Bicarbonate uptake experiment show potential karst carbon sinks transformation into carbon sequestration by terrestrial higher plants

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1. Introduction

For a long time, global major carbon pools, carbon sources, carbon sinks and carbon budgets have attracted much attention (Raymond et al. 2013; Friedlingstein et al. 2020; Luo et al. 2020; Ran et al. 2021). The overall average time of global carbon turnover is 23 (+7) years (95% confidence interval), and carbon turnover in many semiarid regions is faster (Carvalhais et al. 2014). The carbon sinks of exposed areas of carbonate rocks in China account for 177×1011 gCO2/a (Jiang et al. 2014). The carbon sinks of exposed areas of carbonate rock weathering is 6.248×1011 gCO2/a (Liu et al. 2021). Plants can effectively promote long-term carbon storage by directly accumulating and fixing organic carbon in their ecosystems. More than one-third of the CO2 in the atmosphere is exchanged annually with the terrestrial biosphere through stomata and converting it into organic matter. However, the rock exposure rate in karst regions is high, and rainwater enters groundwater from rock crevices, resulting in a two-dimensional structure of water and soil separation, which makes water and soil loss occur easily (Wang et al. 2004; Van Beynen and Townsend 2005; Yan et al. 2012). Due to the unique features of karst regions, plants often root in cracks, resulting in a complex plant-soil-rock structure. The plants are thus exposed to CaCO3 + H2O + CO2 → Ca++ + 2HCO3− for a long time. HCO3− is the main anion present in the soil solution of calcareous soils (Yan et al. 2012). The climate in karst regions of Guizhou Province, China, is humid year-round. In humid and anoxic soil environments, the HCO3− concentration can reach 5 mM or even 10 mM (Boxma 1972; Bloom and Inskeep 1986).

The interaction between CO2 sequestration of plants and karst carbon sinks has a great impact on the carbon pool of terrestrial ecosystems. In karst regions, CO2 + H2O ↔ HCO3− + H+ maintains a dynamic balance, and it is difficult to determine whether karst carbon sinks are transformed into CO2 sequestrations. Previous work has demonstrated the absorption and utilization of bicarbonate at the leaf level (Wu and Xing 2012; Rao and Wu 2017), but more direct evidence from roots is lacking. The two Moraceae species studied here, Broussonetia papyrifera (Bp) and Morus alba L. (Ma), have different adaptability to low nutrient and excess HCO3− environments, and Bp is a karst-adaptable plant because of its good adaptability to karst soil (Zhao and Wu 2017; Yao and Wu 2021). Whether plants can...
absorb and use bicarbonate is one of the key points in proving the transformation of karst carbon sinks into carbon sequestrations of terrestrial higher plants. Moreover, it is of great significance to understand the utilization of inorganic carbon by plants as an additional carbon source for supplementary photosynthesis. This study provides a direct, rapid and nondestructive method for quantifying the bicarbonate absorption capacity of plants and observes the response of plants to photosynthetic CO₂ assimilation during bicarbonate absorption under different photoperiods.

2. Materials and methods

2.1. Plant materials

The experiment was conducted in an artificial climate chamber of the Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, China. Seeds of Bp and Ma were germinated in a greenhouse on a mixture of perlite and vermiculite. The germinated seeds of Bp and Ma were subsequently cultivated in 1/2-strength modified Hoagland solution for 10 months and 5 months, respectively, until the plant height was 52–57 cm. Seedlings with a similar size were used for the experiment. All plants for the experiment were grown under controlled environmental conditions in a greenhouse under a 12-h photoperiod (500 ± 50 μmol m⁻² s⁻¹, photosynthetically active radiation) and a temperature of 25°C/20°C (light/dark). Relative humidity was maintained at 50%–55%.

2.2. Absorption experiment of bidirectional isotope tracer culture

This experiment was divided into 2 labeling groups, 3 treatments, and 3 replicates for each treatment. The absorption solution was 2 L of the modified Hoagland nutrient solution supplemented with 10 mM NaHCO₃ with different δ¹³C values, 4.00‰ (labeled 1) and −27.07‰ (labeled 2). The pH of the absorption solution was adjusted to 8.30 ± 0.05, which can maintain most HCO₃⁻ in the absorption solution was adjusted to 8.30 ± 0.05, and the CO₂ concentration was 380 μmol m⁻² s⁻¹. The water-use efficiency (WUE) was calculated as follows: WUE = Pn/Tr. The 24D treatment group was exposed to 500 ± 50 μmol m⁻² s⁻¹ light for one hour prior to measurement, and the remaining steps were the same.

2.4. Chlorophyll fluorescence

Chlorophyll fluorescence was measured after the determination of leaf gas exchange by a portable photosynthesis system (Li-6400; Li-Cor, Lincoln, NE). First, the minimum initial chlorophyll fluorescence (Fᵢ₀) and maximum chlorophyll fluorescence (Fₚmax) of the plants were measured after dark adaptation for more than 30 min. Subsequently, the maximum fluorescence (Fₚmax) of plants and the steady-state yield of fluorescence (Fᵢ) in the light were measured after the plants were photoactivated for 1 h, at which point the photosynthetic photon flux density (PPFD) was set at 200 μmol m⁻² s⁻¹. The photosystem II potential photochemical quantum efficiency (Fv/Fₚmax), the efficiency of photosystem II photochemistry (qPSII) and the electron transfer rate (ETR) can be calculated (Weis and Berry 1987; Maxwell and Johnson 2000).

2.5. PEPC and Rubisco

The phosphoenolpyruvate carboxylase (PEPC) and ribulose-1,5-bisphosphate carboxylase (Rubisco) contents under the 3 treatments (control, 24L and 24D) were measured by a double antibody one-step cascade enzyme-linked immunosorbent assay (ELISA). Samples, standards and horseradish peroxidase (HRP)-labeled detection antibodies were successively added to micropores precoated with PEPC and Rubisco antibodies, which were incubated and thoroughly washed. Tetramethylbenzidine (TMB) is a chromogenic substrate and changes color from blue to yellow. The intensity of color was positively correlated with the concentrations of PEPC and Rubisco in the sample. The optical density (OD) was measured at 450 nm using a microplate spectrophotometer. The concentrations of PEPC and Rubisco in the sample were then determined by comparing the OD of the samples to the standard curve. The measured samples were fresh roots, stems and leaves of plants stored in a refrigerator at −80°C until analysis.

2.6. Calculation of bicarbonate absorption

The strong fractionation characteristics of stable carbon isotopes facilitate the identification of different inorganic carbon sources. The bivariate isotope-mixture model can be expressed as

\[ \delta_i = \delta_{Ca} - f_{li} \delta_{si} + f_{li} \delta_{Ci} \]  

where \( \delta_i \) is the \( \delta^{13}C \) value of inorganic carbon in the culture medium at a certain period of time in cultured plants, \( \delta_{Ca} \) is the \( \delta^{13}C \) value of inorganic carbon dissolved in the culture solution from CO₂ in the air, \( \delta_{si} \) is the \( \delta^{13}C \) value of bicarbonate in the initial culture solution, and \( f_{li} \) is the proportion of added exogenous bicarbonate accounting for the total inorganic carbon sources in the culture solution at a certain period of time.
For labeled 1, Eq. 1 can be rewritten as follows:

\[ \delta_1 = \delta_{C_1} - f_{b1} \delta_{C_3} + f_{b1} \delta_{C_1} \] (2)

For labeled 2, Eq. 1 can be rewritten as follows:

\[ \delta_2 = \delta_{C_1} - f_{b2} \delta_{C_3} + f_{b2} \delta_{C_2} \] (3)

Comparing Eqs. 2 and 3, \( f_{b1} = f_{b1} = f_{b2} \), thus, Eqs. 2 and 3 can be used for the following calculations:

\[ f_{b1} = \frac{\delta_1 - \delta_2}{\delta_{C_3} - \delta_{C_2}} \] (4)

where \( f_{b1} \) is the proportion of added exogenous bicarbonate ions accounting for the total inorganic carbon sources in the plant culture system at a certain period of time, \( \delta_1 \) is the \( \delta^{13}C \) value of inorganic carbon in the culture medium at a certain period of time in labeled 1, \( \delta_2 \) is the \( \delta^{13}C \) value of inorganic carbon in the culture medium at a certain period of time in labeled 2, \( \delta_{C_3} \) is the \( \delta^{13}C \) value of bicarbonate in the initial culture solution in labeled 1, and \( \delta_{C_2} \) is the \( \delta^{13}C \) value of bicarbonate in the initial culture solution in labeled 2.

In the blank culture system, the added exogenous bicarbonate ions can be exchanged with CO2 in the air. After a period of consumption, the proportion of added exogenous bicarbonates accounting for the total inorganic carbon source in the blank culture system can be represented as follows:

\[ f_{b0} = \frac{\delta_{b1} - \delta_{b2}}{\delta_{C_3} - \delta_{C_2}} \] (5)

In the bidirectional isotope trace plant and blank culture system under the different time periods Eqs. 4 and 5 can be rewritten as

\[ f_{b1} = \frac{\delta_{b1} - \delta_{b2}}{\delta_{C_3} - \delta_{C_2}} \] (6)

\[ f_{b0} = \frac{\delta_{b01} - \delta_{b02}}{\delta_{C_3} - \delta_{C_2}} \] (7)

where \( i \) is the sampling time.

In the bidirectional isotope trace blank culture system under the different time periods, the cumulative bicarbonate consumption amount (\( m_i \)) can be calculated as follows:

\[ m_i = (cv_{afb0} - cv_{afb1}) - d_{i}^{0} \] (8)

where \( c \) is the initial concentration of bicarbonate set at 10 mM, \( v_{af} \) is the volume of the culture solution at different times, \( f_{b0} \) is the proportion of the labeled bicarbonate in the initial culture solution, and \( d_{i}^{0} \) is cumulative sampling consumption.

In the bidirectional isotope trace plant culture system under the different time periods, cumulative bicarbonate consumption amount (\( p_i \)) can be rewritten as

\[ p_i = (cv_{afb0} - cv_{afb1}) - d_{i}^{1} \] (9)

where \( c \) is the initial concentration of bicarbonate set at 10 mM, \( v_{af} \) is the volume of the culture solution at different times, \( f_{b0} \) is the proportion of the labeled bicarbonate in the initial culture solution, and \( d_{i}^{1} \) is cumulative sampling consumption.

In the bidirectional isotope trace blank culture system under the different time periods, \( d_{i}^{0} \) can be calculated as follows:

\[ d_{i}^{0} = d_{i-1}^{0} + cv_{afb0} \] (10)

where \( v_{af} \) is the sampling volume, \( d_{i}^{0} \) is 0, and \( d_{i-1}^{0} \) is the last cumulative sampling consumption.

In the bidirectional isotope trace plant culture system under the different time periods, \( d_{i}^{1} \) can be written as

\[ d_{i}^{1} = d_{i-1}^{1} + cv_{afb1} \] (11)

where \( v_{af} \) is the sampling volume, \( d_{i}^{1} \) is 0, and \( d_{i-1}^{1} \) is the last cumulative sampling consumption.

In the bidirectional isotope trace blank culture system under the different time periods, the cumulative consumption amount of dissolved bicarbonate from the air (\( n_i \)) can be calculated as follows:

\[ n_i = m_i \frac{(1 - f_{b0})}{f_{b0}} \] (12)

In the bidirectional isotope trace blank culture system under the different time periods, the cumulative consumption amount of total bicarbonate (\( a_i \)) can be calculated as follows:

\[ a_i = m_i + n_i \] (13)

In the bidirectional isotope trace plant culture system under the different time periods, the cumulative consumption amount of total bicarbonate (\( b_i \)) can be written as

\[ b_i = p_i + q_i \] (14)

A linear relationship model between \( a_i, b_i \) and absorption time was constructed, and the slope of the model was the consumption rate of the total bicarbonate in the bidirectional isotope trace culture systems. The bicarbonate absorption rate (\( V_b \)) of the plant was calculated as

\[ V_b = V_1 - V_0 \] (16)

where \( V_1 \) is the consumption rate of the total bicarbonate in the bidirectional isotope trace plant culture systems, and \( V_0 \) is the consumption rate of the total bicarbonate in the bidirectional isotope trace blank culture systems.

After the treatment, the shoot fresh weight (\( \text{SFW} \)) was measured. The bicarbonate absorption rate of shoots (\( V_{\text{SFW}} \)) was calculated as follows:

\[ V_{\text{SFW}} = \frac{V_b}{\text{SFW}} \] (17)

### 2.7. Statistical analysis

All measurements were subjected to analysis of variance (ANOVA). The means of the different groups were compared via Duncan’s multiple range tests. Tests were considered significant at the \( p < 0.05 \) level. Data are shown as the mean ± the standard error (SE). Statistical analyses were carried out with SPSS software (version 25, IBM.).
Linear fitting analyses and graphing were carried out with Origin software (version 9.4, OriginLab).

3. Results

3.1. Absorption of bicarbonate by plants

The linear fitting results of the changes in $a_\text{0}$ and $b_\text{0}$ showed discrepancies in the different treatments of the two plant species (Figure 1). In the control, the $V_\text{b}$ of $Bp$ is higher than that of $Ma$. The $V_\text{b}$ of $Bp$ was higher under the control and 24L than 24D, and there was no significant difference between the control and 24L. The $V_\text{b}$ of $Bp$ under the control and 24L were 2.5 times that under 24D. The $V_\text{b}$ of $Ma$ was higher under the control and 24L than 24D, there is no significant difference between the control and 24L, and the $V_\text{b}$ of $Ma$ under the control and 24L were 5 and 7 times that under 24D, respectively. Through the results of the linear fitting analysis (Table 1), there is an excellent linear proportional relationship between $V_{\text{SFW}}$ and absorbed time.

Figure 1. $a_\text{0}$ and $b_\text{0}$ of $Bp$ and $Ma$ in the fitting linear relationship between the cumulative amount of total bicarbonate consumed and absorbed time under the control (A and B), 24L (C and D) and 24D (E and F), respectively.

Note: The shadow part represents the plant absorption rate of bicarbonate ($V_\text{b}$), $a_\text{0}$, the cumulative consumption amount of total bicarbonate in the bidirectional isotope trace blank culture system; $b_\text{0}$, the cumulative consumption amount of total bicarbonate in the bidirectional isotope trace plant culture system.
and the square values of the correlation coefficient ($R^2$) exceed 0.94. In the control, there is no significant difference between the $V_{SFW}$ of $Bp$ and $Ma$. The $V_{SFW}$ of $Bp$ was highest under the control and was 1.5 and 3.8 times that of 24L and 24D, respectively. The $V_{SFW}$ of $Bp$ under 24D was 2.5 times that of 24D. The $V_{SFW}$ of $Ma$ was highest in 24L and was 1.4 and 5.9 times that of the control and 24D, respectively. The $V_{SFW}$ of $Ma$ under the control was 4.3 times that of 24D.

### 3.2. Photosynthetic CO2 assimilation

Leaf gas exchange and chlorophyll fluorescence represent photosynthetic CO2 assimilation. As shown in Table 2, the $Ac$, $gs$, and $Tr$ of $Bp$ were highest, while the WUE was lowest under the control. The $Ac$ of $Bp$ under 24L was lower than that under 24D. $gs$, $Tr$ and WUE of $Bp$ were not significantly different between 24L and 24D. The $Ac$ of $Ma$ was highest under 24D and had no significant differences between the control and 24L. $gs$, $Tr$ and WUE of $Ma$ had no significant differences among the 3 treatments. $\varphi$PSII and ETR of the two plant species had no significant differences among the 3 treatments. The $F_{v}/F_{m}$ of $Bp$ under the control and 24D treatments was significantly higher than that under 24L. The $F_{v}/F_{m}$ of $Ma$ was highest under the control.

### 3.3. PEPC and Rubisco

PEPC and Rubisco were found in roots, stems and leaves under the 3 treatments, but there was a discrepancy between the two plant species under different treatments. As shown in Table 3, the PEPC of the stems and leaves of the two plant species were not significantly different among the treatments. The PEPC of $Bp$ roots was not significantly different among the 3 treatments. The PEPC of $Ma$ roots under 24L was 78% that under 24D. There was more PEPC in the shoots of plants than in the roots. Rubisco in the roots had no significant difference between $Bp$ and $Ma$ among the 3 treatments. Rubisco of $Bp$ stems was lower under 24D than 24L. The Rubisco of $Ma$ stems was higher under the 24L than control. The Rubisco of $Bp$ leaves under 24L was 79% that under 24D. The Rubisco of $Ma$ leaves had no significant difference among the 3 treatments. There was more Rubisco in the shoots of plants than in the roots.

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**Table 1.** The consumption rate of the total bicarbonate in blank and plant culture system under the control, 24L and 24D.

| Equation | $R^2$ | $V_{SFW}$ (μ mol/h.g) |
|----------|-------|-----------------------|
| Blank    |       |                       |
| $Y = 0.1848X$ | 0.99 | 0.19 ± 0.01            |
| $Y = 0.1848X$ | 0.98 |                       |
| $Y = 0.1968X$ | 0.99 |                       |
| Control  |       |                       |
| $Y = 0.3522X$ | 0.96 | 0.34 ± 0.02            |
| $Y = 0.3122X$ | 0.95 | 0.29 ± 0.02            |
| $Y = 0.2885X$ | 0.97 | 6.64 ± 1.16b           |
| $Y = 0.2885X$ | 0.97 | 7.47 ± 0.68b           |
| 24L      |       |                       |
| $Y = 0.2984X$ | 0.99 | 0.34 ± 0.03            |
| $Y = 0.3399X$ | 0.98 | 0.33 ± 0.01            |
| $Y = 0.3171X$ | 0.99 | 4.46 ± 0.82c           |
| $Y = 0.3171X$ | 0.99 | 10.38 ± 0.52a          |
| 24D      |       |                       |
| $Y = 0.2662X$ | 0.98 | 0.25 ± 0.02            |
| $Y = 0.2137X$ | 0.94 | 0.21 ± 0.02            |
| $Y = 0.2267X$ | 0.96 | 1.77 ± 0.76d           |
| $Y = 0.2537X$ | 0.96 | 1.75 ± 1.81d           |

Note: The data are expressed as mean ± standard error (M ± SE). The same letter means no significant difference (Duncan’s multiple range tests, $P < 0.05$). $V_{SFW}$, bicarbonate absorption rate of shoot.

**Table 2.** Leaf gas exchange and chlorophyll fluorescence under the control, 24L and 24D.

| $Bp$ | $Ma$ |
|------|------|
| $Ac$ | $Bp$ | 24L | 24D | $Ma$ | 24L | 24D |
| $gs$ | 0.09 ± 0.03a | 0.01 ± 0.06b | 0.02 ± 0.01b | 0.05 ± 0.01ab | 0.08 ± 0.01a | 0.08 ± 0.01a |
| $Tr$ | 1.86 ± 0.44a | 0.24 ± 0.05c | 0.42 ± 0.12cd | 0.93 ± 0.11bc | 1.37 ± 0.21ab | 1.29 ± 0.10ab |
| WUE | 4.88 ± 0.53b | 5.71 ± 0.85ab | 7.08 ± 0.77a | 6.14 ± 0.68ab | 4.85 ± 0.39b | 6.31 ± 0.43ab |
| $F_{i}/F_{m}$ | 0.70 ± 0.02a | 0.53 ± 0.08b | 0.72 ± 0.01a | 0.73 ± 0.01a | 0.60 ± 0.01b | 0.59 ± 0.03b |
| $\varphi$PSII | 0.62 ± 0.03a | 0.51 ± 0.08a | 0.56 ± 0.03a | 0.56 ± 0.01a | 0.55 ± 0.03a | 0.61 ± 0.01a |
| ETR | 52.19 ± 3.09a | 43.11 ± 6.97a | 47.44 ± 4.26a | 47.30 ± 1.19a | 46.30 ± 3.14a | 41.63 ± 0.84a |

Note: The data are expressed as mean ± standard error (M ± SE). The same letter means no significant difference (Duncan’s multiple range tests, $P < 0.05$). $Ac$, CO2 assimilation rate; $gs$, stomatal conductance; $Tr$, transpiration rate; WUE, water-use efficiency; $F_{i}/F_{m}$, photosystem II potential photochemical quantum efficiency; $\varphi$PSII, efficiency of photosystem II photochemistry; ETR, electron transfer rate. *Values of $Ac$, $gs$ and $Tr$ were reported in $\mu$mol·m$^{-2}$·s$^{-1}$, mol H$_2$O·m$^{-2}$·s$^{-1}$ and mmol H$_2$O·m$^{-2}$·s$^{-1}$, respectively.

**Table 3.** The content of PEPC and Rubisco in roots (R), stems (S) and leaves (L) under the control, 24Land 24D.

| PEPC/L | $Bp$ | $Ma$ |
|--------|------|------|
| $L$    | 0.9 ± 0.12a | 0.82 ± 0.10a | 0.85 ± 0.09a | 0.91 ± 0.13a | 0.87 ± 0.15a |
| $S$    | 0.77 ± 0.08a | 0.67 ± 0.18a | 0.73 ± 0.09ab | 0.81 ± 0.04a | 0.68 ± 0.16a |
| $R$    | 0.49 ± 0.04ab | 0.56 ± 0.07ab | 0.58 ± 0.09ab | 0.50 ± 0.09b | 0.64 ± 0.07a |
| Rubisco/R | $L$ | 0.70 ± 0.04ab | 0.64 ± 0.06b | 0.72 ± 0.16ab | 0.77 ± 0.04ab | 0.75 ± 0.09ab |
| $S$ | 0.64 ± 0.05abc | 0.67 ± 0.04ab | 0.56 ± 0.09c | 0.52 ± 0.05bc | 0.69 ± 0.06a | 0.59 ± 0.11abc |
| $R$ | 0.36 ± 0.05a | 0.32 ± 0.09a | 0.34 ± 0.12a | 0.37 ± 0.03a | 0.37 ± 0.11a | 0.24 ± 0.04a |

Note: The data are expressed as mean ± standard error (M ± SE). The same letter means no significant difference (Duncan’s multiple range tests, $P < 0.05$). *Values of PEPC and Rubisco content were reported in U/g.
4. Discussion

This study demonstrates that plants absorb and utilize considerable amounts of bicarbonate under both light and dark conditions, indicating that karst carbon sinks can be converted into carbon sequestrations by plants during all times of day. The absorption of bicarbonate with first-order reaction kinetics reveals that plants can absorb and utilize root-derived HCO$_3^-$ in addition to use CO$_2$ from the air (Figure 1). Meanwhile, bicarbonate can be absorbed and utilized by the nonphotosynthetic pathway in the dark in addition to via photosynthesis. Inorganic carbon sequestration via these two pathways is a key mechanism for plant adaptation to karst environments. However, the bicarbonate uptake of Bp and Ma was different (Table 1), which may cause discrepancies in the adaptation to the karst environment in the two plant species.

This study also showed that inorganic carbon sequestration from karst carbon sinks was independent of CO$_2$ assimilation. Bp had greater photosynthetic CO$_2$ assimilation than Ma under the control, but there was no significant difference between Ma and Bp in the absorption rate of bicarbonate (Tables 1, 2). The changes in $V_{SVW}$ and photosynthetic CO$_2$ assimilation capabilities of the two plant species under 24L and 24D were inconsistent. Non parallel in bicarbonate uptake and photosynthetic CO$_2$ assimilation of plants indicated that the conversion of karst carbon sinks into photosynthetic carbon sequestrations did not affect photosynthetic CO$_2$ assimilation per unit leaf area.

The absorption and utilization of bicarbonate by plants throughout the day may result from the PEPC and Rubisco contents (Figure 2). It has been reported that DIC carbon fixation in roots is related to PEPC (Vuorinen et al. 1989; Vuorinen et al. 1992). The role of PEPC in root bicarbonate
absorption and utilization is related to the conversion of malic acid. Malic acid is formed from PEPC and/or phosphoenolpyruvate carboxykinase (PEPCK) in the roots (Popp et al. 1982). Bicarbonate is absorbed and utilized by plants via the function of PEPC, which is considered a non-photosynthetic pathway.

A previous study proved that Rubisco in leaves can combine CO₂ from the conversion of root-derived bicarbonate to form carbohydrates during photosynthesis under light (Wu and Xing 2012; Rao and Wu 2017). In this study, we found that there were considerable amounts of Rubisco in the leaves under 24D, even more than under 24L (Table 3), which seemed to question the role of Rubisco in bicarbonate absorption and utilization. However, it has been reported that the expression of the Rubisco chaperone gene in cyanobacterial blooms reached its highest expression during darkness and was downregulated during the daytime (Sandrini et al. 2016). Therefore, Rubisco reacts and is consumed only under light and does not function as carboxylation in the dark. Bicarbonate is absorbed and utilized by plants via the function of Rubisco, which is considered a photosynthetic pathway.

The synergism in the absorption and utilization of photosynthetic pathway and nonphotosynthetic pathway dominated the transformation of the karst carbon sinks of plants. Ma can absorb more bicarbonate than Bp (Table 1). However, bicarbonate is greatly alkaline, and too much absorption and too little utilization by plants will cause damage to plants. In addition to less absorption of bicarbonate than Ma, the absorbed bicarbonate of Bp can be more readily used as a carbon source for photosynthetic organs to further reduce the damage of bicarbonate to plants because the capability of Bp to convert bicarbonate into CO₂ and water by carbonic anhydrase (CA) is stronger than that of Ma. A previous study showed that approximately 30% of the photosynthetic products of Bp come from CO₂ transformed from bicarbonate by CA, while only 0%–15% of those of Ma arise this way (Wu and Xing 2012). The involvement of the photosynthetic pathway vs the nonphotosynthetic pathway in bicarbonate uptake was greater in Bp than in Ma, which prevented the accumulation of organic acids from diminishing the adverse effect of growth in Bp. The moderate absorption and massive utilization of bicarbonate by Bp greatly reduced the damage. Ma is difficult to grow in karst regions, which is related to its high nonphotosynthetic absorption and low photosynthetic utilization of bicarbonate. This result is consistent with previous findings that Bp is a karst-adaptable plant (Zhao and Wu 2018).

The two plant species absorbed and utilized more bicarbonate under 24L than 24D (Table 1), indicating that light can promote the absorption and utilization of bicarbonate by plants, which proves that uptake of bicarbonate occurs through the xylem with transpiration flow to the shoots. It has been previously reported that bicarbonate may be incorporated into xylem proteins and transported to the shoots (Mengel et al. 1994; Wegner and Zimmermann 2004), and HCO₃⁻ in xylem sap may be the carbon source of Rubisco in leaves (Popp et al. 1982; Stringer and Kimmerer 1993).

The greater bicarbonate uptake in Bp with a smaller R/S suggested that the absorption and utilization of bicarbonate is related to transpiration in leaves. Bp with a smaller R/S absorbed more bicarbonate, which will cause more free HCO₃⁻ and increase the toxic effect in the plants (Table 1). Therefore, Bp with a larger R/S has better karst adaptation than that with a smaller R/S.

One of 2 moles of bicarbonate taken up by plants originated from CO₂ in the air during karst corrosion (Figure 2). This part of the carbon flux should be included in the global carbon budget. This pathway may lead to a larger carbon flow during all times of day and play an important role in the global carbon cycle, a role which has previously been underestimated. On the one hand, plants use bicarbonate to drive the corrosion of the carbonate and accelerate the formation of a carbon sink of corrosion, which makes a huge contribution to carbon neutrality. On the other hand, this is conducive to plant growth, increases the carbon sequestration capacity of plants, and provides organic matter and energy for the ecosystem. Karst-adaptable plants are especially able to regulate the entire carbon cycle. Therefore, quantifying the transformation of karst carbon sinks into carbon sequestrations by plants can not only provide new knowledge for the carbon metabolism of plants but also provide basic data for screening karst-adaptable plants and may ultimately provide a new solution for carbon peaks and carbon neutrality. The coupled relationship between the plant inorganic and organic carbon cycles is the key to modifying the carbon circulation model, especially in karst regions.

5. Conclusion

The bidirectional isotope tracer technique can quantify the absorption and utilization of root-derived bicarbonate. The tremendous karst carbon sinks can be transformed into carbon sequestrations by plants via photosynthetic and non-photosynthetic pathway. Karst-adaptable plants can increase carbon sequestrations through the transformation to strengthen both karst and vegetation carbon sinks in the karst ecosystem.

Disclosure statement

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