Accepted: 25 April 2021
DOI: 10.1002/jpln.202100032

REGULAR ARTICLE

# Leaf gas exchange of lowland rice in response to nitrogen source and vapor pressure deficit

Duy Hoang Vu[1,2] | Sabine Stuerz[1] | Alejandro Pieters[1] | Folkard Asch[1]

[1] Institute of Agricultural Sciences in the Tropics (Hans-Ruthenberg-Institute), Management of Crop Water Stress in the Tropics and Subtropics, University of Hohenheim, Stuttgart 70599, Germany

[2] Faculty of Agronomy, Department of Cultivation Science, Vietnam National University of Agriculture (VNUA), Hanoi 131000, Vietnam

**Correspondence**
D. H. Vu, Faculty of Agronomy, Vietnam National University of Agriculture, Hanoi 131000, Vietnam.
Email: vdhoang@vnua.edu.vn

## Abstract

**Background:** In anaerobic lowland fields, ammonium ($NH_4^+$) is the dominant form of nitrogen (N) taken up by rice plants, however, with the large expansion of water-saving irrigation practices, nitrification is favored during drained periods, leading to an increased availability of nitrate ($NO_3^-$).

**Aim:** Since the uptake and assimilation of the two N-sources differ in their demand of photosynthates, leaf gas exchange may be subject to adjustments in response to N-sources, particularly at high evaporative demand, when stomatal conductance ($g_s$) is very sensitive.

**Methods:** Three experiments were carried out to study leaf gas exchange of various lowland rice varieties in response to N-source at low and high vapor pressure deficit (VPD). In the first experiment, seedlings of 12 rice varieties were grown at high VPD for 3 weeks. From this, four rice varieties differing in $g_s$ and $CO_2$ assimilation rate (A) were selected and grown for 2 weeks at low VPD, and after that, they were shifted to high VPD for 1 week, whereas in the third experiment, the same varieties were grown separately at low and high VPD conditions for 2 weeks. In all three experiments, plants were grown hydroponically in nutrient solution with N-sources as sole $NH_4^+$ or $NO_3^-$.

**Results:** At high VPD, $NO_3^-$ nutrition led to a higher $g_s$ and A in four out of 12 varieties (IR64, BT7, NU838, and Nipponbare) relative to $NH_4^+$ nutrition, while no effect was observed at low VPD or after a short-term exposure to high VPD. Further, varieties with a high intrinsic water-use efficiency (WUEi; IR64 and BT7) showed the strongest response to N-source. Higher $g_s$ was partially supported by increased root/shoot ratio, but could not be fully explained by the measured parameters. However, higher A in $NO_3^-$-fed plants did not always result in increased plant dry matter, which is probably related to the higher energy demand for $NO_3^-$ assimilation. Our results suggest that at high VPD, $NO_3^-$ nutrition can improve leaf gas exchange in varieties having a high WUEi, provided a sufficient water supply.

**Conclusion:** Therefore, intensified nitrification under water-saving irrigation measures may improve leaf gas exchange and the growth of rice plants under high transpirational demand. However, choice of variety seems crucial since large varietal differences were observed in response to N-source. Further, breeding strategies for genotypes adapted to

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2021 The Authors. *Journal of Plant Nutrition and Soil Science* published by Wiley-VCH GmbH

aerobic soil conditions should consider responses to $NO_3^-$, potentially using gas exchange measurements as a screening tool.

**KEYWORDS**
assimilation rate, ammonium, nitrate, nitrification, stomatal conductance, water-saving irrigation

## 1 | INTRODUCTION

Ammonium ($NH_4^+$) and nitrate ($NO_3^-$) are the two main nitrogen (N) forms in the soil. While in anaerobic lowland rice fields $NH_4^+$ is predominant, nitrification is favored in aerobic soils, and thus $NO_3^-$ becomes the dominant N-form (Buresh et al., 2008). Chunmei et al. (2020) showed that water-saving irrigation significantly increased the $NO_3^-$ content in the soil due to improved soil aeration, while continuous flooding increases the $NH_4^+$ content. However, some studies demonstrated the existence of substantial quantities of $NO_3^-$ in the rhizosphere even in flooded soils caused by nitrification due to the release of oxygen from the roots (Kirk, 2001; Kirk & Kronzucker, 2005). Therefore, rice plants take up both $NH_4^+$ and $NO_3^-$, with the ratio of the two N-forms depending on the root-associated nitrification activity (Briones et al., 2003; Kirk, 2001; Li et al., 2007) and the nitrification in the soil (Buresh et al., 2008; Chunmei et al., 2020). When both N-sources are provided in equal concentrations in the nutrient solution, rice plants preferentially take up $NH_4^+$; but still, up to 43% of total N is taken up as $NO_3^-$ (Vu et al., 2020). Kirk and Kronzucker (2005) showed that rice roots are exceptionally efficient in the uptake and assimilation of $NO_3^-$, indicating that the uptake of $NO_3^-$ in lowland rice might be comparable to that of $NH_4^+$. With the large expansion of water-saving irrigation technologies, nitrification is intensified during drained periods; consequently, plants may take up a larger share of N as $NO_3^-$ compared to $NH_4^+$.

The energy costs for uptake, transport, and assimilation differ between the two N-forms with higher costs for $NO_3^-$ relative to $NH_4^+$. The total costs of $NO_3^-$ uptake, assimilation, and transport are 32 mol photons $mol^{-1}$ N, while the costs for $NH_4^+$ are only 9.5 mol photons $mol^{-1}$ N (Raven, 1985). $NH_4^+$ supplied to plants would save photo-energy equivalents of 10 mol ATP $mol^{-1}$ N, compared to $NO_3^-$ (Salsac et al., 1987). In barley, $NO_3^-$-fed plants required up to 23% of the energy from root respiration for its absorption and assimilation, while only 14% was needed in $NH_4^+$-fed plants (Bloom et al., 1992), demonstrating the greater demand of photosynthates for the uptake and reduction of $NO_3^-$ relative to $NH_4^+$. Moreover, $NO_3^-$ and $NH_4^+$ are assimilated at different sites, which may lead to differences in the partitioning of photosynthates within the plant. $NH_4^+$ is predominantly assimilated into organic compounds in the root, whereas $NO_3^-$ is readily transported and distributed throughout the plant (Marschner, 2011); therefore, $NH_4^+$ nutrition results in greater depletion of carbohydrates in the root (Bowman & Paul, 1988). However, in sugar beets, $NH_4^+$ treatments also lowered concentrations of starch, sucrose, and maltose in the leaves in comparison to $NO_3^-$ treatments due to carbon skeleton consumption in $NH_4^+$ assimilation (Raab & Terry, 1995).

Since N-forms differ in their assimilation pathways and their demand of photosynthates, the leaf gas exchange of plants may be subject to adjustments in response to N-source. Previous studies showed that N-source influenced photosynthesis, photorespiration, and water uptake of plants; however, variation among species has been reported (S. Guo, Zhou, Shen, & Zhang, 2007). For instance, $NO_3^-$ nutrition increased $CO_2$ assimilation rate (A) and stomatal conductance ($g_s$), compared to $NH_4^+$ in cucumber (Zhou et al., 2011), whereas the opposite was reported for French bean (S. Guo et al., 2002) and tomato (Horchani et al., 2010). In tobacco, Lu et al. (2005) showed a reduction in $g_s$ and transpiration rate caused by $NH_4^+$, compared to $NO_3^-$ and $NH_4^+/NO_3^-$.

Lowland rice is considered $NH_4^+$-tolerant and measured photosynthetic parameters did not differ between plants fed with different N-sources in previous studies under well-watered conditions (S. Guo et al., 2008; Ji & Peng, 2005; Li et al., 2009; Zhou et al., 2011). However, $NO_3^-$ nutrition combined with secondary water stress, induced with polyethylene glycol (PEG), resulted in reduced $g_s$, A, and water consumption in comparison to $NH_4^+$ nutrition (Ding et al., 2015; S. Guo et al., 2008). In contrast, Zhong et al. (2018) showed that the acclimation of photosynthesis to water stress in rice plants could be improved by the uptake and assimilation of $NO_3^-$ since $NO_3^-$ and amino acids could serve as osmotically active substances maintaining leaf turgor; however, varietal differences were observed. The effect of N-source on photosynthesis has been attributed to the adjustment of $g_s$ with subsequent effects of the $CO_2$ supply to chloroplasts (S. Guo et al., 2008; Torralbo et al., 2019). However, $g_s$ (Ohsumi et al., 2008) and water uptake capacity (Vu et al., 2020) of rice varieties are highly sensitive to vapor pressure deficit (VPD); hence, the effect of N-source on leaf gas exchange of rice may differ with changes in VPD and between varieties. Moreover, global VPD increased strongly after the late 1990s due to global warming (Grossiord et al., 2020; Yuan et al., 2019), and it is projected to consistently increase throughout the current century (Yuan et al., 2019); therefore, the effect of N-form on leaf gas exchange of rice plants should be considered under varying VPD conditions.

With the intensification of nitrification during drained periods in water-saving irrigation practices, rice plants take up a larger share of N as $NO_3^-$, which may increase the demand of photosynthates for the uptake and assimilation of N. Since varietal differences in both $CO_2$ assimilation and N uptake and assimilation can be expected, particularly at high VPD, the objectives of this study were: (1) to assess the range of varietal responses to N-source in terms of leaf gas exchange; (2) to investigate the short- and long-term effect of VPD on the response of leaf gas exchange to N-source; and (3) to examine effects

**TABLE 1** Genetic background and growth characteristics of 12 rice varieties used in the study

| No. | Variety | Subspecies | Type | Origin | Characteristics |
|---|---|---|---|---|---|
| 1 | IR64 | Indica | Lowland, inbred variety | The Philippines | High yield, early maturity, susceptible to abiotic stress, and commonly used as an international check variety (Mackill & Khush, 2018) |
| 2 | Bac thom 7 (BT7) | Indica | Lowland, inbred variety | Vietnam | Popular in the North of Vietnam, aromatic, good tillering capacity, and moderate lodging resistance (Pham et al., 2009) |
| 3 | Khang dan 18(KD18) | Indica | Lowland, inbred variety | Vietnam | Popular in the North of Vietnam, moderate tillering capacity, thick and large leaves, and moderate lodging resistance (Pham et al., 2009) |
| 4 | Jasmine 85 | Indica | Lowland, inbred variety | Vietnam | Popular in the South of Vietnam, moderate tillering, susceptibility to drought, and lodging resistance (Pham et al., 2009) |
| 5 | Bao thai | Indica | Lowland, local variety | Vietnam | Popular in the mountainous area, late maturity, and photoperiod sensitivity[a] |
| 6 | Khau nam xit | Indica | Lowland, local variety | Vietnam | Local variety in the mountainous area, large leaves, and tall plant type[a] |
| 7 | Seng cu | Indica | Lowland, local variety | Vietnam | Local variety in the mountainous area and large leaves[a] |
| 8 | Nhi uu 838(NU838) | Indica | Lowland, hybrid variety | China | Popular in the North of Vietnam, high yield, good tillering, large leaves, lodging resistance, and cold tolerance (Pham et al., 2009) |
| 9 | TH3-4 | Indica | Lowland, hybrid variety | Vietnam | High yield, good tillering capacity, large leaves, and lodging resistance (Pham et al., 2009) |
| 10 | J02 | Japonica | Lowland, inbred variety | Vietnam | Newly introduced, high yield, moderate tillering, and moderate lodging resistance[a] |
| 11 | Nipponbare | Japonica | Lowland, inbred variety | Japan | Modern, photoperiod sensitive variety. Has been used as japonica check variety (Hirooka et al., 2018) |
| 12 | Nerica 4 | Interspecific (*Oryza sativa* × *O. glaberrima*) | Upland, inbred variety | Africa Rice | High yield, good tillering, and good response to fertilizers (Kinyumu, 2009) |

[a]Information from local agriculture extension centres.

of N-source on dry matter (DM) production and partitioning in relation to the measured photosynthetic parameters.

## 2 | MATERIAL AND METHODS

### 2.1 | Plant material

Nine common local varieties differing in genetic background and growth characteristics were selected. Seeds were provided by the Vietnam National University of Agriculture and the Vietnam National Seed Group. Additionally, three varieties (IR64, Nipponbare, and Nerica 4) were used as international check varieties in the study. IR64 was provided by the International Rice Research Institute (IRRI) while Nipponbare and Nerica 4 were provided by the Africa Rice Center (AfricaRice). Information on the genetic background and growth characteristics of the 12 varieties are given in Table 1.

### 2.2 | Treatments and growth conditions

Three experiments were carried out in the greenhouse at the Institute of Agricultural Sciences in the Tropics, University of Hohenheim, Germany.

For all three experiments, seeds were germinated in the dark. Germinated seeds were transferred to cleaned (rinsed/washed) sand and irrigated with tap water during the first 4 days and then irrigated with 50% standard YOSHIDA nutrient solution with both N-sources as $NH_4NO_3$ (Yoshida et al., 1976) for the following 4 days. After that, seedlings were transferred to plastic boxes (40 × 30 × 12 cm) containing 8.5 L of 100% standard YOSHIDA nutrient solution at a density of 60 seedlings per box. In this box, seedlings remained for another 10 days.

To provide different N-sources, modified nutrient solutions containing 2.86 mM N in the forms of sole $NH_4^+$ as $(NH_4)_2SO_4$ or $NO_3^-$ as $Ca(NO_3)_2$ and $KNO_3$ were prepared. Other macro- and micro-nutrients were provided in equal amounts in both N-source treatments in the following concentrations: 0.32 mM P, 1.0 mM K, 1.0 mM Ca, 1.6 mM Mg, 35.6 μM Fe-EDTA, 9.5 μM Mn, 18.9 μM B, 0.15 μM Zn, 0.16 μM Cu, 0.52 μM Mo. To inhibit nitrification and ensure identical conditions, 7 μM dicyandiamide ($C_2H_4N_4$) was added to both N treatments (Duan et al., 2006).

All three experiments were conducted with four replications per treatment and artificial light was provided with 12 h photoperiod. The position of boxes and pots was interchanged every day to eliminate the edge effects. Ambient temperature and relative air humidity (RH) were monitored every 10 min with Tinytag data loggers (Gemini data loggers Ltd.), and VPD was calculated. The nutrient solution was renewed every 7 days, while its pH was monitored and adjusted daily to a value of $5.5 \pm 0.1$ using 1N NaOH or HCl.

## 2.3 | Experiment 1—Genotypic variation

To evaluate the variation in leaf gas exchange of 12 varieties in response to N-source, the first experiment was carried out at "standard" greenhouse conditions, without additional humidification or dehumidification. Due to the high air temperature and low RH, it is considered as "high VPD," with average day and night VPD at 2.0 and 0.9 kPa, respectively. Seedlings were transferred to boxes (20 × 15 × 12 cm; 12 plants, each) containing 4.5 L of modified nutrient solution. Leaf gas exchange was measured 3 weeks after the onset of N-source treatment, and then plants were harvested for DM determination.

Four rice varieties (IR64, BT7, KD18, and Jasmine 85) showing different responses in leaf gas exchange to the different N-sources in Experiment 1 were selected and examined in Experiments 2 and 3, which were conducted in self-constructed growth chambers with the possibility to manipulate VPD. After pre-cultivation as described above, individual plants were grown in pots containing 1 L of modified nutrient solution.

## 2.4 | Experiment 2—Acclimation to low VPD

To estimate the effect of a sudden increase in VPD on leaf gas exchange (Experiment 2), plants were grown at low VPD (0.7/0.2 kPa day/night) for the first 2 weeks, and after that, air was dehumidified to increase the VPD (2.4/1.9 kPa day/night) for 1 week. Leaf gas exchange was measured shortly before and 1 week after increasing the VPD.

## 2.5 | Experiment 3—Acclimation to high VPD

The effect of acclimation to high VPD was studied in Experiment 3, where plants were grown in separated chambers differing in VPD. Plants were either exposed to low VPD (0.6 kPa and 0.4 kPa during day and night) or high VPD (2.1 and 1.8 kPa during day and night) for 2 weeks. After that, leaf gas exchange was measured.

In Experiment 3, the day after gas exchange measurements, the same leaves were sampled during the last hours of the light period. The samples were quickly frozen in liquid N and stored at –80°C. Starch concentration was determined with the method described in Pieters et al. (2001). Test tubes of 1.5 mL containing 0.47 $cm^2$ frozen leaf and 0.5 mL of 96% ethanol were incubated at 70°C for 30 min. The ethanolic extract was collected, and extraction was repeated once to remove sucrose. The remaining leaf material was rinsed with distilled water and ground to a fine powder in liquid N using plastic pestles. A 0.5 mL of 50 mM sodium acetate (pH 4.8) containing 1 μL $\alpha$-amylase and 1 μL amyloglucosidase per sample were added and samples were incubated at 37°C for 36 h. An extract of 40 μL was loaded in 96 well-plates with 200 μL assay buffer (100 mM imidazole pH 6.9, 10 mM $MgCl_2$, 33 mM ATP, 12.5 mM NADP). Finally, 1.0 unit of hexokinase was added. Quantification of starch was based on the production of nicotinamide adenine dinucleotide hydrogen phosphate (NADPH) at 340 nm using a microplate reader Infinite (Tecan).

Roots, dead leaves, and green leaf sheaths and blades were separated and oven-dried at 70°C until constant weight for DM determination. Specific leaf area (SLA) was calculated as total green leaf area divided by its dry weight.

## 2.6 | Gas exchange measurement

$g_s$ and A were measured on the youngest fully expanded leaf of the main tiller the following day after the replacement of the nutrient solution. Measurements were performed with a portable gas exchange system GFS-3000 (Heinz Walz GmbH). In the cuvette, the temperature was set to 30°C in all three experiments, while light intensities were set at 1500 and 1200 μmol $m^{-2}$ $s^{-1}$ photosynthetic photon flux density (PPFD; using LED light) in the first and second experiments, respectively, or followed ambient light (700 μmol $m^{-2}$ $s^{-1}$ PPFD) in Experiment 3. Humidity in the cuvette was adjusted to create a similar VPD as outside the cuvette. The growth and cuvette environment are summarized in Table 2. Intrinsic water-use efficiency (WUEi) was defined and calculated as $A/g_s$ (μmol $mmol^{-1}$) (Rashid et al., 2018).

## 2.7 | Data analysis

Data were analyzed with Statistical Analysis System (version 9.4). In Experiments 2 and 3, data analysis was performed for each VPD separately, as VPD was not included as a factor due to the lack of the degree of freedom. A two-way analysis of variance in a linear model (PROC MIXED) was used to examine the effect of variety, N-source, and their interaction on measured parameters. Means were separated using the least significant difference test at $p < 0.05$.

# 3 | RESULTS

In Experiment 1, $g_s$ ranged from 247 mmol $m^{-2}$ $s^{-1}$ in Khau nam xit fed with $NH_4^+$ to 446 mmol $m^{-2}$ $s^{-1}$ in KD18 fed with $NH_4^+$ (Figure 1A).

**TABLE 2** Growth conditions and gas exchange measurement conditions

| Experiment | | Growth conditions: Air temp. (°C) Day | Air temp. (°C) Night | Vapor pressure deficit (VPD; kPa) Day | VPD Night | Light ($\mu$mol $m^{-2}$ $s^{-1}$) | Measurement conditions: VPD (kPa) | Temp. (°C) | Light ($\mu$mol $m^{-2}$ $s^{-1}$) | $CO_2$ (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|
| Experiment 1 | High VPD | 29.6 | 21.1 | 2.0 | 0.9 | 1200 | 2.0 | 30 | 1500 | 400 |
| Experiment 2 | Low VPD | 31.7 | 23.7 | 0.7 | 0.2 | 700 | 1.1 | 30 | 1200 | 400 |
| | High VPD | 31.1 | 25.5 | 2.4 | 1.9 | 700 | 2.5 | 30 | 1200 | 400 |
| Experiment 3 | Low VPD | 30.0 | 24.5 | 0.6 | 0.4 | 700 | 0.9 | 30 | 700 | 400 |
| | High VPD | 28.8 | 25.0 | 2.1 | 1.8 | 700 | 2.5 | 30 | 700 | 400 |

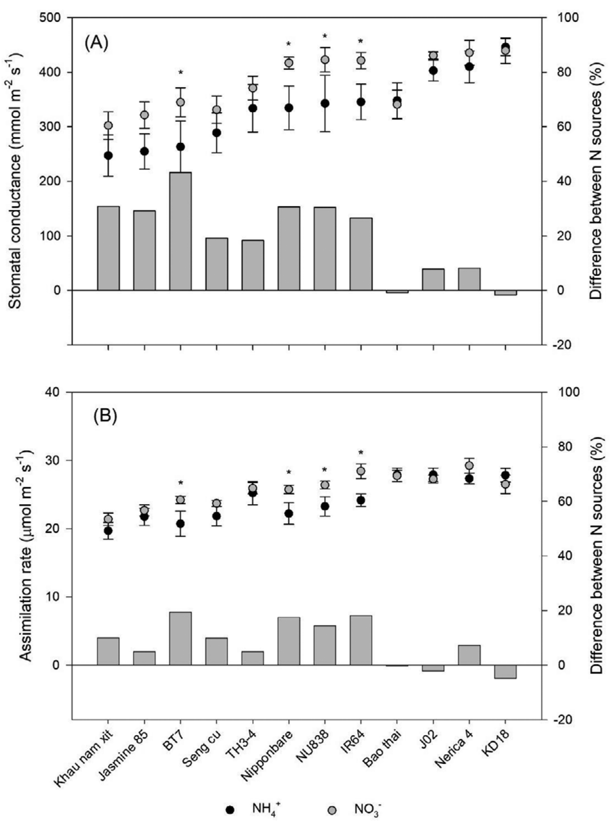


**FIGURE 1** (A) Stomatal conductance ($g_s$) and (B) assimilation rate (A) of 12 rice varieties in response to nitrogen (N) source [ammonium ($NH_4^+$) or nitrate ($NO_3^-$)] at high vapor pressure deficit (VPD; "standard" greenhouse conditions; Experiment 1). Bars show the difference (%) between $NO_3^-$ and $NH_4^+$ treatments. Error bars indicate standard error of means ($n = 4$); * indicates significant differences between N-sources at $p < 0.05$

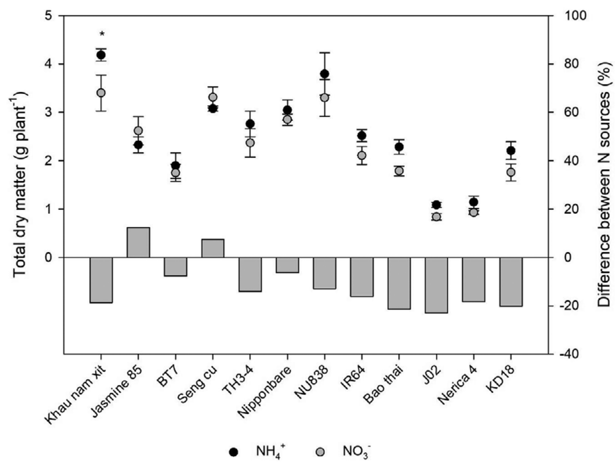


**FIGURE 2** Total dry matter (DM) of 12 rice varieties in response to N-source ($NH_4^+$ or $NO_3^-$) at high VPD ("standard" greenhouse conditions; Experiment 1). Bars show the difference (%) between $NO_3^-$ and $NH_4^+$ treatments. Error bars indicate standard error of means ($n = 4$); * indicates significant differences between N-sources at $p < 0.05$

$NO_3^-$ nutrition led to significantly higher $g_s$ than $NH_4^+$ in IR64, BT7, NU838, and Nipponbare, while for the other varieties, N-form did not affect $g_s$. The largest difference in $g_s$ between plants fed with different N-sources was observed in BT7, which showed a 31% higher $g_s$ fed with $NO_3^-$ than with $NH_4^+$, while the smallest differences were observed in KD18 and Bao thai.

There was a varietal variation in A, ranging from 19.7 to 29.2 $\mu$mol $m^{-2}$ $s^{-1}$ in Khau nam xit fed with $NH_4^+$ and Nerica 4 fed with $NO_3^-$, respectively (Figure 1B). Significant differences in A between N-forms were found in the same varieties, that already showed significant differences in $g_s$, IR64, BT7, NU838, and Nipponbare. The largest difference in A between plants fed with different N-sources was observed in BT7, which showed a 19% higher A in $NO_3^-$ than in $NH_4^+$, followed by IR64 with 18%. In KD18, A was 27.9 $\mu$mol $m^{-2}$ $s^{-1}$, slightly higher in $NH_4^+$ than in $NO_3^-$ with 26.5 $\mu$mol $m^{-2}$ $s^{-1}$. A high positive correlation between $g_s$ and A was found in all varieties (see Figure S1).

Total DM ranged from 0.84 to 4.19 g $plant^{-1}$ in J02 fed with $NO_3^-$ and Khau nam xit fed with $NH_4^+$, respectively (Figure 2). Over all varieties, DM was on average 2.25 g $plant^{-1}$ significantly lower under $NO_3^-$ nutrition than under $NH_4^+$ nutrition with 2.53 g $plant^{-1}$. However, looking at varieties individually, $NO_3^-$ nutrition significantly reduced DM in Khau nam xit alone.

Under low VPD in Experiment 2, N-form did not influence $g_s$ or A significantly, but varietal differences in $g_s$ and A were found (Table 3). One week after shifting the plants from low to high VPD, $g_s$ was strongly decreased in all varieties and for both N-sources (53% on average).

**TABLE 3** Stomatal conductance ($g_s$) and assimilation rate (A) of four rice varieties at low VPD and after 1 week exposure to high VPD, and the difference in $g_s$ and A between low and high VPD (%; Experiment 2). Values are means of four replications. Small letters indicate significant differences between nitrogen (N) sources, while capital letters indicate significant differences between varieties ($p < 0.05$)

| | | $g_s$ (mmol $m^{-2}$ $s^{-1}$) | | | | | | A (μmol $m^{-2}$ $s^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ricevariety | N-source | Low VPD | | High VPD | | | (%)[a] | Low VPD | | High VPD | | | (%)[a] |
| IR64 | Ammonium ($NH_4^+$) | 749.0 | B | 329.5 | b | B | −56.0 | 19.3 | B | 16.9 | | B | −12.6 |
| IR64 | Nitrate ($NO_3^-$) | 786.5 | B | 435.0 | a | B | −44.7 | 19.4 | B | 18.4 | | A | −5.0 |
| BT7 | $NH_4^+$ | 700.5 | B | 273.1 | | B | −61.0 | 19.2 | B | 13.4 | | C | −29.9 |
| BT7 | $NO_3^-$ | 701.7 | B | 302.9 | | C | −56.8 | 18.3 | B | 12.5 | | B | −31.6 |
| KD18 | $NH_4^+$ | 1166.0 | A | 546.7 | | A | −53.1 | 23.3 | A | 19.3 | | A | −17.0 |
| KD18 | $NO_3^-$ | 1279.3 | A | 601.1 | | A | −53.0 | 21.4 | A | 18.1 | | A | −15.3 |
| Jasmine 85 | $NH_4^+$ | 911.4 | B | 482.7 | | A | −47.0 | 19.5 | B | 17.2 | a | B | −12.0 |
| Jasmine 85 | $NO_3^-$ | 1048.8 | A | 503.2 | | B | −52.0 | 18.4 | B | 12.3 | b | B | −33.0 |

[a]Difference between low and high VPD.

At high VPD, $NO_3^-$ nutrition significantly increased $g_s$ in IR64 as compared to $NH_4^+$-fed plants. At both VPD levels and for both N-sources, KD18 showed the highest $g_s$, followed by Jasmine 85, IR64, and BT7. Shifting plants to high VPD also resulted in a strong decrease in A in all varieties and for both N-sources (19% on average). Reductions of more than 30% in A were observed in BT7 under both N-sources and in Jasmine 85 under $NO_3^-$ nutrition, whereas a 5% reduction was observed in $NO_3^-$-fed plants of IR64. At high VPD, A only differed in plants fed with different N-sources in Jasmine 85, where $NO_3^-$ strongly reduced A relative to $NH_4^+$.

$g_s$ and A of four rice varieties grown at low and high VPD are presented in Figure 3 (Experiment 3). At low VPD, N-source only had an effect on IR64, where $NO_3^-$ induced a higher $g_s$ than $NH_4^+$. In contrast, at high VPD, $NO_3^-$ nutrition led to a significantly higher $g_s$ and A in IR64 and BT7, whereas no effect of N-source was observed in KD18 and Jasmine 85. At both VPD levels and in both N-sources, KD18 showed the highest $g_s$.

Comparing $g_s$ and A between VPD levels, both parameters were lower under high VPD with a larger difference in $g_s$ than in A. In comparison to low VPD, $g_s$ and A were on average 26% and 5% lower at high VPD, respectively. The difference between VPD levels was larger in plants fed with $NH_4^+$ than with $NO_3^-$. While in $NH_4^+$, $g_s$ and A were 31% and 8% lower at high VPD than at low VPD, they were 22% and 3% lower when fed with $NO_3^-$.

At low VPD, total DM of IR64 and BT7 did not differ between N-sources, whereas $NH_4^+$ nutrition led to lower total DM in KD18 and Jasmine 85 relative to $NO_3^-$ (Figure 4A). At low VPD and for both N-sources, the highest DM was found in IR64 and BT7 (4.9 and 5.2 g plant$^{-1}$ in IR64, and 4.5 and 4.9 g plant$^{-1}$ in BT7 under $NH_4^+$ and $NO_3^-$, respectively), followed by Jasmine 85 (3.8 and 4.6 g plant$^{-1}$ under $NH_4^+$ and $NO_3^-$, respectively) and KD18 (3.0 and 4.0 g plant$^{-1}$ under $NH_4^+$ and $NO_3^-$, respectively). At high VPD, total DM of IR64 and KD18 did not differ between N-sources, while $NH_4^+$ led to a lower DM in BT7 and Jasmine 85 relative to $NO_3^-$ (Figure 4B). At high VPD and for both N-sources, the highest DM was observed in IR64 (3.8 and 4.1 g plant$^{-1}$ under $NH_4^+$ and $NO_3^-$, respectively), followed by BT7 (2.7 and 3.4 g plant$^{-1}$ under $NH_4^+$ and $NO_3^-$, respectively), Jasmine 85 (2.4 and 3.4 g plant$^{-1}$ under $NH_4^+$ and $NO_3^-$, respectively), and the lowest DM was found in KD18 (1.8 and 2.1 g plant$^{-1}$ under $NH_4^+$ and $NO_3^-$, respectively).

At low VPD, a significant difference in root/shoot (R/S) ratio between plants fed with different N-forms was observed in KD18 alone, where $NO_3^-$ nutrition led to a higher R/S ratio (0.33 g/g) than $NH_4^+$ (0.29 g/g; Figure 5A). At high VPD, $NO_3^-$ significantly increased R/S ratio in all varieties but not in KD18. The largest increase was found in BT7, where R/S ratio was 0.19 g/g in $NH_4^+$ and 0.27 g/g in $NO_3^-$. At both VPD levels and with both N-sources, IR64 showed the highest R/S ratio. At high VPD, R/S ratio was positively correlated with $g_s$ of IR64 and BT7 but not with $g_s$ of KD18 and Jasmine 85 (see Figure S2).

$NO_3^-$ nutrition led to a higher SLA in KD18 and Jasmine 85 at low VPD and in all varieties at high VPD relative to $NH_4^+$ (Figures 5C,D). At both VPD levels and with both N-sources, the lowest SLA was found in KD18.

At low VPD, $NO_3^-$-fed plants showed a significantly higher starch content in the leaves, compared to $NH_4^+$-fed plants, with an exception for Jasmine 85 (Figure 6). At high VPD, $NO_3^-$ nutrition led to significantly higher starch content in BT7 and Jasmine 85. With both N sources, BT7 showed the highest starch content in the leaves at low VPD, while at high VPD, the highest starch content was observed in IR64.

## 4 | DISCUSSION

### 4.1 | $NO_3^-$ induced higher $g_s$ and A relative to $NH_4^+$ at high VPD but not at low VPD

At low VPD, gas exchange parameters were hardly affected by N-source. Among the four rice varieties (IR64, BT7, KD18, and Jasmine 85) at low VPD in Experiments 2 and 3, only $g_s$ of IR64 showed a significant response to N-source in Experiment 3, with $g_s$ being higher in $NO_3^-$ than in $NH_4^+$. In previous studies, N-source did not affect

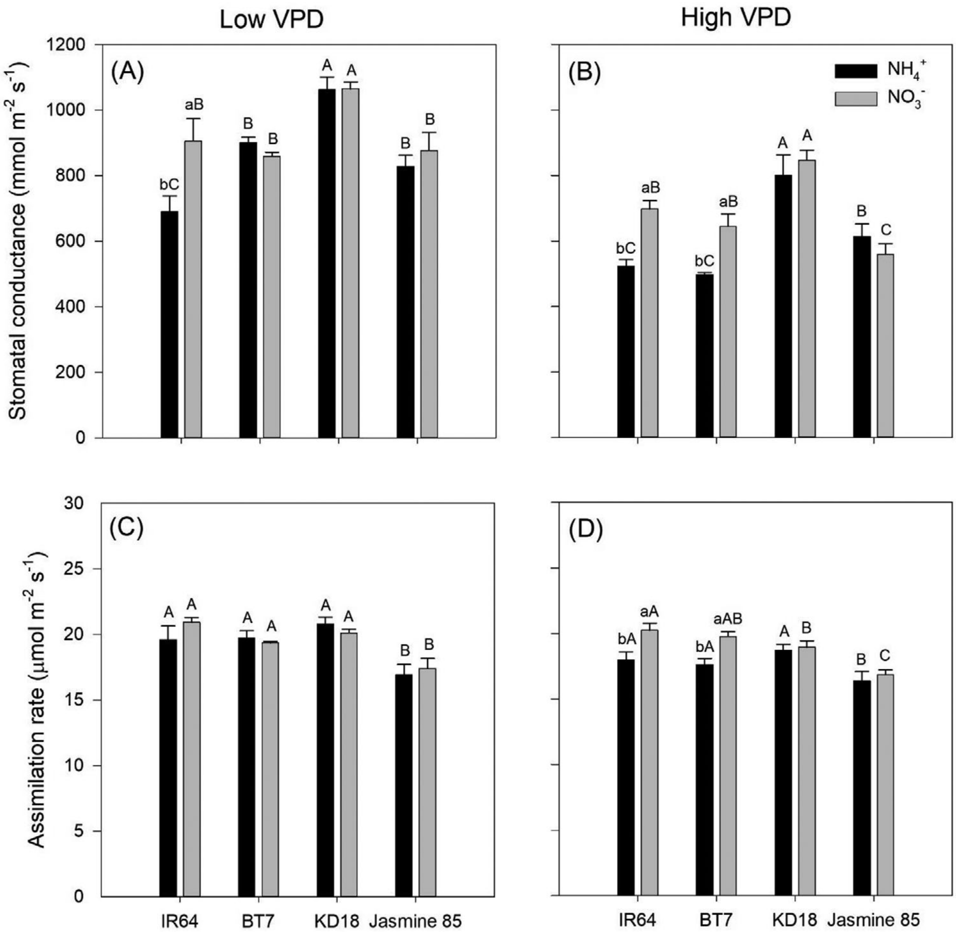


**FIGURE 3** (A, B) $g_s$ and (C, D) A of four rice varieties fed with different N-sources ($NH_4^+$ or $NO_3^-$) at low and high VPD (Experiment 3). Columns and error bars show mean values and standard errors of four replications. Small letters indicate significant differences between N-sources, while capital letters indicate significant differences between varieties, all at $p < 0.05$

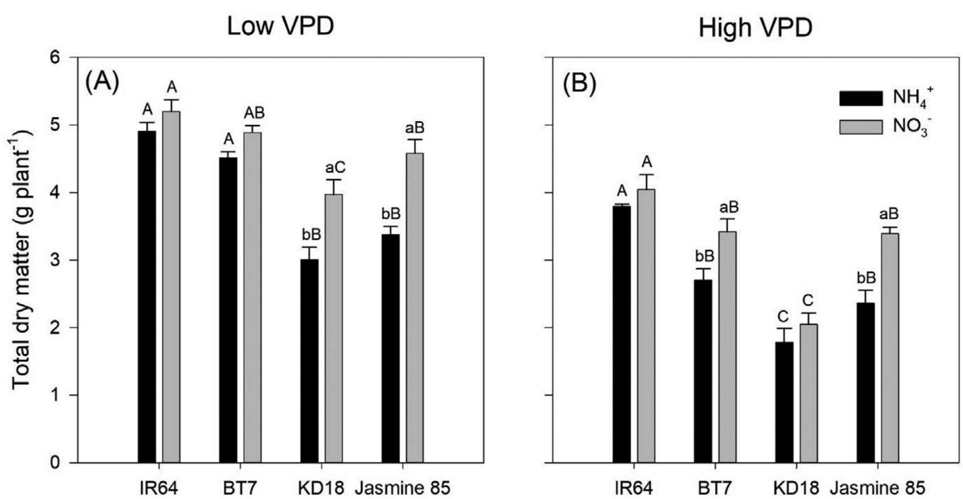


**FIGURE 4** Total DM of four rice varieties fed with different N-sources ($NH_4^+$ or $NO_3^-$) at low and high VPD (A, B) (Experiment 3). Columns and error bars show means and standard errors of four replications. Small letters indicate significant differences between N-sources, while capital letters indicate significant differences between varieties, all at $p < 0.05$

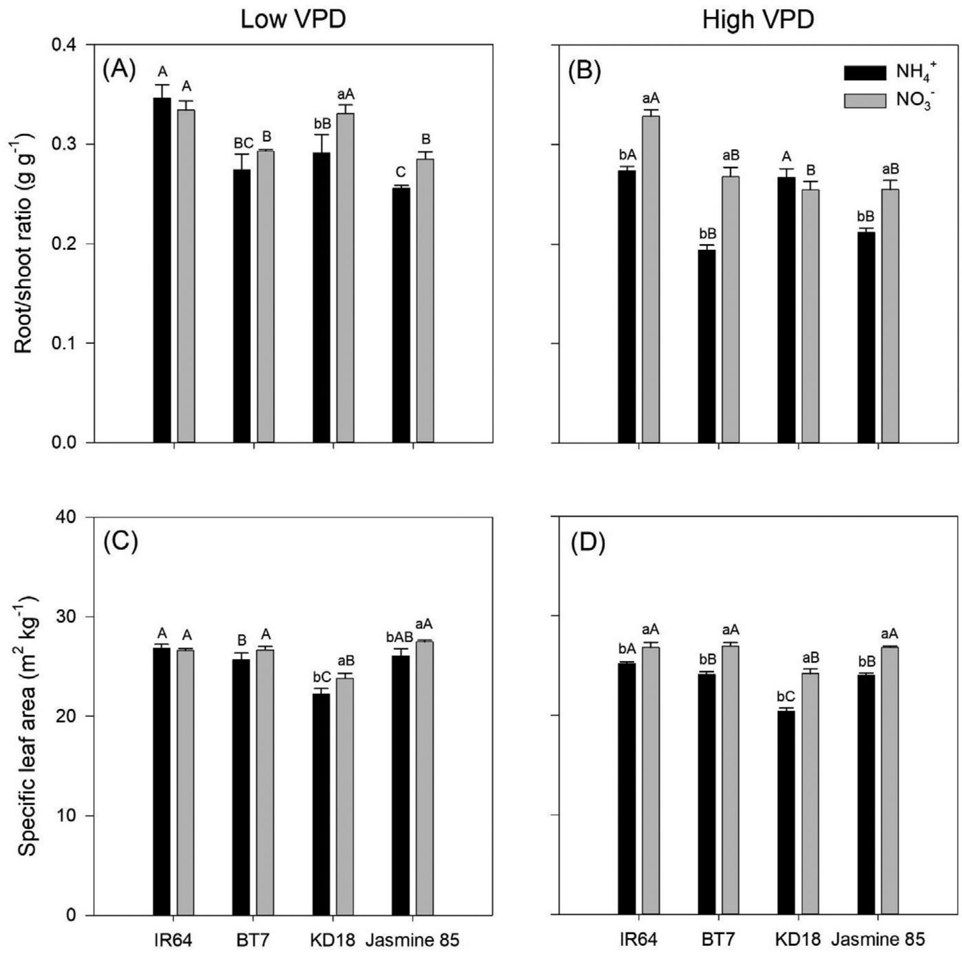


**FIGURE 5** Root/shoot ratio (A, B) and specific leaf area (C, D) of four rice varieties fed with different N-sources ($NH_4^+$ or $NO_3^-$) at low and high VPD (Experiment 3). Columns and error bars show means and standard errors of 4 replications. Small letters indicate significant differences between N-sources, while capital letters indicate significant differences between varieties, all at $p < 0.05$

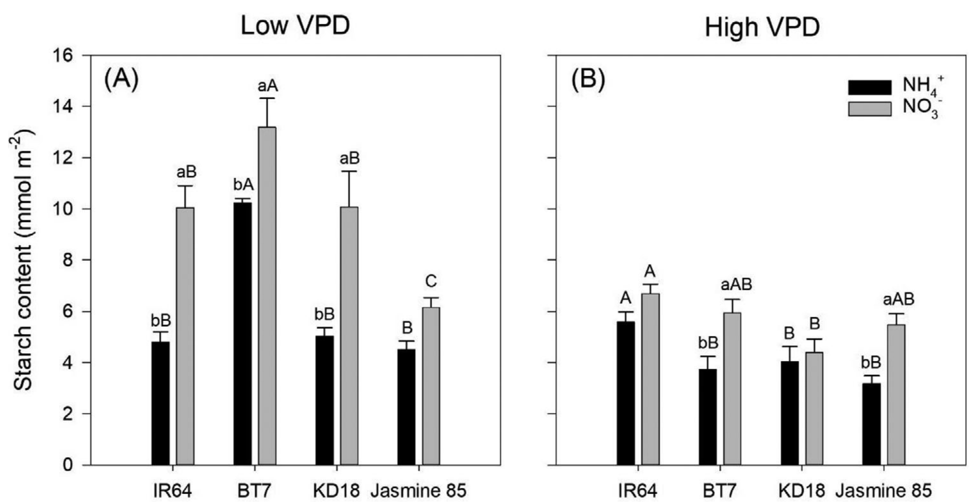


**FIGURE 6** Starch content in leaves of four rice varieties fed with different N-sources ($NH_4^+$ or $NO_3^-$) at low and high VPD (A, B) (Experiment 3). Mean values of four replications are presented. Small letters indicate significant differences between N-sources, while capital letters indicate significant differences between varieties, all at $p < 0.05$

leaf gas exchange of rice under non-water stress conditions (S. Guo et al., 2008; Ji & Peng, 2005; Li et al., 2009; Tran et al., 2015; Zhou et al., 2011), whereas $NH_4^+$ significantly reduced photosynthesis in some other crops, for example, cucumber (Zhou et al., 2011), durum wheat (*Triticum durum;* Torralbo et al., 2019), and eggplant (*Solanum melongena* L.; Claussen & Lenz, 1995). Excessive accumulation of $NH_4^+$ in the cytosol is toxic, leading to tissue necrosis (Marschner, 2011), but a substantial amount of N can be translocated as $NH_4^+$ from the root to the shoot (Schjoerring et al., 2002). In various species, $NH_4^+$ as an exclusive N-source led to toxicity symptoms (Britto & Kronzucker, 2002). Therefore, plants sensitive to $NH_4^+$ decrease $g_s$ as a strategy to reduce $NH_4^+$ transport toward the leaves (Torralbo et al., 2019). However, lowland rice is considered $NH_4^+$-tolerant (Zhou et al., 2011) since activities of N-assimilating enzymes, both in roots and shoots, are much higher than in other more sensitive crops (*e.g.*, tomato, maize; Magalhäes & Huber, 1989). Most of the $NH_4^+$ taken up is assimilated in the root (Marschner, 2011; Tabuchi et al., 2007). However, $NH_4^+$ assimilation consumes carbon skeletons, and a reduction in photosynthate concentration after $NH_4^+$ uptake has been observed in roots (Bowman & Paul, 1988) as well as in leaves (Raab & Terry, 1995). In our study, $NH_4^+$-assimilating enzyme activities were not determined; however, starch concentrations in the leaves were lower in $NH_4^+$-fed plants than in $NO_3^-$-fed plants at low VPD, while A did not differ between plants fed with the two N-sources, suggesting translocation of carbohydrates to the roots, probably for $NH_4^+$ assimilation and detoxification.

In contrast, different N-sources led to significant differences in leaf gas exchange at high VPD under "standard" greenhouse conditions (Experiment 1), with higher $g_s$ and A in four out of 12 varieties, IR64, BT7, NU838, and Nipponbare fed with $NO_3^-$. The same was observed at high VPD in the growth chamber (Experiment 3) where higher $g_s$ and A were found in IR64 and BT7 under $NO_3^-$ nutrition relative to $NH_4^+$. Since $NH_4^+$ and $NO_3^-$ are taken up at different rates (Vu et al., 2020), supplying different N-sources could lead to different N concentrations in the leaves, and thus differences in chlorophyll concentrations. However, chlorophyll concentration was measured directly spectrophotometrically after ethanol extraction, but no differences between N treatments were found (see Figure S3). Several previous studies demonstrated that the effect of N-source on A is associated with $g_s$ (S. Guo, Zhou, Shen, & Zhang, 2007; Torralbo et al., 2019; Zhou et al., 2011). In our study, A was closely correlated with $g_s$, which supports the hypothesis that $g_s$ limits A in response to $NH_4^+$ nutrition. $NO_3^-$ has been linked to $g_s$, as its uptake and accumulation in guard cells induces their depolarization and, eventually, stomatal opening (F.-Q. Guo et al., 2003). Since $NO_3^-$ can be easily transported and stored in the leaves (Marschner, 2011), it can be assumed that $NO_3^-$ concentration in the leaves was higher in $NO_3^-$ relative to $NH_4^+$-treated plants, which consequently enhanced stomatal opening at high VPD maintaining high A. Since $g_s$ limits A more strongly at high transpirational demand (Ohsumi et al., 2008), we hypothesize that the advantageous effects of $NO_3^-$ on $g_s$ and A were only observed at high VPD.

Assimilation of N-sources and water uptake and use seem to be linked since, in previous studies, leaf gas exchange of rice plants did not differ between N-sources under well-water conditions, but $NO_3^-$ nutrition led to reduced $g_s$, A, SPAD, and water consumption under PEG-induced water stress, compared to $NH_4^+$ (Ding et al., 2015; S. Guo et al., 2008). Under water deficit, $NH_4^+$ improved the water relations of rice plants *via* maintaining water uptake (g plant$^{-1}$ ; S. Guo et al., 2008), whereas $NO_3^-$ increased the share of root aerenchyma, leading to decreased water uptake (Yang et al., 2012). In our experiments, a decrease of WUEi ($A/g_s$) under $NO_3^-$ nutrition at high VPD (Table 4) shows high transpirational costs for $CO_2$ assimilation, which will be a disadvantage under conditions of soil water deficit. However, water supply was not a limiting factor in our hydroponic experimental setup.

Moreover, $g_s$ can be seen as an important indicator of the plant's water status (Parkash & Singh, 2020), with plants being considered as water-stressed if $g_s$ is reduced by 40%, compared to well-watered conditions (Perdomo et al., 2016). Under this precondition, plants acclimated to high VPD did not suffer from severe atmospheric water deficit in Experiment 3 since $g_s$ was on average only 27% lower than that of plants at low VPD. Zhong et al. (2018) postulated that the larger stomatal aperture caused by $NO_3^-$ is advantageous for the plant, provided that the leaf water content is not impaired by water deficit, which was observed in Experiment 3. In contrast, in Experiment 2, rice plants were transferred from low to high VPD, and after 1 week, $g_s$ was still reduced by more than 50% on average, implying that the leaf water status was significantly affected, and plants suffered from water stress. Accordingly, A was substantially decreased after transfer of the plants to high VPD in both N treatments, and in Jasmine 85, $NO_3^-$ led to a significantly lower A relative to $NH_4^+$ as observed in previous studies (Ding et al., 2015; S. Guo et al., 2008). Therefore, we hypothesize that $NO_3^-$ nutrition can improve leaf gas exchange in specific rice varieties at high VPD, provided an adequate water supply and sufficient time for acclimation.

The uptake, reduction, and assimilation of $NO_3^-$ consume more energy than that of $NH_4^+$ (Raven, 1985). However, a surplus of NADPH generation during the light reaction is not completely consumed during the assimilation of $CO_2$, and $NO_3^-$ assimilation in the leaves represents an additional NADPH sink, which is not available in $NH_4^+$-fed plants (S. Guo, Zhou, et al., 2007). Therefore, $NO_3^-$ nutrition could protect chloroplasts from photo-damage and maintain the stability of the photosynthetic apparatus when light absorption exceeds the capacity for its utilization during photosynthesis (Zhang et al., 2014). Moreover, NADPH generation is driven by light intensity (Hashida & Kawai-Yamada, 2019), which could explain the higher A in $NO_3^-$-fed plants in our first experiment in greenhouse condition, where light intensity was higher than in the other experiments conducted in growth chambers.

## 4.2 | Varietal variation of leaf gas exchange in response to N-source at high VPD

Rice varieties showed large differences in leaf gas exchange in response to N-source at high VPD where IR64, BT7, NU838, and Nipponbare showed higher $g_s$ and A in $NO_3^-$ relative to $NH_4^+$ nutrition, whereas no significant difference was found between N treatments for the other varieties. Since A was closely correlated with $g_s$ across varieties, the

**TABLE 4** Intrinsic water-use efficiencies (WUEi) of four rice varieties at low and high VPD and the difference in WUEi between the two VPDs (%) in Experiments 2 and 3. Data presented are mean values of four replications. Different small letter indicates the significant differences between N-sources, while different capital letters indicate significant differences between varieties

| | | WUEi (μmol mmol$^{-1}$) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Experiment 2 | | | | | | Experiment 3 | | | | | | | |
| Rice cultivar | N-source | Low VPD | | High VPD | | | (%)[a] | Low VPD | | | High VPD | | | (%)[a] |
| IR64 | $NH_4^+$ | 0.026 | A | 0.051 | a | A | 98.6 | 0.029 | a | A | 0.034 | a | A | 18.6 |
| IR64 | $NO_3^-$ | 0.025 | A | 0.042 | b | A | 71.7 | 0.025 | b | A | 0.028 | b | A | 10.4 |
| BT7 | $NH_4^+$ | 0.027 | A | 0.049 | a | A | 79.9 | 0.022 | | B | 0.037 | a | A | 69.8 |
| BT7 | $NO_3^-$ | 0.026 | A | 0.041 | b | A | 58.6 | 0.023 | | A | 0.031 | b | A | 37.1 |
| KD18 | $NH_4^+$ | 0.020 | B | 0.035 | a | B | 77.1 | 0.020 | | B | 0.024 | | C | 21.4 |
| KD18 | $NO_3^-$ | 0.017 | B | 0.030 | b | B | 80.2 | 0.019 | | B | 0.023 | | B | 19.1 |
| Jasmine 85 | $NH_4^+$ | 0.021 | B | 0.036 | a | B | 66.3 | 0.021 | | B | 0.027 | | B | 28.3 |
| Jasmine 85 | $NO_3^-$ | 0.018 | B | 0.025 | b | B | 39.6 | 0.019 | | B | 0.030 | | A | 59.6 |

[a]Difference between low and high VPD.

difference in A between varieties may be attributed to differences in the response of $g_s$ to N-source at high VPD. After long-term exposure to high VPD in Experiment 3, $NO_3^-$ induced a higher R/S ratio in IR64 and BT7, with a high positive correlation between R/S ratio and $g_s$ (see Figure S2). Varietal differences in root characteristics in response to N-source have been observed before. Song et al. (2011) found that the presence of $NO_3^-$ improved root initiation and root growth in varieties with a high N-use efficiency only. Plant N content was not measured in our experiments; however, N was entirely depleted in the residual nutrition solution after 7 days, and thus we assume that the entire N provided in the nutrient solution was taken up. Since in Experiment 3, IR64, BT7, and Jasmine 85 showed higher biomass than KD18 across N-forms, they probably had higher N-use efficiency. The higher R/S ratio under $NO_3^-$ nutrition in IR64 and BT7 at high VPD could explain the higher $g_s$ since a high R/S ratio supports water uptake, which is consistent with higher transpiration rates in $NO_3^-$-fed plants (see Figure S4). Probably since morphological adaptations need some time to develop, differences in R/S ratio were not observed after short-term exposure to high VPD but only at long-term.

Despite differences in $g_s$ and A, no differences in R/S ratio between N treatments were found in the first experiment, showing that R/S ratio is not the only reason for differences in leaf gas exchange in response to N-form. $NO_3^-$ and amino acids serve as osmotic agents in plant tissues, which are involved in regulating the acclimation of photosynthesis in rice plants; however, varietal differences were observed in the accumulation and assimilation of $NO_3^-$ (Fan et al., 2007; Zhong et al., 2018). There were differences in uptake rate as well as assimilation of $NO_3^-$ between rice varieties at high VPD but not at low VPD (Vu et al., 2020). Moreover, rice varieties also show a large variation in osmotic adjustment as well as dehydration tolerance (Lilley & Ludlow, 1996). Therefore, we hypothesize that the difference in $g_s$ of rice varieties in response to N-form under high VPD may be partly attributed to the varietal difference in osmotic adjustment caused by assimilation and accumulation of $NO_3^-$.

$NO_3^-$ nutrition led to a higher SLA in KD18 and Jasmine 85 at low VPD and in all rice varieties at high VPD in Experiment 3. Several authors have highlighted the advantages of a high SLA for photosynthesis. Evans and Poorter (2001) demonstrated that a high SLA is important for maximum carbon gain per unit leaf mass, especially at low-light conditions (Schmierer et al., 2021). While thicker leaves have more structural material relative to metabolic components and increased internal shading, leading to lower light absorption (Terashima & Hikosaka, 1995), they also slow down intercellular gaseous diffusion, which probably limits $CO_2$ assimilation (Parkhurst, 1994). One reason for higher SLA in $NO_3^-$-fed plants is that $NO_3^-$ nutrition can increase cation uptake and lead to higher concentrations of osmolytes (*i.e.*, $NO_3^-$, cations), resulting in an increased leaf expansion (Chaillou et al., 1986; Raab & Terry, 1995).

IR64 and BT7 showed a higher WUEi than KD18 and Jasmine 85 at both VPDs and in Experiments 2 and 3 (Table 4). Gilbert et al. (2011) demonstrated a wide varietal variation in WUEi, which was attributed to the variation in $g_s$ and the curvature of the relationship between A and $g_s$. Both, reducing $g_s$ per amount of $CO_2$ assimilated or enhancing the A at a given $g_s$ can improve WUEi (Blankenagel et al., 2018). In our study, IR64 and BT7 showed a high WUEi, which is especially beneficial at high transpirational demand. Since both varieties also showed a higher A in $NO_3^-$ treatments, we hypothesize that $NO_3^-$ may increase leaf gas exchange in varieties with high WUEi. As the number of varieties in our study was limited, further research would be needed to confirm this finding.

### 4.3 | Growth response of different rice varieties to N-source at low and high VPD

In most cases, when A of $NO_3^-$-fed plants exceeded A of $NH_4^+$-fed plants, no difference in plant DM was found, showing that the advantage of increased carbon assimilation is not directly translated into growth. Since $NO_3^-$ assimilation has a higher energy demand than the assimilation of $NH_4^+$ (Bloom et al., 1992; Raven, 1985; Salsac et al., 1987), we assume that the higher carbon assimilation in $NO_3^-$-grown plants mainly compensated the higher demand of assimilates for $NO_3^-$ assimilation.

In Experiment 3, total DM was lower in $NH_4^+$- than in $NO_3^-$-treated plants in KD18 and Jasmine 85 at low VPD and in BT7 and Jasmine 85 at high VPD. One reason for this was a large share of dead leaves of KD18 and Jasmine 85 in the $NH_4^+$ treatment at both VPD levels, leading to a relatively low total DM (see Figure S5). Dead leaves showed a striking orange coloration-like symptoms of leaf-oranging, which were probably caused by $NH_4^+$ toxicity as described by Liao et al. (1994). Symptoms of $NH_4^+$ toxicity are attributed to a decline in cation uptake, acidification of the external medium, disturbance of intracellular pH, or changes in the amino acid or organic acid profile of the plants (Britto & Kronzucker, 2002). In our experiment, the pH of the nutrient solution was adjusted daily; however, a large decline in pH was observed in the $NH_4^+$ nutrient solution within the day. Moreover, we assume that these varieties suffered from $NH_4^+$ toxicity even at a moderate concentration since rice plants can accumulate substantial amounts of unmetabolized $NH_4^+$ in the cytoplasm and vacuole in the root even at low external $NH_4^+$ concentrations (Wang et al., 1993). Although rice is $NH_4^+$ tolerant, it can suffer from $NH_4^+$ toxicity due to excessive accumulation of $NH_4^+$ in the leaves (Chen et al., 2013; Liao et al., 1994), which has been associated with two factors. First, a reduction in the activity of assimilating enzymes due to antagonistic effects between $NH_4^+$ and cations, particularly $K^+$, and second, an uptake rate of $NH_4^+$ exceeding the assimilation capacity of the enzymes (Liao et al., 1994). Varieties with a high N-use efficiency showed a greater capacity to resist high $NH_4^+$ concentrations, whereas susceptibility of varieties with low N-use efficiency was related to a relatively poor regulation of plasma-membrane influx of $NH_4^+$ under high $NH_4^+$, accompanied by enhanced root respiration (Chen et al., 2013). Moreover, previous studies have shown that rice plants in flooded soils also take up substantial amounts of $NO_3^-$ instead of $NH_4^+$ through nitrification in the rhizosphere caused by the release of oxygen from the roots (Kirk, 2001; Kirk & Kronzucker, 2005), but this might be marginal in our study as the experiment was conducted in a nutrient solution. However, the mechanism of $NH_4^+$ uptake and the variation in $NH_4^+$ resistance of rice varieties at different N concentrations/contents and diurnal VPDs need to be elucidated.

## 5 | CONCLUSION

The present work evaluated the effect of N-sources on leaf gas exchange and the growth of different rice varieties at low and high VPD. N-sources significantly influenced leaf gas exchange of rice plants at high VPD with a higher A in $NO_3^-$- than in $NH_4^+$-fed plants, which was mainly attributed to higher $g_s$ and supported by a higher R/S ratio. However, this effect varied among rice varieties and was not observed at low VPD or shortly after transferring plants from low to high VPD. With higher transpirational costs for photosynthesis in $NO_3^-$-fed plants, we hypothesize that $NO_3^-$ nutrition can improve leaf gas exchange of rice plants at high VPD in high WUEi varieties, provided a sufficient water supply. Some varieties showed symptoms of $NH_4^+$ toxicity even at moderate concentrations, indicating genotypic diversity in transport and assimilation capacity of $NH_4^+$ among rice varieties. Since a high R/S ratio was not a unique factor improving $g_s$ in $NO_3^-$ supplied plants, further studies are needed to elucidate the role of $NO_3^-$ uptake and assimilation in controlling $g_s$ at high VPD.

## ACKNOWLEDGMENTS

Duy Hoang Vu gratefully acknowledges the support of the *German Academic Exchange Service* (*DAAD*) through providing a PhD-scholarship *via* the Development-Related Postgraduate Course "*Agricultural Economics, Bioeconomy and Rural Development*." The authors thank *VNUA* and *Vinaseed* Group (Vietnam) for kindly providing the seeds of the Vietnamese rice varieties used in this study. The authors thank *Dr. Jens Hartung* (University of Hohenheim) who greatly assisted with the statistical analysis in the paper. The excellent technical assistance of *Julia Asch* is gratefully acknowledged.

Open Access funding enabled and organized by Projekt DEAL.

## DATA AVAILABILITY STATEMENT

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

## REFERENCES

Blankenagel, S., Yang, Z., Avramova, V., Schön, C.-C., & Grill, E. (2018). Generating plants with improved water use efficiency. Agronomy, *8*(9), 194. https://doi.org/10.3390/agronomy8090194

Bloom, A. J., Sukrapanna, S. S., & Warner, R. L. (1992). Root respiration associated with ammonium and nitrate absorption and assimilation by barley. *Plant Physiology*, *99*, 1294–1301.

Bowman, D. C., & Paul, J. L. (1988). Uptake and assimilation of $NO_3^-$ and $NH_4^+$ by nitrogen-deficient perennial ryegrass turf. *Plant Physiology*, *88*, 1303–1309.

Briones, A. M., Okabe, S., Umemiya, Y., Ramsing, N. -B., Reichardt, W., & Okuyama, H. (2003). Ammonia-oxidizing bacteria on root biofilms and their possible contribution to N use efficiency of different rice cultivars. *Plant and Soil*, *250*, 335–348.

Britto, D. T., & Kronzucker, H. J. (2002). $NH_4^+$ toxicity in higher plants: a critical review. *Journal of Plant Physiology*, *159*, 567–584.

Buresh, R. J., Reddy, K. R., & van Kessel, C. (2008). Nitrogen transformations in submerged soils. In J. S. Schepers & W. R. Raun (Eds.), *Nitrogen in Agricultural Systems* (pp. 401–436). American Society of Agronomy.

Chaillou, S., Morot-Gaudry, J. -F., Lesaint, C., Salsac, L., & Jolivet, E. (1986). Nitrate or ammonium nutrition in French bean. *Plant and Soil*, *91*, 363–365.

Chen, G., Guo, S., Kronzucker, H. J., & Shi, W. (2013). Nitrogen use efficiency (NUE) in rice links to $NH_4^+$ toxicity and futile $NH_4^+$ cycling in roots. *Plant and Soil*, *369*, 351–363.

Chunmei, X., Liping, C., Song, C., Guang, C., Danying, W., & Xiufu, Z. (2020). Rhizosphere aeration improves nitrogen transformation in soil, and nitrogen absorption and accumulation in rice plants. >*Rice Science*, *27*, 162–174.

Claussen, W., & Lenz, F. (1995). Effect of ammonium and nitrate on net photosynthesis, flower formation, growth and yield of eggplants (Solanum melongena L.). *Plant and Soil*, *171*, 267–274.

Ding, L., Gao, C., Li, Y., Li, Y., Zhu, Y., Xu, G., Shen, Q., Kaldenhoff, R., Kai, L., & Guo, S. (2015). The enhanced drought tolerance of rice plants under ammonium is related to aquaporin (AQP). *Plant Science*, *234*, 14–21.

Duan, Y. H., Zhang, Y. L., Shen, Q. R., & Wang, S. W. (2006). Nitrate effect on rice growth and nitrogen absorption and assimilation at different growth stages. *Pedosphere*, *16*, 707–717.

Evans, J. R., & Poorter, H. (2001). Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell and Environment*, *24*, 755–767.

Fan, X., Jia, L., Li, Y., Smith, S. J., Miller, A. J., & Shen, Q. (2007). Comparing nitrate storage and remobilization in two rice cultivars that differ in their nitrogen use efficiency. *Journal of Experimental Botany*, *58*, 1729–1740.

Gilbert, M. E., Zwieniecki, M. A., & Holbrook, N. M. (2011). Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought. *Journal of Experimental Botany*, *62*, 2875–2887.

Grossiord, C., Buckley, T. N., Cernusak, L. A., Novick, K. A., Poulter, B., Siegwolf, R. T. W., Sperry, J. S., & McDowell, N. G. (2020). Plant responses to rising vapor pressure deficit. *New Phytologist*, *226*, 1550–1566.

Guo, F. -Q., Young, J., & Crawford, N. M. (2003). The nitrate transporter AtNRT1.1 (CHL1) functions in stomatal opening and contributes to drought susceptibility in Arabidopsis. *Plant Cell*, *15*, 107–117.

Guo, S., Brück, H., & Sattelmacher, B. (2002). Effects of supplied nitrogen form on growth and water uptake of French bean (Phaseolus vulgaris L.) plants. *Plant and Soil*, *239*, 267–275.

Guo, S., Zhou, Y., Gao, Y. -X., Li, Y., & Shen, Q. -R. (2007). New insights into the nitrogen form effect on photosynthesis and photorespiration. *Pedosphere*, *17*, 601–610.

Guo, S., Zhou, Y., Li, Y., Gao, Y., & Shen, Q. (2008). Effects of different nitrogen forms and osmotic stress on water use efficiency of rice (Oryza sativa). *Annals of Applied Biology*, *153*, 127–134.

Guo, S., Zhou, Y., Shen, Q., & Zhang, F. (2007). Effect of ammonium and nitrate nutrition on some physiological processes in higher plants–growth, photosynthesis, photorespiration, and water relations. *Plant Biology*, *9*, 21–29.

Hashida, S., & Kawai-Yamada, M. (2019). Inter-organelle NAD metabolism underpinning light responsive NADP dynamics in plants. *Frontiers in Plant Science*, *10*, 960. https://doi.org/10.3389/fpls.2019.00960

Hirooka, Y., Homma, K., Shiraiwa, T., Makino, Y., Liu, T., Xu, Z., & Tang, L. (2018). Yield and growth characteristics of erect panicle type rice (Oryza sativa L.) cultivar, Shennong265 under various crop management practices in Western Japan. *Plant Production Science*, *21*, 1–7.

Horchani, F., Hajri, R., & Aschi-Smiti, S. (2010). Effect of ammonium or nitrate nutrition on photosynthesis, growth, and nitrogen assimilation in tomato plants. *Journal of Plant Nutrition and Soil Science*, *173*, 610–617.

Ji, X. -M., & Peng, X. -X. (2005). Oxalate accumulation as regulated by nitrogen forms and its relationship to photosynthesis in rice (Oryza sativa L.). *Journal of Integrative Plant Biology*, *47*, 831–838.

Kinyumu, D. M. (2009). Comparative study on the growth and yield of NERICA cultivated with organic and inorganic fertilizers: Participatory on-farm research at Marakwet District in Kenya. *Journal of Developments in Sustainaible Agriculture*, *4*, 106–117.

Kirk, G. J. D. (2001). Plant-mediated processess to acquire nutrients: nitrogen uptake by rice plants. *Plant and Soil*, *232*, 129–134.

Kirk, G. J. D., & Kronzucker, H. (2005). The potential for nitrification and nitrate uptake in the rhizosphere of wetland plants: A modelling study. *Annals of Botany*, *96*, 639–646.

Li, Y., Gao, Y., Ding, L., Shen, Q., & Guo, S. (2009). Ammonium enhances the tolerance of rice seedlings (Oryza sativa L.) to drought condition. *Agricultural Water Management*, *96*, 1746–1750.

Li, Y. L., Zhang, Y. L., Hu, J., & Shen, Q. R. (2007). Contribution of nitrification happened in rhizospheric soil growing with different rice cultivars to N nutrition. *Biology and Fertility of Soils*, *43*, 417–425.

Liao, Z., Woodard, H. J., & Hossner, L. R. (1994). The relationship of soil and leaf nutrients to rice leaf oranging. *Journal of Plant Nutrition*, *17*, 1781–1802.

Lilley, J. M., & Ludlow, M. M. (1996). Expression of osmotic adjustment and dehydration tolerance in diverse rice lines. *Field Crops Research*, *48*, 185–197.

Lu, Y. X., Li, C. J., & Zhang, F. S. (2005). Transpiration, potassium uptake and flow in tobacco as affected by nitrogen forms and nutrient levels. *Annals of Botany*, *95*, 991–998.

Mackill, D. J., & Khush, G. S. (2018). IR64: a high-quality and high-yielding mega variety. *Rice*, *11*, 18. https://doi.org/10.1186/s12284-018-0208-3

Magalhäes, J. R., & Huber, D. M. (1989). Ammonium assimilation in different plant species as affected by nitrogen form and pH control in solution culture. *Fertilizer Research*, *21*, 1–6.

Marschner, P. (2011). *Marschner's mineral nutrition of higher plants*. Academic Press.

Ohsumi, A., Hamasaki, A., Nakagawa, H., Homma, K., Horie, T., & Shiraiwa, T. (2008). Response of leaf photosynthesis to vapor pressure difference in rice (*Oryza sativa* L) varieties in relation to stomatal and leaf internal conductance. *Plant Production Science*, *11*, 184–191.

Parkash, V., & Singh, S. (2020). A review on potential plant-based water stress indicators for vegetable crops. *Sustainability*, *12*(10), 3945. https://doi.org/10.3390/su12103945

Parkhurst, D. F. (1994). Diffusion of $CO_2$ and other gases inside leaves. *New Phytologist*, *126*, 449–479.

Perdomo, J. A., Carmo-Silva, E., Hermida-Carrera, C., Flexas, J., & Galmés, J. (2016). Acclimation of biochemical and diffusive components of photosynthesis in rice, wheat, and maize to heat and water deficit: Implications for modeling photosynthesis. *Frontiers in Plant Science*, *7*, 1719. https://doi.org/10.3389/fpls.2016.01719

Pham D. Q., Nguyen Q. L., Nguyen N. H., Le Q. T., Le V. Đ., Do K. N., Nguyen Q. H., Dinh C. C., Nguyen V. V., Tran T. N., Dao N. C., & Nguyen Q. M. (2009). *966 new crop varieties*. (in Vietnamese). Agriculture Publishing House (APH).

Pieters, A. J., Paul, M. J., & Lawlor, D. W. (2001). Low sink demand limits photosynthesis under Pi deficiency. *Journal of Experimental Botany*, *52*, 1083–1091.

Raab, T. K., & Terry, N. (1995). Carbon, nitrogen, and nutrient interactions in *Beta vulgaris* L. as influenced by nitrogen source, $NO_3^-$ *versus* $NH_4^+$. *Plant Physiology*, *107*, 575–585.

Rashid, M. A., Andersen, M. N., Wollenweber, B., Zhang, X., & Olesen, J. E. (2018). Acclimation to higher VPD and temperature minimized negative effects on assimilation and grain yield of wheat. *Agricultural and Forest Meteorology*, *248*, 119–129.

Raven, J. A. (1985). (Tansley review no. 2) Regulation of pH and generation of osmolarity in vascular plants: A cost-benefit analysis in relation to efficiency of use of energy, nitrogen and water. *New Phytologist*, *101*, 25–77.

Salsac, L., Chaillou, S., Morot-Gaudry, J.-F., Lesaint, C., & Jolivet, E. (1987). Nitrate and ammonium nutrition in plants. *Plant Physiology and Biochemistry*, *25*, 805–812.

Schjoerring, J. K., Husted, S., Mäck, G., & Mattsson, M. (2002). The regulation of ammonium translocation in plants. *Journal of Experimental Botany*, *53*, 883–890.

Schmierer, M., Knopf, O., & Asch, F. (2021). Growth and photosynthesis responses of a super dwarf rice genotype to shade and nitrogen supply. *Rice Science*, *28*, 178–190.

Song, W., Makeen, K., Wang, D., Zhang, C., Xu, Y., Zhao, H., Tu, E., Zhang, Y., Shen, Q., & Xu, G. (2011). Nitrate supply affects root growth differentially in two rice cultivars differing in nitrogen use efficiency. *Plant and Soil*, *343*, 357–368.

Tabuchi, M., Abiko, T., & Yamaya, T. (2007). Assimilation of ammonium ions and reutilization of nitrogen in rice (*Oryza sativa* L.). *Journal of Experimental Botany*, *58*, 2319–2327.

Terashima, I., & Hikosaka, K. (1995). Comparative ecophysiology of leaf and canopy photosynthesis. *Plant, Cell and Environment*, *18*, 1111–1128.

Torralbo, F., González-Moro, M. B., Baroja-Fernández, E., Aranjuelo, I., & González-Murua, C. (2019). Differential regulation of stomatal conductance as a strategy to cope with ammonium fertilizer under ambient *versus* elevated $CO_2$. *Frontiers in Plant Science*, *10*, 597. https://doi.org/10.3389/fpls.2019.00597

Tran, T. T., Kano-Nakata, M., Suralta, R. R., Menge, D., Mitsuya, S., Inukai, Y., & Yamauchi, A. (2015). Root plasticity and its functional roles were triggered by water deficit but not by the resulting changes in the forms of soil N in rice. *Plant and Soil*, *386*, 65–76.

Vu, D. H., Stuerz, S., & Asch, F. (2020). Nutrient uptake and assimilation under varying day and night root zone temperatures in lowland rice. *Journal of Plant Nutrition and Soil Science*, *183*, 602–614.

Wang, M. Y., Siddiqi, M. Y., Ruth, T. J., & Glass, A. (1993). Ammonium uptake by rice roots (I. Fluxes and subcellular distribution of $^{13}NH_4^+$). *Plant Physiology*, *103*, 1249–1258.

Yang, X., Li, Y., Ren, B., Ding, L., Gao, C., Shen, Q., & Guo, S. (2012). Drought-induced root aerenchyma formation restricts water uptake in rice seedlings supplied with nitrate. *Plant & Cell Physiology*, *53*, 495–504.

Yoshida, Shou., Forno, D. A., Cock, J. H., & Gomez, K. A. (1976). *Laboratory manual for physiological studies of rice*. IRRI.

Yuan, W., Zheng, Y., Piao, S., Ciais, P., Lombardozzi, D., Wang, Y., Ryu, Y., Chen, G., Dong, W., Hu, Z., Jain, A. K., Jiang, C., Kato, E., Li, S., Lienert, S., Liu, S., Nabel, J. E. M. S., Qin, Z., Quine, T., … Yang, S. (2019). Increased atmospheric vapor pressure deficit reduces global vegetation growth. *Science Advances*, *5*(8), eaax1396. https://doi.org/10.1126/sciadv.aax1396

Zhang, Q., Wu, S., Chen, C., Shu, L. -Z., Zhou, X. -J., & Zhu, S. -N. (2014). Regulation of nitrogen forms on growth of eggplant under partial root-zone irrigation. *Agricultural Water Management*, *142*, 56–65.

Zhong, C., Cao, X., Bai, Z., Zhang, J., Zhu, L., Huang, J., & Jin, Q. (2018). Nitrogen metabolism correlates with the acclimation of photosynthesis to short-term water stress in rice (*Oryza sativa* L.). *Plant Physiology and Biochemistry*, *125*, 52–62.

Zhou, Y., Zhang, Y., Wang, X., Cui, J., Xia, X., Shi, K., & Yu, J. (2011). Effects of nitrogen form on growth, $CO_2$ assimilation, chlorophyll fluorescence, and photosynthetic electron allocation in cucumber and rice plants. *Journal of Zhejiang University of Science B*, *12*, 126–134.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Vu, D. H., Stuerz, S., Pieters, A., Asch, F. (2021). Leaf gas exchange of lowland rice in response to nitrogen source and vapor pressure deficit. *Journal of Plant Nutrition and Soil Science*, *184*, 448–460. https://doi.org/10.1002/jpln.202100032