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CARBON RELIANCE ON DISSOLVED INORGANIC CARBON OF JUNIOR AND MATURED REED BEDS INDICATED BY STABLE ISOTOPE ANALYSIS

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ABSTRACT

Reed is one of the most widespread and productive wetland plant species worldwide, but few studies have addressed carbon utilization of this plant in natural environments. By collecting reed samples from lakes and ponds in the middle and lower reaches of the Yangtze River, weexamine the association between the carbon isotopic ratio ($\delta^{13}C$) of reed stem and root, and dissolved inorganic carbon concentration (DIC) and $\delta^{13}C_{DIC}$, respectively. Our results suggest that 1) although atmospheric carbon is the dominant pathway, the pathway that uptake DIC through root system also plays an important role in determining the carbon stable isotope signal of reed, and 2) juvenile reedbed incorporates more carbon resources from DIC pool than the matured ones. Our study demonstrated that $\delta^{13}C$ of emergent macrophytes could also be influenced by underwater carbon, especially at their early life stages.

Key words: Carbon utilization; littoral habitat; reed; stable carbon isotope; wetland.

INTRODUCTION

Reeds, *Phragmites,* commonly found in many wetlands worldwide, including fresh and alkaline marshes, pond margins, swamps, ditches, and brackish waters (Choi *et al*., 2005; Eller *et al*., 2017; Packer *et al*., 2017). Reed beds, as important wildlife habitats, maintain and enhance the diversity of other living species, such as birds, fish, and invertebrates (Van der Werff, 1991; Onojeghuo and Blackburn, 2013; Packer *et al*., 2017).

Because they can withstand high levels of environmental stresses, e.g., contamination, salinity, and acidity, and can assimilate heavy metals, nitrogen, and phosphorous (Brix, 1990a; Gustavsson and Engwall, 2012), reeds are increasingly being used as an excellent stabilizer of soil and can store nutrient efficiently through its accumulation of persistent biomass (Baran *et al*., 2002; O'Luanaigh *et al*., 2010; Gustavsson and Engwall, 2012).Previous studies have shown that *Phragmites*spp. effectively retain nitrogen and phosphorus in various wetland ecosystems (Windham and Meyerson, 2003; Palmer-Felgate *et al*., 2013; Shuang *et al*., 2015). However, very few studies have carried out to understand the carbon utilization of reed, which is the base of its production to support its environmental and ecological functions in natural environments.

Reed is characterized by well-developed lacunae, which serve as the convective throughflow mechanism, and accelerates gas exchange between the sediment and the atmosphere (Brix, 1990a; Brix *et al*., 1996). Similar to submerged plants, the root system and the water-covered tissues of emergent species are surrounded by inorganic carbon which is present in three available forms: free CO_2, HCO_3^-, and CO_3^{2-}. For instance, extensive root system of *P. australis* buried in largely anaerobic or anoxic sediments(Choi *et al*., 2005; Tóth, 2016). Thus, it could be expected that *P. australis*might be dependent on inorganic carbon from the water column and/or sediment interstitial water via the root system. Not only the uptake of DIC by roots and rhizomes of *P. australis*, but also the following internal transport and the fixation of photosynthesis were demonstrated by ^{14}C-labelled experiment (Brix, 1990b). However, to our knowledge, there is still no study that has addressed the strategy for carbon utilization of reed in natural environments (Brix, 1990a, 1994; Brix *et al*., 2001).

The major purpose of this study was to assess the strategies for carbon fixation of the common reed (*Phragmitesaustralis*) in littoral habitats of flood plain lakes and ponds by using stable isotope natural abundances. The common reed is submerged at the early life stage (Engloner, 2009). The dissolved inorganic carbon is main source for the plants probably. Later, when the reeds grow bigger, the reeds can depend on the CO_2 in the air (Li *et al*., 2009). Specifically, we hypotheses that 1) although atmospheric carbon is the dominant pathway, the pathway that uptake DIC through root system can also affect the carbon stable isotope signal of reed, and 2) juvenile reedbed incorporates more carbon resources from DIC pool than the matured ones. We also discuss the possible indication of our results to manage or reconstruct reed beds in natural environments.

MATERIALS AND METHODS

Samples of reed were collected from littoral habitats of flood plain lakes and ponds in the middle and lower reaches of the Yangtze River from 2008 to 2015 (Fig. 1). Sampled lakes were from mesotrophic to hypereutrophic systems (Wu *et al.*, 2005). Five independent individuals of reed were harvested by hand and pooled in summer (July – September) at each sampling lake/pond for stable carbon isotope analyses. The sites were selected with water depth between 20cm and 30cm, which allow light illumining the sediment system confirmed by Secchi disc. Reed beds in these sites were categorized according to average individual stem heights of reed, i.e. matured beds with average stem height larger than 1.5m, and juvenile beds with less than 0.5m. Roots and stems of reed were separated as independent specimens. Every specimen was cleaned off mud, debris, epiphytes and dried at 60 ºC to constant weight.

Overlying water samples at the water-sediment interface (depth 0-10cm) for the analysis of DIC concentrations and theδ^{13}C of the DIC were collected from the center of each reed bed with a horizontal water sampler (1120-G45, Dynamic Aqua-Supply Ltd.) and transferred into tightly capped 250-ml glass bottles. Before filling water samples, rinsing each bottle with deionized water and then use the water sample to rinse again. The bottles were leak-proof and adding a few drops of saturated aqueous $HgCl_2$ to avoid algal growth (Deuser and Hunt, 1969). This treatment is assumed to not alter the original DIC concentration and the carbon isotopic signature of DIC significantly (Deuser and Hunt, 1969; Herczeg, 1987).

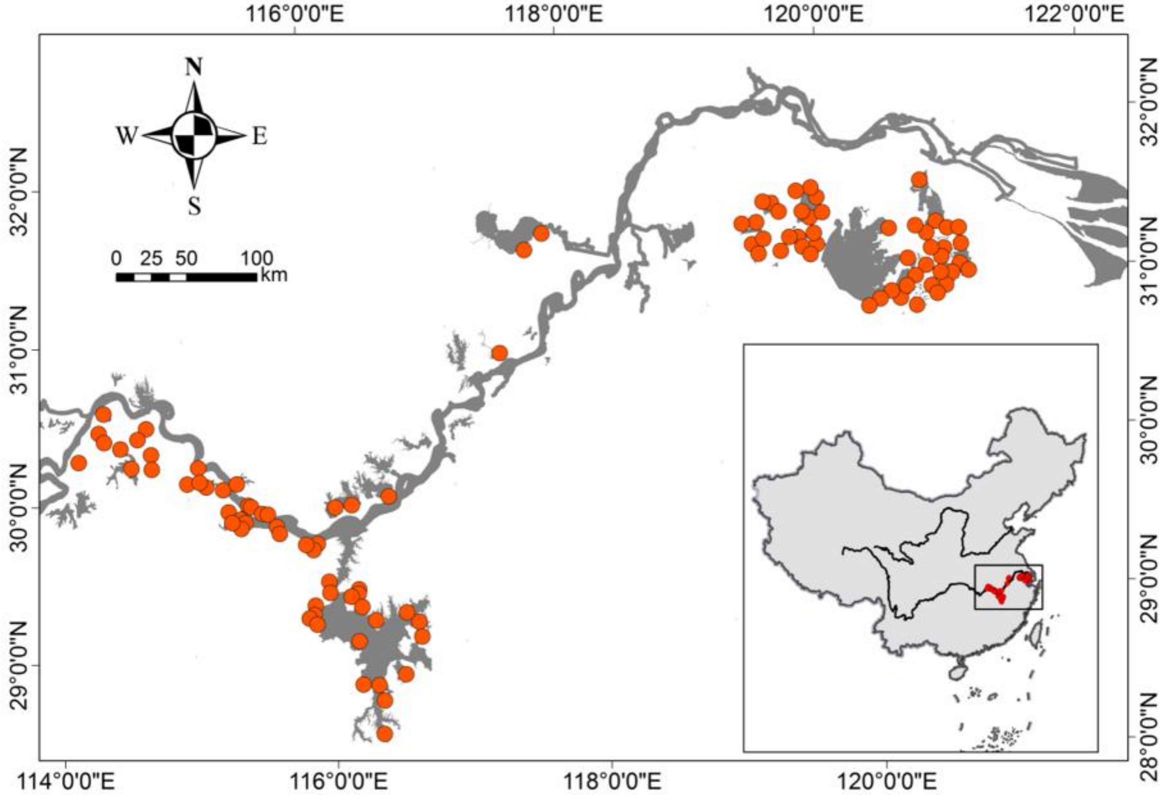

Fig. 1.Locations of studied lakes and ponds. The circular spots are sampling sites in the middle reaches of the Yangtze River from 2008 to 2015.

Water samples for DIC were analyzed on a Shimadzu 5050 TC Analyzer. Water samples were filtered with percombustedWhatman GF/F (450 ºC) before carbon analysis. Samples were first run in total carbon analysis mode to obtain total carbon concentrations in each sample. The samples were acidified to pH of 2 or less to convert inorganic carbon to CO_2 and the interference of inorganic carbon were then be eliminated. Subsequent purging of the sample were done with a vacuum by degassing the CO_2 by volatilization. Purged sample were reanalyzed to obtain dissolved organic carbon concentrations in each sample (Findlay *et al.*, 2010; Laskar *et al.*, 2014). Note that the purging also removes purgeable organic carbon so that the organic carbon measurement made after eliminating inorganic carbon interferences is actually a non-purgeable organic carbon determination. The DIC concentrations were then calculated by minus the total carbon and dissolved organic carbon concentrations. For measurement of carbon concentration in the samples, the phthalate standard curve was used, and there were three interspersed sets of organic carbon standards (potassium phthalate, 2.5, 5, 10, and 20 mg C L^{-1}) and blanks of Nanopure water (Findlay *et al*., 2010). For stable carbon isotope of DIC ($\delta^{13}C_{DIC}$), the water DIC was precipitated as carbonates in the lab. $SrC1_2$-NH_4OH liquor was added to sample in ratio 1 : 9 in a sealed glass bottle to promote precipitation for the $\delta^{13}C_{DIC}$ analysis. Then filtered and dried the carbonate sample and then the precipitated carbonatewas collected by filtering with percombusted (450 ºC) Whatman GF/F to dry for isotopic measurements (Telmer and Veizer, 1999; Taipale and Sonninen, 2009). $SrC1_2$-NH_4OH liquor was added to ≤ l-l sample volume to promote precipitation for the stable carbon isotope analysis of natural water, then sealing the bottle with polyvinylchloride tape, and shaking the bottle well and leaving the contents to precipitate for 48 hours. Then the filtration and drying of the carbonate samples were processed by filtering the precipitate on a glass-fibre filter paper by using a water jet pump in air for 10 min. Then used deionised water to rinse the precipitate, and drying the precipitate in an oven at 110°C for 4 hours. Finally, we scraped the precipitate off filter paper into a small airtight container and store in a desiccator until analysis (Bishop, 1990b).

The dried materials of each sites was ground to fine powder by using electric mill (Mini-Beadbeater-16, Bio Spec Products Inc), and subsamples were used for determination of stable isotope signatures of carbon. The stable carbon isotope was determined using Delta Plus (Finnigan, Germany) continuous flow isotope ratio mass spectrometer coupled to a Carlo Erba NC2500 elemental analyzer. ^{13}C:^{12}C ratios were determined in the mass spectrometer against an internal CO_2 standard. The results are expressed using the δ notation in per mil (‰) as $\delta^{13}C = (R_{sample}:R_{standard} - 1) \times 1000$, where $R = {}^{13}C:{}^{12}C$ and higher δ values denote a greater proportion of the heavy isotope. The international standard is Vienna Pee Dee Belemnite (VPDB). The international reference material is carbonatite (IAEA–NBS18). An internal working standard, urea ($\delta^{13}C$ = -49.44‰). The analytical precision of the isotopic analysis was better than 0.3%.

To test our first hypothesis that although atmospheric carbon is the dominant pathway, the pathway that uptake DIC through root system can also affect the carbon stable isotope signal of reed, we use linearregressions to determine the causal relationship between two variables, i.e. reed $\delta^{13}C$and DIC, and reed $\delta^{13}C$and $\delta^{13}C_{DIC}$.

To test our second hypothesis that juvenilereed is incorporated more carbon resource from DIC than matured reed in natural environments, we compared the scaling parameters (i.e. slopes) between juvenile and matured reed groups to indicate between-group difference. In this case, growth situation is a categorical factor with two levels (i.e. juvenile and matured) while DICor $\delta^{13}C_{DIC}$ is the regressor and$\delta^{13}C_{stem}$or $\delta^{13}C_{root}$ is the response variable. The ANCOVA was used to assess if the regression between$\delta^{13}C_{stem}$or $\delta^{13}C_{root}$ and DICor $\delta^{13}C_{DIC}$ are the comparable between the growth situations. The difference in allometriccarbon reliance on DIC should manifest when a different slope in both regression lines was observed.

We fitted the regression models with a call to the lm() command using the nested structure of DICor $\delta^{13}C_{DIC}$ nested within$\delta^{13}C_{stem}$or $\delta^{13}C_{root}$ and removing the single intercept for the model so that separate intercepts were fit for each equation. We used the aov() command to do an ANCOVA. All these analysis were performed in R for Windows (Version 3.0.3) (Keselman *et al*., 1998; Bates *et al*., 2014).

RESULTS

The changes in the carbon isotope signatures in the reed roots and stems are presented in Fig. 2. The carbon isotope ratios of the reed root and stem varied between -30.4‰ and -28.0 ‰. The carbon isotope ratios of the roots of reed were slightly higher than those of the stems. There was significant positive relationship between $\delta^{13}C_{stem}$and $\delta^{13}C_{root}$, which is not affected by growth conditions (Fig. 2A; $p \leq 0.001$, r=0.99, df=111). Significant difference of reed $\delta^{13}C$ was found between tissues and between growth conditions (Fig. 2B; Tissue: F=33.55, $p \leq 0.001$, n=115; Growth condition: $F = 37.08$, $p \leqslant 0.001$, n=115).

$\delta^{13}C_{stem}$ of junior and mature reed were significantly negativerelated to DIC (junior: R^2= 0.081, $p \leq 0.05$; mature: R^2= 0.1802, $p \leq 0.001$)and different slope was found in both regression lines of growth condition ($F = 7.887$, $p \leq 0.001$, Fig. 3A). $\delta^{13}C_{root}$ of juniorand mature reed were also significantly negativerelated to DIC (junior: R^2= 0.089, $p \leq 0.001$; mature: R^2= 0.3519, $p \leq 0.001$)and different slope was found in both regression lines of growth condition ($F = 16.71$, $p \leq 0.001$, Fig. 3B).

$\delta^{13}C_{stem}$ of junior reedwas significantly positiverelated to $\delta^{13}C_{DIC}$ (junior: R^2= 0.2552, $p \leq 0.001$) and $\delta^{13}C_{stem}$ of mature reed was non-significantly related to $\delta^{13}C_{DIC}$ (p = 0.056) The different slopes was found for the regression lines of growth condition (F = 12.409, $p \leq$ 0.001, Fig. 4A). $\delta^{13}C_{root}$ of juniorand mature reed were also significantly positiverelated to $\delta^{13}C_{DIC}$ (junior: R^2= 0.463, $p \leq 0.001$; mature: R^2= 0.3519, $p \leq 0.001$)and different slope was found in both regression lines of growth condition ($F = 23.63$, $p \leq 0.001$, Fig. 4B).

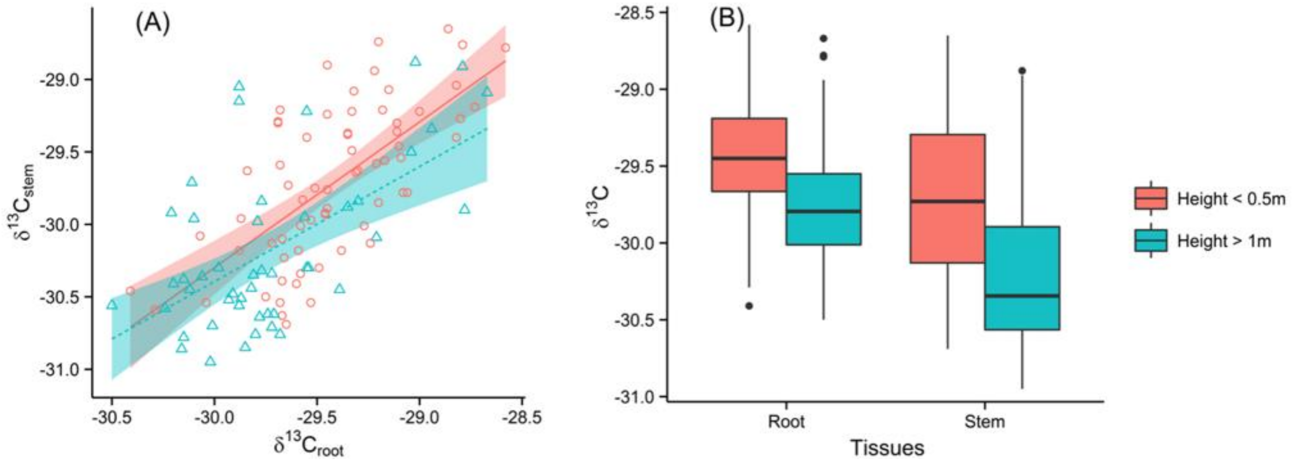

Fig. 2. Relationships and differences of $\delta^{13}C$ between tissues and growth situations. (A)- relationships and differences of $\delta^{13}C$ between stem and root ($p \leq 0.001$, r=0.99, df=111). (B)- relationships and differences of $\delta^{13}C$ between tissues and growth situations (Tissue: F=33.55, $p \leq 0.001$, n=115; Growth condition: F = 37.08, $p \leqslant$ 0.001, n=115).

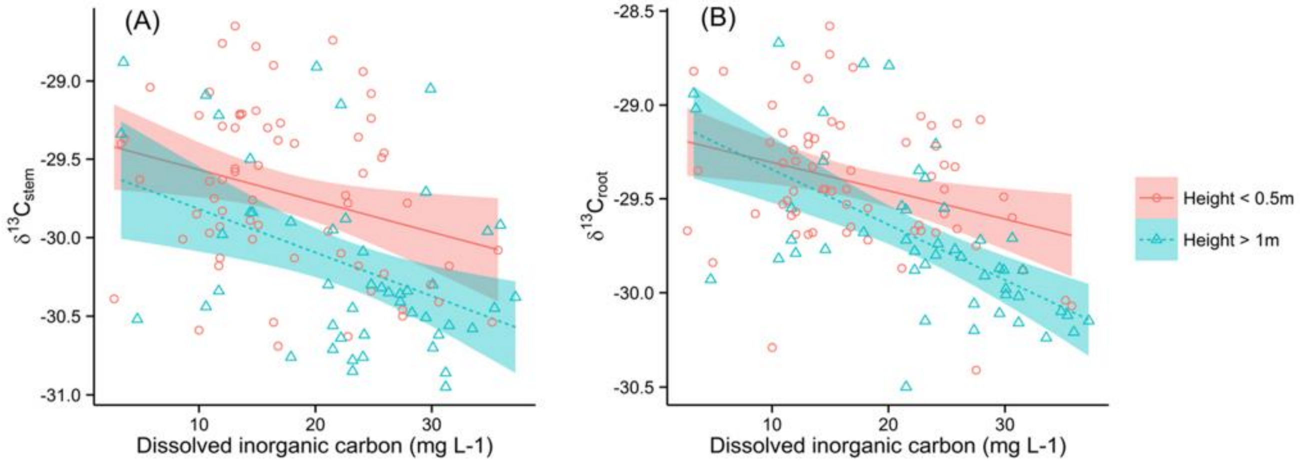

Fig. 3. Correlations between tissues $\delta^{13}C$and dissolved inorganic carbon. (A)-correlation between stem$\delta^{13}C$and dissolved inorganic carbon (junior: R^2= 0.081, $p \leq 0.05$; mature: R^2 = 0.1802, $p \leq 0.001$; F = 7.887, $p \leq$ 0.001). (B)-correlation between root $\delta^{13}C$and dissolved inorganic carbon (junior: R^2= 0.089, $p \leq 0.001$; mature: R^2 = 0.3519, $p \leq 0.001$; F = 16.71, $p \leq 0.001$).

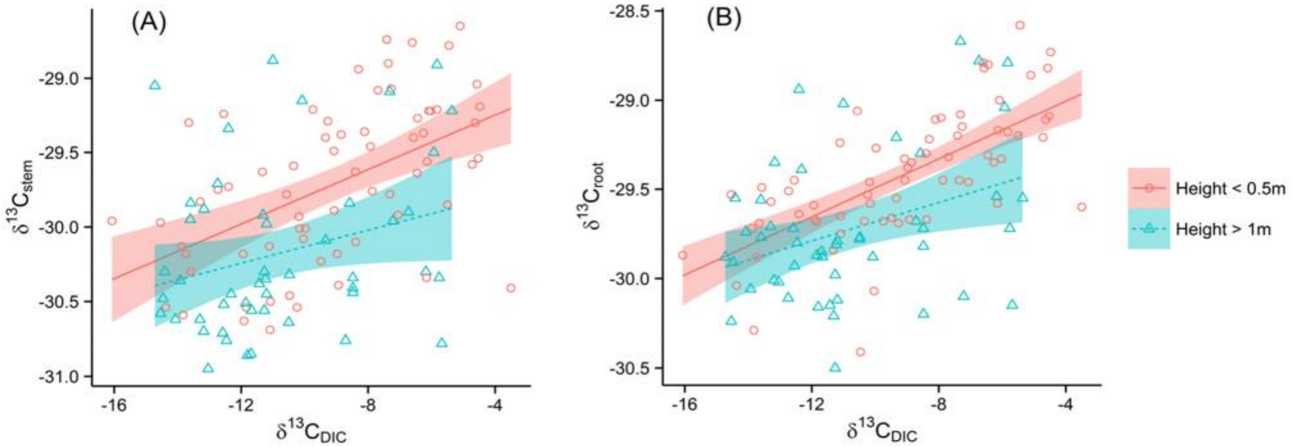

Fig. 4. Relationships between tissues $\delta^{13}C$ with dissolved inorganic carbon$\delta^{13}C$ under growth situation. (A)-relationships between stem $\delta^{13}C$ with dissolved inorganic carbon$\delta^{13}C$ (junior: R^2= 0.2552, $p \leq 0.001$; mature: p = 0.056; F = 12.409, $p \leq$ 0.001). (B)-relationships between root $\delta^{13}C$ with dissolved inorganic carbon$\delta^{13}C$ (junior: R^2= 0.463, $p \leq 0.001$; mature: R^2= 0.3519, $p \leq 0.001$; F = 23.63, $p \leq$ 0.001).

DISCUSSION

The purpose of this study was to assess the strategies for carbon fixation of the common reed (*P. australis*) in littoral habitats of flood plain lakes and ponds by using stable isotope natural abundances. Our results confirm that in natural environments, reed might depend on inorganic carbon from the water column and/or sediment interstitial water via the root system as indicated by the strong correlation between $\delta^{13}C_{root}$ of juniorreed and $\delta^{13}C_{DIC}$. This is in line with the ^{14}C-labelled experiment (Brix, 1990b) that uptake of DIC by roots and rhizomes of reed and the subsequent internal transport and photosynthetic fixation.

Our first hypothesis that, although atmospheric carbon is the dominant pathway, the pathway that uptake DIC through root system also affect the carbon stable isotope of reed was supported by the results. First, the $\delta^{13}C$ of reed samples from littoral areas of studied freshwater lakes generally reflect the C_3 photosynthetic fixation of airborne CO_2 by reed(O'Leary, 1981; Choi *et al*., 2005). This result supported the observation of Antonielli *et al*. (2002) through anatomical and biochemical-physiological characterization of its leaves.The $\delta^{13}C$ values of reed observed in our study were much more negative than those reported for reed in other studies under environmental stresses, e.g., salinity and drought (Farquhar *et al*., 1982; Müller and Voss, 1999; Choi *et al*., 2005; Whitaker *et al*., 2015).

Secondly, we found reed isotope was significantly related with both DIC and $\delta^{13}C_{DIC}$. Reed is characterized by well-developed lacunae, which serve as the convective through flow mechanism, and accelerates gas exchange between the sediment and the atmosphere (Brix, 1990a; Brix *et al*., 1996). It's extensive and deep-penetrating root system buried in largely anoxic or anaerobic sediments (Brix, 1990a, b; Choi *et al*., 2005; Tóth, 2016). Uptake of DIC by roots and rhizomes of reed and the following internal transport and photosynthetic fixation were demonstrated by ^{14}C-labelled experiment (Brix, 1990b). Thus, our results support that reed can accumulate inorganic carbon from the water column and/or sediment interstitial water via the root system in natural environmental systems.

Our second hypothesis that junior reed is incorporated more carbon resources from DIC than the mature reed was also supported by our results. It's probably related to the height of reed. Because junior reed is submerged at the early life stage (Engloner, 2009), and the matured reed grows above the water and can depend on the CO_2 in the air (Li *et al*., 2009). Specifically, matured reed more strongly negatively correlated with DIC concentration, and juvenile reed $\delta^{13}C$ showed more strong relationship with $\delta^{13}C_{DIC}$. From the isotopic point of view, when primary productivity is high, aquatic CO_2 concentration decreases, and ^{13}C in the DIC pool(Takahashi *et al*., 1990).In such environment condition, the strongernegative correlation between $\delta^{13}C$ and DIC may suggested that with increasing DIC, mature common reed became lessenriched in ^{13}C. Thus, it was speculated that the scarcity of DIC led to little isotopic discrimination and enriched ^{13}C of reed which is more manifest in mature reed.Junior reed showed higher correlation with $\delta^{13}C_{DIC}$ suggests that inorganic carbon with enriched ^{13}C in the DIC pool was transported from the root and rhizomes of reed to photosynthetic tissues, e.g., stem, and then was directly fixed by reed (Brix, 1990b), which is more important pathways for the junior reed.

Water bodies in our studied region are under serious eutrophication. Dense algae production intensively affect the exchange between atmospheric CO_2 and DIC(Takahashi *et al*., 1990; Lehmann*et al*., 2004; Gu *et al*., 2006; Zhang *et al*., 2007), severely limit aquatic habitat, and competitively change the carbon fixation strategies of aquatic plants. Reed is one of the most widespread and productive littoral plant species in the region and the damage of reed bed by algal blooms result in adverse effects such as conspicuous withering of bottom leaves, shortened stem length, and decreased biomass is an ongoing problem. It will reduce the nutrient store of reed bed from the water bodies and contribute to the eutrophication probably (O'Luanaigh *et al*., 2010; Gustavsson and Engwall, 2012; Shuang *et al*., 2015). Thus, our study is helpful in understanding reed bed carbon cycling, which is particularly relevant to eutrophic issues such as the reed bed reconstruction,management, and conservation for its environmental and ecological functions.

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