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Stable carbon isotopic composition of submerged plants living in karst water and its eco-environmental importance☆

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ABSTRACT

The stable carbon isotopic composition of submerged plants ($\delta^{13}C_P$) can be controlled by physiological and environmental factors. Herein, we took advantage of a short, natural karst river with an annual mean bicarbonate (HCO_3^-) value of 3.8 mmol L^{-1} to study the stable carbon isotopic composition of submerged plants along the river and the influence of environmental conditions on the $\delta^{13}C_P$ values. The $\delta^{13}C_P$ values of *Ottelia acuminata*, *Potamogeton wrightii*, *Vallisneria natans*, and *Hydrilla verticillata* from upstream to downstream show a gradient and ranged from −34.8‰ to −27.8‰, −36.6‰ to −23.7‰, −35.1‰ to −25.3‰, and −38.6‰ to −26.3‰, respectively and even more depleted values for the first two species at the uppermost site. Diurnal variation of water chemistry and concentration of the dissolved inorganic carbon (DIC) and the stable carbon isotopic composition of DIC ($\delta^{13}C_D$) indicate that the macrophytes and other primary producers in the river have a very high net photosynthetic rate. The gradient of $\delta^{13}C_P$ values was consistent with CO_2 being a declining source of inorganic carbon for photosynthesis in the downstream transect. The results demonstrate that the high DIC concentration with lower negative $\delta^{13}C$ value, particularly in karst water environment has a significant role in controlling the stable carbon isotopic composition of submerged plants living in it.

1. Introduction

Much recent work has focused on the inter-relationships between the ecological, hydrological, and physico-chemical processes in groundwater/surface water interactions (Sophocleous, 2002; Hancock et al., 2009; Bork et al., 2009). One of the most important interactions between surface water and groundwater occurs in spring-fed rivers in which groundwater chemistry controls solute inputs to surface water and represents the initial control on river ecology (Holmes, 2000; Harvey and McCormick, 2009). Surface water function and biodiversity are controlled by interactions between the physical and chemical environments, in addition to the physiological and biochemical acclimation and adaptation of organisms as well as their short-term behavioral responses (Maberly et al., 2015).

Submerged plants are important primary producers, maintaining the ecological balance of aquatic systems and taking part in biogeochemical cycling. However, unlike terrestrial plants, it is a common phenomenon that photosynthesis and growth of aquatic primary producers are strongly restricted to DIC supply (Maberly and Spence, 1989). Because of the low diffusion rates of gases in water and the existence of a well-developed diffusive boundary layer around submerged plant surfaces, CO_2 is generally less available in water than in air. In addition, photosynthesis can also be further limited by the intermittent depletion of CO_2 produced when the rates of photosynthetic demand exceed those of replenishment and by the generation of high concentrations of oxygen that promotes photorespiration (Maberly and Madsen, 2002a; Pedersen et al., 2013). Therefore, submerged plants require high concentrations of DIC to saturate their photosynthesis. A number of submerged macrophytes possess physiological and biochemical features that ameliorate the effect of low carbon availability and minimize the effects of its potential limitation (Spence and Maberly, 1985). Klavsen et al. (2011) summarized that 'avoidance' and 'exploitation' strategies are effective approaches to obtain sufficient CO_2 for photosynthesis. In addition, 'amelioration' strategies based on carbon dioxide concentrating mechanisms (CCMs) can be present, including the ability to use HCO_3^-, crassulacean acid metabolism (CAM) and C_4-like photosynthesis (Maberly and Madsen,

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2002b; Bowes, 2011; Dou et al., 2013). The ability of HCO_3^- utilization is thus particularly advantageous under alkaline conditions, and the most widespread CCM involves the use of HCO_3^- as an alternative carbon source. In terrestrial plants, the $\delta^{13}C_P$ value is closely related to the photosynthetic pathway used in carbon fixation. The $\delta^{13}C_P$ value of freshwater aquatic plant is affected by the type and extent of the CCM and also by the stable isotope signature of the carbon source and has been found to vary from −50‰ to −11‰ (Keeley and Sandquist, 1992).

Ecological processes, particularly the photosynthesis of macrophytes, can significantly impact the hydrochemical characteristic of surface water fed by underground water (de Montety et al., 2011). Photosynthesis of macrophytes is considered to be a crucial biochemical process in controlling the DIC diurnal cycling in spring-fed surface water (Liu et al., 2008). The $\delta^{13}C_D$ value has been used to improve the understanding of the carbon cycle and diel process by macrophytes in the catchment (Heffernan and Cohen, 2010; Parker et al., 2010; Poulson and Sullivan, 2010). The $\delta^{13}C_D$ value in upstream regions is mainly controlled by geochemical processes, while in the downstream, it value is mainly affected by photosynthesis and respiration of macrophytes (Parker et al., 2007; Poulson and Sullivan, 2010). A variety of studies focused on the diurnal variation and the utilization of DIC by macrophytes in spring-fed rivers, particularly on the calculation of submerged macrophyte capacity for a karst carbon sink (Neal et al., 2002). However, it is still unclear for certain submerged plants what the $\delta^{13}C_P$ value is and which carbon sources they tend to use in photosynthetic processes in karst water environment. Therefore, the $\delta^{13}C_P$ values at various sites along the karst river, along with diurnal variations of DIC species and concentration, as well as the $\delta^{13}C_D$ values, were monitored to assess the photosynthetic carbon source and karst impact on the stable carbon isotopic composition of submerged plants.

2. Materials and methods

2.1. Site description

The Zhaidi karst underground river system is located in eastern Guilin Haiyang village Lingchuan County, Guangxi Zhuang Autonomous Region, China (Fig. 1). Its geographic coordinates are 110°32′36″ to 110°37′22″ E, 25°13′59″ to 25°18′19″ N, with approximately 32.7 km^2 of recharge area. The recharge area is mainly comprised of Devonian limestone, which covers 89.5% of the total catchment. In the catchment, underground rivers, karst caves, karst sink holes, underground river skylights, and karst depressions are fully developed (Chen et al., 2013). Meanwhile, the main geomorphology is peak cluster with thin soil; scrub and grass are the dominant vegetation. The main land-use types are farmland and orchard in the depression, whereas in the middle and western regions, the rock desertification is very serious. The area has a subtropical monsoon climate, hot and rainy; the annual mean temperature is about 18–19 °C, and it receives an annual rainfall of about 1650 mm. The rainy season begins in April and ends in August, and this period accounts for approximately 60% of the annual precipitation total. The rainfall is collected in the depression and charges the underground water through underground river skylights, karst funnels, karst sink-holes, karst mountain foot holes. The karst aquifer medium is characterized by two structures, enormous underground pipelines and karst fissures. The groundwater flows through the underground pipeline from north to south and is discharged into the Chaotian River via the Zhaidi River, which is the site of this study (Fig. 1B).

The Zhaidi River has a total length of 512 m and is mostly 2–6 m wide and 0.6–2.2 m deep. The river has been channelized on both sides with a wall. The river sediments, which range in pH value from 8.2 to 8.9, are mainly composed of sand grains with a diameter of 0.075–2 mm, and the organic matter content is between 1.2 and 9.5 g kg^{-1} (unpublished data). The Zhaidi River is colonized by eight species of submerged plants affiliated to four families and six genera (Wang et al., 2015). Among them, *Ottelia acuminata*, *Potamogeton wrightii*, *Vallisneria natans* and *Hydrilla verticillata* are the dominant species, and these species have an average fresh biomass of 526 ± 178 (n = 4), 357 ± 213 (n = 4), 977 ± 460 (n = 4), and 193 ± 156 (n = 4) g m^{-2}, respectively. Dominant algae in Zhaidi River are *Synedra sp.*, *Navicula sp.* and *Pinnularia sp.*, all belonging to *Bacillariophyta*, with an average density of (0.34 ± 0.03) × 10^5 (n = 3) ind. L^{-1}. Epiphytic algae and their density where unfortunately not determined during our study, although they might contribute to the $\delta^{13}C_P$ values of the macrophytes. At the inlet of the Zhaidi River, the chemical composition is dominated by Ca^{2+} and HCO_3^-, with annual mean concentrations of 1.9 mmol L^{-1} and 3.8 mmol L^{-1} respectively (Pei, 2012). The annual discharge at the outlet ranges from 33 to 13000 L s^{-1}.

2.2. Field methods

Temporal variation in water chemistry was assessed at the source of the Zhaidi River and the point upstream from the confluence with the Chaotian River (Fig. 1C). The sampling survey started from 11:00 am on September 10 and lasted to 15:00 pm on September 12, 2014. Water temperature, dissolved oxygen (DO) and pH were monitored and recorded by an oxygen meter (YSI6400, YSI, USA) at 5-min interval at the two locations. The optical DO sensor was calibrated to atmospheric oxygen concentrations before deployment and verified in the laboratory after deployment to be within 3% of 100% saturation. The pH sensor was calibrated at pH 7.00 and pH 4.01 in the laboratory the day before deployment, the drift in pH electrodes after deployment was 0.01 pH unit.

At each site, water samples for stable carbon isotope measurement were collected with a 100 mL disposable sterile syringe at 1-h intervals during the daytime from 5:00 am to 8:00 pm and 3-h intervals overnight. Each water sample was filtered through a Millipore filter of 0.45 μm pore size and preserved in a 50 mL polyethylene bottle without any air-space after injecting three drops of a saturated solution of $HgCl_2$ to prevent microbial alteration. At 6 hourly intervals, water samples were collected at the upstream and downstream site for the analysis of major water elemental components (K^+, Na^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, Cl^-, HCO_3^-, OH^-, and CO_3^{2-}). The water was filtered through a Millipore filter of 0.45 μm pore size and stored in 596 mL plastic bottles without any air-space. The water samples were stored in portable ice boxes until the evening when they were sent back to the laboratory and kept in a refrigerator at 4 °C until analysis.

The submerged plant samples from sites A to F were collected by hand and washed repeatedly with a soft brush to remove adhering material and epiphytic algae from the surface of the leaves. Plant shoots were transferred to Ziplock® polythene bags and stored in portable ice boxes.

2.3. Laboratory analyses

The water samples were analyzed for major cations (K^+, Na^+, Ca^{2+} and Mg^{2+}) with an IRIS Intrepid II XSP (Thermo Scientific, USA). The anions Cl^- and SO_4^{2-} were analyzed with an 861 Advanced Compact Ion Chromatograph (Metrohm, Switzerland). The analytical precision was better than 5% based on duplicate measurements of internal standards. Water pH was measured with SevenMulti pH meter (Mettler Toledo, USA) with a precision of better than 0.01 unit and the concentration of HCO_3^-, OH^- and CO_3^{2-} in a 50 mL sample was titrated within two days of collection by using 0.05 mol L^{-1} HCl that had been standardized against 0.05 mol L^{-1} Na_2CO_3. The error calculated by averaging numerous duplicate samples was ± 0.03 mg L^{-1}. The concentration of free CO_2 was calculated by the geochemical modeling program PHREEQC (Parkhurst and Appelo, 1999).

In the laboratory, all plant samples were removed from the refrigerator, and ultrapure water (Milli-Q, Millipore, Germany) was used to carefully rinse the sample twice. Plants were then dried at 105 °C for 12 h to deactivate enzyme and then dried at 50 °C to a constant weight. Dried samples were ground in an agate mortar and passed through a 100 sieve mesh. A small amount of the powdered