7 \pm 36 a	-1.8 \pm 1.9 a	-17.0 \pm 9.4 a	2.5 \pm 12.0 a	-0.6 \pm 0.6 a	-0.08 \pm 0.27 a	-0.21 \pm 0.35 a	-0.29 \pm 0.30 a			
	LE	4.5 \pm 0.7 b	0.6 \pm 0.3 b	0.03 \pm 0.01 b	-23496 \pm 2186 b	-7143 \pm 810 b	-494.5 \pm 64.3 b	-7812.1 \pm 728.8 b	-2380.9 \pm 270.1 b	-164.8 \pm 21.4 b	-80.61 \pm 4.00 b	-90.91 \pm 3.99 b	-93.39 \pm 3.07 b			
10	LPS	28.3 \pm 0.6 a	8.0 \pm 0.3 a	0.56 \pm 0.03 a	23 \pm 56 a	143 \pm 23 a	10.2 \pm 2.0 a	2.3 \pm 5.6 a	14.3 \pm 2.3 a	1.0 \pm 0.2 a	0.07 \pm 0.21 a	1.81 \pm 0.31 a	1.81 \pm 0.37 a			
	MS	29.9 \pm 0.9 a	8.5 \pm 0.4 a	0.61 \pm 0.03 a	-99 \pm 61 a	40 \pm 139 a	5.8 \pm 10.3 a	-9.9 \pm 6.1 a	4.0 \pm 13.9 a	0.6 \pm 1.0 a	-0.33 \pm 0.21 a	-1.65 \pm 2.77 b	-1.29 \pm 3.02 a			
	LE	-	-	-	-	-	-	-	-	-	-	-	-			
30	LPS	28.1 \pm 0.7 a	8.0 \pm 0.4 a	0.56 \pm 0.03 a	-163 \pm 126 a	-33 \pm 162 a	5.2 \pm 10.1 a	-5.4 \pm 4.2 a	-1.1 \pm 5.4 a	0.2 \pm 0.3 a	-0.54 \pm 0.44 a	1.14 \pm 1.68 a	1.09 \pm 1.91 a			
	MS	29.0 \pm 1.3 a	7.9 \pm 0.7 a	0.56 \pm 0.05 a	-1170 \pm 250 b	-694 \pm 67 b	-62.8 \pm 8.7 b	-39.0 \pm 8.3 b	-23.1 \pm 2.2 b	-2.1 \pm 0.3 b	-3.98 \pm 0.93 b	-7.99 \pm 0.92 b	-9.75 \pm 0.13 b			
	LE	-	-	-	-	-	-	-	-	-	-	-	-			

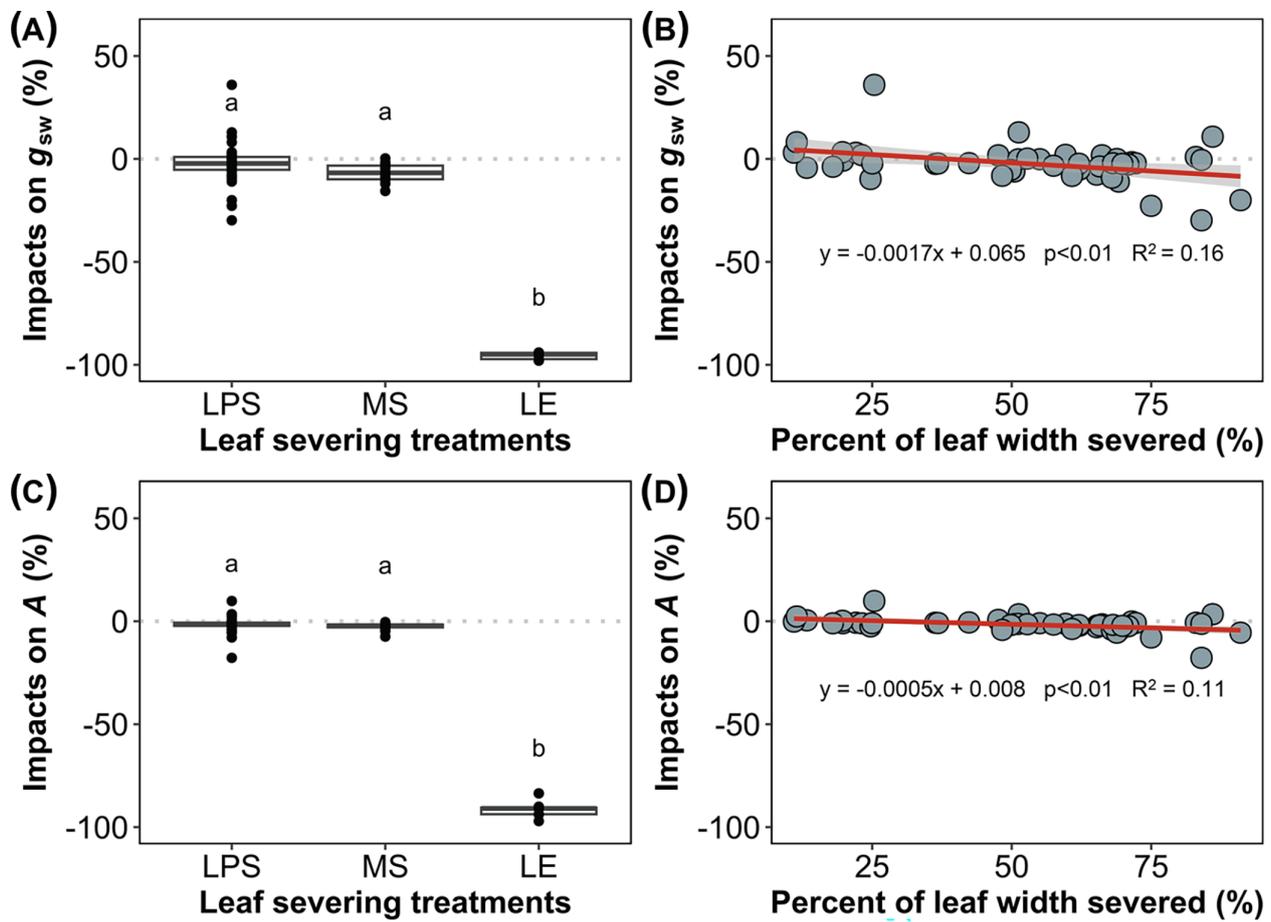


FIGURE 2 The short-term impacts of different leaf severing treatments and the extent of leaf width severance impacts stomatal conductance (g_{sw}) and photosynthetic rate (A) in rice. The leaf severing treatments include leaf partial severing (LPS), midrib severing (MS) and leaf excising (LE). These impacts were quantified by comparing the values 30 minutes post-severance with the mean value prior to severance, using the formula: (post-severance value - mean value prior to severance) / mean value prior to severance. Distinct letters in panels (A) and (C) indicate statistically significant differences among the treatments determining by a two-way ANOVA and LSD test ($P < 0.05$).

4 | DISCUSSION

Disruption of veins caused by damage to leaves is very common in fields, even in intensively managed farmlands (Ristaino et al., 2021; van der Meijden, 2015). It may reduce leaf carbon assimilation rates resulting from the failure to satisfy leaf water demand. Evaluating the reduction of leaf assimilation is of great importance in predicting crop production. Early studies suggested that hierarchical and reticulate venation is profitable to increase the tolerance to vein disruption (Brodribb et al., 2016b; Price and Weitz, 2014). Our study shows that rice leaves with parallel venations are notably tolerant to vein damage. Leaf water demand was satisfied, and leaf photosynthesis was sustained even when all longitudinal veins, excluding the midrib, were severed (Figure 1, 2). In addition, exposed to high water-demand conditions, such as high VPD and high light intensity, vein damaged leaves could still maintain leaf photosynthesis (Figure 3, 4). Under field conditions, severed leaves showed no significant reduction of A and g_{sw} (Figure S5). Our results suggest that leaf hydraulics are not the limiting factor for damaged leaves to maintain leaf assimilation even under field conditions, and the water could be redistributed within the

leaf to satisfy the water demand in different leaf regions. The results are of great significance for stable crop production. It's worth noting that a limitation of the current study lies in its focus on a single species of parallel-veined rice plants. While comparing multiple genotypes or species could offer more comprehensive insights, incorporating such comparisons into one study presents challenges due to the time-intensive nature of these measurements. Future research might consider broadening the scope.

Although the water demand was satisfied, the risk of hydraulic failure was suggested to be different for midrib-severed leaves and other veins-severed leaves. In previous studies, the breakage of the midrib, instead of the high order veins, significantly disrupted the water supply and led to a distinct decrease in photosynthesis in reticulately-veined leaves (Delaney and Higley, 2006; Nardini and Salleo, 2003). However, in the MS treatment of our study, the stomatal conductance at the downstream area of the midrib-severed rice leaves slightly declined by 7.99%. Our results suggest that water bypasses the severed midrib and flows to the downstream area to maintain leaf function in parallel-veined leaves. The suggestion was further supported by the scanned images of the leaves which

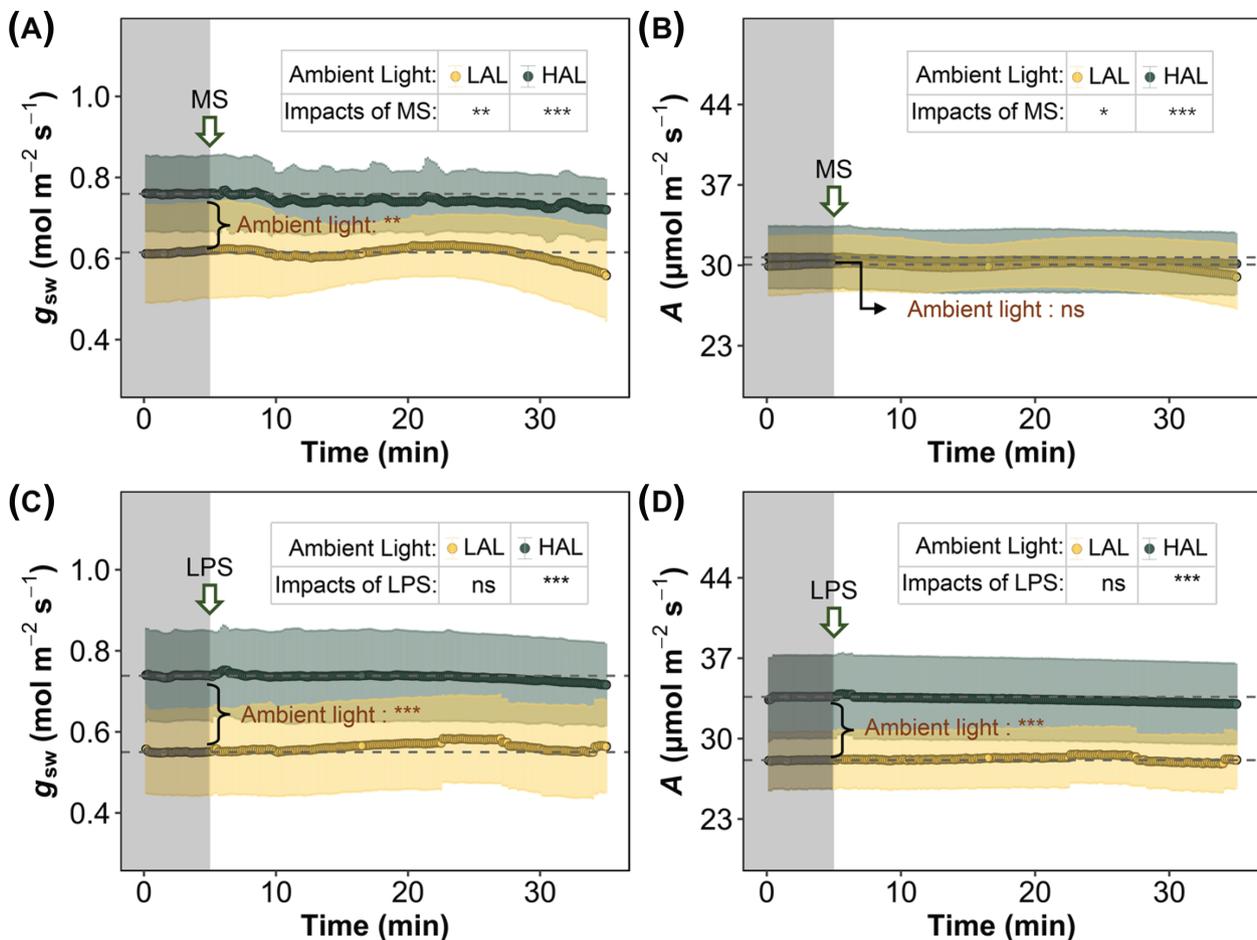


FIGURE 3 The impacts of ambient light conditions on the dynamic change of gas exchange parameters after leaf severing. The dynamic change of (A, C) stomatal conductance (g_{sw}) and (B, D) light-saturated photosynthetic rate (A), following (A, B) midrib severing (MS) or (C, D) leaf partial severing (LPS, the severing percentages ranged from 11.0% to 91.0%), were measured under two ambient light intensities: high ambient light (HAL: $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$) and low ambient light intensities (LAL: $400 \mu\text{mol m}^{-2} \text{s}^{-1}$). Measurements were taken downstream of severed leaf sites. Leaf severing treatments (LPS and MS) were applied five minutes after the gas exchange had reached a steady state, as indicated by an arrow. Mean values and standard deviations are reported in colors. The grey dashed line refers to the mean value of gas exchange before the leaves were severed. The ANOVA was performed to compare the mean gas exchange parameters under different light conditions, as shown in orange text, and the Student's t-test was used to examine the impacts of leaf severing on those parameters, as shown in the inserted table. Significance levels were marked with asterisks: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns = not significant at $p < 0.05$.

distinctly illustrated that some major veins sprout from the midrib (Figure S4). In addition, we found that the closer the damage sites on the midrib were to the leaf base, the more veins were affected and higher reduction of gas exchange rates occurred, which was the same as observed in reticulate-veined leaves (Harayama et al., 2019; Nardini and Salleo, 2003). Severing the midrib at the base of the rice leaf resulted in a 62% reduction in stomatal conductance (Figure S3).

These results clearly showed that the venation pattern in rice leaves incorporates certain hierarchical veins but is different from reticulate venation. When we compared the response of gas exchange parameters in the downstream area of MS treatment to LPS treatment, gas exchange parameters were surprisingly insensitive to other vein severing. Even when all connections were cut off and only the midrib was kept, the water demand was largely satisfied resulting in just a small decrease in gas exchange parameters (Figure 2). It means

that rice leaves were able to buffer the disruption of water supply, resulting from midrib or other vein damages, under mild water-demand conditions. However, when exposed to high VPD, the gas exchange parameters of the midrib severed leaves decreased sharply. These findings highlight the essential function of the midrib in preserving leaf water transport within a parallel-veined system, particularly under conditions of high water-demand. This phenomenon might be attributed to two factors. Firstly, the substantial number of vascular bundles in the midrib of rice leaves proves a large number of water transport paths (Figure S4; Lechthaler et al., 2019; Moulia and Fournier, 1997). Secondly, the midrib's special characteristics, such as their sturdy mechanical structure (Pierantoni et al., 2019), nutrient-deficient composition (e.g. cellulose and xylan) in the constituent cells tend to prevent it from being damaged by insect consumption, The midrib's structure and high cellulose and xylan content further

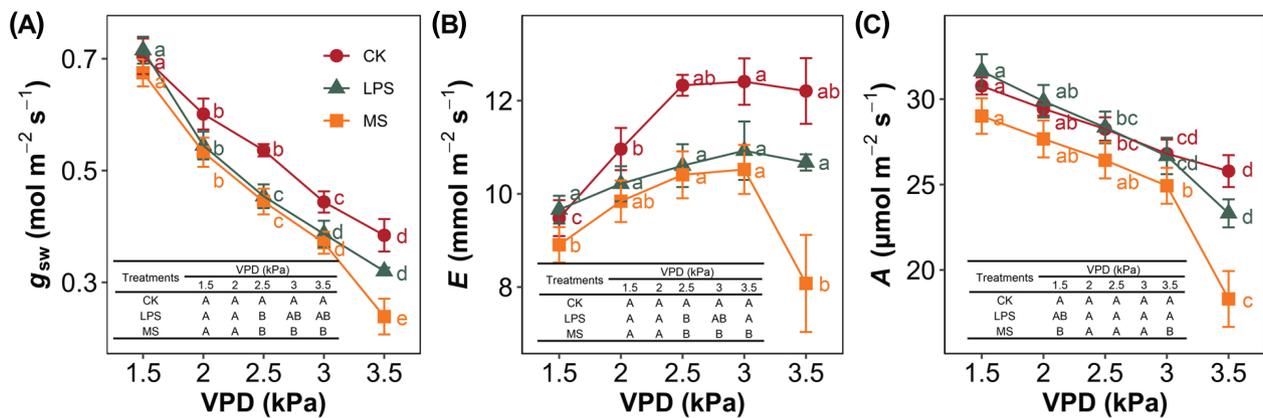


FIGURE 4 Gas exchange parameters of leaves under different leaf severing treatments and their response to increased leaf-to-air pressure deficit (VPD). (A) Stomatal conductance (g_{sw}), (B) Transpiration rate (E), (C) Light-saturated photosynthetic rate (A). Leaf partial severing treatments (LPS, $n = 11$) and midrib severing treatments (MS, $n = 12$) were executed along the longitudinal middle, with measurements taken at the upper one-third site. CK denotes intact leaves without leaf severing ($n = 9$). VPD in the leaf cuvette of Li-Cor 6800 was increased from 1.5 to 3.5 kPa with 0.5 kPa intervals while keeping leaf temperature at 30°C. Mean values and standard deviations are reported. The different lowercase letters in same color near the points indicate that a statistical difference of the estimated parameters exists between different VPD conditions at each leaf severing treatment. The different uppercase letters among the column in table represent statistical difference of the given gas exchange parameters among leaf severing treatments at each VPD condition. Statistical analyses were based on the ANOVA and LSD analysis.

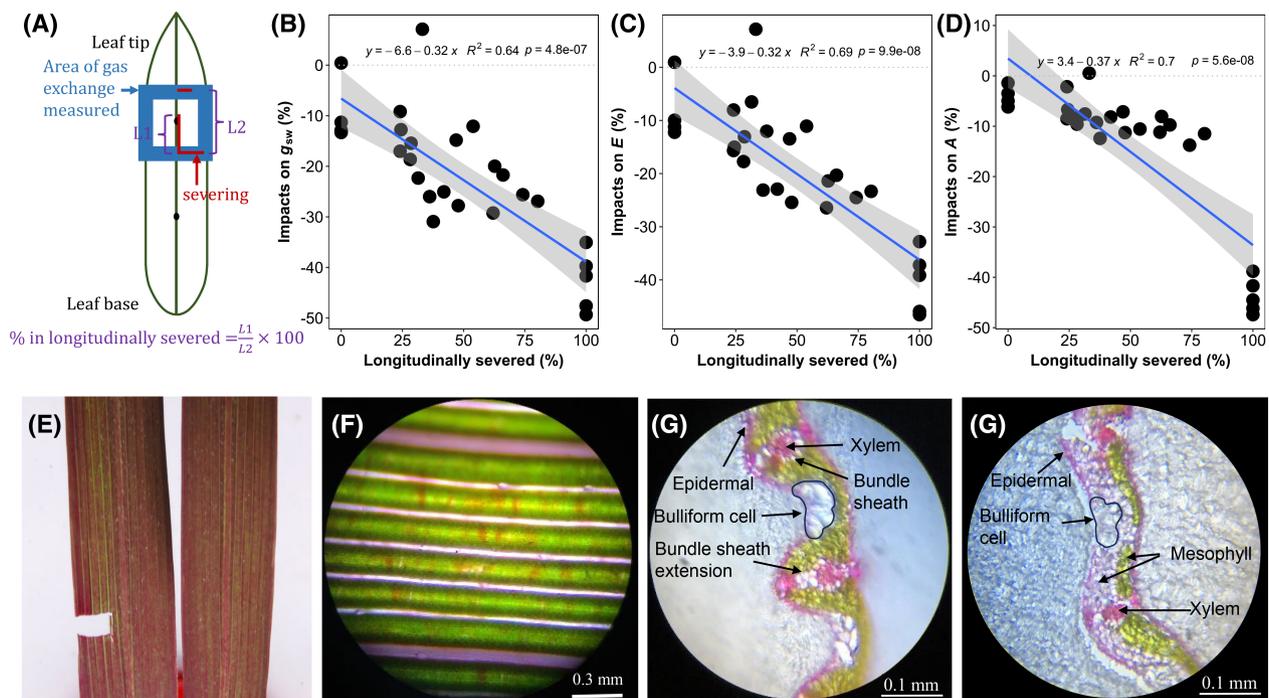


FIGURE 5 The impacts of disruptions in lateral water transport pathway on gas exchange parameters. In the diagram labeled (A), the location of the leaf severance is depicted in red, and the region where gas exchange metrics were recorded is denoted in blue. Subsequent panels (B, C, and D) show the effect of disrupting lateral water transport on (B) stomatal conductance (g_{sw}), (C) the transpiration rate (E), and (D) the light-saturated photosynthetic rate (A). These impacts are quantified by comparing the values 30 minutes after leaf severing with the average values before leaf severing, expressed as a ratio ($n = 27$). The movement of red ink from the incision on the leaf sheath to the blade (E) and examining the spread of red ink in the area beyond the cut under a microscope (F and G). (H) An analysis of a white-leaf rice variety characterized by its partially white mesophyll to reduce the influence of chlorophyll on the visibility of the red ink used in tracking water movement within the leaf.

enhance its water transport capabilities. This underscores the importance of understanding the impact of midrib damage on leaf function due to agricultural activities like topdressing or weeding.

This finding does not necessarily imply that other veins besides the midrib are unimportant; apparently, vascular redundancy provides damage tolerance as an added benefit (Gavrilenko and Katifori, 2019;

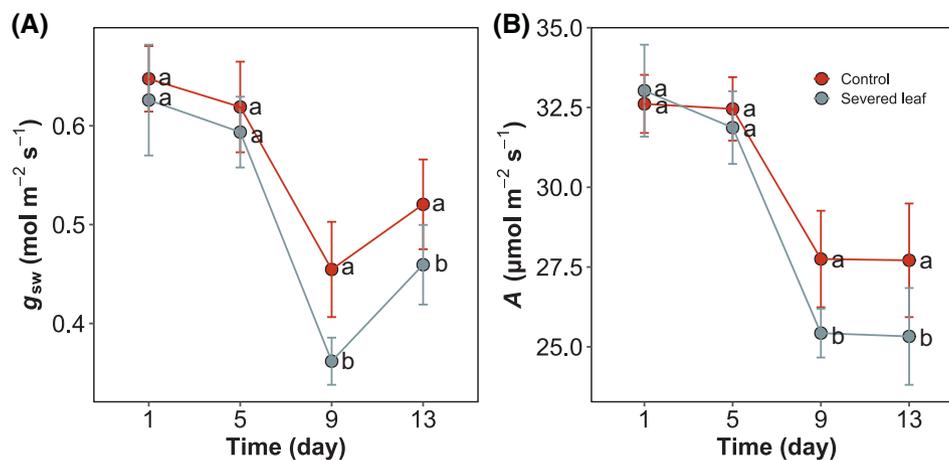


FIGURE 6 The long-term effects of leaf severing on gas exchange. It presents data on (A) stomatal conductance (g_{sw}), and (B) light-saturated photosynthetic rate (A). Initial measurements were made 30 minutes after the leaf was severed, where the leaves were cut at the midpoint, removing half of the leaf's width. These measurements took place at the upper one-third site of a leaf. For comparison, a newly expanded leaf on the same plant acted as a control. Mean values \pm standard errors are presented ($n = 7$). The same letters mean no significant difference between the controlled and severed leaves (two-way ANOVA, LSD test, $P < 0.05$).

Resco de Dios et al., 2021). Indeed, when portions of the leaf veins, other than the midrib were cut, the leaves were largely able to maintain gas exchange (Figure 2, 6; Aldea et al., 2005). However, their photosynthetic rate was significantly lower than that of healthy leaves on the ninth-day post-cutting (Figure 6). This could potentially be due to the severed veins causing a decrease in the transport rate of photosynthetic products, which results in feedback inhibition of photosynthetic system efficiency due to the accumulation of assimilates in photosynthetic cells (Morcuende et al., 1997; Paul and Pellny, 2003). Another possible reason is that the hydraulic system is also adjusted after blade damage, since the transpiration rate and stomatal conductance were also lower in damaged leaves than in healthy leaves. The decrease in hydraulic conductance may be related to hormones produced by leaf damage or may be related to the large production of various substances and regulators required for wound repair (Hua et al., 2021; Verhage, 2021). Clearly, additional research is needed to fully understand the vital role of veins in the transportation of assimilates.

Despite our discovery that not all veins in rice leaves run in parallel, we observed that only a minimal number of veins branch from the midrib in the leaf's middle and tip areas (Figure S4). We indeed discovered a linear relationship between the transpiration flux at the downstream area of the severed vein, and the proportion of connections between that section and the undamaged portion of the leaf (Figure 5). This finding substantiates the notion that water is indeed laterally transported from one vein to another. Consequently, an essential question arises: how is water laterally transported into the area with damaged veins from the undamaged ones, to guarantee thorough hydration of the mesophyll cells downstream of the damaged veins?

Monocot leaves like rice, often described as parallel-veined, frequently exhibit cross-veins, also termed transverse or commissural veins, connecting neighboring longitudinal veins (Altus and Canny, 1985; Dayanandan, 2000; Kuo et al., 1972; Sakaguchi and Fukuda, 2008).

Unexpectedly, the role of commissural veins in water transport has received limited research attention, with previous studies primarily focusing on their developmental processes (Jing et al., 2017; Sakaguchi et al., 2010; Scarpella et al., 2003). While commissural veins are believed to redistribute water after damage to longitudinal veins, their ability to manage hydraulic loads under high water demand remains unproven. In the current study, although we were unable to quantitatively analyze the hydraulic conductivity of commissural veins due to technological limitations, the results of dye tracking experiments and gas exchange measurements indicated their involvement in lateral water transport. We also found that the gas exchange rate could be maintained based on lateral water transport even after longitudinal vein damages. Clearly, further investigation is required to fully understand the variation in the anatomy of commissural veins and their influence on the hydraulic conductance.

Water can also be laterally transported through tissues and cells outside the xylem (Scoffoni, 2015; Zwieniecki et al., 2007). While significant advancements have been made in understanding this intricate process, it remains not entirely comprehended (Buckley et al., 2015; Scoffoni, 2015; Scoffoni et al., 2023). After exiting the xylem, water permeates the bundle sheath cells (Canny, 1990), hydrating the leaf tissue surrounding it. One potential lateral pathway is through the bundle sheath extension and the associated epidermal cells to the neighboring vein (Barbour et al., 2017; Zwieniecki et al., 2007), from where it proceeds to the subsequent vein. This is substantiated by a significant concentration of dye along the densely packed bundle sheath extension and epidermal pathway in the dye tracking test (Figure 5). Another possibility is that water outflowing from the bundle sheath cells reaches the adjacent vein via the mesophyll cells. However, a substantial portion of the rice mesophyll volume is air-space (Niinemets et al., 2007; Xiong et al., 2017; Zait et al., 2021), with minimal cell-to-cell contact, suggesting it may not conduct water at a high rate. This is partially supported by the lighter dye intensity observed in the mesophyll cell wall compared to the epidermal cell

wall. It's important to note that dye-tracking is likely only tracking water transport via the apoplast pathway, not the cell-to-cell pathway. This is because the cell membrane is selectively permeable, preventing the dye from passing through. Indeed, numerous studies have discovered that the aquaporin gene, which is related to water transmembrane transport (Dayer et al., 2017; Reddy et al., 2017; Rodriguez-Gamir et al., 2019), exhibits high expression levels in rice bundle sheath cells and adjacent mesophyll cells (Chen et al., 2018; Ding et al., 2019; Sade et al., 2014). This suggests that the cell-to-cell pathway may play a significant role in water transport.

In conclusion, we discovered that parallel-veined rice leaves can maintain their photosynthetic rates after vein damage, akin to reticulately-veined leaves, even when faced with high water demand conditions, such as increased VPD and light intensity. These findings suggest that the water supply to the areas downstream of the severed leaves site is supported by the commissural veins and by pathways outside the xylem, emphasizing the importance of lateral water transport in the resilience of parallel-veined leaves to damage. These insights shed light on the design of leaf water transport systems and aid in developing models to predict the impacts of herbivore damage on the productivity of grasses.

AUTHOR CONTRIBUTIONS

T.D. and D.X. designed the research. T.D. carried out the research. T.D. and D.X. wrote the paper. T.D., D.X., X.L., J.H. and S.P. substantively revised the paper. All authors read and approved the final manuscript.

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DATA AVAILABILITY STATEMENT

The data will be available from the corresponding author upon reasonable request.

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