Acta Geochim (2018) 37(6):875–885
https://doi.org/10.1007/s11631-018-0296-1

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ORIGINAL ARTICLE

Does bicarbonate affect the nitrate utilization and photosynthesis of *Orychophragmus violaceus*?

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Received: 15 March 2018 / Revised: 9 May 2018 / Accepted: 29 August 2018 / Published online: 6 September 2018
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Abstract The effect of bicarbonate (HCO_3^-) on the growth and development of plants varies by species. To better understand inorganic carbon and nitrogen assimilation changes of karst-adaptable plants under different HCO_3^- treatments, we conducted experiments on seedlings and in vitro plantlets of *Orychophragmus violaceus* (*Ov*). We found that the vital photosynthesis potential (as measured by net photosynthetic rate, actual photochemical efficiency of photosystem-II, photochemical quenching coefficient, and the instantaneous carbon isotope ratio of 3-phosphoglycerate) was consistent under different HCO_3^- treatments of *Ov*. Bicarbonate's lack of effect on carbon assimilation of *Ov* may be related to carbonic anhydrase in *Ov* converting HCO_3^- to H_2O and CO_2. In this way, *Ov* could prevent HCO_3^- ion toxicity and high pH from harming its growth and development under HCO_3^- stress. This study also found that high HCO_3^- concentrations could promote nitrogen assimilation and utilization of *Ov* through changes in related indexes (foliar nitrogen isotope fractionation ratio, stable nitrogen isotope assimilation ratio, foliar stable nitrogen isotope fractionation, nitrate nitrogen utilization efficiency, and nitrate utilization share) under different HCO_3^- treatments. Bicarbonate has different effects on photosynthesis and on inorganic nitrogen assimilation of *Ov*, which may be connected to photosynthesis providing electrons for nitrate/nitrite reduction through the photosynthetic chain.

Keywords Nitrogen assimilation · Photosynthetic capacity · HCO_3^- · *Orychophragmus violaceus*

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1 Introduction

Nitrogen is one of the most important essential nutrients in the growth and development of plants. It is a major component of proteins, nucleic acids, enzymes, and chlorophyll (Raven 2003; George et al. 2008; Hawkesford et al. 2012). Nitrogen also plays vital roles in improving photosynthesis, maintaining life and growth, and promoting plant yield (Cechin et al. 2004). Nitrate and ammonium are the main inorganic nitrogen sources absorbed and used by higher plants.

It is known that inorganic nitrogen assimilation pathways, types and vitalities of key nitrogen assimilation enzymes, and affinity transport systems vary by plant species (Crawford 1995; Evans et al. 1996; Crawford and Glass 1998; Robinson et al. 1998; Campbell 1999; Comstock 2001; Kaiser and Huber 2001; Britto and Kronzucker 2006; George et al. 2008). Nitrate is the main inorganic nitrogen source that most plants tend to utilize (Raven 2003; Wang et al. 2012) due to its benefits for plant nutrition and physiological regulation. Ammonium salt in certain concentrations is toxic to most plants and can inhibit plant growth (Britto and Kronzucker 2002; Bittsánszky et al. 2015). Appropriate types of inorganic nitrogen sources at suitable concentration are vital to the growth of plants (George et al. 2008).

The stable isotope technique can be used to assess stable nitrogen isotope fractionation of plants, since stable nitrogen isotope composition ($\delta^{15}N$) in plants

changes when plants change switch between different nitrogen sources and concentrations (Mariotti et al. 1982). The stable isotope technique can also be used to evaluate nitrogen assimilation capacity (Robinson 2001; Kalcsits et al. 2014, 2015) and to determine inorganic nitrogen utilization preferences of the plants (Kalcsits et al. 2015).

Photosynthesis plays an important role in the growth and development of plants. Its capacity can represent the productivity of plants (Chikov 2008), and can also be used to represent plant growth potential. The variation of chlorophyll fluorescence in photosystem-II complex (PSII) can reflect almost all aspects of photosynthetic activity. Actual photochemical efficiency of photosystem-II (ΦPSII) can indicate the tolerance of plants to environmental stress. The photochemical quenching coefficient (qP) can demonstrate the share of light energy absorbed by the pigment of the PSII antenna, and can be used to represent electron transfer in photochemical reactions and to denote the openness of the PSII reaction center (Maxwell and Johnson 2000; Panda et al. 2008; Chaves et al. 2009). The influence of environmental factors on photosynthesis can therefore be estimated by measuring changes to the photosynthetic parameters (net photosynthetic rate (Pn), ΦPSII, qP).

To date, many studies have investigated the influence of environmental factors on plant photosynthesis by measuring the change in foliar carbon isotope composition ($\delta^{13}C$) under different environmental conditions (Pan et al. 2016). However, foliar $\delta^{13}C$ may be affected by both physiological and environmental conditions as leaves contain many carbon-based substances (cellulose, hemicellulose, lignin, etc.). Meanwhile, considering only when 1,5-ribulose bisphosphate (RuBP) is under the catalysis of ribulose diphosphate carboxylase (Rubisco) and combines with CO_2 to produce 3-phosphoglycerate (3-PGA), can 3-PGA enter the photosynthetic carbon cycle and become the first stable intermediate of the Calvin cycle (Xia et al. 2002; Guo 2014). Thereby, the changes in instantaneous carbon isotope ratio of 3-PGA ($\delta^{13}C_{PGA}$) under different external environmental conditions were determined.

Photosynthesis is closely related to nitrogen metabolism in plants. First, key nitrogen metabolism enzymes such as nitrite reductase (NiR) and glutamine synthetase (GS) are distributed in chloroplasts (Kimata-Ariga and Hase 2014). Second, photosynthesis provides reductants and energy for nitrogen metabolism while the photosynthetic carbon cycle regulates nitrite reduction (Singh et al. 2008). Third, light can indirectly affect nitrate reductase (NR) activity by changing the permeability of cell membranes to nitrate (Nemie-Feyissa et al. 2013). Therefore, investigating the changes in inorganic nitrogen metabolism and photosynthesis under different environmental conditions is the best way to understand the environmental adaptability of plants.

High bicarbonate (HCO_3^-) concentration ($\geq$ 5 mM L^{-1}) (Yan et al. 2012), low–ammonium salt, high-nitrate soil, along with other adverse environmental conditions (low levels of organic matter, phosphorus, potassium, trace elements; and high pH, calcium and magnesium ions, etc.) of the karst region in China, all hinder plant growth (Wu 1997; Jiang 2000) and lead to a relatively simple community structure and an easily destroyed ecosystem (Alcántara et al. 2000; Liu et al. 2011). Previous studies have indicated that *Orychophragmus violaceus* (*Ov*) has developed a special inorganic carbon utilization strategy (Wu et al. 2004; Zhu et al. 2013). *Ov* has high carbonic anhydrase (CA) activity and HCO_3^- utilization capacity (Wu et al. 2011) and its photosynthesis does not experience "midday depression" (Wu et al. 2005). Additionally, *Ov* has higher absorption affinity and low absorbable external concentration for nutrient elements including ammonium nitrogen (Wu et al. 2004) and its soluble sugar, proline, and superoxide dismutase increase under drought stress (Li et al. 2011). Together, these characteristics allow some plant species—known as karst-adaptable plants, including *Ov*—to adapt to the adverse effects of karst environmental conditions (Wu 1997; Wu et al. 2004).

Different plant species exhibit various levels of adaptation to HCO_3^- (Brown et al. 1955). The effects of HCO_3^- (the most typical anionic component in karst soils) on the growth and development of plants are inconsistent. Some scholars believe that high HCO_3^- concentration has an adverse effect on the growth and development of plants, manifested in the following ways: (1) High-concentration HCO_3^- can lead to a high-pH environment, hindering normal growth and essential nutrient absorption (Mendel et al. 1982; Yang et al. 2012). For example, high-concentration HCO_3^- could result in Fe deficiency in barley, sorghum, and corn, resulting from the accumulation of organic acids around roots (Alhendawi et al. 1997). (2) High-concentration HCO_3^- can affect protein synthesis and respiration in plants, inhibiting nutrient absorption and growth and causing adverse symptoms (such as chlorosis) in many plants. For instance, the rootstock of peach [*Prunus persica* (Batsch) L.] suffers chlorosis when the concentration of HCO_3^- exceeds 6 mM·L^{-1} (Yan et al. 2008). (3) High-concentration HCO_3^- can inhibit the normal growth and development of plants by limiting the expression of genes that control the nutrient absorptive capacity of plants (García et al. 2014). (4) High-concentration HCO_3^- can indirectly lead to physiological drought and inhibit the normal growth and development of plants by increasing the osmotic potential of the soil solution (McCutcheon et al. 2005). And (5) high-concentration HCO_3^- can influence growth and development of plants by producing ion toxicity (Covarrubias and Rombolà 2013).

However, other studies have indicated that some level of HCO_3^- promotes plant growth and photosynthesis because HCO_3^- supports the function and stability of PSII. For example, HCO_3^- is an important component of the water-oxygen complex in PSII and is involved in the electron transport process (Klimov and Baranov 2001; Van Rensen 2002; Klimov et al. 2003). Some plant species, such as *Broussonetia papyrifera* (L.), *Ov*, morning glory, and *Lonicera japonica Thunb.,* could provide inorganic carbon and H_2O for photosynthesis by transforming HCO_3^- into CO_2 and H_2O, which may be related to the high CA activity in these plant species (Wu et al. 2011; Zhu et al. 2013).

It is known that HCO_3^- can buffer the circulating soil solution to pH values of 7.5–8.5, drastically reducing Fe solubility and availability and leading to alkaline soil (Donnini et al. 2009). The first step of nitrate assimilation in higher plants and algae is catalyzed by (NR) (Diego et al. 2004). However, different plant species have various N metabolism responses to HCO_3^- levels. On the one hand, the net nitrate uptake rate and root nitrate accumulation of plants show positive correlation with rhizospheric HCO_3^-, and different plant species experience varied enhancement of nitrate uptake in the presence of HCO_3^- (Cramer et al. 1996; Gao and Lips 1997; Vuorinen and Kaiser 1997; Vuorinen 1997; Wanek and Popp 2000). NR activity can also be stimulated by saline conditions (Misra and Dwiverdi 1990; Sagi et al. 1997). On the other hand, some studies report that excessive HCO_3^- has adverse effects on plant growth and N metabolism. Pandey et al. (2006) demonstrated that excessive HCO_3^- is harmful for plant growth because it greatly inhibits saccharide metabolism and protein synthesis. Barhoumi et al. (2007) also indicated that the growth of two cultivars of pea decreased when cultured in NO_3^- nutrition and under ammoniacal treatment in the presence of HCO_3^- (10 mM). Bie et al. (2004) showed that the growth of two butterhead lettuce (*Lactuca sativa* L.) cultivars had decreased growth under $NaHCO_3$ treatment due to HCO_3^- toxicity and high pH. Colla et al. (2012) even observed a significant depression of shoot, root biomass production, and leaf macro- and micro elements (N, P, K, and Fe) in watermelon plants under high pH. Meanwhile, other studies have confirmed that the activity and transcript expression of NR generally decrease under salt stress (Cramer et al. 1985; Rao and Gnaham 1990; Frechill et al. 2001; Parida and Das 2004; Debouba et al. 2013; Yang et al. 2013) In addition, Diego et al. (2004) and Wang et al. (2016) believe nitrate concentration in roots and leaves, and NR activity in plants are little affected by saline conditions.

Taking into consideration that key enzymes such as NR and GS are distributed in leaves (Crawford 1995; Evans et al. 1996; Robinson et al. 1998; Kaiser and Huber 2001), we selected *Ov* to study inorganic nitrogen assimilation and photosynthesis changes of leaves under different HCO_3^- treatments for 21 days to determine the high HCO_3^- concentration adaptive mechanism of karst-adaptable plants.

2 Materials and methods

2.1 Plant materials and experimental treatments

Ov seeds were germinated in a greenhouse using vermiculite as a culture medium. The artificial greenhouse was maintained under a constant cycle of 12 h per day light intensity of 200 μmol m^{-2} s^{-2} PPFD, and 12 h darkness. The daytime temperature was maintained at 25 ± 2 °C, the nighttime temperature was maintained at 20 ± 2 °C, and the relative humidity was maintained around 50%–60%. After germination, *Ov* plants were continuously cultured with deionized water for about 45 days, then switched to nitrogen-free Hoagland nutrient solution (Table 1) until plants grew to the four-leaf stage. About 15 days later, *Ov* seedlings of similar size were selected for experiments.

To study the effect of HCO_3^- on the nitrate utilization preference of *Ov*, the dry leaf weight (LDW), nitrogen content (N), and foliar nitrogen isotope fractionation ratio ($\delta^{15}N_{new}$) of *Ov* seedlings were measured before and after different HCO_3^- treatments (0 and 10 $mM{\cdot}L^{-1}$) as described below.

Treatment 1: Three plants of *Ov* seedling were randomly selected. All the leaves were cut off, washed, drained, covered with foil, and labeled. The leaf samples were placed in a drying oven at 108 °C for 30 min then

Table 1 Nitrogen-free Hoagland nutrient solution

Macroelement (&)	Mole content/mM
$CaCl_2$	4.00
$MgSO_4{\cdot}7H_2O$	2.00
NaH_2PO4	1.00
Fe(Na)EDTA	2.00
Microelement (#)	**Mole content/μM**
KCl	2.00
H_3BO_3	50.00
$MnSO_4{\cdot}4H_2O$	4.00
$ZnSO_4{\cdot}7H_2O$	4.00
$CuSO_4{\cdot}5H_2O$	2.00

Where & represents macroelement in 1L nitrogen-free Hoagland nutrient solution; # represents microelement in 1 L nitrogen-free Hoagland nutrient solution

immediately transferred to an oven at 90 °C for 1–2 days. All leaf samples were used to determine initial data.

Treatment 2: KNO_3 with 16.99‰ $\delta^{15}N$ and NH_4Cl with − 1.2‰ $\delta^{15}N$ were used as nitrate and ammonium nitrogen sources, respectively, with the Hoagland nutrient solution, to create sole-nitrate nutrient solution of 6, 14, and 15 mM·L^{-1} nitrate and sole-ammonium nutrient solution of 1 mM·L^{-1} ammonium. Then, the same quantity of *Ov* seedlings were randomly selected and planted in these solutions for about 21 days (two or three replicates per treatment). The leaf samples were then subjected to the same methods used for Treatment 1. All leaf samples were tested to obtain the final data.

Treatment 3: To investigate the effect of HCO_3^- on the photosynthesis of *Ov*, the following steps were taken. First, Hoagland nutrient solution was made using KNO_3 with 16.99‰ $\delta^{15}N$ and NH_4Cl with − 1.2‰ $\delta^{15}N$. Then, different concentrations of HCO_3^- (0 and 10 mM·L^{-1}) were added to approximate the HCO_3^- concentration of soil in karst areas of Southwest China (Yan et al. 2012). Next, leaves with similar position, orientation, and size were selected and labeled according to HCO_3^- concentration. After 21 days, the photosynthetic indices, chlorophyll fluorescence parameters, and $\delta^{13}C_{PGA}$ of these labeled leaf samples were tested (two or three replicates per treatment).

2.2 Data determination

2.2.1 Determination of net photosynthetic rate and chlorophyll fluorescence parameters

Pn and chlorophyll fluorescence parameters (actual ΦPSII and qP) of *Ov*, cultured in Hoagland nutrient solution under different HCO_3^- treatments (0 and 10 mM·L^{-1}) for 21 days were measured by Li-6400 (three replicates per treatment).

2.2.2 Determination of instantaneous carbon isotope ratio in 3-PGA ($\delta^{13}C_{PGA}$)

The related $\delta^{13}C_{PGA}$ of *Ov* leaf was obtained by mass spectrometer MAT-252 (three replicates per treatment), after the corresponding 3-PGA of *Ov* leaf was isolated and purified by the chromatographic method (Christeller et al. 1976).

2.2.3 Determination of nitrogen content (N) and stable nitrogen isotope ratio ($\delta^{15}N$)

The leaves of *Ov* seedling before and after cultivation in Hoagland nutrient solution, in sole-nitrate nutrient solution, and in sole-ammonium nutrient solution under different HCO_3^- treatments (0 and 10 mM·L^{-1}) for 21 days were dried and ground. Then, the corresponding N and $\delta^{15}N$ ($^{15}N/^{14}N$) values of these leaf samples were determined using an elemental analyzer (Vario MACRO cube, Germany) and a gas isotope mass spectrometer (MAT-253, Germany) (triplicate measurements). The data correction method of MAT-253 is consistent with the Yousfi et al. (2013) method with a precision control of around 0.2‰.

2.2.4 Determination of foliar nitrogen isotope fractionation ($\delta^{15}N_{new}$) and stable nitrogen isotope fractionation ($\Delta^{15}N$)

The dried leaves of *Ov* seedling were weighed and ground, one-by-one; the corresponding N and $^{15}N/^{14}N$ values of these milled leaf samples were determined using an elemental analyzer (Vario MACRO cube, Germany) and isotope mass spectrometry (MAT-253, Germany). The corresponding $\delta^{15}N_{new}$ was calculated according to Eqs. (1) and (2):

$$\delta^{15}N_1 = f\delta^{15}N_0 + (1 - f)\delta^{15}N_{new} \quad (1)$$

$$f = LDW_0N_0/LDW_1N_1 \quad (2)$$

where LDW_0 represents LDW in whole *Ov* plants before testing; N_0 represents the foliar N of *Ov* before testing; $\delta^{15}N_0$ represents the foliar nitrogen isotope fractionation ratio of *Ov* before testing; LDW_1 denotes the LDW in whole *Ov* plants, cultured in sole-nitrate nutrition solution with 15 mM·L^{-1} nitrate under HCO_3^- treatment (10 mM·L^{-1}) for 21 days; $\delta^{15}N_1$ is the foliar nitrogen isotope fractionation ratio of *Ov* cultured in different solutions under HCO_3^- treatment (10 mM·L^{-1}) for 21 days; and *f* represents the share of initial foliar N as a share of the final foliar N of *Ov*.

It is difficult to differentiate nitrate and ammonium nitrogen sources having the same nitrogen isotope ratio through preliminary study. Therefore, our preliminary foliar nitrogen isotope fractionation ratio of *Ov* in vitro plantlets in sole-nitrate culture medium ($\delta^{15}N$ of nitrate source is 8.08‰) with 10, 20, 40, 80, and 120 mM·L^{-1}

Table 2 The foliar $\delta^{15}N$ of *Ov* in vitro plantlets in sole-nitrate culture medium with different nitrate concentrations for 21 days ($\delta^{15}N$ of nitrate source is 8.08‰)

Treatment (mM·L^{-1})	$\delta^{15}N$ (‰)
10	5.23 ± 0.28
20	5.81 ± 0.25
40	6.09 ± 0.57
80	6.28 ± 0.57
120	6.84 ± 0.66

Each value represents the mean ± SE (*n* = 3)

nitrate (Table 2) were converted to "new" results under the nitrate nitrogen source with 16.99‰ $\delta^{15}N$, using the principle that the stable nitrogen isotope assimilation ratio (NS) is constant.

The foliar stable nitrogen isotope fractionation ($\Delta^{15}N$) of *Ov* was calculated using Eq. (3) (Evans et al. 1996):

$$\Delta^{15}N = \delta^{15}N_{substrate} - \delta^{15}N_{product} \quad (3)$$

where $\delta^{15}N_{product}$ is equivalent to $\delta^{15}N_{new}$ in Eq. (1) and $\delta^{15}N_{substrate}$ is $\delta^{15}N_0$.

2.2.5 Determination of stable nitrogen isotope assimilation ratio (NS)

The foliar NS of *Ov* was calculated according to the formula:

$$NS = \delta^{15}N_{new}/\delta^{15}N_{substrate} \quad (4)$$

where $\delta^{15}N_{new}$ represents the foliar nitrogen isotope fractionation of *Ov* under different HCO_3^- treatments, and $\delta^{15}N_{substrate}$ denotes the nitrogen isotope fractionation of the nitrate nitrogen source.

2.2.6 Determination of nitrate nitrogen utilization efficiency (NUE)

NS represents plant nitrate assimilation capacity under different concentrations of substrate nitrate. The NS of *Ov* was calculated through the following steps.

First, the model for the relationship between NS and sole-nitrate concentration of nutrient solution (C) was established, based on the Michaelis–Menten equation:

$$V = V_{max-s}/(K_m + S) \quad (5)$$

in which the principle is that the relationship between NS and C is similar to the relationship between enzyme activity and substrate concentration (*S*) in enzymatic reactions.

The model for the relationship between NS and C was represented as:

$$NS = NS_{max-C}/(K_m + C) \quad (6)$$

where $NS_{max\text{-}C}$ and K_m are Michaelis equation constants.

Second, the corresponding NS calculation equations were derived according to the Lineweaver–Burk double reciprocal mapping method.

Third, the corresponding estimated values of NS (NS_E) were calculated, based on the corresponding NS value calculations.

Meanwhile, nitrate utilization efficiency (NUE) represents the change rate of plant nitrate assimilation capacity with nitrate concentration. NUE of *Ov* leaves was calculated as:

$$NUE = K_m NS_{max-C}/(K_m + C)^2 \quad (7)$$

2.2.7 Determination of nitrate utilization share (f_A)

The inorganic nitrogen utilization preference of *Ov* was judged by nitrate and ammonium nitrogen utility share under HCO_3^- treatments (0 and 10 $mM{\cdot}L^{-1}$). The corresponding values of *Ov* were calculated for different HCO_3^- treatments, in terms of the two end-member mixing model:

$$\delta_T = f_A\delta_A + f_B\delta_B = f_A\delta_A + (1 - f_A)\delta_B \quad (8)$$

where $\boldsymbol{\delta_T}$ represents the foliar stable nitrogen isotope of *Ov*, cultured in mixed nitrogen sources (e.g., Hoagland nutrient solution) under different HCO_3^- treatments (0 and 10 $mM{\cdot}L^{-1}$) for 21 days; δ_A represents the foliar stable nitrogen isotope of *Ov*, cultured in sole-nitrate nutrition solution with 14 $mM{\cdot}L^{-1}$ nitrate under different HCO_3^- treatments (0 and 10 $mM{\cdot}L^{-1}$) for 21 days; δ_B represents the foliar stable nitrogen isotope of *Ov*, cultured in sole-ammonium nutrition solution with 1 $mM{\cdot}L^{-1}$ ammonium under different HCO_3^- treatments (0 and 10 $mM{\cdot}L^{-1}$); f_A denotes the nitrate nitrogen utility share of *Ov*; and f_B denotes the ammonium nitrogen utility share of *Ov* ($f_B = 1 - f_A$).

2.3 Statistical analysis

The statistical data were processed in MS-Excel® and the significance of differences between paired data was analyzed using SPSS. Data are expressed as mean ± standard error (SE). Values signed with the same letter are not significantly different.

3 Results

3.1 The effect of HCO_3^- on photosynthesis of *Ov*

The Pn, ΦPSII, qP, and $\delta^{13}C_{PGA}$ values of *Ov*, cultured in Hoagland nutrient solution under different HCO_3^- treatments (0 and 10 $mM{\cdot}L^{-1}$) for 21 days showed no significant differences (Figs. 1, 2).

3.2 The effect of HCO_3^- on the $\delta^{15}N_{new}$ and $\Delta^{15}N$ of *Ov*

The LDW of *Ov* in sole-nitrate solution with 15 $mM{\cdot}L^{-1}$ nitrate under 10 $mM{\cdot}L^{-1}$ HCO_3^- treatment for 21 days was 14.67 times the value before testing. The leaf grew rapidly even in 10 $mM{\cdot}L^{-1}$ HCO_3^- conditions. With the increase of nitrate concentration in sole-nitrate nutrient solution, the foliar N of *Ov* seedling increased (Table 3).

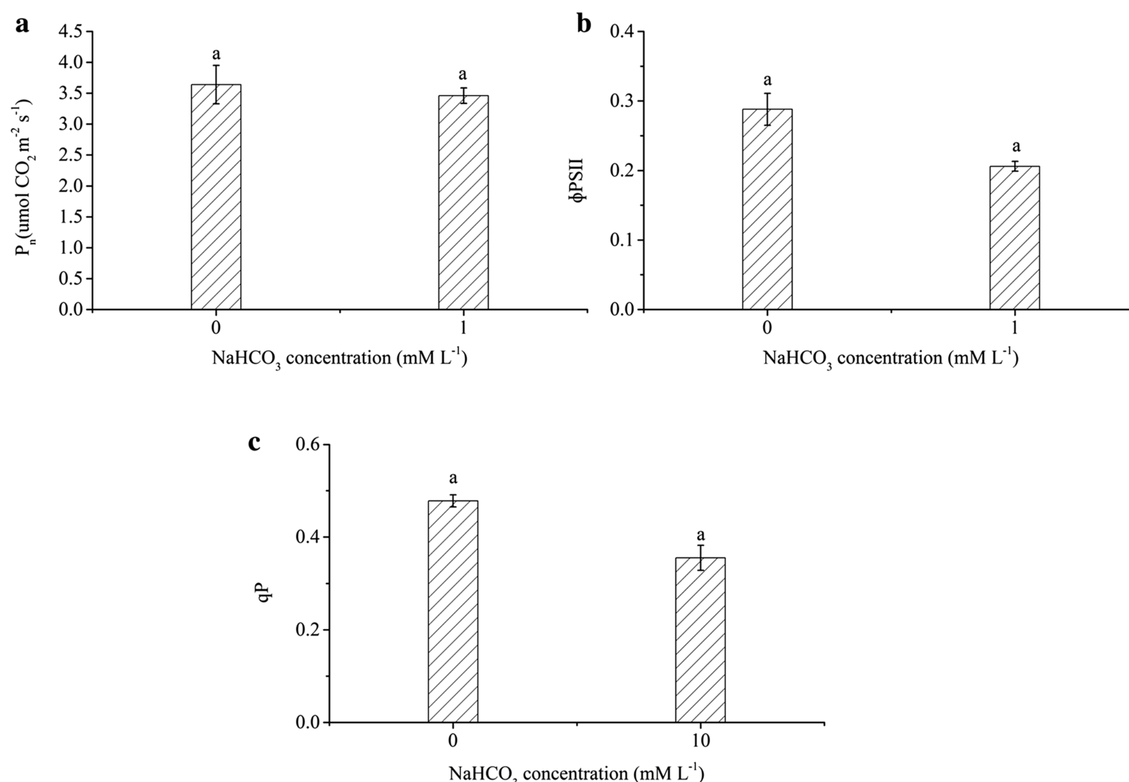

Fig. 1 The effect of HCO_3^- on the main photosynthetic parameters. **a** Pn; **b** ΦPSII; and **c** qP—of *Ov* in Hoagland nutrient solution for 21 days. *Note*: Each value represents the mean ± SE (*n* = 3). Values signed with the same letter are not significantly different as evinced by the use of the independent-samples *t* test ($p > 0.01$)

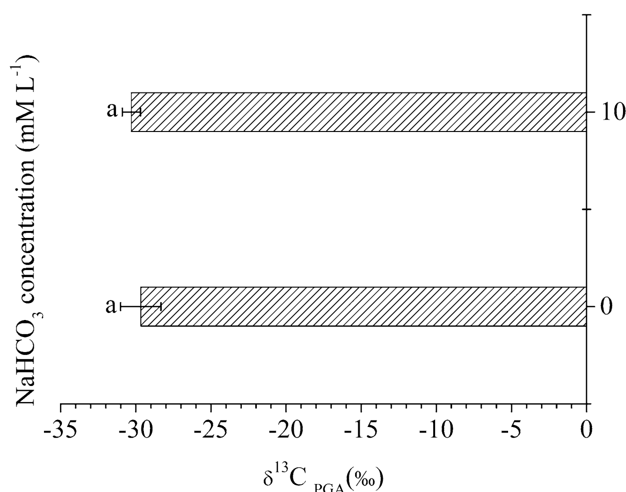

Fig. 2 The effect of HCO_3^- on $\delta^{13}C_{PGA}$ of *Ov* leaf in Hoagland nutrient solution for 21 days. *Note*: Each value represents the mean ± SE (*n* = 3). Values signed with the same letter are not significantly different as evinced by the use of the independent-samples *t*-test ($p > 0.01$)

The corresponding relationship equations of 1/[C] and 1/NS under different HCO_3^- treatments were obtained using the related foliar $\delta^{15}N_{new}$ of *Ov* seedlings. The foliar $\delta^{15}N_{new}$ was obtained by substituting the corresponding LDW, foliar N, and $\delta^{15}N$ of *Ov* seedlings before and after culture in sole-nitrate nutrient solution with 6, 14, and 15 $mM{\cdot}L^{-1}$ nitrate under 10 $mM{\cdot}L^{-1}$ HCO_3^- treatment for 21 days (Table 3) into Eqs. (1) and (2), and by the "new" results of *Ov* in vitro plantlets. The "new" results of *Ov* in vitro plantlets were converted from Table 2. The corresponding relationships between 1/[C] (X) and 1/NS (y) under different HCO_3^- treatments were expressed as: $y = 3.6637X + 1.1936\left(R^2 = 0.945\right)$ under 0 $mM{\cdot}L^{-1}$ HCO3$^-$ treatment, and $y = 1.1851X + 1.0308\left(R^2 = 0.9924\right)$ under 10 $mM{\cdot}L^{-1}$ HCO_3^- treatment. Thus, the corresponding NS of *Ov* leaves under different HCO_3^- treatment were derived according to Eqs. (9) and (10), respectively:

$$NS = (0.838C)/(3.067 + C) \quad \left(\text{under 0 mM L}^{-1}\ HCO_3^- \text{ treatment}\right) \tag{9}$$

$$NS = (0.970C)/(1.149 + C) \quad \left(\text{under 10 mM L}^{-1}\ HCO_3^- \text{ treatment}\right) \tag{10}$$

where C represents the nitrate concentration in the sole-nitrate solution.

The corresponding estimated values of $\delta^{15}N_{new}$ in the sole-nitrate solution with various nitrate concentrations under different HCO_3^- treatments were obtained,

Table 3 The corresponding LDW, N, and $\delta^{15}N$ of *Ov* seedlings leaf before and after cultured in sole-nitrate solution with different nitrate concentrations under HCO_3^- treatment (10 mM·L^{-1}) for 21 days

Treatment (mM·L^{-1})	LDW (g)	N (%)	$\delta^{15}N$ (‰)
Before Experiment	0.006 ± 0.002	3.986 ± 0.559	− 11.47 ± 0.89
6	–	4.549 ± 0.300	12.26 ± 1.84
14	–	4.674 ± 0.303	13.69 ± 0.83
15	0.088 ± 0.009	4.791 ± 0.261	13.65 ± 0.10

– Represents no measure and each value stands for the mean ± SE (*n* = 3)

according to Eqs. (9) and (10). The corresponding $\Delta^{15}N$ values were derived according to Eq. (3) (Table 4).

Measured $\delta^{15}N_{new}$ and estimated $\delta^{15}N_{new}$ ($\delta^{15}N_{new\text{-}E}$) of *Ov* in vitro plantlets cultured in sole-nitrate solution with 10 mM·L^{-1} nitrate for 21 days under no HCO_3^- treatment were 11.00 ± 0.59‰ and 10.89‰, respectively (Table 4). Measured $\delta^{15}N_{new}$ and estimated $\delta^{15}N_{new}$ ($\delta^{15}N_{new\text{-}E}$) of *Ov* seedlings cultured in sole-nitrate solution with 15 mM·L^{-1} nitrate for 21 days under 10 mM·L^{-1} HCO_3^- treatment were 15.23 ± 0.05‰ and 15.29‰, respectively. The similarity between measured foliar $\delta^{15}N_{new}$ of *Ov* and corresponding estimated values ($\delta^{15}N_{new\text{-}E}$) suggests that corresponding $\delta^{15}N_{new\text{-}E}$ changes can be used to evaluate the effect of HCO_3^- on foliar *Ov* $\delta^{15}N_{\text{-}new}$. Meanwhile, foliar $\delta^{15}N_{new\text{-}E}$ of *Ov* increased with nitrate concentration. However, foliar $\Delta^{15}N$ of *Ov* decreased with nitrate concentration.

3.3 The effect of HCO_3^- on stable nitrogen isotope assimilation ratio of *Ov*

Due to the small difference between measured NS and evaluated NS (NS_E) (the relative error was less than 3.80%), the effect of HCO_3^- on the NS of *Ov* in sole-nitrate nutrient solution with different nitrate concentrations under HCO_3^- treatments (0 and 10 mM·L^{-1}) was evaluated by NS_E changes (Table 5). The corresponding measured NS and NS_E were calculated using Eqs. (4), (9), and (10).

NS_E increased with nitrate concentration (Table 5). This indicates that nitrate could promote *Ov* nitrate NUE. Meanwhile, the NS_E of *Ov* under 10 mM·L^{-1} HCO_3^- treatment was higher than under 0 mM·L^{-1} HCO_3^- treatment in the same solution (e.g., the NS_E of *Ov* in sole-nitrate nutrient solution with 6 mM·L^{-1} nitrate under 10 and 0 mM·L^{-1} HCO_3^- treatments were 0.814 and 0.555, respectively; the NS_E of *Ov* in sole-nitrate nutrient solution with 120 mM·L^{-1} nitrate under 10 and 0 mM·L^{-1} HCO_3^- treatments were 0.961 and 0.817, respectively).

3.4 The effect of HCO_3^- on nitrogen use efficiency of *Ov*

According to the equations for nitrogen use efficiency under 0 mM·L^{-1} HCO_3^- treatment:

$$NUE = 2.570/(3.067 + C)^2 \quad (11)$$

and under 10 mM·L^{-1} HCO_3^- treatment Eq. (12),

$$NUE = 1.115/(1.149 + C)^2 \quad (12)$$

the estimated NUE values (NUE_E) of *Ov* under different HCO_3^- treatments were obtained (Table 6).

It can be seen from Table 6 that the NUE_E decreased with increased nitrate. This indicates that HCO_3^- has an

Table 4 The corresponding $\delta^{15}N_{new}$, $\delta^{15}N_{\text{-}newE}$, and $\Delta^{15}N$ of *Ov* leaf in sole-nitrate solution with different nitrate concentrations under different HCO_3^- treatments for 21 days

Treatment (mM·L^{-1})	C_{HCO3}^-=0 mM·L^{-1}			C_{HCO3}^-=10 mM·L^{-1}		
	$\delta^{15}N_{new}$ (‰)	$\delta^{15}N_{\text{-}newE}$ (‰)	$\Delta^{15}N$	$\delta^{15}N_{new}$ (‰)	$\delta^{15}N_{\text{-}newE}$ (‰)	$\Delta^{15}N$
6	–	9.43	7.56	13.83 ± 1.07	13.83	3.16
10	11.00 ± 0.59	10.89	5.99	–	14.78	2.21
14	–	11.67	5.32	15.32 ± 0.82	15.22	1.77
15	–	11.83	5.16	15.23 ± 0.05	15.29	1.70
20	12.22 ± 0.53	12.35	4.77	–	15.58	1.41
40	12.81 ± 1.20	12.81	4.18	–	16.02	0.97
80	13.21 ± 1.20	13.71	3.78	–	16.24	0.75
120	14.38 ± 1.39	13.88	2.61	–	16.33	0.66

– Represents no measure and each value stands for the mean ± SE (*n* = 3)

Table 5 The corresponding NS and NS_E of *Ov* seedlings (plantlets)cultured in sole-nitrate nutrient solution with different nitrate concentrations under HCO_3^- treatments for 21 days

Treatment ($mM\cdot L^{-1}$)	C^-_{HCO3}=0 $mM\cdot L^{-1}$			C^-_{HCO3}=10 $mM\cdot L^{-1}$		
	NS	NS_E	Relative Error (%)	NS	NS_E	Relative Error (%)
6	–	0.555	–	0.814 ± 0.063	0.814	0.000
10	0.647 ± 0.035	0.641	0.927	–	0.870	–
14	–	0.687	–	0.902 ± 0.048	0.896	0.665
15	–	0.696	–	0.896 ± 0.003	0.900	0.446
20	0.719 ± 0.031	0.727	1.113	–	0.917	–
40	0.754 ± 0.071	0.778	3.183	–	0.943	–
80	0.778 ± 0.071	0.807	3.728	–	0.956	–
120	0.846 ± 0.082	0.817	3.429	–	0.961	–

– Represents no measure and each value stands for the mean ± SE (n = 3)

Table 6 The NUE_E of *Ov* in sole-nitrate nutrient solution with different nitrate concentrations under different HCO_3^- treatments for 21 days

Treatment ($mM\cdot L^{-1}$)	C^-_{HCO3} = 0 $mM\cdot L^{-1}$ NUE_E	C^-_{HCO3}=10 $mM\cdot L^{-1}$ NUE_E
6	0.0313	0.02182
10	0.0151	0.00897
14	0.0088	0.00486
15	0.0079	0.00428
20	0.0048	0.00249
40	0.0014	0.00066
80	0.0004	0.00017
120	0.0002	0.000076

adverse effect on the NUE of *Ov*. For instance, NUE_E of *Ov* under 10 $mM\cdot L^{-1}$ HCO_3^- treatment in sole-nitrate nutrient solution for 21 days was smaller than under 0 $mM\cdot L^{-1}$ HCO_3^- treatment.

3.5 The effect of HCO_3^- on nitrate utilization share (f_A) of *Ov*

We supposed that HCO_3^- has no significant effect on foliar $\delta^{15}N_{new}$ of *Ov* in sole-nitrate nutrient solution with 14 $mM\cdot L^{-1}$ nitrate and in sole-ammonium nutrient solution with 1 $mM\cdot L^{-1}$ ammonium for 21 days. The foliar $\delta^{15}N_{new}$ values of O*v*, cultured in Hoagland nutrient solution under 0 and 10 $mM\cdot L^{-1}$ HCO_3^- treatments in sole-nitrate nutrient and in sole-ammonium nutrient solution under 10 $mM\cdot L^{-1}$ HCO_3^- treatment for 21 days were 12.51 ± 0.83‰, 14.42 ± 0.68‰, 15.32 ± 0.82‰, and 5.21 ± 0.00‰, respectively. Therefore, by substituting the corresponding foliar $\delta^{15}N_{new}$ values into Eq. (8), the

Table 7 The corresponding nitrate utilisation share (f_A) and ammonium utilisation share (f_B) of *Ov* under different HCO_3^- treatments for 21 days

Treatments ($mM\cdot L^{-1}$)	f_A	f_B
0	0.722	0.278
10	0.911	0.089

related f_A values of *Ov* under different HCO_3^- treatments (0 and 10 $mM\cdot L^{-1}$) were calculated (Table 7). It can be seen from Table 7 that HCO_3^- had a positive effect on the f_A of *Ov*.

4 Discussion

Plant stomata may close and initial ribulose-1,5-diphosphate carboxylase activity decrease as soon as external conditions inhibit the normal growth and development of plants (Jia et al. 2000; Sun et al. 2004). Adverse environmental conditions can also damage the photosynthetic organs, reducing mesophyll cell photosynthetic activity (Gilmore and Yamamoto 1991) and photosynthesis rate of plants. However, our study found that Pn, ΦPSII, qP, and $\delta^{13}C_{PGA}$ of *Ov* under different HCO_3^- treatments for 21 days exhibited no significant difference, indicating that HCO_3^- has no effect on the photosynthetic system or on the rate of photosynthesis in *Ov*. In addition, these results suggest that the opening of the PSII reaction center, the fluorescence yields of the PSII reaction center, and the process of photosynthesis in *Ov,* are not affected by HCO_3^-.

Our study results are consistent with Brown et al.'s (1955) conclusion that the effect of HCO_3^- on the growth and development of plants is dependent on the HCO_3^-

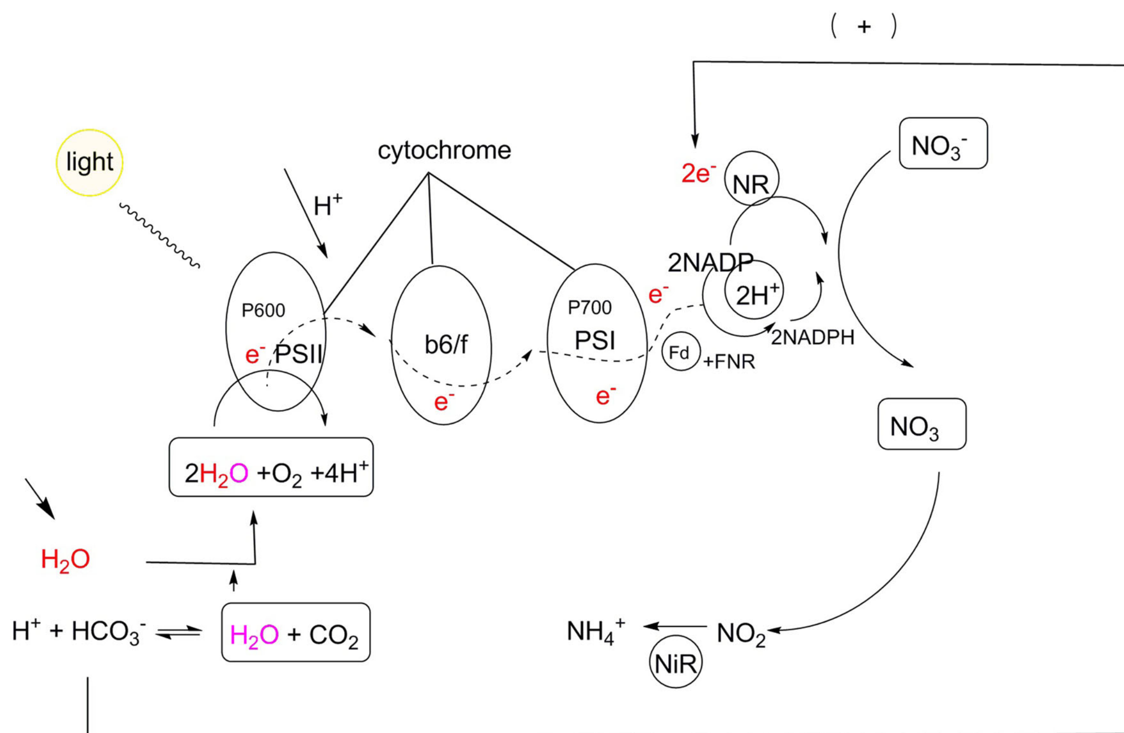

Fig. 3 The promoting mechanism of HCO_3^- on inorganic nitrogen process in chloroplast

adaptability of the plant species. Meanwhile, our study confirmed the conclusion that *Ov* can grow and develop normally in karst areas even with surface water HCO_3^- concentrations as high as 5 $mM{\cdot}L^{-1}$ (Wu et al. 2004; Yan et al. 2012).

The HCO_3^- utilization capacity of terrestrial plants is mainly promoted by CA (Wu et al. 2010, 2011). Therefore, our study results that the photosynthesis of *Ov* is not inhibited by HCO_3^- may relate to high-efficiency HCO_3^- utilization, a counter-effect of HCO_3^- of *Ov*. Specifically, *Ov* could prevent HCO_3^- ion toxicity and high-pH effects to maintain stable and effective photosynthesis by converting HCO_3^- into H_2O and CO_2 for its high CA activity (Hu et al. 2010; Wu 2011; Zhao and Wu 2017).

Based on the negative correlation between $\Delta^{15}N$ with nitrogen assimilation, and the positive correlation of NS with nitrate metabolism of plants (Pate et al. 1993; Kalcsits et al. 2013), our study results indicate that HCO_3^- exerts promotional effects on inorganic nitrogen assimilation of *Ov*.

NR and NiR are vital enzymes in nitrogen assimilation. The activity of NR or NiR can affect plant nitrogen assimilation capacity (Pate et al. 1993; Kalcsits et al. 2013). Our study found that *Ov*'s strong nitrogen assimilation capacity under 10 $mM{\cdot}L^{-1}$ HCO_3^- may be related to the activating effect of HCO_3^- on NR or NiR activity.

Our study further found that HCO_3^- exerts a promotional effect on inorganic nitrogen assimilation but has no influence on the inorganic carbon metabolism of *Ov*. This opposite effect of HCO_3^- on inorganic nitrogen and inorganic carbon metabolism may confirm that the close relationship between photosynthesis and inorganic nitrogen metabolism is due to photosynthesis providing the necessary electron sources for nitrate and nitrite reduction through the photosynthetic chain. Specifically, the nitrate and nitrite reduction capacity may strengthen NR or NiR activity and the number of electrons provided for nitrate and nitrite reduction increase rapidly as long as certain HCO_3^- levels exist (Fig. 3).

Acknowledgements This work was supported by the National Key Research and development Program of China (2016YFC0502602), the National Natural Science Foundation of China (U1612441), and the project of high-level innovative talents of Guizhou Province [2015(4035)].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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