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ORIGINAL PAPER

Using variations in the stable carbon isotope composition of macrophyte remains to quantify nutrient dynamics in lakes

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Abstract The apparent isotope enrichment factor $\varepsilon_{\mathrm{macrophyte}}$ of submerged plants ($\varepsilon_{\mathrm{macrophyte-DIC}} = \delta^{13}\mathrm{C}_{\mathrm{macrophyte}} - \delta^{13}\mathrm{C}_{\mathrm{DIC}}$) is indicative of dissolved inorganic carbon (DIC) supply in neutral to alkaline waters and is related to variations in aquatic productivity (Papadimitriou et al. in Limnol Oceanogr 50:1084–1095, 2005). This paper aims to evaluate the usage of $\varepsilon_{\mathrm{macrophyte}}$ inferred from isotopic analyses of submerged plant fossils in addition to analyses of lake carbonate as a palaeolimnological proxy for former $\mathrm{HCO_3}^-$ concentrations. Stable carbon isotopic analysis of modern *Potamogeton pectinatus* leaves and its host water DIC from the Tibetan Plateau and Central Yakutia (Russia) yielded values between −23.3 and +0.4‰ and between +14.0 and +6.5‰, respectively. Values of $\varepsilon_{Potamogeton\text{–}\mathrm{DIC}}$ (range −15.4 to +1.1‰) from these lakes are significantly correlated with host water $\mathrm{HCO_3}^-$ concentration (range 78–2,200 mg/l) ($r = -0.86$; $P < 0.001$), thus allowing for the development of a transfer function. Palaeo-$\varepsilon_{Potamogeton\text{–}\mathrm{ostracods}}$ values from Luanhaizi Lake on the NE Tibetan Plateau, as inferred from the stable carbon isotope measurement of fossil *Potamogeton pectinatus* seeds (range −24 to +2.8‰) and ostracods (range −7.8 to +7.5%) range between −14.8 and 1.6‰. Phases of assumed disequilibrium between $\delta^{13}\mathrm{C}_{\mathrm{DIC}}$ and $\delta^{13}\mathrm{C}_{\mathrm{ostracods}}$ known to occur in charophyte swards (as indicated by the deposition of charophyte fossils) were excluded from the analysis of palaeo-ε. The application of the $\varepsilon_{Potamogeton\text{–}\mathrm{DIC}}$-$\mathrm{HCO_3}^-$ transfer function yielded a median palaeo-$\mathrm{HCO_3}^-$-concentration of 290 mg/l. Variations in the dissolved organic carbon supply compare well with aquatic plant productivity changes and lake level variability as inferred from a multiproxy study of the same record including analyses of plant macrofossils, ostracods, carbonate and organic content.

Keywords Submerged aquatic plants · Macrophyte macrofossils · Stable carbon isotopes ·

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Tibetan Plateau · Hydrogen carbonate uptake · Lake carbon cycle · Productivity

Introduction

Proxies for lake productivity are crucial to the understanding of past ecosystem development. Information about palaeo-productivity is often inferred from stable carbon isotope analyses of bulk organic samples assuming that the organic matter in lake sediments is predominantly of aquatic origin (Lücke et al. 2003; Parplies et al. 2008; Wu et al. 2008). In contrast to terrestrial plants, aquatic plants such as algae and submerged macrophytes are often capable of utilising HCO_3^- for photosynthesis at times of $CO_{2(aq)}$ shortage (Raven 1970) resulting in the enrichment of ^{13}C in their organic matter. The theory for numerically linking the $\delta^{13}C$ signal of lake organic matter ($\delta^{13}C_{organic}$) to the dissolved inorganic carbon (DIC) supply was first suggested several years ago (Oana and Deevey 1960; Hollander and McKenzie 1991). Several experiments and field studies have been undertaken to establish a proxy that will enable the derivation of information about former carbon availability from marine and lacustrine sediments (Freeman and Hayes 1992; Burkhardt et al. 1999; Bade et al. 2006). Whilst these studies enabled us to form an understanding of the basic information about the mechanisms of carbon fractionation in phytoplankton, no reliable transfer function has yet been developed. In addition to other factors that hinder the establishment of $\delta^{13}C_{organic}$ as a reliable proxy, one primary limitation is that sedimentary organic matter is derived from a mixture of organisms that differ in their capabilities to utilise bicarbonate for photosynthesis. Brenner et al. (1999) and Bade et al. (2006) advise palaeolimnologists to refrain from using a transfer function that directly relates changes in bulk organic sediment ^{13}C to changes in productivity or palaeo-CO_2 levels.

To overcome this problem, isotopic analyses can be performed on separate identified fossil leaves and seeds of submerged macrophytes that are preserved in coastal and lacustrine sediments. In comparison to land plants, the interpretation of stable carbon isotope values of submerged aquatic plants ($\delta^{13}C_{macrophyte}$) is complex due to the influence of a variety of factors. The relationship between environmental conditions and $\delta^{13}C_{macrophyte}$ has scarcely been investigated to date, and no study has yet focused on the application of stable carbon isotope compositions of organic macrophyte fossils for palaeoecological reconstructions (but see Street-Perrott et al. 2004).

Three main mechanisms might considerably influence the $\delta^{13}C$ values of submerged aquatic plants: (1) the switch from $CO_{2(aq)}$ to HCO_3^- uptake for photosynthesis during periods of $CO_{2(aq)}$ limitation; (2) variation in ^{13}C in the DIC source within the lake; and (3) the occurrence and magnitude of the plant's isotopic fractionation as a function of lake water inorganic carbon supply.

Many submerged plant species such as *Elodea canadensis*, several *Potamogeton* species, *Zannichellia palustris*, *Ceratophyllum demersum*, *Ranunculus aquatilis* and *Myriophyllum spicatum* have the ability to utilise HCO_3^- as an additional carbon source for photosynthesis (Sand-Jensen 1983). The physiological mechanism of HCO_3^- uptake of submerged macrophytes has been investigated in many studies (Steemann Nielsen 1947; Lucas 1983; Maberly and Spence 1983; Elzenga and Prins 1988; Prins and Elezenga 1989; Maberly and Madsen, 1998). Across the range of temperatures commonly encountered by aquatic plants, the $\delta^{13}CHCO_3^-$ will be 7–12‰ enriched compared to $\delta^{13}C_{CO_2(aq)}$ (Mook et al. 1974; Romanek et al. 1992; Zhang et al. 1995). Therefore, the proportion of carbon assimilation arising from the active uptake of HCO_3^- will affect $\delta^{13}C_{macrophyte}$. Accordingly, Keeley and Sandquist (1992) reported that aquatic plants exhibit a wide range of $\delta^{13}C$ values from −33 to −11‰. The proportion of the two carbon species that is assimilated is dependent upon species-specific differences the in the capacity for active transport of the HCO_3^- ion and on the proportion of CO_2 to HCO_3^- in the boundary layer of the leaf (Keeley and Sandquist 1992). The ratio of CO_2 to HCO_3^- in the water is largely a function of ambient pH (Stumm and Morgan 1970). For comparison: at pH 5.5, 80% of the inorganic carbon occurs as $CO_{2\ (aq)}$, whereas at pH 8.5, $CO_{2(aq)}$ accounts for <1% and at pH 10, HCO_3^- accounts for <50%, while most of the inorganic carbon occurs in form of CO_3^{2-}, which is not available for uptake by plants.

The species-specific relationship of photosynthesis rates and dissolved inorganic carbon source is traditionally tested in pH-drift experiments in laboratory cultures. The photosynthesis-pH curve of *Potamogeton pectinatus* for instance indicates that this species can use HCO_3^- very effectively, but that it still has a higher affinity for $CO_{2(aq)}$ (Sand-Jensen 1983). Stable carbon isotope ratios of −25.0 and −25.7‰ for *Potamogeton pectinatus* collected from flowing water of pH 7.0 and 7.5 in Finland (Osmond et al. 1981) confirm that the plants mostly utilised CO_2 for photosynthesis at neutral pH and high $CO_{2(aq)}$ levels. However in shallow productive lakes, pH usually rises above 8 (Pentecost et al. 2006) and $CO_{2(aq)}$ concentration approaches zero (Stumm and Morgan 1970). Under such conditions, *Potamogeton pectinatus* relies solely on HCO_3^- uptake as inferred from pH-drift experiments under comparable conditions (Sand-Jensen 1983).

The stable carbon isotope signatures of submerged plants are, to a certain extent, influenced by the isotopic composition of the carbon source (Papadimitriou et al. 2005). Accordingly, the $\delta^{13}C$ signals of *Potamogeton pectinatus*, when growing in neutral to alkaline lakes, are a function of lake-water $\delta^{13}C_{DIC}$. In contrast to modern studies, direct measurements of the stable carbon isotope composition of DIC are not possible for palaeolimnological studies of lake sediments, and analyses of various carbonate materials such as authigenic carbonates, ostracods, or molluscs can be taken as proxies for former $\delta^{13}C_{DIC}$. Regardless of the carbonate component which is used in palaeolimnological studies, the detailed interpretation of stable carbon isotope records from lakes is still complicated due to the variety of factors that affect the carbon isotopic composition of lake waters (Leng 2004). Furthermore, a detailed carbonate-isotope study by Pentecost et al. (2006) revealed that pH in dense charophyte swards can increase to ~11, allowing atmospheric CO_2 to directly combine with the OH^- ions of water which results in strong isotopic disequilibrium between charophyte carbonate and HCO_3^-. Whether or not, under such conditions, other biogenic carbonates such as mollusc and ostracod shells are also affected by isotopic disequilibrium was not investigated in this study, although it would be reasonable to assume so. In this instance, the reliability of $\delta^{13}C_{ostracods/molluscs}$ as a proxy of former $\delta^{13}C_{DIC}$ is reduced and in palaeolimnological studies, such disequilibrium phases between $\delta^{13}C_{DIC}$ and $\delta^{13}C_{ostracods/molluscs}$, typically occurring in dense calcified charophyte mats, have to be identified and excluded prior to relating $\delta^{13}C_{ostracods/molluscs}$ to $\delta^{13}C_{macrophyte}$. These complications could be one reason why stable carbon isotopic records of organic matter and carbonate have rarely been related to each other. However, it can be concluded that when $\delta^{13}C$ determined from aquatic macrophytes fossils from shallow lake sediments co-varies with $\delta^{13}C_{carbonate}$, it probably reflects former changes in $\delta^{13}C_{DIC}$.

In previous palaeolimnological studies, little attention has been paid to the constraints of $\delta^{13}C$ values of organic matter to former HCO_3^- availability. This is presumably due to the fact that inorganic carbon has been considered an inexhaustible resource because of continuous supply from the atmosphere (Vadstrup and Madsen 1995). However, the short-term exchange of CO_2 across the air–water interface is slow, and may be inadequate to keep pace with the photosynthetic removal of $CO_{2(aq)}$ and HCO_3^- by aquatic photoautotrophic organisms (Vadstrup and Madsen 1995). Thus, in very productive lakes and in lakes of high pH, HCO_3^- concentrations drop below the level needed to saturate photosynthesis (Maberly, 1996). According to conceptual models and empirical studies, the isotopic fractionation between inorganic carbon and organic carbon in biological tissue is smaller when carbon demand exceeds supply, and the isotopic composition of the biomass may approach the value of DIC at very low HCO_3^- concentrations (Fogel and Cifuentes 1993).

The occurrence and magnitude of carbon isotope fractionation related to biological carbon supply can be explored by calculating the apparent isotope enrichment factor of the submerged plant relative to external DIC ($\varepsilon_{macrophyte–DIC}$). Details and equations for the underlying conceptual model are given in Burkhardt et al. (1999) and Papadimitriou et al. (2005, 2006).

When solely utilizing HCO_3^-, values for $\varepsilon_{macrophyte–DIC}$ range between $\varepsilon_{CO_2(aq)-HCO_3^-}$ (=temperature-dependent equilibrium isotopic difference between $CO_{2(aq)}$ and HCO_3^-: −12 to −9‰ in the temperature range of 0 and 25°C) and zero (=maximum ^{13}C in enrichment in new biomass occurring during periods of fast growth rate, when high carbon demand relative to its supply can hinder isotopic fractionation during assimilation). Papadimitriou et al. (2005, 2006) showed that the variability of $\varepsilon_{Zostera–DIC}$ reflects the imbalance between inorganic carbon supply and plant demand caused by spatially and seasonally varying productivity. Their studies represent, to a certain extent, the ground-truthing for the usage of palaeo-ε as a proxy of former carbon supply influenced by productivity changes.

This paper has three objectives: Firstly we determine whether modern $\varepsilon_{Potamogeton\text{–}\mathrm{DIC}}$ are significantly related to DIC supply. Secondly, we apply the inferred relationship to palaeo-$e_{Potamogeton\text{–}\mathrm{ostracod/molluscs}}$ from a small Tibetan Lake in order to reconstruct former DIC concentration quantitatively and thirdly we verify this information with macrophyte community reconstruction as inferred from the plant macrofossil record. *Potamogeton pectinatus* (*Stuckenia pectinata* [L.] Börner) was selected as the reference species for stable carbon isotope analysis as it is a common submerged aquatic plant native to all continents except Antarctica (Van Wijk 1988). It often forms dense mats in shallow neutral to alkaline water bodies. Compared with other aquatic plant species, seeds of *Potamogeton pectinatus* are often preserved in the plant macrofossil records of lakes.

The Tibetan Plateau and Central Yakutia have been selected as study areas for two reasons. Firstly, they are rich in shallow lakes of variable chemical and stable carbon isotopic composition and have only been minimally influenced by anthropogenic activity. Secondly, the submerged macrophyte diversity is low or often monospecific in most lakes, which makes it easier to identify the causes of variations in $\delta^{13}C_{Potamogeton}$.

Luanhaizi Lake is located on the NE Tibetan Plateau (37.59°N; 101.35°E; 3200 m a.s.l.; Fig. 1). This freshwater lake (area $\sim$1.5 km^2) has a present-day maximum depth of $\sim$0.4 m; it is fed by a number of small streams (catchment area $\sim$33 km^2). The lake is situated in the eastern–central part of the Qilian Mountains (NE margin of the Tibetan Plateau) which are characterised by a semi-humid climate (mean annual precipitation $\sim$500 mm, mean annual temperature -1°C). The majority of the results from a multi-proxy study of the sediment core have already been published elsewhere (Herzschuh et al. 2005, 2006; Mischke et al. 2005).

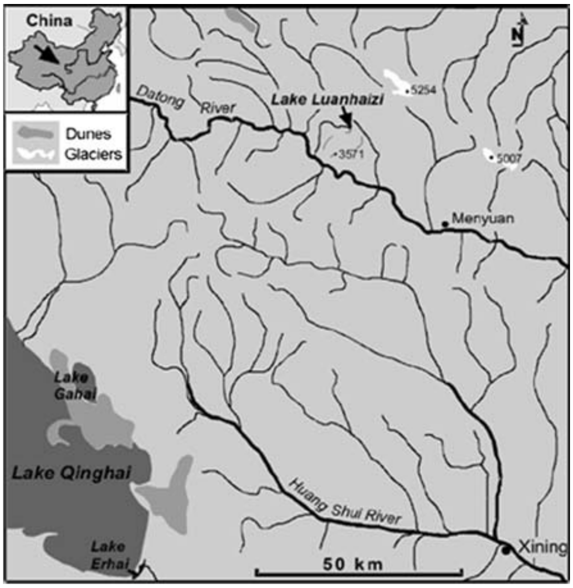

Fig. 1 Location of Luanhaizi Lake on the north-eastern Tibetan Plateau (China)

Materials and methods

Recent plant material was collected from shallow lakes (median lake depth: 0.5 m) on the central and northeastern Tibetan Plateau and in Central Yakutia, Russia (ESM 1). The majority of lakes are closed systems fed by surface run-off. Individual specimens of *Potamogeton pectinatus*, preferentially those bearing fruits, were collected at monospecific sites. Plant material was dried using tissue paper. Modern submerged vegetation coverage was qualitatively investigated at all plant sample points and the *Potamogeton* coverage was estimated on a three part scale (loose <50%, dense 50–90, very dense >90%) at 8 sites. Water samples were collected for chemical analysis at 0.3 m below water surface immediately before the plant samples were obtained. Electrical conductivity (EC), pH, dissolved oxygen content and water temperatures were measured using a portable field instrument (WTW Multi 340i) at 0.3 m water depth. Alkalinity was measured in the field using the Alkalinity AL 7 titration test kit of Macherey–Nagel. HCO_3^- concentration was calculated using pH and alkalinity values $(HCO_3^-) = (2 \times \text{alkalinity-}10^{-14+\mathrm{pH}})/(1 + 2 \times K_2^{10 \times \mathrm{pH}})$ and was corrected for ionic strength. Further information on the methods used for water analysis is provided in Mischke et al. (2007).

A core of 13.94 m in length was retrieved from the central part of Luanhaizi Lake The sediments were dated by means of ^{14}C AMS and U/Th dating. Some general information on the recovered sediments is provided in Fig. 2. Abundances of carbonate-, organic- and plant fossil-rich sediments are indicative of lake phases. Core sections, which are dominated by gravelly sands and silts, indicate a rapid accumulation of flash-flood or river sediments lacking fossils. As the data presented here will not be evaluated for its chronostratigraphic significance, a generic age scale is shown in Fig. 2. Details concerning the age-depth model are described in Mischke et al. (2005). For plant macrofossil analysis, approximately 50 g of dry sediment was prepared and investigated according to Birks (2001). Detailed information on macrofossil sample preparation and a complete plant macrofossil diagram of the core are presented in Herzschuh et al. (2005).

To avoid the problem of carbonate contamination for isotopic measurement, recent and fossil plant material was treated with HCl (2%) for 3 h. The modern leave material was already very clean before treatment and HCl yielded no reactions in most cases. The visible reaction of HCl with carbonate at *Potamogeton* seeds (bubbling) ended mostly after few seconds. Sample material was then rinsed several times with H_2O. Analyses of $\delta^{13}C$ were performed using a Finnigan Mat Delta-S isotope ratio mass spectrometer. The sample is combusted at 950°C under O_2 supply and the organic carbon quantitatively transferred to CO_2, which enters the mass spectrometer through an elemental analyser and CONFLO III gas mixing system.

We will use stable carbon isotope values of ostracods and mollusc as a proxy on former DIC. Molluscs foreseen for carbon stable isotope determination were picked from the plant macrofossil samples. Information regarding the preparation of ostracod samples and the complete ostracod record are reported in Mischke et al. (2005). DIC was precipitated using $BaCl_2$ directly after sampling in the field. Its stable carbon isotope composition was analysed using a Finnigan Delta Plus XL at GeoForschungsZentrum Potsdam. Mollusc shells (genus *Gyraulus*) and ostracod valves were reacted with

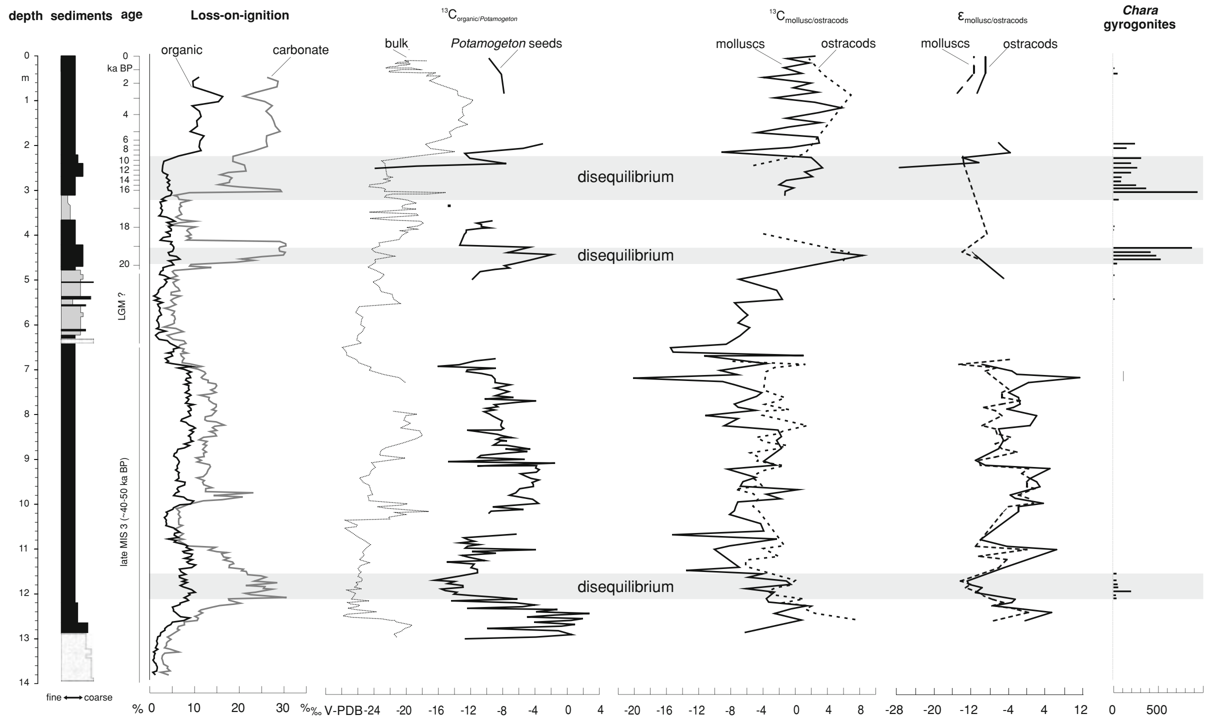

Fig. 2 Results of stable carbon isotope measurements on *Potamogeton pectinatus* ($\delta^{13}C_{Potamogeton}$) and mollusc and ostracod shells ($\delta^{13}C_{ostracods/molluscs}$) from Luanhaizi Lake profile. Information on age, sediment characteristics, organic and carbonate content and $\delta^{13}C$ of bulk organic are also given (from Herzschuh et al. 2005; age-depth-profile from Herzschuh et al. 2006). Phases of probably disequilibrium between $\delta^{13}C_{DIC}$ and $\delta^{13}C_{ostracods}/\delta^{13}C_{molluscs}$, indicated by abundant *Chara* gyrogonites (for further identification criteria see text), were *shaded* in *grey*

100% phosphoric acid at +75°C in a Kiel III (Kiel IV at Geoforschungszentrum) online carbonate preparation line connected to a ThermoFinnigan 252 mass spectrometer at the Institute of Geology and Mineralogy, University of Erlangen (molluscs) and Geoforschungszentrum Potsdam (ostracods). All carbon isotope ratios were determined relative to laboratory standards of known isotopic composition. All values are reported in ‰ relative to V-PDB by assigning a δ^{13}C value of +1.95‰ to NBS19. Reproducibility was checked by replicate analysis of a laboratory standard (IAEA NBS19) and is better than 0.05‰ (1σ) for δ^{13}C.

Results

Stable carbon isotope analyses of modern *Potamogeton* material, DIC and ostracods

Stable carbon isotope measurements on modern *Potamogeton pectinatus* leaves ($\delta^{13}C_{Potamogeton}$) from shallow Tibetan and Yakutian lakes yielded values ranging between −23.3 and +0.4% ($n = 68$; median$_{\text{all}}$: −12.1%; median$_{\text{Tibet}}$; −12.67% median$_{\text{Yakutian}}$: −9.76‰ ESM 1). The difference between the stable carbon isotope values of leaves and seeds, determined from 11 single modern *Potamogeton pectinatus* plants was not significant (median difference between leaves and seeds: 0.3‰; r^2: 0.97).

The host waters of sampled *Potamogeton pectinatus* plants cover a wide range of the key chemical parameters such as pH, alkalinity, and conductivity (ESM 1). Host water $\delta^{13}C_{\text{DIC}}$ measurements, available from 23 sites, range between −14.0‰ and +6.5‰ ($n = 23$), while $\delta^{13}C_{\text{ostracods}}$ values were available only from 7 sites. Compared to the large total range, the isotopic differences between both carbonates were relatively small. For samples from two lakes (CTP-13; CTP-16) with the most depleted $\delta^{13}C_{\text{DIC}}$, high pH ($\sim 10$), and some calcified *Chara* in the submerged vegetation the difference between $\delta^{13}C_{\text{DIC}}$ and $\delta^{13}C_{\text{ostracods}}$ is as large as −6.0 and −13.0%, respectively, indicating disequilibrium during shell calcification. Fractionation processes in these two lakes are considered to be atypical and were therefore excluded from further analysis. $\delta^{13}C_{Potamogeton}$ of all other lakes shows moderate correlation with $\delta^{13}C_{\text{DIC}}$ ($r = 0.59$; $r^2 = 0.35$).

The apparent isotope enrichment factor $\varepsilon_{Potamogeton-\text{DIC}}$, the difference between $\delta^{13}C_{Potamogeton}$ and $\delta^{13}C_{\text{DIC}}$, ranges between −15.4 and +1.1%. $\varepsilon_{Potamogeton-\text{DIC}}$ is negatively correlated to the HCO_3^- concentration of the host water (range 78–2200 mg/l; Fig. 3). Regression analysis yielded an r^2 value of 0.74 ($P < 0.00001$) after the identification (2σ) and exclusion of one outlier (Yak-18). However the reliability of the transfer function is reduced at the upper and lower ends of the ε_{DIC} range. *Potamogeton pectinatus* leaf tissue from Tibetan Lakes, which are largely very shallow and alkaline, was more enriched in ^{13}C relative to DIC (median ε_{DIC}: −4.3%) than that from deeper and less alkaline Yakutian Lakes (median ε_{DIC}: −11.0%).

As information regarding plant coverage is available for only eight sites, we cannot statistically determine whether HCO_3^- concentration, *Potamogeton pectinatus* coverage and $\varepsilon_{Potamogeton-\text{DIC}}$ are significantly related to each other. A slight trend of decreasing HCO_3^- concentrations with increasing *Potamogeton* coverage may be inferred Fig. 4.

Stable carbon isotope analyses of fossil material from Luanhaizi Lake

Stable carbon isotope measurements of fossil *Potamogeton pectinatus* seeds from the Luanhaizi Lake profile range between −24.0 and +2.8% ($n = 158$, median: −8.9%). The values vary strongly between

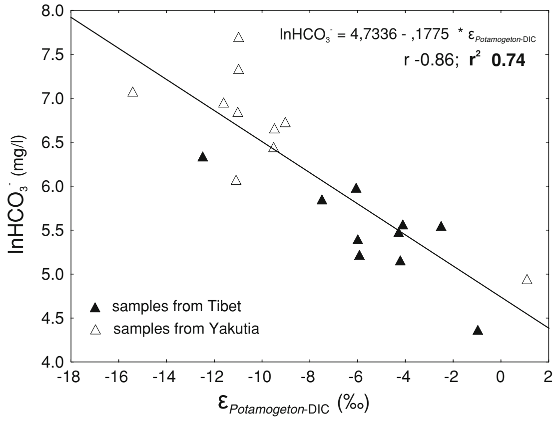

Fig. 3 Plot between $\varepsilon_{Potamogeton-\text{DIC}}$ (as inferred from analysis of modern leaves of *Potamogeton pectinatus* and host-water DIC) and host-water $\ln HCO_3^-$ from shallow lakes on the eastern Tibetan Plateau and Central Yakutia. Correlation coefficient (r), coefficient of determination (r^2) and inferred transfer function were indicated as well

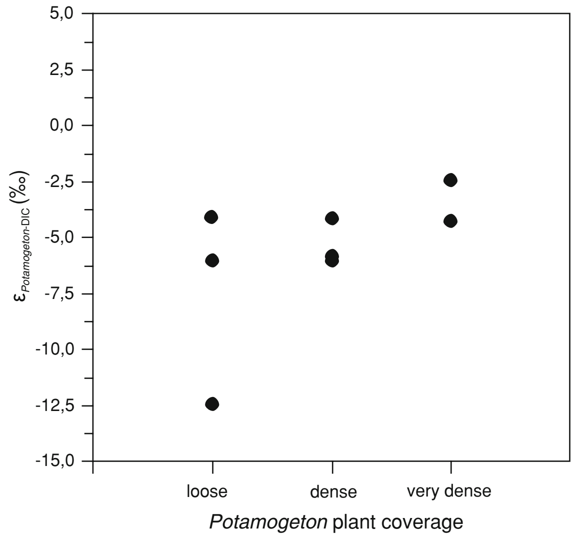

Fig. 4 Plot showing the relationship between modern $\varepsilon_{Potamogeton}$–DIC and *Potamogeton* coverage from 6 monospecific lakes on the eastern Tibetan Plateau

the different sections of the core but are quite similar within one single section.

$\delta^{13}C_{\mathrm{ostracods/molluscs}}$ was taken as a proxy of former $\delta^{13}C_{\mathrm{DIC}}$ to infer palaeo-$\varepsilon_{\mathrm{Potamogeton–ostracods/molluscs}}$. Stable carbon isotope values from ostracod valves and fossil mollusc shells (*Gyraulus* sp.) from the same core range between −7.8 and +7.5% and between −20.2 and +8.4%, respectively, indicating co-variation throughout the core (Fig. 2).

Prior to the calculation of palaeo-$\varepsilon_{\mathrm{Potamogeton–ostracods/molluscs}}$, we assessed the Luanhaizi Lake record for phases of disequilibrium (Pentecost et al. 2006) between former $\delta^{13}C_{\mathrm{DIC}}$ and $\delta^{13}C_{\mathrm{ostracods/molluscs}}$ by applying the following criteria: (1) abundant charophytic remains (gyrogonites, stem encrustations) in the macrofossil record (Herzschuh et al. 2005; Fig. 2); (2) increased carbonate content compared with the organic content (Mischke et al. 2005, Fig. 2), and (3) strongly depleted $\delta^{13}C_{Potamogeton}$ (Fig. 2). The core sections 12.10–11.50 m, 4.60–4.30 m and 3.20–1.95 m show these characteristics and were therefore excluded from the calculation of palaeo-$\varepsilon_{\mathrm{Potamogeton–ostracods/molluscs}}$.

Outside the sections of probable disequilibrium, values of $\delta^{13}C_{Potamogeton}$ from the Luanhaizi Lake core are weakly correlated with $\delta^{13}C_{\mathrm{ostracods}}$ ($r = 0.33$). Palaeo-$\varepsilon_{\mathrm{Potamogeton–ostracods}}$ outside these sections ranges between −14.7 and 1.6% and palaeo-$\varepsilon_{\mathrm{Potamogeton–molluscs}}$ ranges between −10.9 and +6.2%. Both curves show similar trends (Fig. 2). The $\varepsilon_{Potamogeton\,\mathrm{–DIC}}$-lnHCO$_3^-$ transfer function inferred from the modern data set was applied to palaeo-$\varepsilon_{Potamogeton\mathrm{–ostracods}}$ values in order to quantify palaeo-HCO$_3^-$ concentrations (Fig. 5). Reconstructed lnHCO$_3^-$ concentration shows considerable variation throughout the core (lnHCO$_3^-$ range 4.45–7.36 mg/l, median: 5.68 mg/l). Sections with low (10.0–9.1 m), intermediate (12.6–12.1; 8.9–8.3; 7.8–7.4 m) and high HCO$_3^-$ concentrations (11.5–10.9; 7.1–6.8; 9.1–8.9; 8.3–7.8; 4.3–3.9; 0.9–0 m) alternate throughout the core.

Discussion

Modern $\delta^{13}C_{\mathrm{Potamogeton}}$ and inferred $\varepsilon_{\mathrm{Potamogeton–DIC}}$

Measurements of $\delta^{13}C_{Potamogeton}$ yielded an extremely wide range for modern plant leaves (−23.3 to +0.4%). A comparable $\delta^{13}C$ range has never been reported for any other plant species. Morrill et al. (2006) used *Potamogeton* seeds (probably *Potamogeton pectinatus*) from a shallow lake on the Tibetan Plateau for radiocarbon dating and provided $\delta^{13}C$ values of *Potamogeton* but without any discussion of the $\delta^{13}C_{Potamogeton}$ results. Although the $\delta^{13}C$ range of fossil *Potamogeton* in this lake is narrower (from −18.0 to −5.0%; $n = 50$), these data generally confirm the wide range of our observed $\delta^{13}C_{Potamogeton}$ values.

We initially checked whether a consistent offset existed between the $\delta^{13}C$ values of leaves and seeds from modern plants. We expected seeds to be more depleted than leaves as more synthesis steps are involved in leaf formation, which could increase the total fractionation. However when compared with the wide data range for $\delta^{13}C_{Potamogeton}$, the observed differences between the $\delta^{13}C_{Potamogeton}$ values of leaves and seeds are negligible. Modern $\delta^{13}C_{Potamogeton}$ measurements of leaves can therefore be directly compared with values obtained from fossil seeds.

The studied lakes on the Tibetan Plateau and in Central Yakutia had pH values between 8 and 11 (median$_{\mathrm{Tibet}}$: 9.8 median$_{\mathrm{Yakutia}}$: 8.6; ESM 1). In such lakes, HCO$_3^-$ is the only carbon which is available for uptake by plants (Zhang et al. 1995). When compared with the values of *Potamogeton pectinatus* growing in neutral flowing water in Finland (Osmond

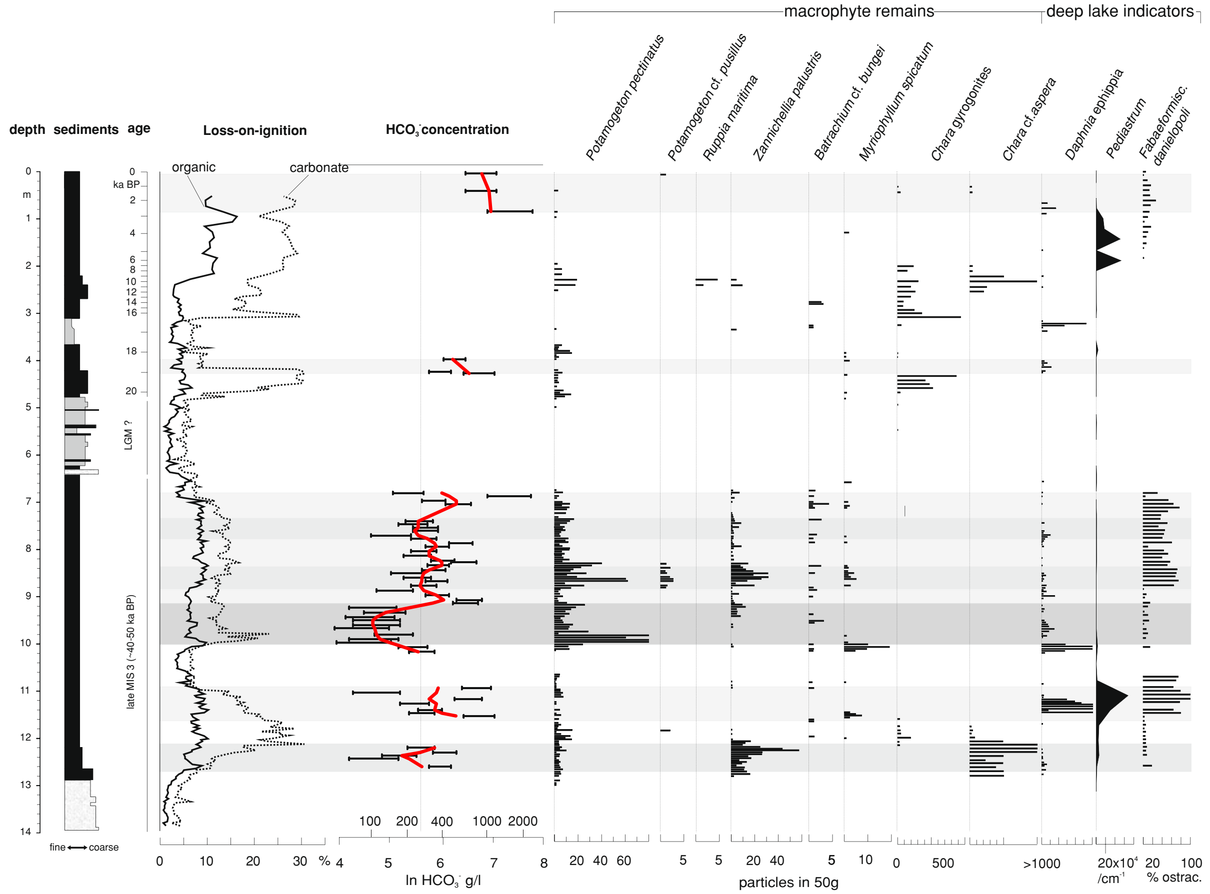

Fig. 5 Reconstructed $\ln HCO_3^-$ for Lake Luanhaizi (NE Tibetan Plateau) as inferred from the application of the $\varepsilon_{Potamogeton\text{–}DIC}$-$\ln HCO_3^-$ transfer function to palaeo-$\varepsilon_{Potamogeton}$–ostracods. Phases of low, intermediate, and high HCO_3^- concentration are *shaded* in *dark grey*, *grey*, and *light gray*, respectively. For comparison, information on lake development concerning macrophyte composition and productivity (as indicated by the fossil macrophyte record after Herzschuh et al. 2005) lake depth and stability (as indicated by the ostracod *Fabaeformiscandona danielopoli* and by the carbonate content after from Mischke et al. 2005), and algae productivity (indicated by *Pediastrum* concentration after Herzschuh et al. 2005)

et al. 1981), our modern $\delta^{13}C_{Potamogeton}$ are enriched in ^{13}C.

In alkaline lakes, $\delta^{13}C_{macrophyte}$ is assumed to partly reflect the $\delta^{13}C_{DIC}$ and partly reflect the kinetics of assimilation of carbon isotopes (Hemminga and Mateo 1996; Papadimitriou et al. 2005). Approximately 35% of the site-to-site variability in $\delta^{13}C_{Potamogeton}$ from Tibetan and Yakutian Lakes can be explained by its modest but significant correlation with $\delta^{13}C_{DIC}$. A similar relationship was observed for intra- and inter-annual variations of $\delta^{13}C_{Zostera}$ and $\delta^{13}C_{DIC}$ in *Z. marina* and *Z. noltii* meadows at the Danish and Welsh coasts (Papadimitriou et al. 2005, 2006). Our data set, composed of information from various small closed lake systems has a much wider range in $\delta^{13}C_{DIC}$ and $\delta^{13}C_{macrophyte}$ than the samples from different coastal habitats. This is due to the nature of the macrophyte's host waters being more homogeneous in coastal areas but highly variable in each closed lake system due to variation in hydrological conditions (Leng and Marshall 2004).

The specific kinetics of the assimilation of carbon by aquatic plants yields that fractionation during phases of strong growth rates and limited carbon supply is suppressed, leading to isotopically enriched biomass compared with the biomass produced during phases of carbon excess (Burkhardt et al. 1999). The calculated apparent isotope enrichment factors $\varepsilon_{Potamogeton\text{–}DIC}$ of Tibetan and Yakutian plants indicates the occurrence and magnitude of the growth related biological carbon isotope effect. The observed range of $\varepsilon_{Potamogeton\text{–}DIC}$ covers the entire possible range of $-12 < \varepsilon_{Potamogeton\text{–}DIC} < 0$. It is larger than the $\varepsilon_{Zostera\text{–}DIC}$ range from *Zostera marina* meadows on the Danish coast (Papadimitriou et al. 2005). This indicates that we sampled both habitats with optimal growing conditions for *Potamogeton pectinatus* in terms of salinity, depth and temperature and also sites at the tolerance boundaries where productivity is reduced. The studies of Papadimitriou et al. (2005, 2006) have shown that seasonal variability of isotopic fractionation of macrophytes is a function of productivity-driven carbon supply. Our inferred $\varepsilon_{Potamogeton\text{–}DIC}$ are also significantly related to $\ln HCO_3^-$ concentration ($r - 0.86$ r^2 0.74; $P < 0.00001$). However, several factors may reduce the reliability of the proposed transfer function:

1. The residuals between the observed values and those estimated by the transfer function increase considerably at $\varepsilon_{Potamogeton\text{–}DIC}$ values below −10% (=$\ln HCO_3^-$ 6.51 mg/l). This is reasonable as such a strong depletion in ^{13}C of the biomass relative to the DIC probably indicates that plants at these sites have not solely relied on HCO_3^- as a carbon source but that some isotopically light CO_2 was assimilated as well.
2. In our study, stable carbon isotope measurements performed on *Potamogeton pectinatus* leaves were related to the $\delta^{13}C_{DIC}$ of the host water. The time windows for lake water sampling (to infer HCO_3^- concentration) and leaf formation differ strongly: DIC uptake during leaf formation is an integration of values over time during the growing period whilst HCO_3^- concentration in sampled host waters is just a snapshot of the conditions at that particular moment.
3. Information concerning *Potamogeton pectinatus* coverage for our modern sample set was incomplete and lacked data on *Potamogeton pectinatus* productivity. Hence, the relationship between productivity and HCO_3^- concentration is not established in our data set. This would represent an important piece of evidence when using $\varepsilon_{Potamogeton\text{–}DIC}$ as a proxy of former productivity. However, it has been proven by many studies that HCO_3^- concentration decreases with increased macrophyte productivity (Van den Berg et al. 2002).
4. The number of lakes sampled for DIC and hence the number of data points available for setting up the transfer function was comparatively low ($n = 20$).
5. Modern $\varepsilon_{Potamogeton\text{–}DIC}$ values originate from two different regions, the Tibetan Plateau and Central Yakutia. These regions differ markedly in heights a.s.l. by about 4000 m (Table S1. Electronic Supplementary Material) meaning large differences in atmospheric CO_2 partial pressures (pCO_2) which could have an effect on isotopic fractionation during plant uptake. Benthien et al. (2007) found in a laboratory study that even the isotopic signals of marine algae, which were assumed to rely on $CO_2(aq)$ rather than on HCO_3^- such as *Potamogeton pectinatus*, were negligibly effected by artificial variations of atmospheric pCO_2.
6. The plasticity of *Potamogeton pectinatus* has to be taken into account when applying the proposed transfer function to fossil plant material from further regions (Van Wijk et al. 1988; Pilon et al. 2003) although we found no obvious regional difference in the ability of plants to assimilate inorganic carbon.

Nevertheless, the significance of the relationship between $\ln HCO_3^-$ and $\varepsilon_{Potamogeton\text{–}DIC}$ and the consistency between the results and the conceptual model justifies the application of the inferred transfer function to palaeo-$\varepsilon_{Potamogeton\text{-ostracods/molluscs}}$.

Changes in palaeo-ε, HCO_3^- and productivity at Luanhaizi Lake

The reliability of the $\varepsilon_{Potamogeton\text{–DIC}}$-$\ln HCO_3^-$ transfer function was applied to the stable carbon isotope record from Luanhaizi Lake. As with modern leaves, measurements of $\delta^{13}C_{Potamogeton}$ for fossil seeds yielded an extremely wide range (−24.0 to +2.8%). The curves for $\delta^{13}C_{Potamogeton}$ and $\delta^{13}C_{organic}$ (from Herzschuh et al. 2005) show no co-variation and their absolute ranges show only slight overlap (Fig. 2). Bulk organic matter at Luanhaizi Lake has various origins (terrestrial plants, algae, submerged plants) characterised by different stable carbon isotopic fingerprints (Herzschuh et al. 2005).

Calculation of palaeo-$\varepsilon_{\text{ostracods/molluscs-}Potamogeton}$ and the application of the $\varepsilon_{Potamogeton\text{-DIC}}$-$\ln HCO_3^-$

transfer function yielded reasonable results. The information obtained can be compared to a variety of environmental variables providing a comprehensive environmental history. These variables include aquatic plant macrofossils, non-pollen palynomorphs, $\delta^{13}C_{organic}$, C/N ratios, *n*-alkane distribution (Herzschuh et al. 2005), ostracods, element concentrations, (Mischke et al. 2005) and terrestrial pollen (Herzschuh et al. 2006).

Phases of strongly enriched $\delta^{13}C_{Potamogeton}$ relative to $\delta^{13}C_{ostracods/molluscs}$ (high palaeo-ε, Fig. 2) and reconstructed low $\ln HCO_3^-$ concentration (Fig. 5) are largely related to sections of high macrophyte fossil abundance in the sediment (taken from Herzschuh et al. 2005). Submerged plant macrofossils are considered to represent a reliable proxy of local macrophyte colonization and productivity (Birks 1973; Zhao et al. 2006).

Intermediate $\ln HCO_3^-$ concentrations were recorded between 12.6 and 12.1 m. Beside *Potamogeton pectinatus* seeds, the most frequent macrofossils in that part of the core are fruits of *Zannichellia palustris* and oospores of *Chara* cf. *aspera* (Fig. 5). Hence, dense macrophyte meadows probably led to the limitation of HCO_3^- in the water. A modern analogue for the former situation in Luanhaizi Lake may be the recent vegetation conditions in Veluwemeer (Netherlands), which is a shallow alkaline lake dominated by *Potamogeton pectinatus* and *Chara aspera* (Van den Berg et al. 2002). Detailed investigations of the relationship between HCO_3^- availability and vegetation composition in Veluwemeer showed that very low HCO_3^- concentrations were observed in areas dominated by *Chara*, while the areas dominated by *Potamogeton* had higher HCO_3^- concentrations. In mixed stands, the growth rates of both species suffer from low HCO_3^- concentrations. *Chara aspera* seems to exploit the HCO_3^- reservoir more efficiently under conditions of low HCO_3^- supply than *Potamogeton pectinatus*. It is probable that the considerable variations of reconstructed $\ln HCO_3^-$ in the core section 12.6–12.1 m from Luanhaizi Lake can be attributed to local changes in the relative abundances of the single macrophyte species. In contrast to the observations from Veluwemeer, the lowest $\ln HCO_3^-$ concentrations of the whole Luanhaizi Lake core were recorded during a phase of almost monospecific dense *Potamogeton pectinatus* mats as inferred from the macrofossil record between 10.0 and 9.1 m.

Phases of low palaeo-ε/high $\ln HCO_3^-$ occur mostly during phases of deep stable lake conditions as indicated by the ostracod species *Fabaeformiscandona danielopoli* (Fig. 5; the complete ostracod record is given in Mischke et al. 2005) and/or high planktonic productivity as indicated by *Pediastrum* and *Daphnia* ephippia abundances (Herzschuh et al. 2005).

To conclude, absolute values of reconstructed HCO_3^- concentrations are confirmed by modern analogues. Variations can reasonably be related to changing conditions within the lake as suggested by other proxies. However, several factors may reduce the reliability of palaeo-ε reconstruction.

Conclusions

The modern and fossil data support our hypothesis that the combined interpretation of stable carbon isotope data from submerged macrophyte fossils and from lake carbonates could in general provide information about former lake DIC concentrations and about former lake productivity. Our investigations of modern *Potamogeton pectinatus* from Tibetan Plateau in relation to lakewater environmental variables demonstrate that $\delta^{13}C_{Potamogeton}$ is mainly influenced by the ability of the species to utilise HCO_3^- uptake for photosynthesis, by the stable carbon isotope composition of DIC in the lake and by a growth-rate dependent enrichment. The growth-rate dependent apparent isotope enrichment factor can be approximated by the difference between $\delta^{13}C_{Potamogeton}$ and $\delta^{13}C_{DIC}$. Calculated $\varepsilon_{Potamogeton\text{–}DIC}$ from modern Tibetan and Yakutian Lakes is significantly related to HCO_3^-concentration and is probably driven by submerged plant productivity.

Palaeo-HCO_3^- supply was reconstructed for Luanhaizi Lake by applying the $\varepsilon_{Potamogeton\text{–}DIC}$-$\ln HCO_3^-$ transfer function to palaeo $\varepsilon_{Potamogeton\text{–}ostracods}$ data. Our results can be reasonably well explained in terms of palaeo-productivity changes known from other lines of evidence (e.g. plant macrofossils, ostradods). From a palaeolimnological perspective, the calculation of palaeo-$\varepsilon_{macrophyte}$ is a potential new proxy for inferring former hydrochemical conditions in neutral and alkaline lakes. Future results from similar studies can be directly compared to our investigation as *Potamogeton pectinatus* has a world-wide distribution. The former availability of carbon, a main nutrient element for plants, can be reconstructed using $\delta^{13}C_{Potamogeton}$. The proxy should be capable of tracing ecosystem state changes and main changes in the aquatic submerged plant community in cases where no comprehensive macrofossil record is available. Our investigation shows how complex the interpretation for single species $\delta^{13}C$ is and that it is almost impossible to identify former productivity/carbon availability changes from bulk organic analysis. The stable carbon analysis of submerged plant biomarkers (e.g. mid-chain *n*-alkanes) could possibly be used when macrofossils are not preserved in lake sediment records.

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