Assimilation of ancient organic carbon by zooplankton in Tibetan Plateau lakes is depending on watershed characteristics

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Abstract

Ancient (i.e., radiocarbon depleted) organic carbon (OC) is exported from ice sheet, glacier, and permafrost systems and may be buried, respired, or assimilated in downstream aquatic systems. Few studies have explored the potential use of this ancient OC in lake food webs. We combined natural abundance radiocarbon and stable carbon isotope data (Δ^{14}C and δ^{13}C) to study ancient OC utilization by zooplankton in six lakes covering a large climate gradient on the central and peripheral Tibetan Plateau. A depleted Δ^{14}C signature of dissolved and particulate OC was found in the inflowing streams and lakes, ranging from – 49‰ to – 569‰, corresponding to radiocarbon ages between 403 yr and 6757 yr. The Δ^{14}C values for zooplankton in the lakes ranged from – 45‰ to – 264‰, reflecting that zooplankton obtain ^{14}C-depleted signatures through assimilation of ancient OC and/or indirectly through consumption of phytoplankton or aquatic plant utilizing ^{14}C-depleted inorganic carbon. Moreover, ancient OC from inflowing streams contributed more to zooplankton diets in the temperate glacier area than in the cold glacier area. Assimilation of ancient OC by zooplankton in lakes is not only affected by drainage basin characteristics, such as the recharge coefficient of the lake, but also by the biogeochemical properties of OC. Use of ancient OC by zooplankton in high-altitude lakes may constitute an important link between the contemporary aquatic food webs and the glaciated watersheds. Our findings have important implications for the contribution of ancient carbon to the modern lake food webs of high-altitude and polar lakes.

Global warming is predicted to alter the ice-free season length, permafrost active layer thickness, and terrestrial inputs in unproductive high-elevation ecosystems (Sommaruga-Wögrath et al. 1997; Holzapfel and Vinebrooke 2005). A key challenge, ecologists facing, is to comprehend how such changes will affect the structure and functioning of aquatic ecosystems. The ongoing glacier mass loss and permafrost thawing have important implications for the quantity, age, and reactivity of organic carbon (OC) in proglacial freshwater (Hood and Scott 2008; Caraco et al. 2010; Mann et al. 2015; Qu et al. 2017). By measuring natural abundance of radiocarbon (^{14}C), highly aged (^{14}C depleted) organic material has been found in some rivers and lakes, from Arctic (Schell 1983; Guo et al. 2007), tropical (Mayorga et al. 2005)

to temperate rivers and lakes (Raymond et al. 2004; Drenzek et al. 2009; Keaveney et al. 2015a,b; Zigah et al. 2017), and Tibetan alpine streams and lakes (Spencer et al. 2014; Hu et al. 2016). A few studies have provided compelling evidence that ancient allochthonous OC may be an important food source for microbial heterotrophs (Cherrier et al. 1999; Singer et al. 2012; Mann et al. 2015; Bellamy and Bauer 2017), invertebrates, and higher trophic consumers such as fish and waterfowl (Schell 1983; Hågvar and Ohlson 2013; Fellman et al. 2015) and that it may be a significant resource for food webs in rivers and estuaries (McCallister et al. 2004; Caraco et al. 2010). The ancient OC support of aquatic consumers indicates that a shortage of modern primary production in low-productive aquatic ecosystems may not necessarily limit or constrain the food supply to the consumers. However, some studies (Zigah et al. 2011, 2012a) in Lake Superior revealed that radiocarbon signatures of mesozooplankton tracked those of dissolved inorganic carbon (DIC) instead of particulate organic matter (POM). Since aquatic

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autotrophic organisms, such as phytoplankton and submersed plants, primarily utilize DIC as the carbon source for photosynthesis (Raymond et al. 2004; Ishikawa et al. 2013, 2014), this suggests that zooplankton prefers recently synthesized autochthonous material rather than POM with ^{14}C-depleted signatures (Zigah et al. 2012*b*; Kruger et al. 2016). Accordingly, whether the incorporation of old carbon into aquatic food webs is a universal phenomenon has not been well assessed due to the limited availability of data on the ^{14}C content of consumers in aquatic ecosystems such as lakes (Zigah et al. 2012*b*; Guillemette et al. 2017), particularly high-altitude lakes.

Stable isotopes of carbon have been widely used for identifying carbon sources for metazoan consumers. However, isotopic overlap of different resources, high-temporal variability, and lack of data at the base of the food web may hamper detailed exploration of food-web dynamics (Middelburg 2014). Natural abundance radiocarbon has emerged as a promising complementary approach and provides sensitive means for differentiating OC sources and identifying the ancient components (Caraco et al. 2010; Fellman et al. 2015). A combination of Δ^{14}C and δ^{13}C is especially suitable for tracking the incorporation of old organic matter into the aquatic food web (Zigah et al. 2014, 2017; Kruger et al. 2016; Bellamy et al. 2017; Guillemette et al. 2017).

The Tibetan Plateau is the world's highest plateau and has the largest mass ($\sim$ 49,873 km^2) of ice sheets (Yao et al. 2007), except for those of Greenland and Antarctica, and has an extremely large permafrost area (1.26 $\times$ 10^6 km^2) (Cheng and Jin 2013). Moreover, the Tibetan Plateau hosts large lakes with a total surface area of $\sim$ 51,000 km^2 (Liu et al. 2009), amounting to 51% of the total lake area in China. The warming rate on the Tibetan Plateau was 0.3°C/10 yr during the past three decades, which was twice as high as that of global warming (Xu et al. 2009). A dramatic recession ($\sim$ 17% coverage) of Tibetan glaciers has occurred in the past 30 yr owing to climate warming (Qiu 2010). While climate change on the short term will lead to increased runoff and thus input of allochthonous OC, on the longer term runoff will decline again (Walvoord and Striegl 2007; Kang et al. 2010; Yao et al. 2012; Aiken et al. 2014). In addition, it is estimated that there is a huge carbon store of 12.3 Pg-C in the permafrost regions on the Tibetan Plateau. The permafrost active layer thickness on the Tibetan Plateau has increased by 1.33 cm/yr since 1980 due to climate change (Zhao et al. 2004; Wu and Zhang 2010). Melting of permafrost may lead to the release of huge amounts of soil OC, which will be transported into lakes with rivers and/or groundwater (Guo et al. 2007; Qu et al. 2017). These processes tend to cause changes in land cover at local and regional scales when glaciers and tundra are replaced by forested ecosystems. In turn, this transition will alter the dominant sources and reactivity of OC inputs to downstream ecosystems (Fellman et al. 2015). For example, the

bioavailable fraction of dissolved organic carbon (DOC) in glacial streams is as much as 2–5$\times$ more labile than in forested and wetland streams (Hood et al. 2009). Mount Nyainqentanglha and Karola glaciers and glacier streams in Tibet contain ancient DOC (radiocarbon ages 749–2350 yr before present, ybp) with high bioavailability; being significantly higher than in downstream Lakes Namtso and Yamdrok (46–69% and 23–26%, respectively; Spencer et al. 2014). However, little information is available on the bioavailability of ancient particulate organic carbon (POC), and this deserves further investigations, because POC can be directly utilized by metazoan consumers in lakes.

To date, signals of ancient OC in high-altitude lakes and its contribution to the modern aquatic food webs have only rarely been studied. Here, we investigated the importance and the possible cycling of ancient OC in the lacustrine food webs by analyzing the natural abundance of Δ^{14}C and δ^{13}C of planktonic crustaceans and their potential carbon sources. We focused on planktonic crustaceans as they play significant roles in the carbon flow in aquatic ecosystems and link primary producers and microorganisms with higher level consumers in lake food webs (Taipale et al. 2008). We selected six alpine lakes (Chentso, Basongtso, Yueqiatso, Namtso, Heihai, and Huanghai; Fig. 1), covering a large climate gradient from the north to the southeast and contrasting catchment conditions resulting in different inflowing streamwater characteristics (Supporting Information Table S1) and different riverine transit times from glaciers to downstream lakes. DOC and POC are therefore anticipated to have undergone differential biological or photochemical alteration before entering the lakes. Specifically, we tested the hypotheses that ^{14}C-depleted DOC and POC are widespread in high-altitude lakes, which both can be incorporated into consumer diets and that the old carbon contribution to consumers depends on drainage basin characteristics.

Methods

Study sites

The lakes on Tibetan Plateau—Lake Chentso (90°33′E, 28°58′N), Lake Basongtso (93°58′E, 30°01′N), Lake Yueqiatso (88°37′E, 30°28′N), Lake Heihai (100°04′E, 27°21′N), Lake Huanghai (100°04′E, 27°20′N), and Lake Namtso (90°16′–91°03′E, 30°30′–30°55′N)—included in this study are located in the central or on the edge of the Tibetan Plateau (Fig. 1). The lakes are situated in a harsh natural environment with strong radiation and low temperatures and have been little affected by human activities. Lakes Chentso, Yueqiatso, and Namtso are located in the continental climate zone with a dry, cold climate, and surrounded by alpine meadows, whereas Lakes Heihai, Huanghai, and Basongtso are located in a temperate glacier area. All the lakes are primarily fed by surface runoff including melted ice and snow and atmospheric precipitation. The general geographical and chemical
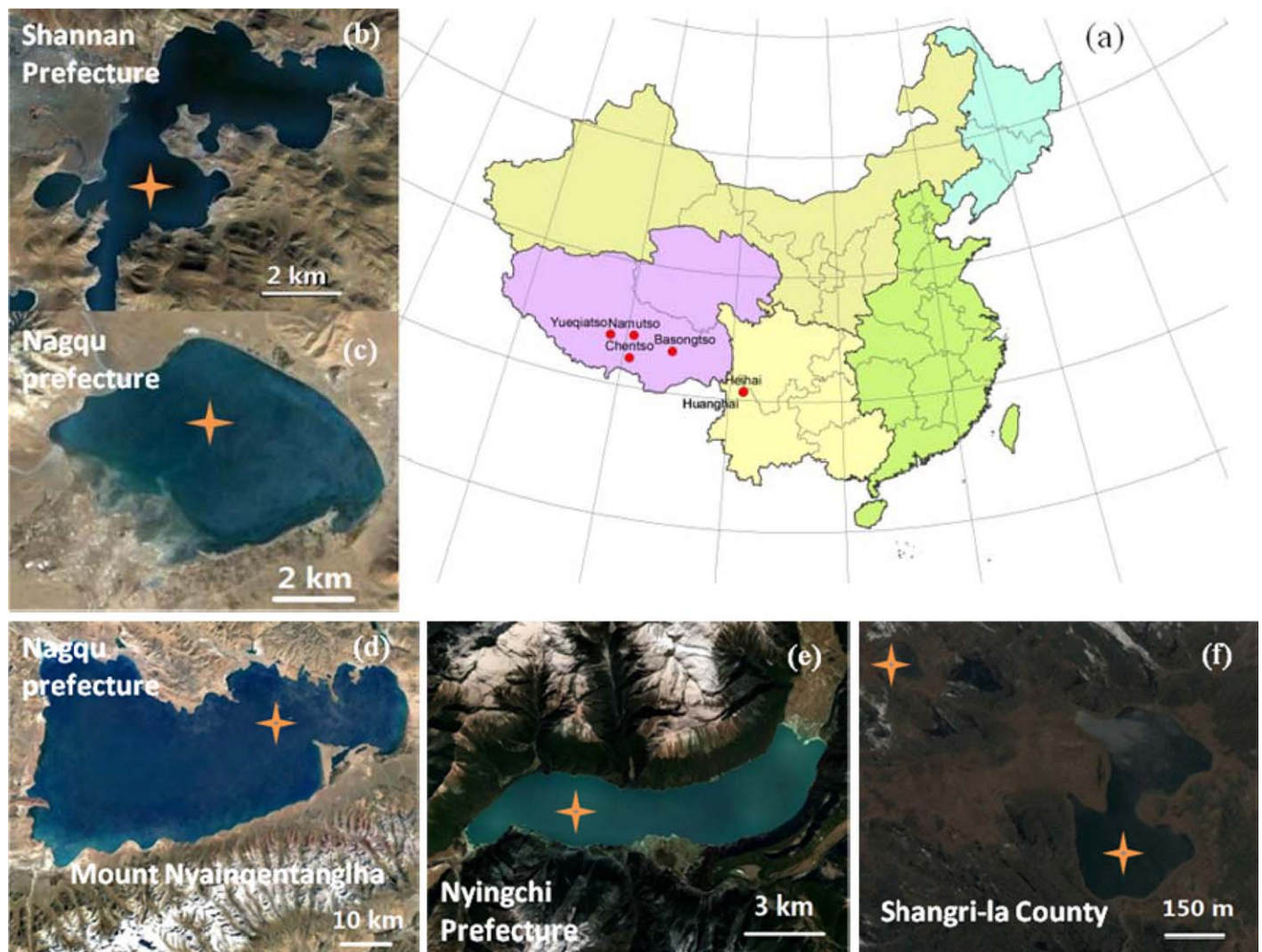
Fig. 1. Maps of study areas (**a**) and approximate location of sampling sites in Lake Chentso (**b**), Lake Yueqiatso (**c**), Lake Namtso (**d**), Lake Basongtso (**e**), Lake Heihai, and Lake Huanghai (**f**).

characteristics of the lakes and their inflowing streams are presented in Supporting Information Table S1.

Sample collection

During May and August 2015 water from six lakes (0–8 m mixed water) and inflowing glacier streams (surface water) were sampled with Niskin samplers and transferred to 5 L acid-washed polypropylene plastic bottles. The samples were transported quickly back to the lab where they were immediately filtered for further analysis. We collected zooplankton—including calanoid copepod and cladoceran—aquatic plants, and ambient terrestrial plants. The taxon classification is presented in Supporting Information Table S2.

Zooplankton were collected by vertical tows through the water column (0–8 m) using 140 μm plankton nets and left for 6 h in distilled water to void their guts. Subdivision and

selection of the studied genera of zooplankton were performed using a binocular microscope. Dominant aquatic and terrestrial plants were hand collected and cleaned with deionized water to remove detritus. Shore-side soil could be washed into the lake during rainy season; therefore, ambient surface soils within 5 m of the shore adjacent to the inflowing streams were sampled with a shovel to analyze the source of old carbon.

POC samples were obtained by filtering water (0–8 m for lakes and surface water for inflowing streams, respectively) through a 0.7 μm Whatman GF/F filter precombusted at 450°C for 4 h. The samples were placed in precombusted aluminum foil and stored at − 20°C. A 1-L subsample of the filtered water was prepared for ^{13}C and ^{14}C analysis of DIC and stored in pre-cleaned (10% HCl and deionized water) gastight polypropylene Nalgene bottles with zero headspace

and stabilized with addition of 100 μL saturated $HgCl_2$ solution (Fellman et al. 2015). DOC samples were obtained from the filtered water after being acidified to pH = 2 with HCl and stored in amber glass bottles at $-20°C$. A 50-mL DOC sample was used for measuring stable carbon isotopes, while a 2-L DOC sample was freeze dried to obtain powdery sample, and then oxidized by CuO to CO_2 and the CO_2 was analyzed for measuring radiocarbon isotope of DOC. All particulate samples were freeze dried before analysis.

Analyses

Water quality parameters

Lake water temperature was recorded using a multi-parameter water quality measuring instrument (YSI 556 MPS). Total nitrogen (TN) was determined by alkaline potassium persulphate digestion of the sample followed by ultraviolet spectrophotometry. Samples for total phosphorus (TP) were digested with potassium persulfate and all the forms of phosphorus were oxidized to orthophosphate under neutral conditions. Orthophosphate concentration was quantified using the molybdenum blue method. The ratio of dissolved organic carbon to nitrogen (DOC/DON) was quantified using an elemental analyzer. Transparency was measured with a Secchi disk. Glacier area was determined based on the second Chinese Glacier Inventory, and the distance between lake and glacier terminus was determined from large numbers of aerial photographs, large-scale topographic maps, and remote sensing images.

Radiocarbon and stable carbon isotope analysis

All the solid samples were sulfurous acid fumed to remove inorganic carbon prior to stable isotope radiocarbon analysis. The stable isotope ($\delta^{13}C$) composition of samples was measured using a DeltaPlus Advantage mass spectrometer (Finnigan MAT) connected to a Flash EA1112 elemental analyzer. The $\delta^{13}C$ is calculated as parts-per-thousand (‰) deviations from international standards (Vienna PeeDee Belemnite for $\delta^{13}C$):

$$\delta^{13}C\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 10^3$$

where, $R = {}^{13}C/{}^{12}C$. The analytical error ranged within ± 0.1‰. Radiocarbon isotope ($\Delta^{14}C$) measurements were conducted by the accelerator mass spectrometry facility at the Beta Analytic Radiocarbon Dating Laboratory (Miami, Florida) and Xi'an Accelerator Mass Spectrometry Center (Xi'an, China). The $\Delta^{14}C$ value is calculated according to the following equation:

$$\Delta^{14}C\ (‰) = \delta^{14}C - 2(\delta^{13}C + 25)\ (1 + \delta^{14}C/1000)$$
$$\delta^{14}C\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 10^3$$

where, $R = {}^{14}C/{}^{12}C$ and $R_{standard}$ are obtained from the ^{14}C activity of oxalic acid II. The $\Delta^{14}C$ value is corrected for isotopic fractionation since $\delta^{13}C$ is in the definition. The analytical precision of $\Delta^{14}C$ analyses averaged ± 3.7‰. The $\Delta^{14}C$

values of aquatic plants were obtained from plant leaves. The $\Delta^{14}C$ of DIC was applied as a substitute for that of phytoplankton because the $\Delta^{14}C$ of algae biomass production tracks in situ DIC (Zigah et al. 2011, 2012a). The $\delta^{13}C$ value of phytoplankton in lakes was estimated using phospholipid fatty acids (PLFAs) biomarkers of phytoplankton groups. PLFAs were extracted from riverine and lake POC samples by a modified Bligh and Dyer procedure in a dichloromethane (DCM)–methanol (MeOH)–phosphate (5 : 10 : 4 v/v/v) solution and separated by silica gel column chromatography (Dickson et al. 2009). PLFAs were derivatized by mild alkaline transmethylation to generate fatty acid methyl esters (FAMEs) (Boschker et al. 2005). An internal FAME (19 : 0) standard was concurrently added to the extracts. Gas chromatography–mass spectrometry (GC-MS) (Thermo Finnigan Trace) was applied to identify compounds, based on retention times and mass spectra. The $\delta^{13}C$ of individual FAMEs was determined by GC combustion isotope ratio mass spectrometry (GC-c-IRMS, using a Trace GC Ultra GC apparatus, Thermo Finnigan). The Trace GC Ultra was fitted with an Agilent HP-5 column (50 m, 0.2 mm i.d.). Identification of peaks was based on retention times. In this study, PLFAs ($18:3\omega3$ and/or $20:5\omega3$) were attributed to algae in lakes on the Tibetan Plateau. Their concentration-weighted $\delta^{13}C$ was corrected for an offset (+ 3‰) between the $\delta^{13}C$ of PLFA and that of the total cell, and then used as a proxy for the $\delta^{13}C$ of phytoplankton (Middelburg 2014).

Isotope modeling and statistical analysis

An isotope mixing model using $\delta^{13}C$ and $\Delta^{14}C$ values and a Bayesian approach (R package SIAR (Parnell et al. 2008) was used to estimate the relative contribution of different potential carbon sources. This isotope-mixing model can cope with strong variation, yield robust estimates of diets, and provide a frequency distribution of the contribution of aged carbon to zooplankton (Parnell et al. 2010; Bellamy et al. 2017). Our modeling is based on natural abundance stable carbon and radiocarbon isotopes, and the endmembers vary from conventional sources used in natural abundance stable isotope modeling (Zigah et al. 2012b).

The potential carbon sources of autochthonous (phytoplankton and aquatic plants) and allochthonous (terrestrial plants and OC from inflowing glacier streams) input were chosen as endmembers. We acknowledge that POC from inflowing rivers is probably a mixture of algae, detritus, soil, material from glacier, etc. Similarly, riverine DOC has multiple sources. In this study, OC from inflowing glacier streams comprises a true aged fraction and an apparently ancient fraction from phytoplankton and aquatic plants that have fixed ^{14}C-depleted DIC in lake water through photosynthesis.

To elucidate the robustness of our findings, we explored two alternative models to test the effect of including soils in the analysis. One model involved the use of soil data rather than POC and another included both POC and soil data. The results of the first alternative showed that the

contribution of soils to zooplankton varied from 0% to 2.5% in six studied lakes, which is much lower than the contribution of riverine POC.

The second alternative model did not yield feasible solutions for some lakes, e.g., Lakes Chentso and Namtso. We infer that it is because the vast majority of DOC and POC released from soils has been transferred into rivers, hence the contribution of riverine POC and DOC to lake food webs implicitly includes soil-derived organic matter (Mann et al. 2015).

One mixing model was run for each individual lake and only sources from each respective site were used in each model run. Individual plant taxa were lumped into either the category terrestrial or aquatic plants, because these taxa showed similar δ^{13}C and Δ^{14}C values, hence, their respective mean values were used to run the mixing models.

Statistical Program for Social Sciences (SPSS) 16.0 software was used for statistical analysis. The significance of differences in the contribution of aged carbon between lakes in temperate and cold glacier areas was examined with an independent samples *t*-test using a *p* value of 0.05. Linear regression analysis was applied to assess the correlation between lake and drainage basin parameters and the contribution of riverine OC to zooplankton. The main drainage basin parameters and the biogeochemical properties of OC include the lake recharge coefficient, DOC/DON, Δ^{14}C of riverine OC, and distance between lake and glacier terminus.

Results

Stable carbon isotopes

We concentrated on five potential carbon sources for zooplankton: modern terrestrial plants, POC and DOC from inflowing rivers (POC_{inflow} and DOC_{inflow}), phytoplankton, and aquatic plants within the lakes. Among these sources, modern terrestrial plants had relatively uniform δ^{13}C values among different sampling sites (– 28.4‰ to – 25.9‰; Fig. 2a).

POC_{inflow} and DOC_{inflow} had δ^{13}C values from – 29.8‰ to – 11.3‰ and from – 29.1‰ to – 23.3‰, respectively (Fig. 2a). The most enriched δ^{13}C value (– 11.3‰) occurred in the POC_{inflow} for Lake Chentso, resembling the value of soil from the riverbank (– 12.3‰; Supporting Information Table S1), which may be attributed to permafrost thawing and ground collapse along the riverbank of Lake Chentso observed during sampling. In addition, the ratio of DOC/DON (C/N) in the inflowing rivers varied from 7.2 (Huanghai) to 8.3 (Basongtso) (Supporting Information Table S1).

POC within the lakes (POC_{lake}) had δ^{13}C values ranging from – 26.7‰ to – 24.2‰ (Supporting Information Fig. S1). DOC within the lakes (DOC_{lake}) had δ^{13}C values ranging from – 28.8‰ to – 22.2‰, which were slightly enriched compared with those of DOC_{inflow}. The δ^{13}C values of POC_{lake} and DOC_{lake} from the lakes Heihai and Huanghai in the

Yunnan province were generally more depleted than those in the Tibetan lakes.

Phytoplankton δ^{13}C values ranged from – 28‰ to – 22.7‰. DIC in the lakes had δ^{13}C values ranging between – 10.2‰ and 3.0‰ (Supporting Information Table S1). Consequently, phytoplankton fractionation calculated following Finlay (2004) was estimated to range from – 27.6‰ to – 22.3‰ (Fig. 2a). Aquatic plants δ^{13}C values varied from – 27.7‰ to – 8.9‰.

Interestingly, zooplankton had more depleted δ^{13}C values (– 37.7‰ to – 26.3‰) than their potential sources (Fig. 2a). This is particularly remarkable for Lake Heihai (– 38.2‰ for copepods and – 37.1‰ for cladocerans) and Huanghai (– 31.7‰ for copepods and – 33.0‰ for cladocerans), being located closer to a glacier than the four Tibetan lakes. Zooplankton in the Tibetan lakes had similar δ^{13}C values, ranging from – 28.9‰ to – 26.3‰ for copepods and from – 28.8‰ to – 27.8‰ for cladocerans.

Radiocarbon isotopes

Unsurprisingly, alpine terrestrial plants showed a positive Δ^{14}C value (26.2–38.9‰) (Fig. 2b). POC_{inflow}, however, had strongly depleted Δ^{14}C values that ranged from – 568.8‰ (Chentso) to – 114.1‰ (Huanghai), corresponding to conventional radiocarbon ages of 910 ybp and 6757 ybp. The lowest Δ^{14}C value of POC_{inflow} occurred in the inflowing river to Lake Chentso, close to Δ^{14}C value of soils along the river bank (Supporting Information Table S1), likely reflecting severe erosion of the river bank. The Δ^{14}C values of DOC_{inflow} varied from – 163.2‰ (Huanghai) to – 48.9‰ (Basongtso).

The Δ^{14}C values of POC_{lake} ranged from – 249.3‰ to – 98.5‰ (Supporting Information Fig. S1). Lakes Heihai and Huanghai were the least depleted in radiocarbon, whereas POC_{lake} for the four Tibetan lakes showed similar depleted Δ^{14}C values (mean value – 234.1‰). The Δ^{14}C value of DOC_{lake} varied from – 159.2‰ to – 70.2‰. The Δ^{14}C values of DIC (proxy for phytoplankton) and aquatic plants also exhibited depleted ^{14}C signatures (– 265.9‰ to – 36.1‰ and – 251.4‰ to – 82.0‰, respectively) with relatively high values in Lake Namtso and low values in Lakes Basongtso and Yueqiatso (Fig. 2b).

Zooplankton had depleted Δ^{14}C values of – 45.3‰ to – 293.6‰ corresponding to radiocarbon ages from 310 ybp to 2392 ybp (Fig. 2b). Like phytoplankton and aquatic plants, the highest value appeared in Lake Namtso (– 45.3‰ for copepods) and the lowest in Lakes Basongtso (– 289.6‰ for copepods) and Yueqiatso (– 220.4‰ for copepods and – 218.5‰ for cladocerans). In Lake Chentso, copepods and cladocerans had Δ^{14}C values of – 81.9‰ and – 89.0‰, respectively. In addition, similar Δ^{14}C values for copepods (– 117.4‰ in Huanghai and – 118.5‰ in Heihai) and for cladocerans (– 127.2‰ in Huanghai and – 120.6‰ in Heihai) were found. Overall, the Δ^{14}C values of zooplankton are
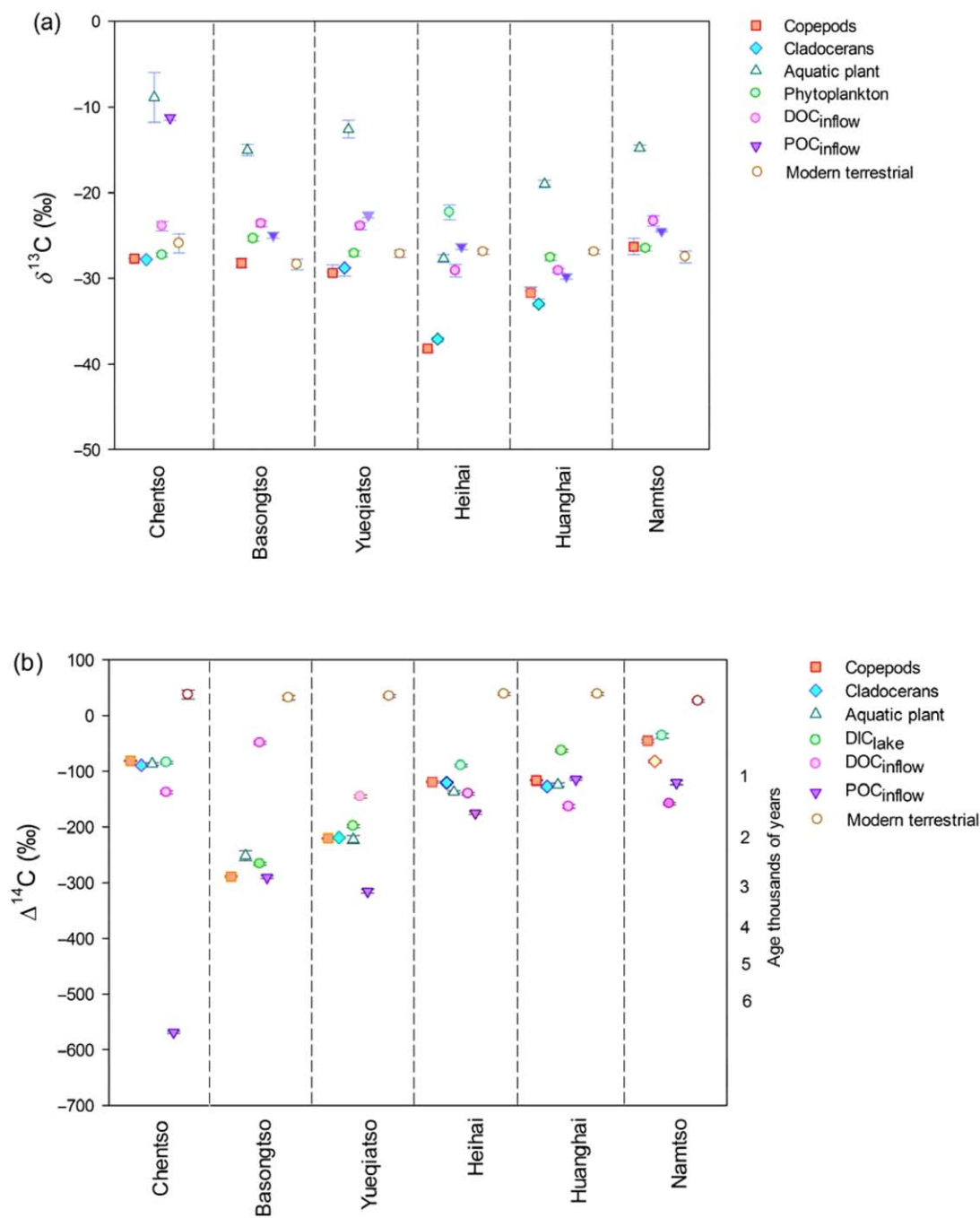
Fig. 2. Isotope plots of δ13C (**a**) and Δ14C and conventional 14C age (**b**) for zooplankton (copepods and cladocerans) and their potential organic matter sources in the studied lakes. DOCinflow and POCinflow represent dissolved and POC in inflowing stream, respectively. DIClake represents DIC in lake and the Δ14C of DIClake is applied as a substitute for that of phytoplankton. Data of aquatic and terrestrial plants in Lake Namtso are from Hu et al. (2016).

intermediate between those of terrestrial plants and other potential carbon sources.

Contribution of potential carbon sources to zooplankton

The Bayesian isotopic mixing model exercise based on $\delta^{13}C$ and $\Delta^{14}C$ values showed that the contribution of

phytoplankton and aquatic plant-derived OC to the carbon source for zooplankton in the six lakes varied from 4.1% to 29.1% and 17.6% to 30.0%, respectively (Table 1). Contemporary terrestrial plants were important in Lakes Chentso and Namtso (24.9–32.6%) but less important (1.5–9.7%) in Lakes Basongtso, Heihai, Huanghai, and Yueqiatso. On

Table 1. Bayesian isotopic mixing model results showing the percentage contribution of five organic matter sources to zooplankton for six studied lakes, based on δ^{13}C and Δ^{14}C values. The results include potential sources shown as the mean and (1–99) percentiles.

Lake	Zooplankton species	Phytoplankton	Aquatic plant	Terrestrial plant	Riverine POC	Riverine DOC
Chentso	Copepods	25.2 (12–28)	24.7 (10–25)	28.3 (18–30)	4.6 (4–10)	17.2 (6–20)
	Cladocerans	25.9 (13–29)	25.5 (14–29)	24.9 (14–28)	5.1 (4–11)	18.6 (5–21)
Yueqiatso	Copepods	23.4 (11–26)	27.8 (13–28)	7.5 (3–12)	25.5 (16–30)	15.9 (8–23)
	Cladocerans	20.6 (10–22)	27.2 (13–28)	6.9 (4–12)	30.7 (18–32)	14.6 (6–22)
Namtso	Copepods	28.0 (14–39)	17.6 (10–21)	32.6 (19–43)	12.2 (8–26)	9.6 (8–20)
Basongtso	Copepods	29.1 (16–35)	18.7 (16–23)	1.9 (0–3)	47.7 (35–62)	2.7 (0–5)
Huanghai	Copepods	8.0 (3–9)	30.0 (12–33)	3.2 (0–5)	23.3 (12–27)	35.6 (29–55)
	Cladocerans	4.1 (3–6)	22.0 (10–28)	1.5 (0–3)	13.4 (12–20)	58.9 (40–75)
Heihai	Copepods	21.4 (11–28)	24.2 (12–29)	9.7 (6–15)	20.8 (15–25)	24.0 (13–29)
	Cladocerans	20.3 (12–26)	24.7 (13–29)	8.9 (6–14)	21.6 (16–26)	24.5 (13–28)

average, POC$_{inflow}$ contributed 4.9% (Chentso, 4.6% for copepods and 5.1% for cladocerans) to 47.7% (Basongtso, copepods) and DOC$_{inflow}$ 2.7% (Basongtso) to 47.3% (Huanghai, 35.6% for copepods and 58.9% for cladocerans) to the carbon source for zooplankton. Generally, the contribution of upstream riverine carbon (POC$_{inflow}$ and DOC$_{inflow}$) to zooplankton in Lakes Basongtso, Heihai, and Huanghai, situated in the temperate glacier area, was significantly higher (mean value 53.8%) than in the other three lakes in the continental climate area having a colder climate (mean value 29.3%, $p < 0.001$).

Discussion

Our findings clearly showed that in all studied lakes on the Tibetan Plateau, both modern and ^{14}C-depleted OC resources were present and assimilated by zooplankton. Moreover, zooplankton use of ancient OC differed among the lakes.

Radiocarbon-depleted zooplankton

There are two major pathways toward radiocarbon-depleted zooplankton: (1) through the consumption of ancient OC originating from the glaciated watershed as well as (2) through the consumption of contemporary OC that was produced locally using a ^{14}C-depleted DIC reservoir (Ishikawa et al. 2014). The DIC pool may become radiocarbon depleted, either because of dissolution of limestone in the bedrock and soils of the watershed, or because of a significant contribution of carbon dioxide generated by respiration of ancient OC from the glaciated watershed. Algae and aquatic plants can fix ^{14}C-depleted DIC in lake water via photosynthesis and thus exhibit a ^{14}C-depleted signature and appear ancient despite their contemporary source. In this way, ancient DIC can be incorporated into the food-web

carbon cycling via primary production and trophic transfer (Raymond et al. 2004; Ishikawa et al. 2014).

The two sources can be partly disentangled by using the combined ^{14}C and ^{13}C signatures of DIC. For Lakes Heihai, Huanghai, and Namtso, the depleted Δ^{14}C and negative δ^{13}C signatures of DIC suggest that the ^{14}C-depleted DIC mainly derives from heterotrophic respiration of ancient OC. In contrast, the depleted Δ^{14}C combined with a δ^{13}C-DIC of $\sim$ 0‰ for Lakes Chentso, Basongtso, and Yueqiatso likely reflects a substantial contribution of limestone bedrock weathering within the lake watershed. Since many lakes on the Tibetan Plateau show lake reservoir effects regarding ^{14}C of DIC (Hou et al. 2012; Mischke et al. 2013), the apparent ages of organic matter and organisms in these lakes cannot directly be linked to assimilation of ancient, external OC. However, through the combination of ^{13}C and ^{14}C data on OC sources and consumers with an isotope mixing model, we can nevertheless elucidate the contribution of external, ancient OC to zooplankton diets.

The estimated 21.8–72.3% contribution of riverine carbon (the sum of POC$_{inflow}$ and DOC$_{inflow}$) to zooplankton diets supports our hypothesis that bioavailable OC released from glacial and/or permafrost ecosystems is an important resource for the zooplankton food web. POC and DOC from glacial runoff are readily metabolized, not only at low-trophic levels (Hood et al. 2009; Singer et al. 2012) but also at higher trophic levels in downstream lakes (Fellman et al. 2015). As previously mentioned, the apparent ages of lake OC are influenced by various sources, and our results may not fully elucidate the uptake pathway of the ancient fraction by zooplankton. However, they provide strong evidence that OC from glacial and/or permafrost ecosystems have the potential to support the downstream ecosystem productivity in lakes. Ancient POC$_{inflow}$ may be directly ingested by some cladocerans and copepods (Cottingham 1999; Caraco et al.

2010). Besides, ancient DOC_{inflow} may sustain heterotrophic microbial production, and heterotrophic microbes are subsequently used by the zooplankton. Cherrier et al. (1999) found that oceanic bacteria assimilated a fraction of old DOC components in the eastern North Pacific. Likewise, ancient DOC in northern temperate lakes and streams can be respired and converted to POC by bacteria (McCallister and Del Giorgio 2012).

The $\Delta^{14}C$ signatures of zooplankton in Lakes Basongtso, Heihai, and Hunghai $\Delta^{14}C$ values appeared to track those of POC_{inflow} or DOC_{inflow}, in contrast, zooplankton in Lakes Chentso, Yueqiatso, and Namtso exhibited $\Delta^{14}C$ signatures rather similar to those of DIC. These results may be interpreted that phytoplankton dominated carbon supply to zooplankton in Lakes Chentso, Yueqiatso, and Namtso. However, it was evident that zooplankton in the six studied lakes had more ^{14}C-depleted signatures (with an exception of copepods in Lake Chentso) relative to phytoplankton (based on DIC $\Delta^{14}C$) and modern terrestrial plant. This suggests that zooplankton should assimilate at least one OC sources that is more ^{14}C depleted than phytoplankton. The summed contribution of POC_{inflow} and DOC_{inflow} to zooplankton in Lakes Chentso, Yueqiatso, and Namtso reached as much as 21.8–45.3% (Table 1).

The large portion of ^{14}C-depleted DOC_{inflow} assimilated in consumer tissues in Lakes Chentso, Heihai, and Huanghai might reflect higher bioavailability of DOC_{inflow} than of POC_{inflow} in these three ecosystems. The high bioavailability of DOC_{inflow} in this work is comparable with that recorded in samples from Alaska and the European Alps (Hood et al. 2009; Singer et al. 2012). Fellman et al. (2015) reported that ancient DOC was more likely to enter the diet of consumers than POC in proglacial stream food webs in the upper Herbert River, located close to Juneau in coastal southeast Alaska. This is probably because riverine POC in some watersheds to a large extent is derived from glacier sedimentary rock material with large particle size, which is regarded as an unpalatable food source for consumers (Clitherow et al. 2013; Fellman et al. 2015). In the Hudson River (USA) zooplankton had a more ^{14}C-depleted signature than "average" POC, but similar $\Delta^{14}C$ values as POC with smaller particle size, which is likely because large particles ($> 35 \mu m$) are unpalatable to the zooplankton (Caraco et al. 2010). However, the greater contribution of ancient POC_{inflow} than DOC_{inflow} incorporated in zooplankton in Lake Basongtso may be partly ascribed to the absolute dominance of copepods, which usually favor larger particles than cladocerans (Cottingham 1999).

It is interesting to note that the $\delta^{13}C$ value (– 11.3‰) of POC_{inflow} for Lake Chentso resembled the value of soils from the riverbank (– 12.3‰), which is also close to the value of the C_4 plants. Generally, C_3 and C_4 plants have $\delta^{13}C$ values ranging from – 35‰ to – 22‰ and from – 19‰ to – 9‰, respectively (Cifuentes and Salata 2001). The C_4 species are mainly distributed below ~ 3000 m as extremely low temperature at high altitudes restrains the photosynthesis process of C_4 species (Tieszen et al. 1979; Zhang et al. 2013).
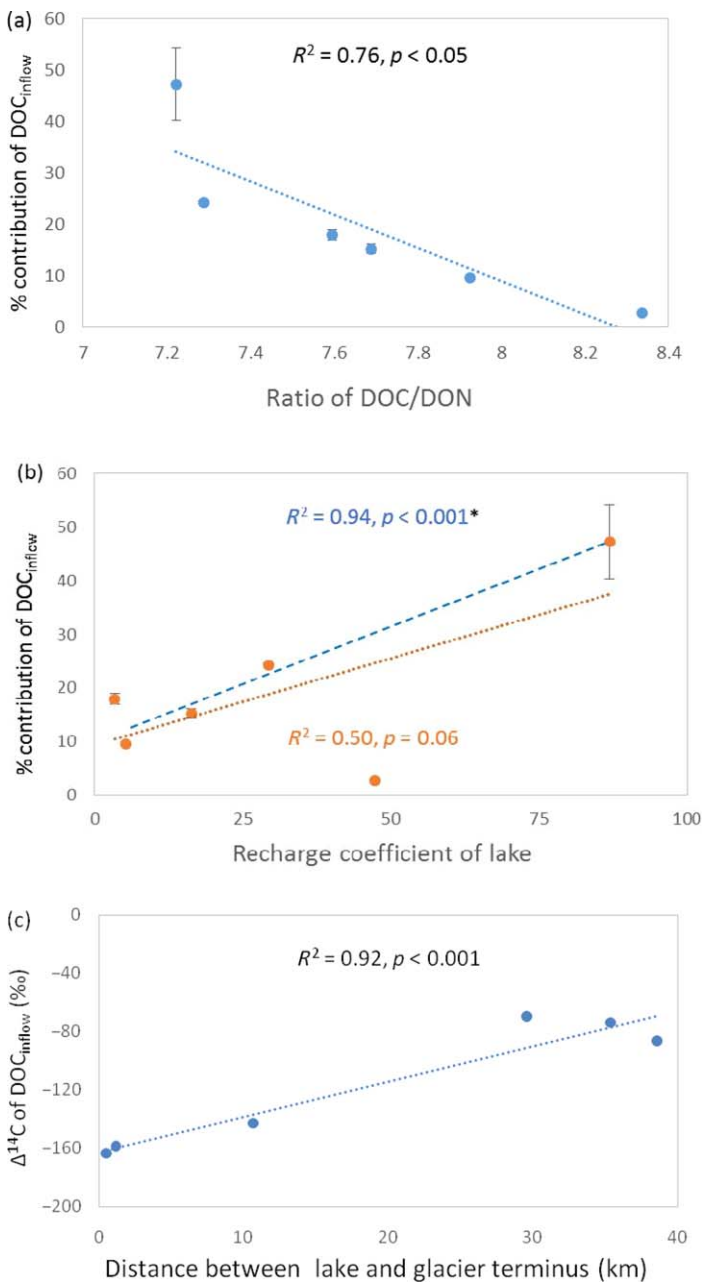
Drainage basin and lake characteristics mediate the contribution of carbon sources to zooplankton

The contribution of ancient riverine OC_{inflow} is likely governed by different geographic and lacustrine variables across the watershed. Among the investigated variables, the C/N ratio in the inflowing rivers on the Tibetan Plateau showed a strong negative correlation with the contribution of DOC_{inflow} to the food sources of zooplankton ($R^2 = 0.76$, $p < 0.05$; Fig. 3a). A low C/N ratio corresponds to high bioavailability of dissolved organic matter (Thurman 1985; Wiegner et al. 2006). These findings provide evidence that the most bioavailable fraction of DOC could be derived from pre-aged DOC as well as young DOC. Our results contradict the prevalent view that the most bioavailable fraction of DOC_{inflow} is dominated by young, freshly produced DOC (Spencer et al. 2014). Despite that the apparent ages of lake OC can be affected by a mixture of ^{14}C-depleted OC sources, the relatively old ages of the Tibetan lake DOC are primarily caused by inputs of pre-aged DOC with glacial runoff (Spencer et al. 2014).

The recharge coefficient of the lake, i.e., the ratio of watershed area to lake surface area, provides information on potential terrestrial input to the lake. A higher recharge coefficient of the lake probably implies that more old DOC can be delivered to the lakes with runoff and thus accounts for the higher contribution of DOC_{inflow} to zooplankton ($R^2 = 0.50$, $p = 0.06$; Fig. 3b). Zigah et al. (2012b) compared the carbon sources of zooplankton in the Hudson River, five small lakes in southern Quebec and different sites in the North Pacific Ocean to the carbon resources for zooplankton in Lake Superior. They found that the difference between $\Delta^{14}C_{Zoop}$ and $\Delta^{14}C_{DIC}$ increases with the recharge coefficient of the lake, and thus concluded that the contribution of phytoplankton to zooplankton decreased with increasing recharge coefficient of the lake (Zigah et al. 2012b). Note that in the recharge coefficient-DOC_{inflow} contribution correlation plot (Fig. 3b), the contribution of DOC_{inflow} to five lakes except for Basongtso is above the general trend line (orange dotted line) and has a slightly steeper slope relative to the recharge coefficient (blue dotted line; $R^2 = 0.94$, $p < 0.001$). Lake Basongtso is surrounded by thick pinewood, evergreen broad-leaf and spruce forest, and the glacier tongue even extends to forests. Therefore, this result suggests that the recharge coefficient of a lake should be more efficient in supplying bioavailable DOC_{inflow} to the watersheds with less forest coverage.

The lake and watershed areas were highly variable among the studied lakes. Lakes Chentso, Yuequiatso, and Namtso in the colder climate were on average larger than the other three lakes in the temperate climate (39.1–1982 km^2 vs.

Fig. 3. Drainage basin and lake characteristics mediate the contribution of carbon sources to zooplankton: ratio of DOC/DON with DOCinflow contribution (**a**), recharge coefficient of lake with DOCinflow contribution (**b**), and Δ14C of DOCinflow with the distance from glacier terminus to lake (**c**). The blue dotted line in (b) means linear correlation for the data of five lakes except for Lake Basongtso ($p < 0.001$, $R2 = 0.94$). DON. Recharge coefficient of lake = watershed/lake surface area. The contribution of carbon sources to zooplankton was the mean value calculated from different species of zooplankton in respective lake. Further statistical analyses are shown in Supporting Information Fig. S2. [Color figure can be viewed at wileyonlinelibrary.com]

0.01–25.5 km^2). However, no strong correlation was found between lake area and the contribution of either POC$_{inflow}$ ($R^2 = 0.05$, $p > 0.5$) or DOC$_{inflow}$ ($R^2 = 0.09$, $p > 0.4$) to

zooplankton (Supporting Information Fig. S2). Our riverine POC and DOC end-members are usually based on one or two streams for each lake and in a large watershed this introduces uncertainty and potentially bias. For instance, there are more than six inflowing rivers for Lake Namtso watershed but we only sampled one eastern stream. Moreover, lakes were samples at one location and large lakes may have spatial gradients. Our sampling strategy was, however, designed to limit potential bias due to spatial heterogeneity. For instance, the sampling site in Lake Chentso was chosen far from the estuary of the sole inflowing river, and the sampling in Lake Yueqiatso was conducted in the open waters near to the middle of the lake. The calculated contribution of aged OC$_{inflow}$ to zooplankton was relatively high in Lakes Basongtso and Yueqiatso having relatively large lake areas. For these reasons, we anticipate that the recharge coefficient of the lake rather than lake area better explains the contribution of aged carbon to zooplankton.

Furthermore, the Δ^{14}C values of DOC$_{inflow}$ are significantly related to the distance from glacier terminus to lake (the length of the inflowing stream) ($R^2 = 0.92$, $p < 0.001$; Fig. 3c). Shorter stream transit times implies that DOC carried by streams to lakes tends to be more bioavailable and contributes significantly to the sustenance of bacterial carbon biomass with less biological or photochemical alteration (Hood et al. 2009). For instance, Lakes Heihai and Huanghai are close to glacier terminus (0.53 km for Huanghai and 1.15 km for Heihai); melt water from snow and ice sheet flows into these lakes with short stream transit times. This perhaps leads to more ^{14}C-depleted signatures of zooplankton through utilizing ^{14}C-depleted bacterial carbon (McCallister et al. 2004; Caraco et al. 2010).

Trends discussed in this work provide a broad insight into ecosystem functioning across the ratio of watershed area to lake area gradient in Tibetan Plateau lakes. More data are critically needed to elucidate the relationship between the recharge coefficient of the lake and zooplankton ecology on a wider scale.

Implications for metazoan food webs of lakes

Widespread glacier recession for several decades has affected the runoff from the Tibetan Plateau and polar regions. Glacial meltwater runoff increased, on average, from 5.2×10^8 m^3 in the 1960s to 795×10^8 m^3 in the 2000s in China (Ren et al. 2011). During 1976–2009, the lake area of Namtso increased by 80.7 km^2 and the lake water volume increased by 84.2×10^8 m^3 (Zhang et al. 2011). Yao et al. (2010) estimated that the negative glacier mass balance accounted for over 1.5 m of the 3 m elevated water level of lakes in the Yarlung Tsangpo River catchment. However, glacier volume loss will reduce downstream runoff in the long term (Yao et al. 2012; Aiken et al. 2014), which may lead to a reduced proportion of bioavailable OC entering the streams.

Our findings suggest that OC released from glacial and permafrost ecosystems can contribute to the carbon sources of metazoan zooplankton in downstream lakes that receive glacier runoff and permafrost meltwater. Moreover, the summed contribution of POC_{inflow} and DOC_{inflow} to zooplankton in the lakes in the temperate glacier area was significantly higher than in the lakes in cold glacier area ($p < 0.001$). We infer that it is mainly because the influence of glacier retreat and permafrost thaw on OC input is more prominent in the temperate glacier area than in the cold glacier area due to larger amplitude of glacier and permafrost change in the former (Yao et al. 2013). However, it remains a challenge to predict how the magnitude of bioavailable OC influences the assimilation of ancient OC by zooplankton during an ecosystems shift.

Conclusions

Our results provide evidence that ancient OC is ubiquitous in the glaciated aquatic ecosystems and that glacier OC could be a driver of secondary production via consumption of ancient OC originating from the glaciated watershed. However, metazoan zooplankton may also appear ancient because of consumption of contemporary organic matter that inherited its ^{14}C-depletion from DIC. Assimilation of ancient OC by zooplankton and higher trophic level consumers in lakes is not only affected by drainage basin characteristics but also by the biogeochemical properties of OC. The influence of glacier retreat and permafrost thaw on OC input is more prominent in the temperate glacier area than in the cold glacier area. In this context, the food-web structure in the temperate glacier area will be mainly sustained by contemporary primary production instead of ancient OC in the long term. This may have important impacts on the stability and biodiversity of high-altitude and polar lake ecosystems. Though ancient OC has been shown to play a role for aquatic ecosystems, the processes and the fate are poorly understood. Elucidation of quantitative and qualitative incorporation of ancient OC into lake food webs is required to improve predictions of how the aquatic ecosystems will respond to future climate change.

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Acknowledgments

Conflict of Interest

None declared.