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Bicarbonate stimulates non-structural carbohydrate pools of *Camptotheca acuminata*

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The role of root-derived dissolved inorganic carbon (DIC) has been emphasized lately, as it can provide an alternative source of carbon for photosynthesis. The fate of newly fixed DIC and its effect on non-structural carbohydrate (NSC) pools has not been thoroughly elucidated to date. To this end, we used ^{13}C ($NaHCO_3$) as a substrate tracer to investigate the incorporation of newly fixed bicarbonate into the plant organs and NSC compounds of *Camptotheca acuminata* seedlings for 24 and 72 h. NSC levels across the organs were all markedly increased within 24 h of labeling treatment and afterward only decreased in stems at 72 h. The variation range of NSC concentrations in roots was considerably smaller than in the stem and leaves. As time passed, the $\delta^{13}C$ in NSC compounds was significantly affected by ^{13}C labeling and was more positive in the roots than in the stem and leaves. Starch was more ^{13}C-enriched than was soluble carbohydrate, and the $\delta^{13}C$ of root starch was as high as −4.70‰. Bicarbonate incorporation into newly formed NSC compounds contributed up to 0.24% of the root starch within 72 h. These data provided strong evidence that bicarbonate not only acted as a C source that contributed slightly to the NSC pools but also stimulated the increase in NSC pools. The present study expands our understanding of the rapid change of NSC pools across the organs in response to bicarbonate.

Introduction

The role of root-derived dissolved inorganic carbon (DIC) has been emphasized lately, as it can provide an alternative source of carbon for photosynthesis and contribute to the total C gain (Ford et al. 2007, Aubrey and Teskey 2009, Wu and Xing 2012, Rao and Wu 2017). Soil DIC may contain CO_2, H_2CO_3, HCO_3^- or CO_3^{2-}, depending on the solution pH. In general, HCO_3^-, resulting from hydration of CO_2 or ionization of bicarbonate, is more stable and abundant than CO_2 and CO_3^{2-} at pH 7–9. A small quantity of CO_2 or HCO_3^- is absorbed by roots and transported to aboveground tissues along the transpiration stream (Stringer and Kimmerer 1993, Epron et al. 2012, Bloemen et al. 2016). The delivery of DIC through the xylem is notably fast. An earlier study revealed that HCO_3^- with ^{14}C labeling was transferred to the leaves of willow plants within 5 h and reached the highest level after a 10 h labeling period (Vuorinen et al. 1989). In tomato, the amount of ^{14}C was 61% in the roots and 39% in the shoots and leaves after 72 h of labeling (Bialczyk and Lechowski 1992). The xylem DIC efflux was reported to range from 0.03 to 0.35 μmol m^{-2} s^{-1} in certain woody plants (Levy et al.

Abbreviations – DIC, dissolved inorganic carbon; DW, dry weight; g_s, stomatal conductance; MCW, methanol, chloroform and water; NSCs, non-structural carbohydrates; PDB, Pee Dee Belemnite; PEPC, phosphoenolpyruvate carboxylase; *Pn*, net photosynthetic rate; PPFD, photosynthetic photon flux density; *Tr*, transpiration rate.

1999, Aubrey and Teskey 2009). DIC fixation was also associated with the catalysis of phosphoenolpyruvate carboxylase (PEPC) to generate malic acid and citric acid in the roots (Shahabi et al. 2005, Msilini et al. 2009, Covarrubias and Rombolà 2013). It is likely that malic acid was decarboxylated again, transported to the leaves and then refixed in the carboxylation site of the chloroplast (Rombolà et al. 2005). The proportion of CO_2 supplied from root-derived DIC accounted for 0.8–8% of the total C gain, and an even greater proportion (approximately 20%) was observed when encountering stressors (Ford et al. 2007, Aubrey and Teskey 2009, Rao and Wu 2017). Previous studies demonstrated that most ^{13}C derived from root-uptake DIC was allocated in the roots (Rombolà et al. 2005, Ford et al. 2007), but did not reveal whether basipetal photosynthate transport was supplied from DIC. We once speculated that the $\delta^{13}C$ of roots was more likely influenced by the new photosynthates, rather than ^{13}C tracer retained in the roots (Rao and Wu 2017), but direct evidence was lacking. To date, little is known regarding the fate of recently fixed DIC. Taken together, these above-mentioned studies renewed the interest in photosynthates supplied from DIC, as well as addressing the need for understanding post-photosynthetic C partitioning.

As we know, photosynthates are primarily starch, soluble sugars and sorbitol (Moing et al. 1992, Raines 2011). During daytime, a fraction of the carbohydrates remain in the chloroplast to synthesize transitory starch (Thalmann and Santelia 2017). This starch is then degraded and converted to soluble sugars (mainly sucrose) to supply the plants with carbohydrates during the following night (Smith and Stitt 2007, Zeeman et al. 2010). Most studies only used $^{13}CO_2$ labeling to track the partitioning of newly fixed C into different tissues (Meng et al. 2013, Bloemen et al. 2015, Li et al. 2016). The pattern of soil DIC assimilation, with respect to post-photosynthate allocation, should be similar to that of atmospheric CO_2. Therefore, it is also feasible to determine the photosynthates supplied from DIC allocated into different compartments. Nevertheless, the $\delta^{13}C$ of bulk tissues or organs after ^{13}C labeling often provide imprecise information, as they contain many types of metabolites with different isotope signatures (Eglin et al. 2009). In addition, it is highly difficult to distinguish within an organ between the upstream C fluxes from those downstream, as previously stated. It is thus necessary to determine the concentration and C-isotope composition at the level of compounds.

The non-structural carbohydrates (NSCs), of which more than 80% are soluble carbohydrate (sucrose, glucose and fructose) and starch, exist in leaves, branches, stem and roots (Hoch et al. 2003, Göttlicher et al. 2006, Carbone et al. 2013). As a C reserve, NSC is basically required for growth, transport, osmoregulation, defense and reproduction (Chapin et al. 1990, Zeeman et al. 2010, Hartmann and Trumbore 2016). NSC concentration variations usually reflect the current C supply status (Hoch et al. 2003). Studies from individuals to the ecosystem scale have shown that NSC concentrations are dynamic in response to varying environmental cues at different time scales (Chapin et al. 1990, Rosas et al. 2013, Quentin et al. 2015). Nevertheless, no conclusive pattern is apparent in the change of NSC concentration when plants are exposed to environmental stressors (reviewed by Thalmann and Santelia 2017). The transfer of NSC from the canopy to the soil compartments is rapid in some species, usually within 1 day (Ruehr et al. 2009, Gavrichkova et al. 2011). However, drought or other stresses were observed to prolong the transfer of recently assimilated NSC (Ruehr et al. 2009, Blessing et al. 2015). To date, the dynamic of NSC allocation under bicarbonate treatment remains poorly investigated. The effect of bicarbonate on plants seems to be associated with its concentration. Low level of bicarbonate supply usually leads to a plant growth increase (Enoch and Olesen 1993), while high concentration of bicarbonate often induces growth inhibition, stomatal closure and a decreased photosynthetic C uptake (Peiter et al. 2001, Cambrollé et al. 2014, Rao and Wu 2017). Under this circumstance, NSC concentration is expected to show great variations due to negative carbohydrate balances. In addition, bicarbonate itself acts as a C source that may also affect the NSC level. Therefore, an understanding of NSC dynamics is very important for predicting post-photosynthetic C partitioning in response to bicarbonate. In this regard, we hypothesized that the NSC concentration would increase in all organs, and more starch would accumulate in the roots under bicarbonate stress.

Camptotheca acuminata is native to China and mainly used for extraction of Camptothecin (Ma et al. 2012). This tree is adapted to most soil conditions, such as lightly waterlogged or drought, alkaline or acid soil (Wang et al. 2009, Ying et al. 2015). In addition, we chose this species to extend the research on bicarbonate utilization from our previous study (Rao and Wu 2017). In this study, we used the combination of a hydroponic ^{13}C label and monitoring NSC dynamics to trace the fate of newly fixed DIC in *C. acuminata* seedlings. The objectives of this work were to (1) study the effect of bicarbonate on the dynamics of NSC concentration; (2) investigate the incorporation of ^{13}C into plant organs and NSC compounds and (3) determine whether the root NSC was a major C sink pool.

Materials and methods

Plant materials

The experiment was conducted in the phytotron of the Institute of Geochemistry, Chinese Academy of Science, Guiyang, China. The phytotron was $10 \times 5 \times 4$ m in length, width and height, respectively (LabTech, Beijing, China). Uniform *C. acuminata* seedlings were selected after 2 months of growth. All plant materials were incubated in a photoperiod of 16/8 h of light/dark conditions. The temperature was set at 25/19°C in light/dark and the relative humidity was maintained at 55–60%. Light was supplied by metal halide lamps (Philips, Amsterdam, The Netherlands), with 500 ± 50 μmol m^{-2} s^{-1} of photosynthetic photon flux density (PPFD).

^{13}C labeling

To manipulate the precise ^{13}C labeling, 10 mg of high ^{13}C-abundance $NaHCO_3$ (98% atm., Merck KGaA, Darmstadt, Germany) and 98.7 mg of low ^{13}C-abundance $NaHCO_3$ (1.08% atm.) were weighed, dissolved and mixed in the same bottle filled with 250 ml of modified Hoagland solution to generate 5 mM of 10% (atm.) $NaH^{13}CO_3$. Thus, the modified Hoagland solution contained 5 mM $NaH^{13}CO_3$, 2 mM $Ca(NO_3)_2$, 2.5 mM KNO_3, 0.5 mM NH_4NO_3, 0.125 mM KH_2PO_4, 1 mM $MgSO_4$ and micronutrients. The solution pH was adjusted to 7.5 ± 0.2. The experiment started when the seedlings were exposed to the nutrient medium in the flask. The treatment groups included 24 and 72 h of labeling. Each treatment contained four replicates, with one replicate (seedling) in each bottle. The flasks were quickly sealed and CO_2-free air (trapped by NaOH) was pumped from the top of the flask into the solution for root respiration. The outlet air was driven through plastic tubing out of the phytotron. After 24 and 72 h, plants from both treatment groups were harvested.

Measurement of leaf gas exchange

Leaf gas exchange was determined by a portable photosynthesis system (Li-6400; Li-Cor, Lincoln, NE). The PPFD was set at 500 μmol m^{-2} s^{-1} and the CO_2 concentration at 380 μmol m^{-2} s^{-1}. The net photosynthetic rate (*Pn*), stomatal conductance (g_s) and transpiration rate (*Tr*) were measured from 09:00 to 11:00.

Determination of dry weight and C content

At the final harvest, the root, stem and leaf samples were dried at 80°C for 3 days, weighed and ground for analysis of C content. The C content measurement was performed on an elemental analyzer (vario MACRO cube; Elementar, Frankfurt, Germany).

Preparation of carbon fractions

The purifications of soluble carbohydrate and starch were prepared according to the method of Wanek et al. (2001) and the modified protocol of Richter et al. (2009). Briefly, 100 mg of plant materials were extracted with 1 ml methanol:chloroform:water (MCW; v/v/v 12:5:3) at 70°C for 30 min. After cooling, the samples were centrifuged at 12000*g* for 5 min. The suspension (0.65 ml) was transferred to another centrifuge tube with the addition of 0.2 ml of chloroform and 0.7 ml of deionized water. Next, the tube was centrifuged at 12000*g* for 5 min. A methanol and water phase (upper) was used to extract the soluble carbohydrate via ion exchange resin (Dow Chemical, Midland, MI). The purification method contained cation exchange resin (0.5 g, Dowex 50×8, 50–100 mesh, H^+ form) and anion exchange resin (0.6 g, Dowex 1×8, 50–100 mesh, $HCOO^-$ form). The efflux was collected, transferred into tin capsules and stored in an oven at 60°C overnight (Galiano et al. 2017) for the measurement of the C-isotope composition. After the above MCW extraction steps, the insoluble substance was resuspended in 1 ml MCW, incubated at 70°C for 30 min, and then centrifuged at 12 000 *g* for 5 min. The precipitate was washed three times with deionized water and hydrolyzed with a pre-ultrafiltered α-amylase solution. The product of the starch hydrolysis was collected and processed like soluble carbohydrate.

Determination of the NSC concentration

The concentrations of soluble carbohydrate and starch hydrolysate were measured by high-performance liquid chromatography (LC-20A; Shimadzu, Kyoto, Japan) equipped with a refractive index detector. The compounds were separated on a 150×4.6 mm Inertsil NH_2 column (Shimadzu, Kyoto, Japan). The soluble carbohydrate referred to the sum of sucrose, glucose and fructose.

Carbon isotope composition analysis

The dried organs and prepared C fractions were further processed for isotope composition determination performed by a continuous-flow isotope ratio mass spectrometer (MAT 253; Thermo Fisher Scientific, Waltham, MA). The natural abundance of ^{13}C in the samples is expressed as

$$\delta^{13}C\ (‰) = \left[\left(R_{Sample}/R_{Standard}\right) - 1\right] \times 1000 \quad (1)$$

where R_{Sample} and $R_{Standard}$ are the $^{13}C/^{12}C$ ratios of the samples and the Pee Dee Belemnite (PDB), respectively. The SD of repeated measurements of the laboratory standards was 0.10‰ for $\delta^{13}C$.

^{13}C allocation

The ^{13}C enrichment in plant samples is expressed as the ^{13}C atom% excess. Therefore, $\delta^{13}C$ was first converted to atom% values as follows:

$$\text{atom\%} = \frac{100 \cdot 0.0112372 \cdot \left(\frac{\delta}{1000} + 1\right)}{1 + 0.0112372 \cdot \left(\frac{\delta}{1000} + 1\right)} \quad (2)$$

where 0.0112372 is the standard value for the isotopic $^{13}C/^{12}C$ ratio of the PDB standard. The excess ^{13}C incorporated into plant organs was determined as follows:

$$\text{excess}^{13}\text{C} = \frac{\text{atom\%}_s\text{-atom\%}_b}{100} \cdot \text{DW} \cdot \frac{\text{C\%}}{100} \quad (3)$$

where $\text{atom\%}_s$ and $\text{atom\%}_b$ are the atom% of the labeled samples and unlabeled background, respectively; DW is the dry weight of the organs and C% is the C content of the organs. The relative distribution of ^{13}C allocated in different organs is expressed as

$$^{13}\text{C distribution\%} = \frac{100 \cdot \text{excess } ^{13}\text{C}_o}{\text{excess}^{13}\text{C}_t} \quad (4)$$

where excess $^{13}C_o$ is the amount of excess ^{13}C in a specific organ and excess $^{13}C_t$ is the total amount of excess ^{13}C in the whole plant.

In addition, we introduced the two end-member mixing model to roughly estimate the contribution of bicarbonate to the $\delta^{13}C$ in different compartments after ^{13}C labeling, which is expressed as

$$\delta_N = f_B \cdot \delta_B + \left(1\text{-}f_B\right) \cdot \delta_C \quad (5)$$

where δ_N is the new $\delta^{13}C$ value of the compartment after mixing; f_B is the fraction of bicarbonate contributing to the C-isotope composition of the mixed compartment; δ_B is $\delta^{13}C$ value of the label ($NaHCO_3$) and δ_C is the $\delta^{13}C$ value of the control. The fractionation of ^{13}C label occurring in transport or the reactions was very small in relative to $\delta^{13}C$ of the label and was thus neglected.

Statistical analysis

All measured variables were characterized by descriptive statistics (means and SE, n = 4). The effect of bicarbonate with different labeling times on photosynthesis, DW, C content, NSC concentration, $\delta^{13}C$ of organs and NSC compounds, ^{13}C allocation and f_B were assessed by analysis of variance (general linear model). Significant differences between the means were calculated with Duncan's multiple range tests. Pearson's correlation coefficient was calculated between the NSC concentration and the $\delta^{13}C$ of the NSC compounds. Tests were considered to be significant at $P < 0.05$. Statistical analyses were carried out with SAS version 9.4 statistical package (SAS Institute, Cary, NC).

Results

Photosynthesis and biomass

Overall, bicarbonate supply had a significant effect on leaf gas exchange (Table 1). The *Pn* decreased by 26.6 and 41.1% after 24 and 72 h labeling, respectively, compared to the control. Similar decreasing trends were observed for g_s and *Tr*. The DW of different organs showed no change while only the C content of the stem was influenced and slightly decreased ($P = 0.006$).

Soluble carbohydrate and starch concentrations

Variations in NSC concentration differed strongly from organ to organ, and soluble carbohydrate concentration was generally higher than starch under the same treatment (Fig. 1). Soluble carbohydrate concentration increased significantly in all organs after 24 h labeling

Table 1. Means and SE of leaf gas exchange and DW and C content of organs for the control, 24 and 72 h labeling. The *P* values were obtained from multiple comparison analysis; r^2 represented the adjusted coefficients of determination. *Values of *Pn*, g_s and *Tr* were reported in μmol m^{-2} s^{-1}, mol H_2O m^{-2} s^{-1} and mmol H_2O m^{-2} s^{-1}, respectively. Bold values indicated significant differences at $P < 0.05$.

	Photosynthetic parameters*			DW (g)			C content (%)		
	Pn	g_s	*Tr*	Roots	Stem	Leaves	Roots	Stem	Leaves
Control	7.62 (0.53)	0.05 (0.00)	1.43 (0.08)	0.96 (0.06)	1.99 (0.07)	3.06 (0.12)	33.43 (0.55)	40.53 (0.31)	40.08 (0.40)
24 h	5.59 (0.43)	0.03 (0.01)	0.87 (0.16)	0.92 (0.04)	1.96 (0.04)	3.05 (0.07)	34.44 (0.48)	38.71 (0.36)	40.20 (0.27)
72 h	4.49 (0.27)	0.02 (0.00)	0.41 (0.05)	0.89 (0.02)	1.89 (0.06)	2.95 (0.08)	34.71 (0.68)	39.08 (0.27)	40.28 (0.93)
P	**0.002**	**<0.001**	**<0.001**	0.537	0.439	0.653	0.303	**0.006**	0.973
r^2	0.757	0.830	0.837	0.129	0.167	0.091	0.233	0.645	0.006

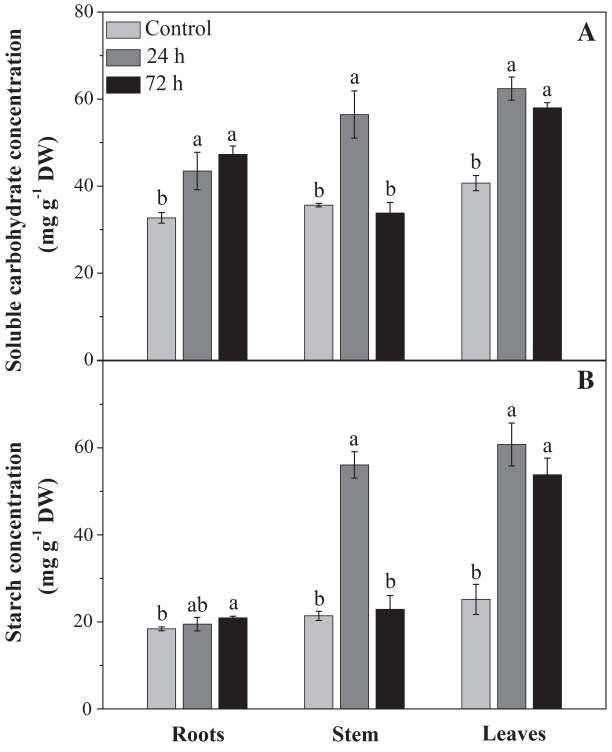

Fig. 1. Concentration (mg g^{-1} DW) of (A) soluble carbohydrate and (B) starch in different organs for the control, 24 and 72 h labeling in *Camptotheca acuminata* seedlings. Different lowercase letters indicate significant differences among the labeling time ($P < 0.05$).

relative to the control, which showed an increase by 33.0, 58.5 and 53.3% in the roots, stem and leaves, respectively (Fig. 1A). After 72 h of treatment, soluble carbohydrate in the roots reached the highest level (47.31 mg g^{-1}). Nevertheless, the stem and leaves showed different change patterns, in particular, a large decline in the stem after 72 h labeling. Starch concentrations in all organs were lower in the control, with a mean value of 21.66 mg g^{-1} (Fig. 1B). Notably, the bicarbonate-induced stress resulted in the generation of starch, leading to 2.6- and 2.4-fold increases in the stem and leaves within 24 h. After 72 h labeling, the starch concentration remained high in the leaves while returning to the control level in the stem. During the whole treatment period, the starch content showed a slight increase in the roots with less variation than in the stem and leaves.

C-isotope composition

The δ^{13}C of the roots, stem and leaves were all ^{13}C-enriched after labeling compared to the control, with an approximately 10‰ increase in the δ^{13}C of roots after 24 h labeling (Fig. 2A). As time passed, the

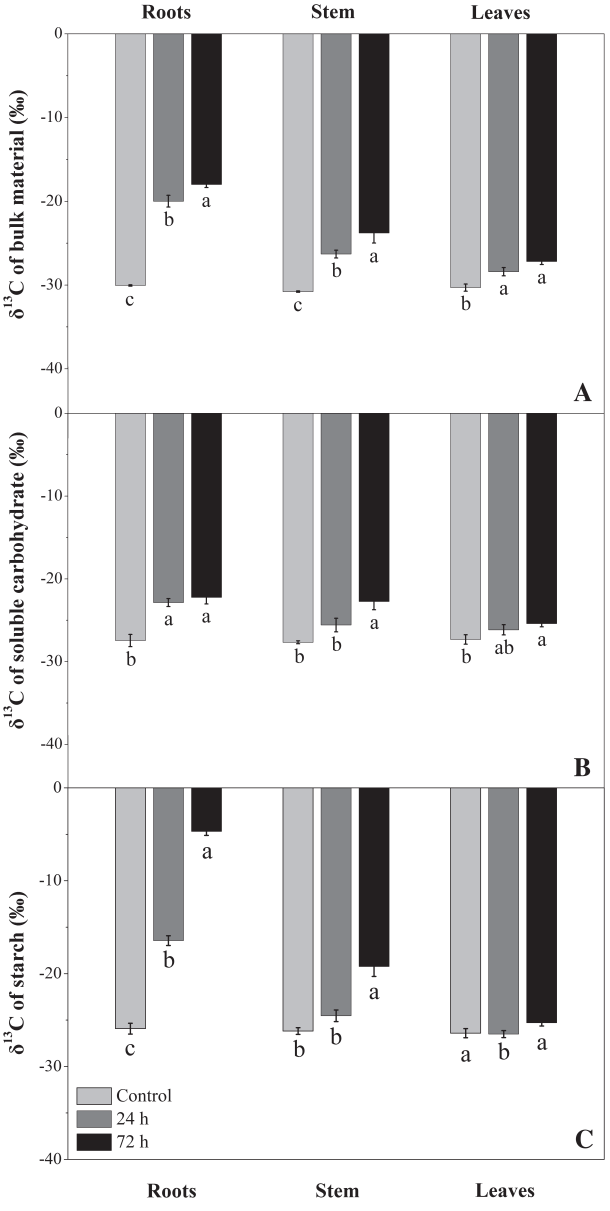

Fig. 2. δ^{13}C of (a) bulk material, (b) soluble carbohydrate and (c) starch in different organs for the control, 24 h labeling and 72 h labeling in *Camptotheca acuminata* seedlings. Different lowercase letters show significant differences among labeling time ($P < 0.05$).

δ^{13}C in the roots showed greater variations than in the stem and leaves. After 72 h labeling, −17.98, −23.78 and −27.18‰ were observed in the δ^{13}C of the roots, stem and leaves, respectively.

A compound-specific C-isotope composition was determined in the different organs (Fig. 2B, C). The δ^{13}C of soluble carbohydrate in all organs was significantly affected by ^{13}C labeling but was never positive than −20‰. Under 72 h labeling, approximately 5.21, 4.93 and 1.92‰ more ^{13}C-enriched soluble carbohydrates

Table 2. Pearson's correlation between NSC concentration and $\delta^{13}C$ of NSC among the different organs. Bold values indicated significant differences at $P < 0.05$.

	Soluble carbohydrates			Starch		
	Roots	Stem	Leaves	Roots	Stem	Leaves
r	0.848	0.026	0.567	0.705	−0.155	0.142
P	**0.001**	0.935	0.055	**0.011**	0.631	0.659

were observed in the roots, stem and leaves, respectively, than in the control. The $\delta^{13}C$ of starch in roots was significantly positive compared to the control, with values of −16.45 and −4.70‰ after 24 and 72 h of treatment, respectively. Nevertheless, the $\delta^{13}C$ of starch in the stem and leaves showed less variation than in the roots. In addition, the correlation between $\delta^{13}C$ and the NSC concentration (Table 2) was only positively significant in the roots ($r = 0.848$ for soluble carbohydrate and $r = 0.705$ for starch, respectively).

^{13}C allocation and contribution to the $\delta^{13}C$ of different compartments

The ^{13}C dynamic among different organs revealed the ^{13}C allocation pattern. Under 24 h of treatment, the distribution of ^{13}C was on average approximately 36.48, 38.41 and 25.10% in the roots, stem and leaves, respectively. However, the ^{13}C allocation showed no difference among the organs after 72 h labeling, indicating a slow transfer of ^{13}C.

Greater f_B was observed in the roots than in the stem and leaves whether the compartments were bulk organs or NSC compounds (Table 3). In bulk organs, only the roots showed a continuing increase in f_B after 72 h labeling ($P = 0.047$). In addition, soluble carbohydrate exhibited no change in f_B between the 24 and 72 h labeling periods in all organs analyzed. Starch in the roots and stem showed significant increases in f_B after 72 h labeling.

Discussion

This study was the first attempt to track the fate of recently fixed photoassimilates supplied from bicarbonate together with monitoring the NSC dynamics and C-isotope composition across the organs. The hydroponic ^{13}C labeling was much different from the short-term pulse $^{13}CO_2$ labeling (usually a few hours), in which the ^{13}C enrichment from the latter could surpass 1000‰ in the leaves within 1 day (Ruehr et al. 2009, Galiano et al. 2017). Nevertheless, DIC labeling was continuous and required a longer time, varying from 5 h to 10 days (Vuorinen et al. 1989,Rombolà et al. 2005 , Ford et al. 2007). Similar to our study, previous work showed that the ^{13}C enrichment in leaves was less than −20‰ throughout the experiment (Rombolà et al. 2005, Ford et al. 2007). Even though a small amount of bicarbonate was assimilated, the NSC level and C-isotope composition were greatly affected across the organs after exposure to bicarbonate for 24 h. Our study revealed that the photosynthates supplied from bicarbonate were incorporated into recently formed NSC and then allocated to C sink pools in a short time as a rapid response to bicarbonate.

Dynamics of NSC level within organs

The NSC levels across the organs showed significant increases within 24 h but decreased in the stem after 72 h labeling. This finding was in keeping with results obtained regarding the drought stress effect, in which the NSC level increased in the early stage (40 days since the drought inception) and eventually declined (McDowell 2011, Hoch 2015). Several studies also exhibited a slow change in NSC levels in response to variable drought stress (Hummel et al. 2010, Hartmann et al. 2013, Galiano et al. 2017). This phenomenon was observed because drought stress resulted from gradual declines in soil water availability (Chaves and Oliveira 2004), thereby imposing an osmotic stress that could lead to turgor loss (Krasensky and Jonak 2012). However, our study differed from prior results in that plants seemed to respond quicker to bicarbonate than to water deficit, because roots were in direct contact with high concentration of bicarbonate. The alkaline solution usually affects ion balance and osmotic adjustment, and subsequently results in water loss, stomatal

Table 3. Contribution of bicarbonate (f_B) to the $\delta^{13}C$ of different compartments after 24 and 72 h labeling. The *P* values were obtained from multiple comparison analysis; r^2 represented the adjusted coefficients of determination. Bold values indicated significant differences at $P < 0.05$.

	Organs (%)			Soluble carbohydrates (%)			Starch (%)		
	Roots	Stem	Leaves	Roots	Stem	Leaves	Roots	Stem	Leaves
24 h	0.11 (0.01)	0.05 (0.01)	0.02 (0.01)	0.05 (0.01)	0.02 (0.01)	0.01 (0.01)	0.11 (0.01)	0.02 (0.01)	0 (0.00)
72 h	0.14 (0.00)	0.08 (0.01)	0.03 (0.00)	0.06 (0.01)	0.06 (0.01)	0.02 (0.00)	0.24 (0.00)	0.08 (0.01)	0.01 (0.00)
P	**0.047**	0.137	0.107	0.628	0.060	0.356	**0.001**	**0.005**	0.094
r^2	0.509	0.329	0.375	0.042	0.471	0.143	0.987	0.759	0.397

closure and toxic effect because of the accumulation of Na^+ (Shahabi et al. 2005, Yang et al. 2008, Aroca et al. 2012). In the present experiment, the adverse conditions hampered photosynthesis and the water supply more severely but largely facilitated the formation of NSC within 24 h (Table 1; Fig. 1). The newly formed NSC was mainly composed of three parts originating from remobilization of stored C reserve, atmospheric CO_2 and dissolved bicarbonate. Stomatal limitations reduced photosynthesis, which seemed to conflict with the increase in NSC concentration. Presumably, this finding was observed because large stored NSC pools in the leaves and stem remained accessible to plant tissues (Hoch et al. 2003, Carbone et al. 2013, Hoch 2015). These NSC pools became autonomous from the stored C reserve to overcome adversity when the C demand and supply were unbalanced (Hoch et al. 2003). The increasing NSC was regarded as an active NSC accumulation at the expense of growth (Krasensky and Jonak 2012, Sala et al. 2012, Galiano et al. 2017), which was preferentially consumed for osmotic adjustment, restoration and respiration rather than used for other functions (Zeeman et al. 2010, McDowell 2011). Specifically, bicarbonate utilization also resulted in the increase in NSC. The ^{13}C enrichment of NSC in all organs supported this claim (Fig. 2). In addition, rough estimates of f_B also showed that bicarbonate assimilation and incorporation into the total NSC compounds contributed up to 0.24% within 72 h, double that after 24 h of labeling (Table 3). The present work clearly demonstrated that the assimilated bicarbonate had become a part of the NSC.

Variability of $\delta^{13}C$ in organs and NSC compounds

As a single proxy, the ^{13}C tracer provided more precise evidence for monitoring C mobilization than NSC dynamics. The $\delta^{13}C$ of bulk organs and NSC compounds was observed to be continuously ^{13}C-enriched, indicating a bottom-up transport of DIC and downward translocation of photosynthates within 24 h (Fig. 2). The entire process was achievable because it only took 2–6 h to transport DIC and 3–27 h to allocate recently fixed photosynthates from the leaves to the roots in many species (Vapaavuori and Pelkonen 1985, Ruehr et al. 2009, Epron et al. 2012, Blessing et al. 2015). We observed that the $\delta^{13}C$ in bulk organs were isotopically heavier in the roots than in the stem and leaves (Fig. 2) due to DW differences between the organs (dilute effect, Table 1). It could also be explained by several other factors: (1) a fraction of the absorbed $H^{13}CO_3^-$ ion was retained in the roots. This is reliable because the $\delta^{13}C$ of the label was far more positive than that of the background in this study (approximately 8887 vs −30‰). Rombolà et al. (2005) divided the belowground tissues into main roots and fine roots, which showed that the $\delta^{13}C$ of the latter sharply increased to 2500‰, whereas the main roots only reached 10‰ under 10 days of high abundance ^{13}C labeling (20% atm.). This finding might be observed due to different functions between the main roots (xylem transport) and the fine roots (uptake); (2) bicarbonate was directly catalyzed by PEPC to generate organic acids in the roots; (3) newly fixed photosynthates supplied from bicarbonate were allocated to the stem and roots. The data showed that photosynthates supplied from bicarbonate had a greater influence on the $\delta^{13}C$ of NSC compounds in the roots than in the stem and leaves (Tables 2 and 3). Both soluble carbohydrate and starch in the roots were more ^{13}C-enriched and thus contributed to the more positive $\delta^{13}C$ value of the roots. The present work differed from our previous experiment carried out with 14 days of labeling, which indicated that $\delta^{13}C$ in the roots was more likely influenced by the new photosynthates allocated to the roots (Rao and Wu 2017). In the present experiment, the NSC content only represented 2–6% of the root DW (Fig. 1). If NSC compounds were all starch with a $\delta^{13}C$ value of −4.70‰ (Fig. 2), according to the isotope mixing model, the $\delta^{13}C$ of the roots would not change compared to the control.

Effect of bicarbonate on the NSC pools

The variation range of NSC concentrations among the organs exhibited different patterns, which reflected the organ-specific differences of the NSC pool sizes (Sala et al. 2012, Hoch 2015). This finding was consistent with several studies showing that the NSC concentration and variation in the roots were generally less than in the stem and leaves (Hartmann et al. 2013, Galiano et al. 2017). Interestingly, while the NSC concentration in the stem and leaves increased, it only slightly accumulated in the roots after 72 h labeling. This was different from our assumption. Thus, it was difficult to see that the root NSC served as a major C sink pool, at least in the short term. Two factors could potentially explain this phenomenon: (1) the export of leaf transitory starch was limited because starch degradation and sucrose export to the phloem generally proceed at night (Zeeman et al. 2010). In addition, the phloem transport of photosynthetic products was insufficient due to hydraulic constraints (Ruehr et al. 2009, Ainsworth and Bush 2011, Zang et al. 2014). The slow change in $\delta^{13}C$ and ^{13}C allocation between the 24 and 72 h labeling periods supported this argument (Fig. 2); (2) root growth, respiration and exudates were also part of the C sink pools, even though photosynthesis

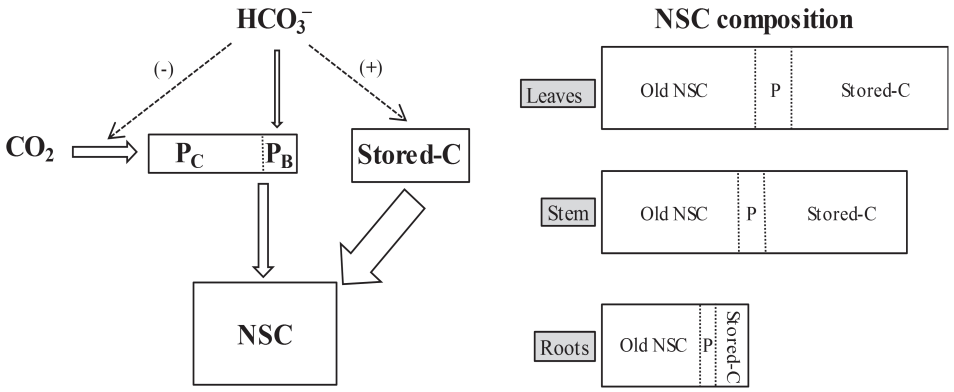

Fig. 3. Schematic concept for the effect of bicarbonate supply on the NSC pools of *Camptotheca acuminata*. Box and arrow sizes are roughly proportional to the NSC pool sizes and fluxes among the organs. P means photosynthates. P_C and P_B represent newly fixed photosynthates supplied from CO_2 and bicarbonate, respectively. The direction (dotted arrow) of the bicarbonate stress effect on fluxes or pools are indicated by + (increase) and – (decrease).

was curtailed (McDowell 2011, Hartmann and Trumbore 2016). To elucidate the effect of the bicarbonate supply on the NSC pools of all organs, a schematic concept is introduced here (Fig. 3). Initially, bicarbonate-induced stress led to the decline of g_s and thus reduced photosynthetic CO_2 uptake. Then, the stored C reserve was triggered and became mobilized NSC in different organs. At the same time, photosynthates supplied from bicarbonate and CO_2, as well as a part of mobilized NSC, were both transported downward but were constrained by bicarbonate to some extent. Finally, the mixture of old NSC, newly fixed photosynthates and stored C reserve shaped the size of the new NSC pools across the organs.

Conclusions

Bicarbonate triggered a dramatic decrease in photosynthesis but an increase in NSC concentration across the organs. The majority of the NSC was derived from stored C reserves, presumably being consumed for osmotic regulation, restoration and respiration. The combination of ^{13}C labeling and NSC dynamics accurately recorded the fate of recently fixed bicarbonate in *C. acuminata* seedlings, which affected the $\delta^{13}C$ values of organs and NSC compounds. Nevertheless, the increased NSC in the leaves and stem only slightly accumulated in the roots, suggesting that root NSC might not be a major C sink pool. We acknowledge that our findings are limited to several days of observations and bulk roots rather than separation between fine and coarse roots. Long-term NSC allocation into subdivided C sink pools, such as coarse roots and phloem sap, needs to be investigated in future studies.

Author contributions

S.R. designed and undertook the experiments and wrote the manuscript. Prof. Y. W. and Dr R. W. conceptualized and supervised the manuscript.

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