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## Macrophytes and periphyton carbon subsidies to bacterioplankton and zooplankton in a shallow eutrophic lake in tropical China

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### Abstract

The subsidy of carbon derived from macrophytes and associated periphyton to bacterioplankton and zooplankton in subtropical shallow eutrophic Huizhou West Lake in China was analyzed using carbon stable isotope signatures. A restored part of the lake dominated by macrophytes was compared with an unrestored phytoplankton-dominated part. Macrophytes, periphyton, seston, and zooplankton were sampled every two months to determine natural-abundance carbon isotope ratios ( $\delta^{13}\text{C}$ ). The  $\delta^{13}\text{C}$  of phytoplankton and bacterioplankton was determined from  $\delta^{13}\text{C}$  of fatty acid biomarkers. Macrophytes and associated periphyton had similar  $\delta^{13}\text{C}$  values and were the most enriched in  $^{13}\text{C}$  of all measured organic carbon pools. A macrophyte–periphyton carbon isotopic signal was detected in particulate organic carbon, bacterioplankton, and zooplankton in the macrophyte-dominated lake part, which was demonstrated by a significant enrichment in  $^{13}\text{C}$  compared with the unrestored part, while phytoplankton and dissolved organic carbon had similar  $\delta^{13}\text{C}$  values in both lake parts. A two-source (macrophytes–periphyton and phytoplankton) mixing model showed that macrophytes–periphyton potentially contributed 14–85% (average 55%) to bacterioplankton in the macrophyte-dominated lake part, depending on season. The macrophytes–periphyton contribution to zooplankton seasonally varied between 26% and 86%, with an average of 47%. The contribution of macrophytes–periphyton to bacterioplankton increased with increasing macrophyte biomass relative to phytoplankton biomass (indicated by chlorophyll *a*). Carbon from macrophytes with associated periphyton subsidizes bacterioplankton and zooplankton, likely enhancing the cascading effects of planktonic food webs, providing an additional explanation for the stability of a clear-water state in shallow lakes dominated by macrophytes.

Macrophytes play a central role in shallow lakes, which often exhibit two possible states: a turbid-water state dominated by phytoplankton and a clear-water state dominated by macrophytes (Scheffer et al. 1993). Macrophytes maintain the clear water by a number of mechanisms; they retain nutrients via incorporation into plant biomass and enhance sedimentation and reduce sediment resuspension because of their submerged roots (Carpenter and Lodge 1986). Macro-

phytes control phytoplankton through nutrient competition and allelopathy (Van Donk and Van De Bund 2002). Meanwhile, increases in abundance and body sizes of zooplankton and hence an enhanced grazing effect on phytoplankton have also been observed in macrophyte beds, which has been recognized as another main mechanism of sustaining clear water by macrophytes (Jeppesen et al. 1997, 1998, 2002). These effects have been attributed mainly to the structuring role of macrophytes which provide refuges for zooplankters against fish predation (Jeppesen et al. 1998). However, top-down control including the grazing effect on phytoplankton by zooplankton in pelagic food webs may be augmented if a benthic energy pathway exists (Vander Zanden et al. 2005).

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Macrophytes and associated periphyton can potentially serve as a carbon source for lower trophic levels in lake systems. Macrophytes are known to release part of their organic carbon as dissolved organic carbon (DOC) (Penhale and Smith 1977; Søndergaard 1981), which is subsequently consumed by microbial members of the periphyton or by bacterioplankton (Findlay et al. 1986). Higher specific growth rates of bacterioplankton in lakes with macrophytes compared with those without macrophytes have been observed in several studies in Danish lakes (Søndergaard et al. 1998; Theil-Nielsen and Søndergaard 1999; Jeppesen et al. 2002), Austrian lakes (Reitner et al. 1999), and Canadian lakes (Rooney and Kalff 2003). These studies suggest direct bacterial support from macrophytes and associated periphyton, but it remains a challenge to distinguish the direct support from the indirect support due to changes in nutrients and pelagic food-web structure.

Zooplankton feed on organic particles of different origin and size depending on species, feeding mode, and substrate availability. Phytoplankton is considered the primary food source for zooplankton, but zooplankton can also obtain energy from allochthonous carbon (Cole et al. 2011) and bacteria (Wylie and Currie 1991). Large-bodied zooplankters, especially *Daphnia* spp., have been shown to be effective bacterivores (Pace et al. 1983). Copepods are considered more selective feeders that consume larger particles and generally do not prey directly on bacterioplankton, but they do graze on bacterivores such as protists (Sanders and Wickham 1993). Hence, macrophytes and associated periphyton could support zooplankton production via bacterioplankton and the microbial food web. In addition, zooplankton (*Daphnia* spp.) can graze on periphyton directly, which has been shown in a laboratory experiment (Siehoff et al. 2009). A field study in shallow European lakes also indicated that periphyton can be a carbon source for zooplankton and higher trophic levels (Jones and Waldron 2003). The latter study used carbon stable isotope ratios ( $\delta^{13}\text{C}$ ) to discriminate between periphyton and seston as macrophytes and associated periphyton are usually enriched in  $\delta^{13}\text{C}$  compared with phytoplankton (Fry 2006). A relative enrichment in  $\delta^{13}\text{C}$  in zooplankton during periods of high macrophyte coverage has also been observed in shallow European lakes by Boll et al. (2012). Although the authors suggested that this  $\delta^{13}\text{C}$  enrichment was due to changes in the  $\delta^{13}\text{C}$  of phytoplankton, it could also be indicative of carbon subsidy by macrophytes and associated periphyton.

Few studies have investigated the direct carbon subsidy by macrophytes and associated periphyton to bacterioplankton and zooplankton (Jones and Waldron 2003). Such a subsidy to lower trophic levels strengthens trophic linkages, lengthens the food chains, and thus increases the complexity and stability of lake food webs (Layman et al. 2007). Subsidies have been shown to enhance cascading effects in ecosystems (Nakano et al. 1999; Vander Zanden et al. 2005; Leroux and Loreau 2008). Thus, subsidies of macrophytes–periphyton to

pelagic food webs likely enhance zooplankton growth and, with it, the cascading effect on phytoplankton, constituting an additional mechanism of maintaining clear water in macrophyte-dominated lakes.

Here, we undertook a natural-abundance carbon stable-isotope analysis to examine the carbon subsidy from macrophytes and associated periphyton to bacterioplankton and zooplankton in a restored part of Huizhou West Lake, a shallow, eutrophic, tropical lake in southern China. The restored part of this lake was dominated by macrophytes, whereas the un-restored part was phytoplankton dominated. We hypothesized that macrophytes and associated periphyton would be an important carbon source for bacterioplankton and zooplankton in a macrophyte-dominated system. To test this hypothesis, the concentrations and isotopic composition ( $\delta^{13}\text{C}$ ) of all major carbon pools were monitored over a year in both the restored and the un-restored part. The isotopic composition of phytoplankton and bacteria was derived from stable isotope values of polar lipid fatty acids (PLFA) biomarkers specific for phytoplankton and bacteria (Boschker and Middelburg 2002). Using a two-source isotope mixing model, we were able to determine the carbon contributions from macrophyte–periphyton and phytoplankton to bacterioplankton and zooplankton.

## Methods

### Site description

Huizhou West Lake is a tropical urban lake in the city of Huizhou in southern China (23°06'N, 114°23'E). The total surface area of the lake is about 1.6 km<sup>2</sup> and the mean depth is about 1.6 m. The lake consists of several basins that are connected via channels. Due to increased wastewater inputs in the 1970s and the 1980s, the lake became eutrophic, and submerged macrophytes disappeared in the 1980s (Li et al. 2007). To improve water quality, a large-scale biomanipulation of Huizhou West Lake was carried out in one of its basins (area 0.12 km<sup>2</sup>) in May 2007. This biomanipulation measures included fish removal, followed by transplantation of submerged macrophytes (*Hydrilla verticillata*, *Vallisneria spiralis*, and *Myriophyllum spicatum*), and in June 2010, the biomass of submerged macrophytes had reached 253 g dry weight m<sup>-2</sup> in the biomanipulated lake (macrophyte-dominated part, M+) (Gao et al. 2014). The phytoplankton community in Huizhou West Lake was dominated by cyanobacteria whose abundance was significantly lower in M+ than in the un-restored lake part, which had no macrophytes and was dominated by phytoplankton (M–) (Chen et al. 2010). Copepods were the main crustacean in both M+ and M–, the number of cladoceran being extremely low (Chen 2012). The un-restored lake part was dominated by cyclopoid copepods such as *Thermocyclop stathokouensis* and the restored lake by calanoid copepods, for instance, *Neodiaptomus schmackeri*. Cladocerans in M– were mainly *Moina micrura* and *Bosmina* spp., and in M+ *Diaphanosoma*

*brachyurum* was occasionally observed (Chen 2012). The species richness, abundance, biomass, and biodiversity of crustacean plankton were all higher in the restored lake part than in the unrestored one (Chen 2012).

### Sampling

The restored (M+) and the unrestored (M–) lake parts were sampled every two months from February 2010 to February 2011 at a single central sampling point. In both lake parts, triplicate and single water samples were taken 0.5 m below the surface (lake fully mixed) using a five liter plexi-glass water sampler for carbon stable isotope analyses and water quality analyses, respectively. Macrophyte biomass was determined from May 2010 to March 2011 in another independent study (Gao et al. 2014). For both biomass and stable isotope analyses, plants were collected from 12 randomly selected locations using a quantitative iron clamp with an area of 0.06 m<sup>2</sup>. From June 2010 to February 2011, a five liter depth-integrated water sample was gathered through a 20 μm mesh-size net after which the sample was fixed in 5% formalin to determine the abundance of zooplankton. For stable isotope analyses, zooplankton were collected with a 63 μm mesh-size net. All samples were stored in a cooler box and transported back to the laboratory.

### Laboratory analyses

The single water sample was used for analyses of chlorophyll *a* (Chl *a*), total nitrogen (TN), and total phosphorus (TP). The Chl *a* concentration was determined spectrophotometrically after filtering a subsample of 20 mL through cellulose acetate filters and extraction of the filtered material into 90% acetone. TP and TN concentrations in the lake water samples were determined spectrophotometrically after digestion with persulfate (Ebina et al. 1983).

The triplicate lake water samples were subdivided (without prefiltration) for analyses of stable isotopic composition and concentrations of particulate organic carbon (POC), DOC, dissolved inorganic carbon (DIC), and fatty acids. Particulate organic matter (POM) in seston was measured by filtering one liter of lake water through preweighted and precombusted GF/F filters (Whatman, nominal pore size 0.7 μm), which were subsequently dried at 105°C for 24 h. For determination of fatty acids in seston, two-liter lake water was concentrated through precombusted GF/F filters, and the filters were subsequently freeze dried. From the filtrate of each sample, samples were collected for the analyses of DIC and DOC pools. Gas-tight vials (20 mL) were carefully filled and sealed for isotope analyses of DIC. Mercury chloride was added for preservation, and the samples were stored at room temperature. Clean pony vials of 20 mL were filled for DOC analyses and stored frozen.

The quantitatively collected submerged macrophytes were dried at 105°C for 24 h, and weighed to determine their biomass. Quantitative zooplankton samples were prepared by carefully removing water so that the sample volume was

reduced to 10 mL and then crustaceans present in two 2-mL subsamples were counted under 40X magnification.

For stable isotope analyses, macrophyte samples were sorted into species, rinsed with distilled water, and dried at 60°C for 48 h. Periphyton was collected from macrophytes with a wire or nylon brush in a plastic container filled with distilled water. All visible nonperiphyton particles were removed manually and the periphyton samples were then filtered through a 100 μm mesh sieve, followed by filtration onto precombusted GF/F filters which were subsequently dried at 60°C for 48 h. The dried samples of macrophytes and periphyton were then ground with mortar and pestle for stable isotope analyses. Upon return to the lab, zooplankton were transferred to beakers with demineralized water to empty their guts for two hours and were subsequently sorted into genera, handpicked and transferred to precombusted tin cups, which were subsequently freeze dried.

### Stable isotope analyses

Stable isotope ratios are expressed in the delta (δ) notation, defined as parts per thousand (per mil, ‰) deviation from a certified standard;  $\delta^{13}\text{C} = ([R_{\text{sample}}/R_{\text{standard}}] - 1) \times 1000$ , and *R* is the ratio <sup>13</sup>C:<sup>12</sup>C. The standard for δ<sup>13</sup>C was Vienna Pee Dee Belemnite. All collected samples of POM, macrophytes, periphyton, and zooplankton were analyzed for δ<sup>13</sup>C on a Thermo Electron Flash EA 1112 analyzer (EA) coupled to a Delta V isotope ratio mass spectrometer (IRMS).

For DIC-δ<sup>13</sup>C analyses, a helium headspace was created in the gas-tight vials and samples were then acidified with phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) solution. After equilibration, the CO<sub>2</sub> concentration and isotope ratio in the headspace were measured using EA-IRMS. For DOC-δ<sup>13</sup>C analyses, the samples were acidified with H<sub>3</sub>PO<sub>4</sub>, flushed with helium to remove DIC, and subsequently oxidized with sodium persulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub>); the produced isotopes were measured using high-performance liquid chromatography–isotope ratio mass spectrometry (Boschker et al. 2008).

Lipids were extracted using a modified Bligh and Dyer method (Bligh and Dyer 1959; Middelburg et al. 2000). The lipids were fractionated in different polarity classes by column separation on a heat-activated silicic acid column and subsequent elution with chloroform, acetone, and methanol. The methanol fractions, containing most of the PLFA, were collected and derivatized to fatty acid methyl esters. The standards 12:0 and 19:0 were used as internal standards. Concentrations and δ<sup>13</sup>C of individual PLFA were measured using gas chromatography–combustion isotope ratio mass spectrometry a HP G1530 GC (Hewlett Packard) connected to Delta-plus IRMS via a type-III combustion interface from Thermo Finnigan (Bremen) (Middelburg et al. 2000).

### Data analyses

PLFA are structural lipids in cell membranes and therefore show less variation in concentration per cell than, for instance, storage lipids. Moreover, they have rapid turnover

and are more likely to represent living biomass (Boschker and Middelburg 2002). PLFA can be used as chemotaxonomic markers for both phytoplankton and heterotrophic bacteria (bacterioplankton). The most abundant branched fatty acids in both M+ and M– were i14:0, i15:0, and ai15:0, which are characteristic for heterotrophic bacteria (Kaneda 1991). The sum of concentrations was used as proxy for bacterioplankton biomass. The isotopic composition of bacterioplankton was estimated by weighting the  $\delta^{13}\text{C}$  values with their respective concentrations. The most abundant polyunsaturated fatty acids in both M+ and M– were 18:3 $\omega$ 3, 18:4 $\omega$ 3, 20:5 $\omega$ 3, and 22:6 $\omega$ 3, which are markers for phytoplankton (both cyanobacteria and eukaryotic algae) (De Kluijver et al. 2012). Their concentration-weighted  $\delta^{13}\text{C}$  was used as a proxy for  $\delta^{13}\text{C}$  of phytoplankton. Because isotope values of fatty acids are generally depleted relative to other structural components, a fractionation factor of +3‰ was added to  $\delta^{13}\text{C}$  values of PLFA to obtain  $\delta^{13}\text{C}$  values for whole phytoplankton cells (Hayes 2001). For bacterioplankton, Burke et al. (2003) suggested that in the field samples the depletion of fatty acids to whole cells would be 0‰, so we applied no correction on bacterial  $\delta^{13}\text{C}$ . The carbon biomass of phytoplankton was calculated from Chl *a* concentrations assuming a C:Chl *a* ratio of 40 (Middelburg et al. 2000).

Stable isotope mixing equations can be used to infer the diet of consumers, provided that carbon sources have distinct signatures and that there are only two sources in case of one isotope (Phillips and Gregg 2003). One option to overcome this limitation is to a priori aggregate food sources, under the condition that sources have similar isotopic values and are logically related (Phillips et al. 2005). In M+, macrophyte and periphyton carbon isotope data were very similar and distinct from that of phytoplankton (see Results). Macrophyte- and periphyton-derived carbon was therefore considered as one source.

The contribution of allochthonous organic carbon to bacterioplankton and zooplankton were also not included in the isotope mixing model. Huizhou West Lake, and particularly the restored part, is an urban lake and has a small catchment with limited terrestrial vegetation (Li et al. 2007). Therefore, the allochthonous organic carbon inputs into the lake and thus their contribution to the food webs in the restored macrophyte-dominated lake is likely negligible.

The contributions of macrophytes–periphyton and phytoplankton as carbon source for zooplankton ( $f_{\text{macro-peri-zoo}}$  and  $f_{\text{phyto-zoo}}$ , respectively) and bacterioplankton ( $f_{\text{macro-peri-bac}}$  and  $f_{\text{phyto-peri-bac}}$ , respectively) in the restored lake were calculated for each sampling month using an algebraic isotope mixing model:

$$f_{\text{macro-peri}}(\%) = \frac{\delta^{13}\text{C}_{\text{phyto}} - \delta^{13}\text{C}_{\text{consumer}}}{\delta^{13}\text{C}_{\text{phyto}} - \delta^{13}\text{C}_{\text{macro-peri}}} \times 100 \quad (1)$$

$$f_{\text{phyto}}(\%) = 100 - f_{\text{macro-peri}}$$

$\delta^{13}\text{C}_{\text{phyto}}$ ,  $\delta^{13}\text{C}_{\text{macro-peri}}$ , and  $\delta^{13}\text{C}_{\text{consumer}}$  are the carbon isotope values (‰) of phytoplankton, macrophytes–periphyton,

and consumers, respectively. The uncertainties of  $\delta^{13}\text{C}$  in carbon sources and in consumers were considered in the calculations using random sampling ( $n = 1000$ ) from a normal distribution. The normal distribution was created from the mean  $\delta^{13}\text{C}$  value  $\pm$  standard deviation (SD) of sources and consumers. For bacterioplankton and phytoplankton, the mean  $\delta^{13}\text{C}$  value  $\pm$  SD of triplicate samples was used. For macrophytes, the unweighted mean value of  $\delta^{13}\text{C} \pm$  SD of all macrophyte genera and periphyton was used. Data on biomass composition of macrophyte genera and periphyton were not available, so a weighted mean of the different species could not be used. Error bars in the analyses were used to cover this uncertainty. Although different zooplankton genera were abundant in each isotope sample, the average  $\delta^{13}\text{C}$  value  $\pm$  SD of total zooplankton was used in the isotope mixing modeling. Bacterial and zooplankton consumers both had  $\delta^{13}\text{C}$  values in between those of phytoplankton and macrophytes–periphyton. From the set of possible solutions, only outcomes between 0% and 100% were accepted and contributions are presented as average  $\pm$  SD of the accepted outcomes.

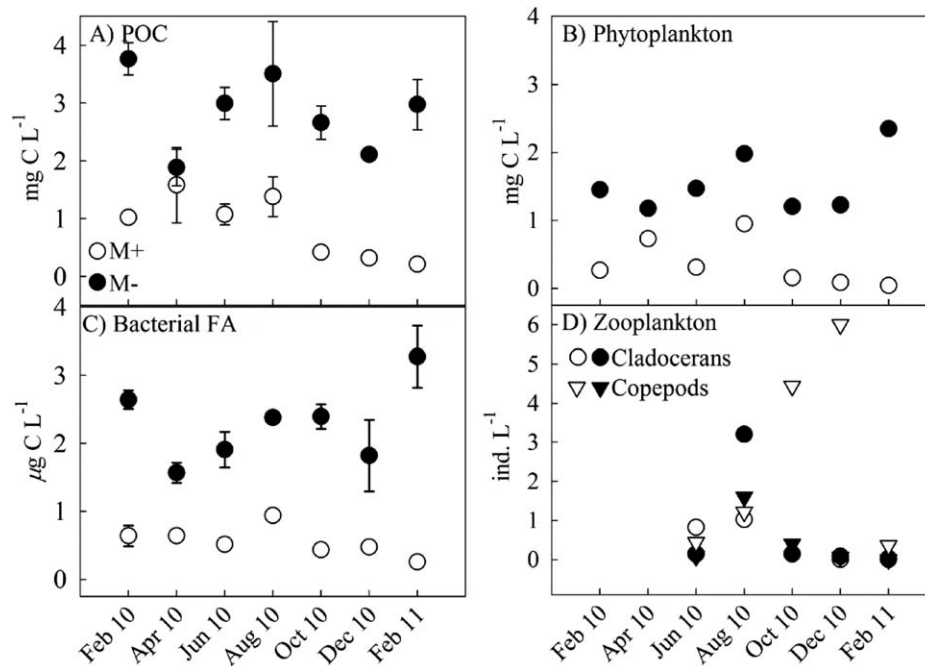
### Statistics

Monthly data are shown as mean  $\pm$  SD of triplicate samples. Total annual averages are presented as mean  $\pm$  SD of the means of each sampling event ( $n = 7$ ). Total annual averages were analyzed for normal distribution using Shapiro tests and the differences between M+ and M– were statistically tested with paired student *t*-tests for normally distributed data and with nonparametric sign tests for non-normally distributed data or if M+ and M– had data of unequal *n*. The relations between macrophyte–periphyton contributions to consumers and relative macrophyte biomass (macrophyte biomass to phytoplankton Chl *a* ratio) over the year were statistically tested with Pearson product-moment correlation. Correlation coefficients were also determined for phytoplankton biomass and  $\delta^{13}\text{C}$  values of phytoplankton in M+ and M–. Food source calculations and statistical analyses were done in R software (R Core Team 2014).

### Results

#### Concentrations of nutrients and POC, and biomasses of phytoplankton, macrophytes, bacterioplankton, and zooplankton

Total annual average concentrations of TP, TN, and Chl *a* were significantly lower in M+ than in M–. TP was  $0.019 \pm 0.008 \text{ mg L}^{-1}$  in M+ vs.  $0.102 \pm 0.041 \text{ mg L}^{-1}$  in M– ( $t = -5.18$ ,  $df = 6$ ,  $p < 0.01$ ), and TN was  $0.79 \pm 0.35 \text{ mg L}^{-1}$  in M+ vs.  $1.64 \pm 0.50 \text{ mg L}^{-1}$  in M– ( $t = -2.66$ ,  $df = 6$ ,  $p < 0.05$ ). Chl *a* was  $9.1 \pm 8.6 \mu\text{g L}^{-1}$  in M+ vs.  $38.8 \pm 11.2 \mu\text{g L}^{-1}$  in M– ( $t = -5.66$ ,  $df = 6$ ,  $p < 0.01$ ). Macrophyte biomass (measured from May onward) ranged from 213 to 346 g dry weight  $\text{m}^{-2}$ . Biomasses of both phytoplankton (derived from Chl *a*) and



**Fig. 1.** Concentrations of (A) POC, (B) phytoplankton (Chl *a* based), (C) bacteria FA in seston, and (D) abundance of zooplankton in the restored (M+, open circles) and unrestored (M-, closed circles) lake parts. Data points in (A) and (B) indicate the average  $\pm$  SD ( $n=3$ ).

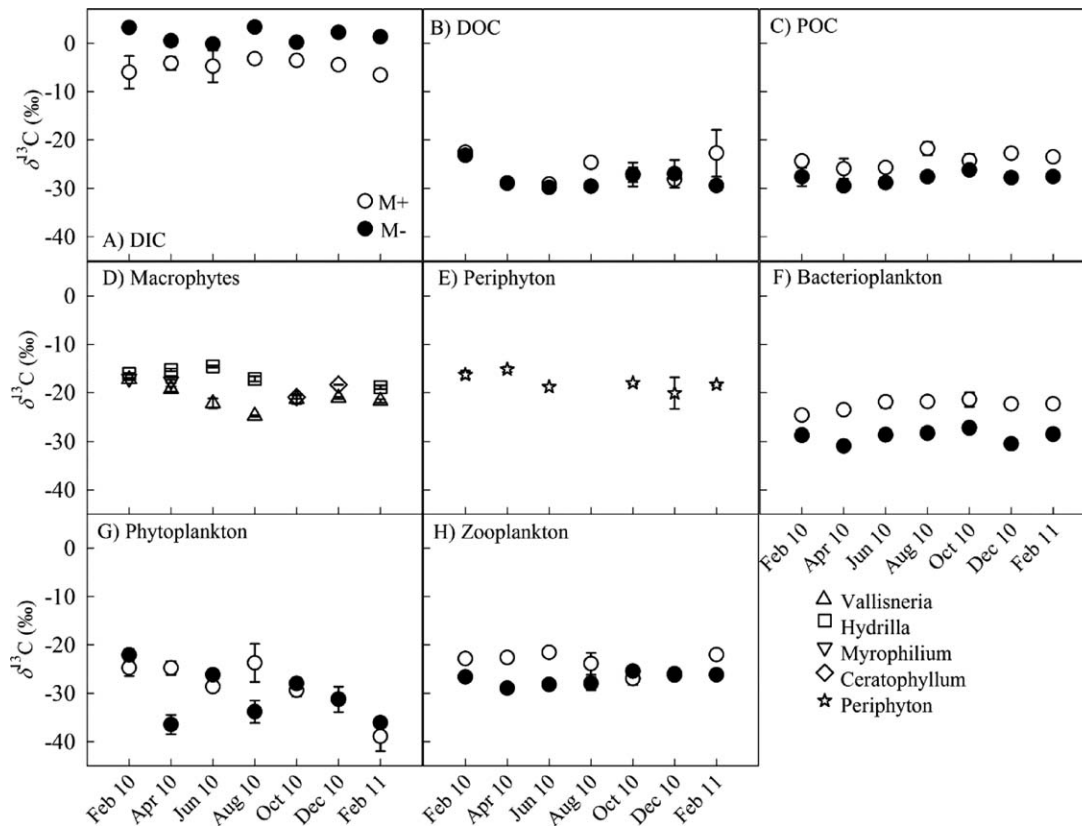
macrophytes were higher in summer (June and August) than in winter (December and February) in M+, but the relative macrophyte biomass (macrophyte-to-Chl *a*-ratio) was higher in winter than in summer. POC concentrations in seston were  $2.0 \pm 0.8 \text{ mg C L}^{-1}$  lower in M+ than in M- (Fig. 1A), which can mainly be attributed to the difference in phytoplankton biomass, which was  $1.2 \pm 0.6 \text{ mg C L}^{-1}$  lower in M+ than in M- (Fig. 1B). Phytoplankton made up  $13 \pm 14\%$  (M+) and  $23 \pm 12\%$  (M-) of total POC. Bacterioplankton biomass (expressed by concentrations of fatty acids) was  $1.7 \pm 0.7$

times higher in M- than in M+ (Fig. 1C). However, the biomass of bacterioplankton relative to phytoplankton (bacteria: phytoplankton ratio) was higher in M+ (29%) than in M- (15%). Average numbers of zooplankton (from June onward) were generally low and not significantly different between M+ and M-:  $2.9 \pm 2.3 \text{ ind. L}^{-1}$  in M+ and  $1.1 \pm 2.1 \text{ ind. L}^{-1}$  in M- (sign test, Fig. 1D). Copepods showed a peak in abundance in October and December (2010) in M+, while cladoceran abundance remained low (Fig. 1D). In M-, copepods and cladocerans density peaked in August (Fig. 1D).

**Table 1.** Total annual average (tot. av.) carbon isotope ratios  $\pm$  SD ( $\delta^{13}\text{C}$ ) of analyzed organic and inorganic carbon pools in restored (M+) and unrestored (M-) parts of Huizhou West Lake.  $n$  gives the number of measurements over the year, and  $p$  gives the significance level of differences between M+ and M- from paired  $t$ -tests or sign tests

	Tot. av. M+	Tot. av. M-	$n$	Test	$t$ -value	$p$
DIC	$-4.7 \pm 1.2$	$1.5 \pm 1.3$	7,7	Sign test		*
DOC	$-26.2 \pm 2.8$	$-27.9 \pm 2.4$	7,7	Paired $t$ -test	1.51	NS
POC	$-24.1 \pm 1.5$	$-27.9 \pm 1.1$	7,7	Paired $t$ -test	7.85	**
Macrophytes	$-17.8 \pm 1.8$		7,-			—
Periphyton	$-19.2 \pm 1.7$		7,-			—
Bacterioplankton	$-22.6 \pm 1.1$	$-29.0 \pm 1.3$	7,7	Paired $t$ -test	13.40	**
Phytoplankton	$-28.8 \pm 5.3$	$-30.6 \pm 5.4$	7,7	Paired $t$ -test	0.75	NS
Copepods	$-24.1 \pm 2.0$	$-26.6 \pm 1.3$	7,5	Sign test		*
Cladocerans	$-24.0$	$-28.4 \pm 1.0$	1,4			—
Total zooplankton	$-23.7 \pm 2.0$	$-27.1 \pm 1.3$	7,7	Paired $t$ -test	2.98	*

\*  $p < 0.05$ , \*\*  $p < 0.001$ . NS means nonsignificant.



**Fig. 2.** Carbon isotope ( $\delta^{13}\text{C}$ ) values (‰) of (A) DIC, (B) DOC, (C) POC, (F) bacterioplankton, (G) phytoplankton, and (H) zooplankton in the restored (M+, open circles) and unrestored (M-, closed circles) lake. (D) and (E) show  $\delta^{13}\text{C}$  (‰) values in different macrophyte genera and periphyton in M+, respectively. Each data point shows average  $\pm$  SD ( $n=3$ ).

### Carbon stable isotope composition

The DIC pool, which forms the substrate for phytoplankton, was significantly more  $^{13}\text{C}$  depleted in M+ than in M- (Fig. 2A; Table 1). There was no overall enrichment in  $^{13}\text{C}$  of DOC in M+ (Fig. 2B); however, the annual average  $\delta^{13}\text{C}$  value of POC was significantly higher in M+ than in M- (Fig. 2C, Table 1).

Macrophytes and attached periphyton were the most  $^{13}\text{C}$  enriched of all the organic carbon pools, while phytoplankton were the most depleted and all other organic carbon pools had intermediate isotopic values (Table 1). The  $\delta^{13}\text{C}$  signature of macrophytes varied among the different species: *Hydrilla verticillata* being the most  $^{13}\text{C}$  enriched and *Vallisneria natans* the most depleted. *Ceratophyllum demersum*, *Myriophyllum spicatum*, and periphyton had intermediate values (Fig. 2D,E).

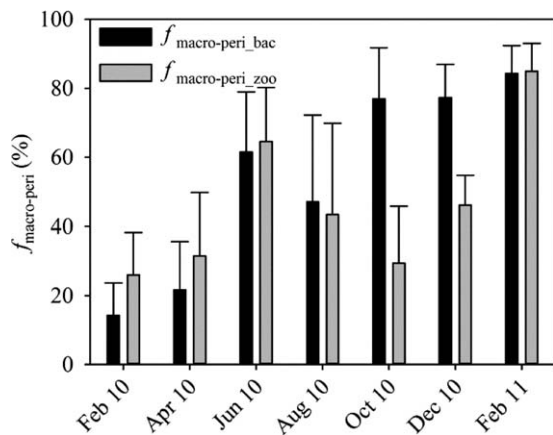
Analyses of FA showed that the annual average of bacterioplankton was significantly more  $^{13}\text{C}$  enriched in M+ than in M- (Fig. 2F; Table 1). Next to macrophytes including periphyton, bacterioplankton was the most  $^{13}\text{C}$ -enriched organic carbon pool in M+ (Table 1). The  $\delta^{13}\text{C}$  values of phytoplankton did not statistically differ between M+ and M- (Fig. 2G; Table 1). In M-,  $\delta^{13}\text{C}$  values of phytoplankton were unrelated to phytoplankton biomass, but in M+, a

weak relation between  $\delta^{13}\text{C}$  values and biomass of phytoplankton was observed ( $r=0.74$ ,  $t=2.47$   $p=0.06$ ).

Because of low cladoceran abundance in M+ (Fig. 1D), their isotopic composition was only obtained in April, while isotope signatures of copepods could be determined at each sampling date. Hence,  $\delta^{13}\text{C}$  values of total zooplankton in M+ represent the  $\delta^{13}\text{C}$  of copepods, except in April when  $\delta^{13}\text{C}$  in zooplankton represents cladocerans and copepods (Table 1). In M-, there was no significant difference between the  $\delta^{13}\text{C}$  values of copepods and that of cladocerans (Table 1). The annual average  $\delta^{13}\text{C}$  value of zooplankton was significantly higher in M+ than in M- (Table 1). Zooplankton were more enriched in  $^{13}\text{C}$  in M+ than in M- during most of the sampling period except in October and December when  $\delta^{13}\text{C}$  values in M+ and M- were rather similar (Fig. 2H). During this period, the zooplankton community in M+ showed a peak in abundance (Fig. 1D). Zooplankton had  $\delta^{13}\text{C}$  values similar to those of POC (Table 1).

### Macrophytes–periphyton as a carbon source

Average  $\delta^{13}\text{C}$  of macrophytes was not statistically different from average  $\delta^{13}\text{C}$  of periphyton (sign test), so these two



**Fig. 3.** The contribution (%) of macrophyte–periphyton carbon in each sampled month to bacteria (solid black) and zooplankton (shaded gray) in the restored lake (M+); the other carbon source is phytoplankton and together they total 100%. The error bars indicate the uncertainty of the calculations.

sources could not be separated based on isotope signature and were therefore combined in the isotope mixing model as a single source as macrophytes–periphyton. The contribution of macrophytes–periphyton to bacterioplankton ( $f_{\text{macro-peri\_bac}}$ ) was on average  $55 \pm 28\%$ , varying seasonally (Fig. 3). The highest  $f_{\text{macro-peri\_bac}}$  value (85%) was observed in February 2011 and the lowest (14%) in February 2010 (Fig. 3).  $f_{\text{macro-peri\_bac}}$  was positively related to relative macrophyte biomass to phytoplankton, expressed as the macrophyte-to-Chl *a*-ratio ( $r = 0.87$ ,  $t = 3.09$ ,  $p = 0.05$ ) (Fig. 4).

The average contribution of macrophytes together with periphyton to zooplankton carbon ( $f_{\text{macro-peri\_zoo}}$ ) was  $47 \pm 21\%$ , ranging from 26% in February (2010) to 86% in February (2011) (Fig. 4). Except in autumn (October and December),  $f_{\text{macro-peri\_bac}}$  and  $f_{\text{macro-peri\_zoo}}$  were rather similar. In the autumn period,  $f_{\text{macro-peri\_zoo}}$  was much lower than  $f_{\text{macro-peri\_bac}}$  (Fig. 3). The macrophytes–periphyton carbon contribution to the zooplankton community was independent of the relative macrophyte biomass to phytoplankton (macrophyte-to-Chl *a*-ratio) (Fig. 4).

### Discussion

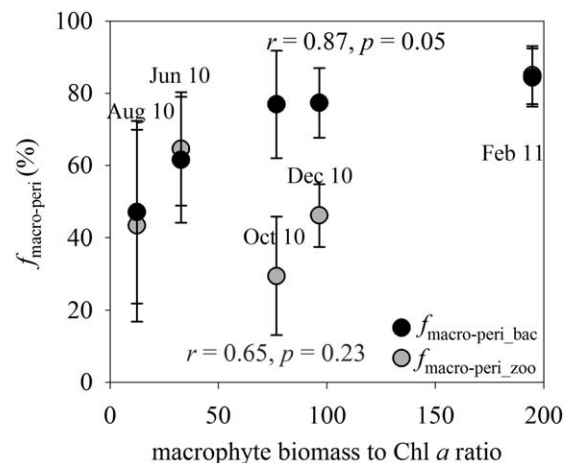
The goal of this study was to investigate macrophyte–periphyton carbon subsidies to lower trophic levels, bacteria, and zooplankton, in a restored part of a shallow lake in China. Carbon isotope analyses showed that the  $^{13}\text{C}$ -enriched macrophytes with associated periphyton in the restored lake resulted in a significant increase in  $\delta^{13}\text{C}$  values of POC, zooplankton, and bacterioplankton, but not of phytoplankton, compared to those in the unrestored lake part. Isotope mixing results indicated that macrophyte–periphyton contributed substantially to the diet of bacterioplankton and zooplankton in the restored lake.

### Restoration effects on biomasses and nutrient concentrations

Lake restoration in Huizhou West Lake led to dominance of macrophytes and resulted in lower TN and TP concentrations, lower seston POC, and lower phytoplankton biomass in the restored lake part (Fig. 1A,B), see details about the restoration in Gao et al. (2014).

The increase of macrophytes also resulted in a decrease in bacterioplankton biomass (Fig. 1C), probably due to the decrease in phytoplankton and POM in general. In addition, the observed decrease in TN and TP in M+ could have induced nutrient limitation in bacterioplankton due to nutrient competition with macrophytes as demonstrated by Huss and Wehr (2004). The decrease in bacterioplankton biomass can also be due to a strong grazing pressure of zooplankton (top-down control). Biomanipulation studies in temperate lakes show that decreases in bacterioplankton can be caused by an increase in *Daphnia* abundance, grazing being the main factor controlling bacterial abundance (Jürgens and Jeppesen 1998; Søndergaard et al. 1998; Jeppesen et al. 2002). However, in Huizhou West Lake, zooplankton numbers were generally low and were only higher in M+ than in M– in October and December (Fig. 1D). Furthermore, the zooplankton community in M+ was dominated by (calanoid) copepods (Fig. 1D) and a strong grazing control of zooplankton on bacterioplankton biomass is thus not expected (Burns and Schallenberg 1996).

Despite an overall lower biomass, the ratio of bacterioplankton to phytoplankton biomass was higher in M+ than in M–, implying that bacterioplankton does not completely depend on phytoplankton as the carbon source and macrophytes–periphyton carbon subsidies are likely responsible for the relatively high bacterioplankton biomass (Reitner et al.



**Fig. 4.** Relationships with correlation coefficients between relative macrophyte biomass (macrophyte biomass to Chl *a* ratio) and contributions of macrophyte–periphyton to bacteria ( $f_{\text{macro-peri\_bac}}$ ) and zooplankton ( $f_{\text{macro-peri\_zp}}$ ) in the restored part of Huizhou West Lake.



1999; Theil-Nielsen and Søndergaard 1999; Jeppesen et al. 2002).

### Isotopic composition

DIC in M+ was significantly depleted in  $^{13}\text{C}$  compared to DIC in M– (Fig. 2A; Table 1), which can probably be attributed to a higher production to respiration ratio in phytoplankton-dominated M–. Respiration of organic matter (OM) causes depletion in  $\delta^{13}\text{C}$  of DIC due to addition of  $^{13}\text{C}$ -depleted OM-derived C, while primary production causes relative enrichment of  $^{13}\text{C}$  in the DIC pool due to preferential uptake of  $^{12}\text{C}$  from this pool by phytoplankton (Bade et al. 2004; Bontes et al. 2006). The higher phytoplankton biomass and by inference higher primary production in M– may therefore explain the enrichment with  $^{13}\text{C}$  of the DIC pool.

Even though macrophytes are known to release DOC (Penhale and Smith 1977; Søndergaard 1981; Demarty and Prairie 2009), a macrophyte signal in the DOC pool (i.e.,  $^{13}\text{C}$  enrichment) was not detected for most of the sampling dates (Fig. 2B). The most enriched DOC was observed in February 2011 when relative macrophyte coverage was at its highest (Figs. 2B, 4). It is likely that DOC release by macrophytes was masked by a large refractory DOC pool derived from phytoplankton. The contribution of macrophytes and associated periphyton was more discernible in POC, which may be explained by sloughing of periphyton from macrophytes into the water column. Other mechanisms resulting in enriched POC could be aggregation or bacterial conversion of macrophyte DOC into POC, release of macrophyte particles (detritus) into the water (Mann 1988), and a relative lower contribution of phytoplankton.

There was no significant difference in  $\delta^{13}\text{C}$  composition of phytoplankton (Table 1; Fig. 2G). A detailed evaluation of phytoplankton  $\delta^{13}\text{C}$  is beyond the scope of this study, but our results show that phytoplankton  $\delta^{13}\text{C}$  is not related to absolute phytoplankton biomass (Chl *a*) as has been demonstrated in other investigations (Laws et al. 1995; De Kluijver et al. 2014). Also, in a study of lake restoration by biomanipulation in the Netherlands, Bontes et al. (2006) did not find any difference in  $\delta^{13}\text{C}$  for phytoplankton between unrestored and restored parts of a eutrophic lake despite significant differences in phytoplankton biomass.

In the restored lake, bacterioplankton and zooplankton were more enriched in  $\delta^{13}\text{C}$  (3.4‰ and 6.4‰, respectively) than in the unrestored lake (Table 1; Fig. 2F,H). The clear isotopic signature of macrophytes–periphyton in bacterioplankton and zooplankton indicates carbon subsidies by the former and to the latter. Isotope mixing calculations suggest that macrophytes and associated periphyton contributed a substantial amount of carbon to bacterioplankton and zooplankton, although phytoplankton was the main source for zooplankton in most months, except in June and February (2011) (Fig 3).

Note that the estimates of the macrophyte–periphyton contribution to bacterioplankton are rather conservative because of the assumption that  $\delta^{13}\text{C}$  of bacterial FA represents  $\delta^{13}\text{C}$  of bacterial cells. If a correction of +3‰ for  $\Delta\delta^{13}\text{C}_{\text{FA-cell}}$  was applied as suggested by Hayes (2001), the  $\delta^{13}\text{C}$  of bacterioplankton would have been more enriched (–19.6‰) and average  $f_{\text{macro-bac}}$  would have been  $74 \pm 18\%$ , ranging from 45% to 94%.

### Carbon subsidy in food webs

There are several potential pathways for carbon transfer from macrophytes and associated periphyton to bacterioplankton and zooplankton. Bacterioplankton can grow on DOC derived from macrophytes and the attached periphyton during detritus formation (Findlay et al. 1986; Theil-Nielsen and Søndergaard 1999). Another mechanism is DOC release from bacterioplankton growing on macrophytes and the attached periphyton (Theil-Nielsen and Søndergaard 1999). The close relationship between relative macrophyte abundance and the contribution of macrophytes–periphyton to bacterioplankton in this study indicates a strong coupling between macrophytes–periphyton substrate availability and bacterioplankton growth (Fig. 4). The differences in carbon subsidies between February 2010 and February 2011 can be attributed to differences in the timing of the onset of the phytoplankton bloom. In February 2010, phytoplankton biomass in M+ was higher than in February 2011, indicating earlier bloom development (Fig. 1B). The low phytoplankton biomass in February 2011 was associated with much depleted  $^{13}\text{C}$  (Fig. 2G), which was not reflected in bacterioplankton and zooplankton (Fig. 2F,H).

In most months,  $f_{\text{macro-peri-bac}}$  and  $f_{\text{macro-peri-zoo}}$  were relatively similar, which may indicate bacterioplankton-mediated carbon flows from macrophytes and periphyton to zooplankton (Fig. 3). We suggest that direct grazing of copepods on bacterioplankton may be limited as seen in several grazing studies with labeled bacterioplankton (Sanders and Wickham 1993; Jeppesen et al. 1996). A more likely pathway is the transfer of bacterioplankton carbon via trophic intermediates, such as protists (Sanders and Wickham 1993). In marine systems, ciliates have been found to support ~30% of the daily carbon consumption of copepods (Calbet and Saiz 2005). Another possible pathway is direct grazing of zooplankton on periphyton or on the macrophyte-derived particles in seston. A direct consumption of periphyton by *Daphnia magna* has been demonstrated in a laboratory study (Siehoff et al. 2009) and was also suggested by Jeppesen et al. (2002). The latter study showed that zooplankton grazing largely exceeded phytoplankton production in macrophyte-dominated enclosures, while grazing was only a fraction of phytoplankton production in enclosures without macrophytes (Jeppesen et al. 2002).

In October and December when copepods reached a peak in numbers (Fig. 1D), there was a strong decoupling between

$f_{\text{macro-peri\_bac}}$  and  $f_{\text{macro-peri\_zoo}}$  (Fig. 3). Here,  $f_{\text{macro-peri\_zoo}}$  was low and the  $\delta^{13}\text{C}$  values of zooplankton and phytoplankton in the M+ and M– lake parts were very similar (Fig. 2G,H), suggesting that zooplankton relied more on phytoplankton and less on the carbon subsidy from macrophyte–periphyton in M+. This is in agreement with the general consensus that copepods are selective feeders that generally feed on larger particles of high quality, such as phytoplankton (Wylie and Currie 1991).

Carbon subsidies, primarily from organic material of allochthonous origin, have been found to consolidate cascading effects in ecosystems (Nakano et al. 1999; Leroux and Loreau 2008). Within lake ecosystems, carbon subsidies from benthic sources can have an important effect on pelagic ecosystems (Vander Zanden et al. 2005). Our study provides evidence that macrophytes and the associated periphyton subsidize zooplankton, hence supporting zooplankton growth. However, in Huizhou West Lake, zooplankton abundances were not significantly higher in the restored lake where the zooplankton was subsidized by macrophytes–periphyton than in the unrestored lake. In the restored lake, Gao et al. (2014) found that the fish community was dominated by omnivores and the fish biomass expressed as catch per unit effort was similar to that in the unrestored lake. The recovery of the fish community after fish removal is likely due to more frequent and earlier reproduction in warm lakes (Teixeira-de Mello et al. 2009; Jeppesen et al. 2010). These abundant omnivorous fish are efficient zooplankton feeders, especially in young stages (Gao 2013), and likely exert a high predation pressure on the zooplankton in the restored lake part in Huizhou West Lake. However, the subsidy to zooplankton by macrophytes–periphyton documented in our study has the potential to increase the zooplankton biomass and thus the zooplankton to phytoplankton ratios in macrophyte-dominated lakes, contributing to strengthening the top-down effects on phytoplankton. Thus, restoring benthic energy pathways to pelagic food webs via re-establishing submerged macrophytes (and thus associated periphyton) is one of the key measures of the restoration of eutrophic lakes as controlling phytoplankton is the primary goal.

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