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ORIGINAL ARTICLE

Quantification of photosynthetic inorganic carbon utilisation via a bidirectional stable carbon isotope tracer

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Abstract The amount of bicarbonate utilised by plants is usually ignored because of limited measurement methods. Accordingly, this study quantified the photosynthetic assimilation of inorganic carbon (CO_2 and HCO_3^-) by plants. The net photosynthetic CO_2 assimilation (P_N), the photosynthetic assimilation of CO_2 and bicarbonate (P_N'), the proportion of increased leaf area (f_{LA}) and the stable carbon isotope composition ($\delta^{13}C$) of *Orychophragmus violaceus* (*Ov*) and *Brassica juncea* (*Bj*) under three bicarbonate levels (5, 10 and 15 mm $NaHCO_3$) were examined to determine the relationship among P_N, P_N' and f_{LA}. P_N', not P_N, changed synchronously with f_{LA}. Moreover, the proportions of exogenous bicarbonate and total bicarbonate (including exogenous bicarbonate and dissolved CO_2-generated bicarbonate) utilised by *Ov* were 2.27 % and 5.28 % at 5 mm bicarbonate, 7.06 % and 13.28 % at 10 mm bicarbonate, and 8.55 % and 17.31 % at 15 mm bicarbonate, respectively. Meanwhile, the proportions of exogenous bicarbonate and total bicarbonate utilised by *Bj* were 1.77 % and 3.28 % at 5 mm bicarbonate, 2.11 % and 3.10 % at 10 mm bicarbonate, and 2.36 % and 3.09 % at 15 mm bicarbonate, respectively. Therefore, the dissolved CO_2-generated bicarbonate and exogenous bicarbonate are important sources of inorganic carbon for plants.

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1 Introduction

In general, terrestrial plants prioritize the use of atmospheric CO_2 as their principal inorganic carbon source for photosynthesis. In karst regions, karst rocks mainly develop in limestone ($CaCO_3$) during the dynamic chemical dissolution of calcium carbonate [$CaCO_3 + H_2O + CO_2 \rightarrow Ca^{2+} + HCO_3^-$], where water and atmospheric CO_2 are consumed. A large amount of dissolved inorganic carbon (DIC) in the form of HCO_3^- exists in the surface runoff. Therefore, plants growing in the karst regions can utilise both atmospheric CO_2 and dissolved HCO_3^- for photosynthesis (Waele et al. 2009; Palmer 1991; Wu and Xing 2012; Yan et al. 2012). The photosynthetic assimilation of CO_2 in plants and the chemical dissolution of carbonate rocks are important CO_2 sinks. Thus, research on the photosynthetic assimilation of inorganic carbon (atmospheric CO_2 and dissolved HCO_3^-) in plants is crucial for providing evidence on plant productivity and carbon sinks in the karst regions.

Photosynthetic activities, which include the net assimilation of CO_2, indicate the potential growth and productivity of plants. The photosynthetic rate reflecting the photosynthetic assimilation of CO_2 in plants can be accurately determined using an open gas-exchange system (Long and Bernacchi 2003). Several studies indicated that plants can utilise exogenous bicarbonate as an alternative inorganic carbon source for photosynthesis when sources of the exogenous inorganic carbon change (Raven 1970; Shelp and Canvin 1980). However, the photosynthetic assimilation of bicarbonate in plants cannot be determined using an open gas exchange system. Some plants utilise exogenous bicarbonate (Wu and Xing 2012; Raven et al. 1982; Price et al. 1985), whereas others utilise bicarbonate generated from dissolved CO_2. However, the dissolution of carbonate rocks currently remains unclear.

The stable carbon isotope technique is commonly used to identify various carbon sources utilised by plants. The stable carbon isotope ratio ($\delta^{13}C$) can be applied as an index for carbon metabolic processes, such as photosynthesis (Farquhar et al. 1989). Labelling the stable carbon isotope in exogenous bicarbonate can trace whether or not plants utilise CO_2 from the conversion of exogenous bicarbonate for photosynthesis. Therefore, the changes of the $\delta^{13}C$ of plant tissues in different culture environments can reflect the sources of inorganic carbon for photosynthesis, as carbon metabolic pathways change when plants are exposed to various environmental conditions.

Orychophragmus violaceus (L.) and *Brassica juncea* (L.) Czern.et Coss. cv. Zangyou No.8 are cruciferous plants commonly used as experimental materials because of their high tolerance to bicarbonate stress in Southwest China (Wu et al. 2005; Wang et al. 2014). In this study, the photosynthetic and growth parameters of *O. violaceus* and *B. juncea* under different levels of exogenous bicarbonate were examined to determine their potential productivity. Moreover, the $\delta^{13}C$ values of leaves and culture solutions were measured to determine the photosynthetic assimilation of inorganic carbon (atmospheric CO_2 or HCO_3^-) under water culture conditions. Furthermore, the relationship between the $\delta^{13}C$ and photosynthesis of the two plant species was studied to explore the capacity of the plants to utilise bicarbonate and the 'missing carbon sink' involved in the chemical dissolution of carbonate rocks.

2 Materials and methods

2.1 Plant materials and experimental treatments

The *O. violaceus* and *B. juncea* were obtained from the Institute of Geochemistry, Chinese Academy of Sciences and the Guizhou Institute of Rapeseed, respectively. Seeds of these plants were germinated in 12-hole trays with perlites in a greenhouse at a 12 h light cycle (200 µmol m^{-2} s^{-1}, PPFD), a day/night temperature range of 25 °C/18 °C, and a relative humidity range of 50 %–60 % in the laboratory of the Institute of Geochemistry, Chinese Academy of Sciences, Guizhou Province, China (26.57° N, 106.72° E, altitude of 1045 m). Seedlings which germinated in a uniform size were selected and cultured with half-strength Hoagland nutrient solution (Hoagland and Arnon 1950). 5, 10, 15 mm $NaHCO_3$ labelled with $\delta^{13}C$ value of −2.45 ‰ were added into the Hoagland nutrient solution to simulate three bicarbonate levels to culture two-old seedlings which germinated healthily and uniformly from each plant species. Meanwhile, three levels of bicarbonate labelled with $\delta^{13}C$ value of −24.409 ‰ were used to culture two-old seedlings that germinated healthily and uniformly. Eighteen healthy and uniform seedlings from each plant species were subjected to both treatments. The modified Hoagland nutrient solution was changed daily to maintain consistency for each treatment. All measurements were conducted in triplicates.

2.2 Leaf gas exchange

Leaf gas exchange was determined between 09:00 and 11:00 am by using an open gas-exchange system (Li-6400, *Li–Cor*, *Lincoln*, NE, USA). Photosynthesis was induced with light (200 µmol m^{-2} s^{-1}, PPFD) and ambient CO_2 concentration (400 µmol mol^{-1}). The net photosynthetic rate (P_N, µmol CO_2 m^{-2} s^{-1}), the transpiration rate (E, mmol H_2O m^{-2} s^{-1}), and the stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) were measured on the youngest fully expanded leaf from the top of all the tested plants on day 7 after the onset of bicarbonate treatment. Water use efficiency (WUE) was calculated using the following equation:

$$\mathrm{WUE} = P_N/E$$

where P_N is the net photosynthetic rate and E is the transpiration rate.

2.3 Determination of leaf biomass

The leaf biomass was estimated to determine the leaf area (LA, mm^2) of the youngest fully expanded leaf of both plants from each bicarbonate treatment (Evans and Poorter 2001). After 1, 3, 5, 7, 9, 11, and 13 days of water culture, the leaf length (X_L, mm) and the maximum leaf width (X_W, mm) of the youngest fully expanded leaves from each bicarbonate treatment were determined using a portable digital caliper. Leaves in varying sizes of each plant species were randomly selected to determine the LA, leaf length, and maximum leaf width on day 7. The values of LA were estimated using the leaf length and the maximum leaf width of all the leaves on the basis of the following *power* curve equation:

$$\mathrm{LA} = b_0 \times (X_L \times X_W)^{b1} \quad (1)$$

where LA(mm^2) is the value of the LA of each plant at different bicarbonate treatments on day 7, and b_0 and b_1 are constants.

To eliminate the physiological errors produced by the initial leaf, the initial leaf length and maximum leaf width of each plant species under different bicarbonate treatments were calibrated using the followed logistic equation:

$$LA' = Y_0 + A / \left(1 + B \times e^{-k\times t}\right) \quad (2)$$

where LA' is the LA of each plant treated with bicarbonate; A, B, and K are constants; and t is the culture time ($t = 0$, 1, 3, 5, 7, 9, 11, 13 days).

Furthermore, the proportion of increased biomass (f_{LA}) during bicarbonate treatment can be calculated as

$$f_{LA} = (LA'_i - LA'_0) / LA'_i \quad (3)$$

where LA'_0 and LA'_i are the initial LA ($t = 0$ day) and final LA ($t = i$ day) during bicarbonate treatments, respectively.

2.4 Determination of the stable carbon isotope composition in leaves

The stable carbon isotope ratios ($\delta^{13}C_L$) of the first youngest fully expanded leaf from the top of each tested plants at each bicarbonate treatment level was determined via gas isotope ratio mass spectrometry (MAT-252, *Finnigan* MAT, Bremen, Germany). The $\delta^{13}C$ values of leaves from three seedlings in each plant species under each bicarbonate level were determined on day 7 after the bicarbonate treatment. The stable carbon isotope ratios ($\delta^{13}C$) in all samples were calculated using a standard equation (Pee Dee Belemnite, PDB) and expressed as Eq. 4. The accuracy of the analysis was ± 0.1 ‰.

$$\delta^{13}C\,(permil) = \left[\left({}^{13}C/{}^{12}C\right)_{sample} / \left({}^{13}C/{}^{12}C\right)_{standard} - 1\right] \times 1000 \quad (4)$$

where $({}^{13}C/{}^{12}C)_{sample}$ and $({}^{13}C/{}^{12}C)_{standard}$ are the ratios of height to light isotopes of the sample and the standard, respectively.

2.5 Determination of the proportion of exogenous bicarbonate in the nutrient solution

The stable carbon isotope ratios ($\delta^{13}C_{NS}$) in the nutrient solution from each plant under each treatment were determined 1 day after the bicarbonate treatment via gas isotope ratio mass spectrometry.

According to the bivariate isotope-mixture model,

$$\delta_{NSi} = \delta_a - f_{BNSi}\delta_a + f_{BNSi}\delta_{Ci} \quad (5)$$

where δ_{NSi} is the $\delta^{13}C$ value of the nutrient solution, δ_a is the $\delta^{13}C$ value of the bicarbonate generated from atmospheric carbon dioxide, δ_{Ci} is the $\delta^{13}C$ value of the initial nutrient solution added with exogenous $NaHCO_3$, and f_{BNSi} is the proportion of exogenous bicarbonate in the total inorganic carbon sources in the nutrient solution.

For the exogenous $NaHCO_3$ labelled with a $\delta^{13}C$ value of −2.45 ‰PDB, Eq. 5 can be changed to

$$\delta_{NS1} = \delta_a - f_{BNS1}\delta_a + f_{BNS1}\delta_{C1} \quad (6)$$

where δ_{NS1} is the $\delta^{13}C$ value of the nutrient solution, δ_a is the $\delta^{13}C$ value of the bicarbonate generated from atmospheric carbon dioxide, δ_{C1} is the $\delta^{13}C$ value of the initial nutrient solution added with exogenous $NaHCO_3$ and labelled with a $\delta^{13}C$ value of −2.45 ‰PDB, and f_{BNS1} is the proportion of exogenous bicarbonate in the total inorganic carbon sources in the nutrient solution. Similarly, for the exogenous $NaHCO_3$ labelled with a $\delta^{13}C$ value of −24.409 ‰PDB, Eq. 5 can be changed to

$$\delta_{NS2} = \delta_a - f_{BNS2}\delta_a + f_{BNS2}\delta_{C2} \quad (7)$$

where δ_{NS2} is the $\delta^{13}C$ value of the nutrient solution, δ_a is the $\delta^{13}C$ value of the bicarbonate generated from atmospheric carbon dioxide, δ_{C2} is the $\delta^{13}C$ value of the initial nutrient solution added with exogenous $NaHCO_3$ and labelled with a $\delta^{13}C$ value of −24.409 ‰PDB, and f_{BNS2} is the proportion of exogenous bicarbonate in the total inorganic carbon sources in the nutrient solution.

Plant seedlings with uniform sizes were randomly selected for analysis; thus, f_{BNS1} can be equal to f_{BNS2}.-Comparing Eq. 6 with Eq. 7, we can calculate f_{BNS} as

$$f_{BNS} = f_{BNS1} = f_{BNS2} = (\delta_{NS1} - \delta_{NS2}) / (\delta_{C1} - \delta_{C2}) \quad (8)$$

where δ_{NS1} is the $\delta^{13}C$ value of the bicarbonate treatment solution added with $NaHCO_3$ and labelled with a $\delta^{13}C$ value of −2.45 ‰PDB, δ_{NS2} is the $\delta^{13}C$ value of the bicarbonate treatment solution added with $NaHCO_3$ and labelled with a $\delta^{13}C$ value of −24.409 ‰PDB, and f_{BNS} is the proportion of exogenous bicarbonate in the total inorganic carbon sources in the nutrient solution.

2.6 Calculations of the bicarbonate utilisation proportion and corrected photosynthetic rate

For the bivariate isotope-mixture model,

$$\delta_L = \delta_A(1 - f_{BL}) + \delta_B f_{BL} \quad (9)$$

δ_L is the $\delta^{13}C$ value of the leaves in the tested plants cultivated with $NaHCO_3$ and labelled with an $\delta^{13}C$ value of −2.45 ‰ or −24.409 ‰PDB, δ_A is the $\delta^{13}C$ value of the leaf in the tested plants with atmospheric CO_2 as the sole carbon source, δ_B is the $\delta^{13}C$ value of the leaf of the tested plants with exogenous $NaHCO_3$ as the sole carbon source, and f_{BL} is the proportion of exogenous bicarbonate utilised by the tested plants under each bicarbonate treatment.

For the exogenous $NaHCO_3$ labelled with a $\delta^{13}C$ value of −2.45 ‰PDB, Eq. 9 can be changed to

$$\delta_{L1} = \delta_{A1}(1 - f_{BL1}) + \delta_{B1} f_{BL1} \quad (10)$$

where δ_{L1} is the $\delta^{13}C$ value of the leaves in the tested plants cultivated with $NaHCO_3$ and labelled with a $\delta^{13}C$ value of −2.45 ‰PDB, δ_{A1} is the $\delta^{13}C$ value of the leaf in the tested plants with atmospheric CO_2 as the sole carbon source, δ_{B1} is the $\delta^{13}C$ value of the leaf of the tested plants with exogenous $NaHCO_3$ as the sole carbon source, and f_{BL1} is the proportion of exogenous bicarbonate utilised by the tested plants under each bicarbonate treatment.

Similarly, for the exogenous $NaHCO_3$ labelled with a $\delta^{13}C$ value of −24.409 ‰PDB, Eq. 9 can be changed to

$$\delta_{L2} = \delta_{A2}(1 - f_{BL2}) + \delta_{B2} f_{BL2} \quad (11)$$

where δ_{L2} is the $\delta^{13}C$ value of the leaves in the tested plants cultivated with $NaHCO_3$ and labelled with a $\delta^{13}C$ value of −24.409 ‰PDB, δ_{A2} is the $\delta^{13}C$ value of the leaf in the tested plants with atmospheric CO_2 as the sole carbon source, δ_{B2} is the $\delta^{13}C$ value of the leaf in the tested plants with exogenous $NaHCO_3$ as the sole carbon source, and f_{BL2} is the proportion of exogenous bicarbonate utilised by the tested plants under each bicarbonate treatment.

In this study, plant seedlings with uniform sizes were randomly selected for analysis. Thus, δ_{A1} could be equal to δ_{A2}, and f_{BL1} could be equal to f_{BL2}. Comparing Eq. 10 with Eq. 11, we can calculate the proportion of utilised exogenous bicarbonate (f_{BL}) as

$$f_{BL} = f_{BL1} = f_{BL2} = (\delta_{L1} - \delta_{L2}) / (\delta_{B1} - \delta_{B2}) \quad (12)$$

For (δ_{B1}–δ_{B2}) in Eq. 12, the difference can be replaced with (δ_{C1}–δ_{C2}), where δ_{C1} and δ_{C2} represent the $\delta^{13}C$ values of $NaHCO_3$ labelled with −2.45 ‰ and −24.409 ‰PDB, respectively. Thus, Eq. 12 can be changed to

$$f_{BL} = (\delta_{L1} - \delta_{L2}) / (\delta_{C1} - \delta_{C2}) \quad (13)$$

where f_{BL} only represents the proportion of exogenous bicarbonate used by the plants (in total leaf biomass) as a photosynthetic substance during the bicarbonate treatment. Thus, the bicarbonate utilisation proportion (f_b) of the increased leaf biomass of each plant during the bicarbonate treatment can be calculated as

$$f_b = f_{BL} / f_{LA} \quad (14)$$

where f_b is the proportion of utilised exogenous bicarbonate with increased leaf biomass (LA'$_i$–LA'$_0$) during the bicarbonate treatment, f_{BL} is the proportion of exogenous bicarbonate used by the plants (in total leaf biomass) as a photosynthetic substance during the bicarbonate treatment, and f_{LA} is the proportion of increased biomass during the bicarbonate treatment.

We used Eq. 14 to determine the proportion of exogenous $NaHCO_3$ used by the plants (in increased leaf biomass) as a photosynthetic inorganic carbon during the bicarbonate treatment. However, the addition of both exogenous bicarbonate and dissolved atmospheric CO_2 in the nutrient solution can trigger a reversible chemical reaction because of the bicarbonate treatment. We assumed that f_{BNS0} and f_{BNS} are the proportions of exogenous bicarbonate in the initial and final nutrient solutions, respectively. In this study, the value of f_{BNS0} can be considered as 1, and the value of f_{BNS} can be calculated using Eq. 8. Furthermore, the proportion of total bicarbonate (f_b') utilised by each plant during the bicarbonate treatment can be calculated with

$$f_b' = 2 f_{BL} / f_{LA} (1 + f_{BNS}) \quad (15)$$

The bicarbonate utilisation capacity (BUC) and the corrected photosynthetic rate (P_N') were calculated with

$$\mathrm{BUC} = P_N \times f_b' / (1 - f_b') \quad (16)$$

$$P_N' = P_N + \mathrm{BUC} \quad (17)$$

where P_N is the net photosynthetic rate of the plants with CO_2 as the sole carbon source for photosynthesis, f_b' is the proportion of total bicarbonate utilised by the plants, and BUC is the photosynthetic rate of the plants which catalysed bicarbonate into CO_2 for photosynthesis [7].

2.7 Data analysis

Data were subjected to ANOVA to determine significant differences (defined as $P \leq 0.05$) between group means. Data are shown as mean ± standard error (SE) via factorial analysis with SPSS (version 20.0). The mean results were compared via a Duncan post hoc test at the 5 % significance level ($P \leq 0.05$).

3 Results

3.1 Gas exchange

The *O. violaceus* and *B. juncea* exhibited different changes in gas exchange characteristics, such as P_N, g_s, and WUE, under various bicarbonate levels (Fig. 1). P_N significantly decreased in *O. violaceus* but increased in *B. juncea* after the bicarbonate treatment. However, both plants showed no significant changes in g_s after treatments with different bicarbonate levels. Furthermore, the WUE values of both plants significantly increased after the bicarbonate treatment, and the highest WUE was achieved at 10 mm bicarbonate.

3.2 Leaf biomass

To estimate accurately the leaf biomass, 15 leaves of varying sizes from each plant species were randomly selected to determine the LA, leaf length and maximum

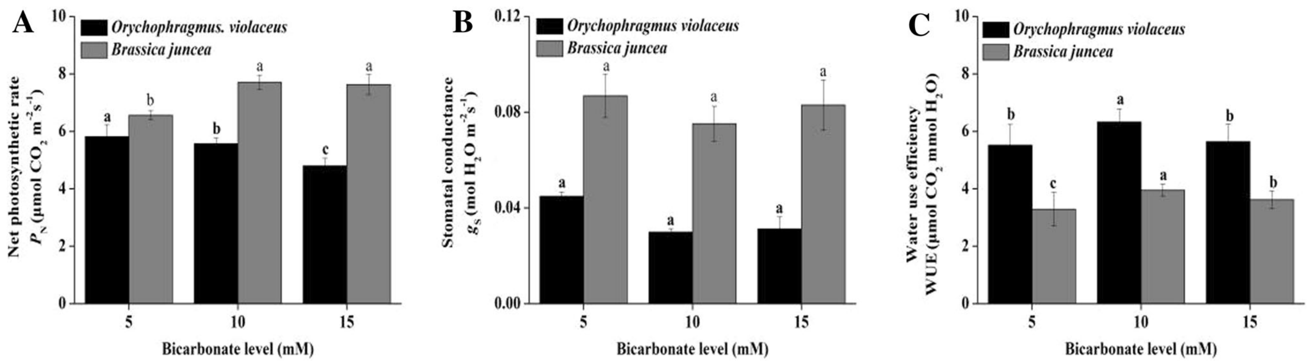

Fig. 1 Net photosynthetic rate (**a**, P_N), stomatal conductance (**b**, g_s) and water use efficiency (**c**, WUE) of *Orychophragmus violaceus* and *Brassica juncea* under bicarbonate treatments. The mean ± SE ($n = 9$) followed by *different letters* in the same plant species differ significantly at $p \leq 0.05$ subjected to one-way ANOVA and *t*-test

Table 1 Leaf area estimation model

Plant	Leaf area (LA) and product of leaf length and leaf width ($X_L \times X_D$) model
Ov	$Y_{LA} = 2.5479(X_L \times X_D)^{0.8557}$ $R^2 = 0.974$ $P = 0.000$ $n = 15$
Bj	$Y_{LA} = 0.6037(X_L \times X_D)^{1.0487}$ $R^2 = 0.954$ $P = 0.001$ $n = 15$

Table 2 The model of growth rate (leaf area) with time (*t*)

Plant	Bicarbonate level	Leaf area ($Y_{LA'}$) and time (*t*) model
Ov	5 mm	$Y_{LA'} = -194.644 + 1798.284/(1 + 2.446\exp^{-0.150t})$ $R^2 = 0.993$ $P = 0.000$ $n = 8$
	10 mm	$Y_{LA'} = -199.405 + 2933.572/(1 + 4.437\exp^{-0.144t})$ $R^2 = 0.996$ $P = 0.001$ $n = 8$
	15 mm	$Y_{LA'} = -9079.365 + 10359.304/(1 + 0.107\exp^{-0.058t})$ $R^2 = 0.990$ $P = 0.000$ $n = 8$
Bj	5 mm	$Y_{LA'} = -578.022 + 11799.457/(1 + 14.063\exp^{-0.186t})$ $R^2 = 0.998$ $P = 0.000$ $n = 8$
	10 mm	$Y_{LA'} = -454.682 + 5161.412/(1 + 7.453\exp^{-0.254t})$ $R^2 = 0.999$ $P = 0.000$ $n = 8$
	15 mm	$Y_{LA'} = -753.655 + 9538.045/(1 + 10.055\exp^{-0.207t})$ $R^2 = 0.998$ $P = 0.001$ $n = 8$

leaf width. The LAs can be estimated using the power curve equation (Table 1). Furthermore, the leaf area of both plants in the hydroponic culture with bicarbonates for various culturing times can be fitted using the logistic growth model equation (Table 2). The initial leaf, initial leaf length, and maximum leaf width of each plant species under various bicarbonate treatments were calibrated on the basis of the logistic growth equation to eliminate physiological errors.

The *O. violaceus* and *B. juncea* exhibited different changes in f_{LA} values under different bicarbonate levels (Fig. 2). The highest and lowest f_{LA} values in *O. violaceus* were under 10 and 15 mm bicarbonate levels, respectively. Those of *B. juncea* were under 10 and 5 mm bicarbonate levels, respectively.

3.3 Leaf stable carbon isotope ratios

The $\delta^{13}C$ values of the leaves varied with plant species and bicarbonate treatments (Table 3). The $\delta^{13}C$ values of *O. violaceus* were higher than those of *B. juncea*. Moreover, the $\delta^{13}C$ values of *O. violaceus* and *B. juncea* were higher under treatment with 10 mm bicarbonate than under treatments with other bicarbonate levels.

3.4 Stable carbon isotope rations in the nutrient solution

Similarly, the $\delta^{13}C$ values in the bicarbonate treatment solutions varied with plant species and bicarbonate levels (Table 4). When the bicarbonate concentration was increased, the $\delta^{13}C$ values in the initial nutrient solutions (δ_1 and δ_2) used to culture the plants for 1 day were similar to those of the exogenous bicarbonate (δ_{C1} and δ_{C2}).

3.5 BUC and corrected photosynthetic rates

The seedlings of the two plants were cultured for 7 d under treatments with different levels of exogenous $NaHCO_3$ and labelled with $\delta^{13}C$ values of −24.409 ‰ and −2.45 ‰PDB. The f_{BL} and f_b' of both plants were calculated using Eqs. 13 and 15, respectively. The f_{BL} and f_b' values of *O. violaceus* were significantly increased with the increasing concentration of bicarbonate, while those in *B. juncea* had no significant change (Fig. 3a). The f_{BL} and f_b' values of *B. juncea* were lower than those of *O. violaceus* under each bicarbonate treatment. The photosynthetic inorganic carbon assimilation capacities (BUC and P_N') were calculated using Eqs. 16 and 17, respectively. *O. violaceus* had higher BUC than *B. juncea* under the same bicarbonate treatment (Fig. 3b). However, the P_N' of *O. violaceus* were lower than that of *B. juncea* under each treatment. Moreover, both plants had the highest P_N' values under the 10 mm bicarbonate level.

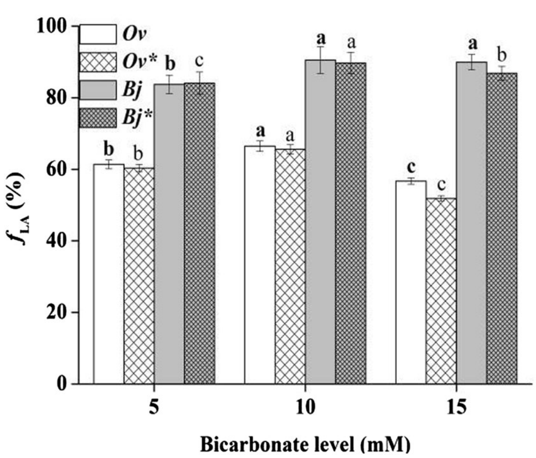

Fig. 2 The proportion of the increased leaf biomass (f_{LA}) during bicarbonate treatment, *asterisk* represents the calibrated f_{LA} values in the same parameters. *Ov—Orychophragmus violaceus*, *Bj—Brassica juncea*. The mean ± SE ($n = 9$) followed by *different letters* in the same plant species differ significantly at $p \leq 0.05$ subjected to one-way ANOVA and *t*-test

4 Discussion

Terrestrial plants utilise atmospheric CO_2 as their principal inorganic carbon source for photosynthesis. However, these plants can also utilise exogenous bicarbonate as an alternative inorganic carbon source for photosynthesis when sources of exogenous inorganic carbon change (Raven 1970; Shelp and Canvin 1980). In karst regions, during the chemical dissolution of carbonate rocks ($CaCO_3 + H_2O + CO_2 \rightarrow$ -$Ca^{2+} + HCO_3^-$), the majority of DIC is involved in the formation of bicarbonate. In the presence of plants, the dissolution of carbonate rocks can be accelerated by various biological effects. Therefore, plants growing in karst regions have access to both atmospheric CO_2 and bicarbonate for photosynthesis (Waele et al. 2009; Yan et al. 2012; Raven 1970).

P_N reflects the photosynthetic CO_2 assimilation and thus the potential growth and productivity of plants. This index can be determined using the open gas exchange system. Several studies determined the bicarbonate utilisation capacities of particular plants under hydroponic culture conditions via the stable carbon isotope technique; however, the fact that the bicarbonate generated from the dissolved CO_2 is utilised by the plants was ignored (Long and Bernacchi 2003; Wu and Xing 2012). In natural environments, quantifying the photosynthetic assimilation of bicarbonate in plants is difficult. Based on previous studies, the present study developed a new and improved method to quantify the bicarbonate utilisation capacity of plants under various bicarbonate levels via the stable carbon isotope technique in hydroponic culture.

The P_N and LA of both plants under various bicarbonate levels were examined in this study (Figs. 1a, 2). When the bicarbonate levels increased, the P_N of *O. violaceus* significantly decreased, whereas that of the *B. juncea*

Table 3 $\delta^{13}C$ values of the leaves of *Orychophragmus violaceus* and *Brassica juncea* under bicarbonate treatments

Parameter	Plant	Bicarbonate treatment		
		5 mm	10 mm	15 mm
$\delta^{13}C$ (‰)	*Ov*–2	−31.68 ± 0.11	−30.78 ± 0.10	−31.14 ± 0.06
	Ov–24	−31.74 ± 0.08	−32.33 ± 0.09	−31.86 ± 0.13
	Bj–2	−33.52 ± 0.15	−33.12 ± 0.06	−33.13 ± 0.03
	Bj–24	−33.75 ± 0.07	−33.33 ± 0.04	−33.56 ± 0.08

Data are presented as mean ± SE ($n = 3$). *Ov*–2 and *Bj*–2 represent *O. violaceus* and *B. juncea* treated with $NaHCO_3$ labelled with a $\delta^{13}C$ value of −2.45 ‰; *Ov*–24 and *Bj*–24 represent *O. violaceus* and *B. juncea* treated with $NaHCO_3$ labelled with a $\delta^{13}C$ value of −24.409 ‰

Table 4 $\delta^{13}C$ values in the nutrient solutions under bicarbonate treatments

Parameter	Plant	Bicarbonate treatment		
		5 mm	10 mm	15 mm
δ_{C1} (‰)	*Ov*	−1.53 ± 0.12	−1.53 ± 0.12	−1.53 ± 0.12
	Bj	−1.53 ± 0.12	−1.53 ± 0.12	−1.53 ± 0.12
δ_{C2} (‰)	*Ov*	−28.87 ± 0.25	−28.87 ± 0.25	−28.87 ± 0.25
	Bj	−28.87 ± 0.25	−28.87 ± 0.25	−28.87 ± 0.25
δ_1 (‰)	*Ov*	−6.69 ± 0.04	− 4.70 ± 0.02	−2.25 ± 0.05
	Bj	−10.04 ± 0.10	−6.93 ± 0.05	−2.99 ± 0.01
δ_2 (‰)	*Ov*	−17.57 ± 0.09	−21.05 ± 0.15	−22.40 ± 0.12
	Bj	−18.01 ± 0.08	−20.68 ± 0.10	−22.22 ± 0.23
f_{BNS} (%)	*Ov*	39.79 ± 2.15c	59.79 ± 3.24b	73.67 ± 3.98a
	Bj	29.16 ± 1.02c	50.27 ± 2.35b	70.35 ± 2.98a

Data are presented as mean ± SE ($n = 3$). f_{BNS} is the proportion of exogenous bicarbonate in the final nutrient solution. The mean ± SE ($n = 3$) followed by different letters in the f_{BNS} values of each plant species differ significantly at $p \leq 0.05$ subjected to one-way ANOVA and *t* test

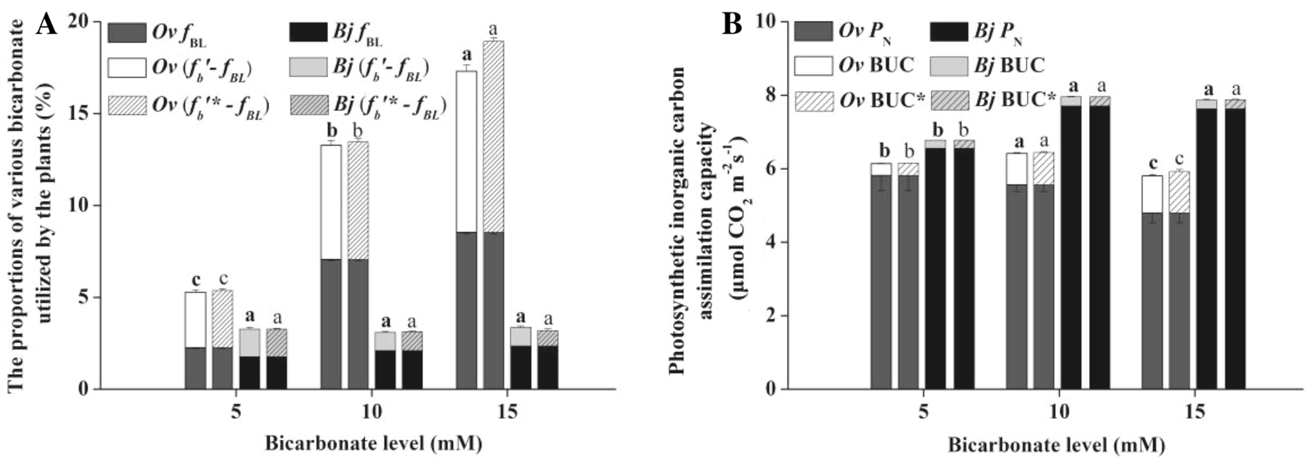

Fig. 3 The various forms and proportions of bicarbonate utilized by the plants (**a**) and photosynthetic inorganic carbon assimilation capacities (**b**) of both plants among bicarbonate treatment. f_{BL} is the proportion of exogenous bicarbonate utilised by the plants, f_b' is the proportion of total bicarbonate utilised by the plants, (f_b'−f_{BL}) is the proportion of the bicarbonate generated by the dissolved CO_2 utilized by the plants. P_N is net photosynthetic rate and BUC is bicarbonate–utilisation capacity of the plants. *Asterik* represents the calibrated values in the same parameters. *Ov—Orychophragmus violaceus*, *Bj—Brassica juncea*. The mean ± SE ($n = 9$) followed by *different letters* in the same plant species differ significantly at $p \leq 0.05$ subjected to one-way ANOVA and *t*-test

significantly increased. The increased f_{LA} in *O. violaceus* changed non–synchronously with P_N as the bicarbonate treatment intensified (Fig. 4a). Meanwhile, the f_{LA} values in the leaves of the *B. juncea* changed synchronously with the P_N in response to various bicarbonate treatments. The deviation between the f_{LA} and P_N of the *O. violaceus* revealed that the P_N values determined using the open gas exchange system did not reflect the true response to bicarbonate treatments and that some errors of the f_{LA} values were caused by the difference in the properties of the initial leaves. Thus, an LA growth logistical model was established to eliminate these errors under each bicarbonate level. To eliminate the physiological errors caused by the initial leaf, we assumed that the calibrated values of LA in the initial leaves were 287 mm^2 in *O. violaceus* and 152 mm^2 in *B. juncea*. Similarly, the f_{LA}* of *O. violaceus* still changed non-synchronously with P_N. Therefore, we hypothesised that the P_N does not reflect the real growth state (f_{LA} or f_{LA}*) under bicarbonate treatments. Furthermore, the change in $\delta^{13}C$ values in the leaves and culture solutions treated with different bicarbonate levels can reflect that the plants can utilise both exogenous bicarbonate ($NaHCO_3$) and dissolved atmospheric CO_2-generated bicarbonate.

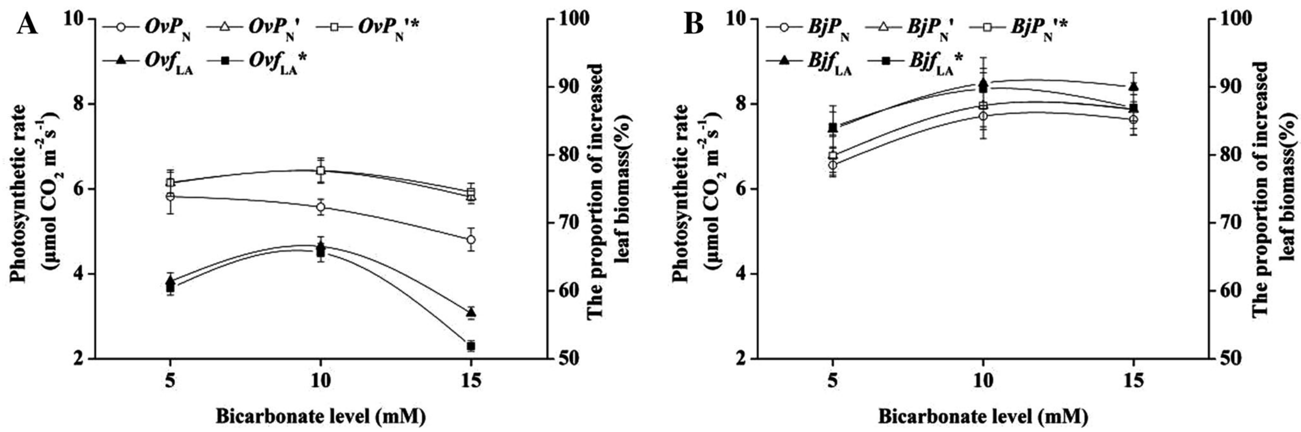

Fig. 4 The relationship of photosynthetic rate (P_N, P_N' or P_N'*) and the proportion of increased leaf biomass (f_{LA} or f_{LA}*) of *Orychophragmus violaceus* (**a**) and *Brassica juncea* (**b**) during the bicarbonate treatment

The P_N' and P_N'* changed synchronously with f_{LA} and f_{LA}* in the leaves of both plants under various bicarbonate levels (Fig. 4). During the bicarbonate treatment period (7 days), the proportions of exogenous $NaHCO_3$ and total bicarbonate (including exogenous bicarbonate and the bicarbonate generated by the dissolved CO_2) utilised by *O. violaceus* were 2.27 % and 5.28 % at 5 mm bicarbonate, 7.06 % and 13.28 % at 10 mm bicarbonate, and 8.55 % and 17.31 % at 15 mm bicarbonate, respectively. Meanwhile, the proportions of exogenous $NaHCO_3$ and total bicarbonate utilised by *B. Juncea* were 1.77 % and 3.28 % at 5 mm bicarbonate, 2.11 % and 3.10 % at 10 mm bicarbonate, and 2.36 % and 3.09 % at 15 mm bicarbonate, respectively. When the amount of exogenous bicarbonate was increased, the amounts of exogenous bicarbonate and total bicarbonate utilised increased in *O. violaceus* but did not significantly increase in *B. juncea*. The plants consumed a considerably large amount of bicarbonate generated from the dissolved CO_2. The results of this study can be used to explore the potential productivity of the plants and the 'missing carbon sink' produced by the dissolution of carbonate rocks.

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