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Use of Multi-Carbon Sources by Zooplankton in an Oligotrophic Lake in the Tibetan Plateau

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Abstract: We applied natural abundance stable isotope $\delta^{13}C$ and radiocarbon $\Delta^{14}C$ analyses to investigate trophic linkages between zooplankton and their potential food sources (phytoplankton, submersed plants, and allochthonous organic carbon) in Lake Nam Co, one of the largest oligosaline and oligotrophic lakes in the Tibetan Plateau, in south-west China. The $\delta^{13}C$ and $\Delta^{14}C$ levels of the calanoid copepod *Arctodiaptomus altissimus pectinatus* indicate that it uses different carbon sources. Thus, based on a two-isotope mixing model, our results suggested that recently synthesized but ^{14}C-depleted primary producers (phytoplankton and submersed plants) were the most important sources of carbon, together contributing 92.2% of the zooplankton biomass. Allochthonous organic carbon and dissolved organic carbon constituted 4.7% and 3.1% of the carbon in the diet of zooplankton, respectively. Our findings from Lake Nam Co suggest that the carbon in the food webs of lakes located in a glaciated environment originates from various sources of different ages.

Keywords: zooplankton; carbon sources; stable carbon isotope; radiocarbon isotope; Lake Nam Co; Tibetan Plateau

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1. Introduction

Zooplankton have significant impacts on the flow of material and energy in aquatic ecosystems and constitute important links in lake food webs between primary producers, microorganisms, and higher level consumers [1,2]. Traditionally, zooplankton are considered to mainly feed on organic matter derived from contemporary photosynthesis because of its lability and palatability. Phytoplankton is the primary food source and is often grazed upon directly by the zooplankton. In some lakes, macrophytes may also support zooplankton production via bacterioplankton and the microbial food web [3]. Recent research has, however, suggested that zooplankton often exploit a wider variety of food sources, including terrestrial organic carbon (t-OC) [4]. Stable isotope analysis and lake ^{13}C labeling techniques have thus demonstrated a significant contribution by t-OC to zooplankton biomass [5–7]. Zooplankton are capable of assimilating allochthonous material of terrestrial origin [8,9], either through consumption of micro-heterotrophic organisms using dissolved organic carbon (DOC) or by direct exploitation of particulate organic carbon (POC) [4,6].

Glaciated environments have dynamic and reactive organic carbon systems [10–12]. Recent studies have revealed that modern glacier runoffs export organic matter to aquatic ecosystems [13–15]. This organic matter is a potentially important carbon source to heterotrophic microbes [16,17], invertebrates [18,19], and some consumers at higher trophic levels such as fish and waterfowl [19,20].

The Tibetan Plateau (TiP) is a source and sink region of some large rivers and lakes. In the past several decades, the impact of climate warming on ecosystems has been more pronounced in TiP than in other regions of the Earth, including phenomena such as increasing/faster retreat of glaciers and a degrading permafrost soil horizon [21,22]. Driven by climate warming, organic materials may be released and transported to the downstream lakes [15]. Given the severe energy limitations in proglacial lakes, t-OC from glaciated watersheds is proposed to be a particularly important cross-ecosystem subsidy that helps buffer energy limitations set by contemporary primary production. Here, we tested the hypothesis that proglacial lake food webs are sustained by t-OC released from glacial ecosystems by using natural abundance stable isotope ($\delta^{13}C$) and radiocarbon ($\Delta^{14}C$) analyses of zooplankton and potential carbon sources in Lake Nam Co, a typical mid-latitude and high-altitude lake in TiP. Moreover, an IsoSource mixing model was used to estimate the relative contribution of potential carbon sources to zooplankton.

2. Materials and Methods

2.1. Study Site Description

Lake Nam Co (Figure 1, Table 1), one of the largest oligosaline and oligotrophic lakes in China, is located in central TiP. It has a surface area of about 1982 km^2 and a water depth exceeding 90 m [23]. It is a closed lake that is mainly supplied by precipitation and glacier melt water from the south-eastern Nyainqen Tanglha Mountains. The lake is a unique habitat and only insignificantly impacted by human activities due to its harsh, high-altitude natural environment characterized by strong radiation and low temperatures.

Table 1. Water characteristics of Lake Nam Co.

Altitude	pH	Total Nitrogen	Total Phosphorus	Chlorophyll-a	Salinity	POC	DOC
4730 m a.s.l.	9.9	0.37 mg/L	0.003 mg/L	0.26 μg/L	0.98 g/L	1.32 mg/L	11.93 mg/L

2.2. Sample Collection

A 1 L water sample was taken from Lake Nam Co and filtered through pre-combusted Whatman GF/F glass fiber filters (450 °C for 4 h; 0.7 μm nominal pore size) for ^{14}C analysis of DIC (Dissolved Inorganic Carbon). The DIC samples were stored in pre-cleaned gas-tight polypropylene Nalgene bottles containing 100 μL saturated $HgCl_2$ solution in the dark at room temperature. POC samples were taken by filtering 10 L of water through pre-combusted Whatman GF/F glass fiber filters for analysis of fatty acids. The filters were subsequently placed in pre-combusted aluminum foil and freeze dried. The submersed plant *Ceratophyllum demersum* was collected by hand and rinsed with deionized water to remove detritus. Zooplankton (the calanoid copepod *Arctodiaptomus altissimus pectinatus*, which was the dominant zooplankton species in the lake) were collected by vertical tows through the water column with a 140 μm plankton net and subsequently left for 6 h in filtered lake water to allow gut evacuation. The zooplankton samples were freeze dried and preserved in pre-combusted aluminum foil or pre-cleaned cryogenic vials for further treatment. To account for the fact that terrestrially produced OC could be delivered to the lake during summer flows, POC samples from one main inflowing (Figure 1) stream were also taken to represent allochthonous carbon sources.

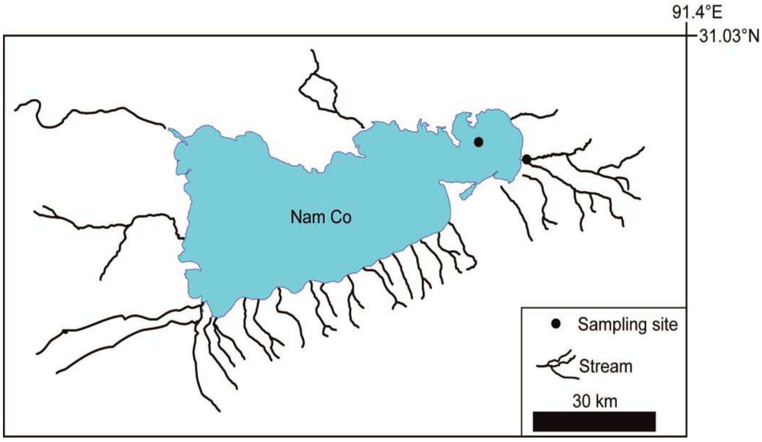

Figure 1. Location of Lake Nam Co and the sampling sites.

2.3. Radiocarbon and Stable Carbon Isotope Analysis

Stable ^{13}C isotope analysis was performed using a DeltaPlus Advantage mass spectrometer (Finnigan MAT) connected to a Flash EA1112 elemental analyzer at Nanjing Institute of Geography and Limnology, Nanjing, China. The stable C isotope value is calculated as δ^{13}C (‰) = $(R_{sample}/R_{standard} - 1) \times 10^3$, i.e., parts-per-thousand (‰) deviations from international standards (PeeDee Belemnite for δ^{13}C), where $R = {}^{13}C/{}^{12}C$. The analytical error between repeated measurements was typically within ±0.1‰ [24].

Radiocarbon ^{14}C isotope measurements were performed by the Accelerator Mass Spectrometry Facility at the Beta Analytic Radiocarbon Dating Laboratory, which is an ISO 17025-accredited radiocarbon dating laboratory in Miami, Florida. The Δ^{14}C value is expressed as $\Delta^{14}C\ (‰) = \delta^{14}C - 2(\delta^{13}C + 25)\ (1 + \delta^{14}C/1000)$, i.e., the deviation in parts per thousand (‰) from the ^{14}C activity of 1950s oxalic acid, where $\delta^{14}C\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 10^3$, $R = {}^{14}C/{}^{12}C$. The analytical precision for ^{14}C analyses averaged ±3.73‰ [25].

We estimated the δ^{13}C value of phytoplankton using phospholipid fatty acids (PLFAs), which represent specific biomarkers for phytoplankton groups [9]. Separation and extraction of main PLFAs from POC samples were performed. In brief, PLFAs were extracted according to 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 [26]. PLFAs were derivatized by mild alkaline transmethylation to form FA methyl esters (FAME) [27]. Concurrently, an internal FAME (19:0) standard was added to the extracts. GC-MS (Gas Chromatography–Mass Spectrometer) was used to identify compounds based on retention times and mass spectra. The δ^{13}C measurements of individual FAMEs of total PLFA biomarkers were analyzed by GC combustion isotope ratio mass spectrometry coupled to a GC (Gas Chromatography) PAL auto-sampler. 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 light of PLFA spectra, PLFAs (20:5ω3 and 22:5ω3) were attributed to algae in Lake Nam Co and their concentration-weighted δ^{13}C was used as a proxy for the δ^{13}C of phytoplankton.

2.4. Isotope Modeling for Multiple Food Sources

Based on δ^{13}C and Δ^{14}C values, the IsoSource model was used to estimate the relative contribution of identifiable sources (autochthonous phytoplankton and submersed plants as well as allochthonous POC and DOC from the inflowing stream) to the biomass of zooplankton in Lake Nam Co. This isotope-mixing model solves iteratively for possible carbon source composition and provides a frequency distribution of the contribution of each carbon source to the consumer [28]. We used tolerance parameters of 1‰–3‰, reflecting the high uncertainty of the sources, no trophic enrichment factor for Δ^{14}C (because of its definition), and a trophic adjustment of 0.4‰ for δ^{13}C [24,25]. The Δ^{14}C of DIC was used as a surrogate for the $\Delta^{14}C$ of phytoplankton since algae biomass production is based on in situ DIC and therefore tracks that of DIC [29–31].

3. Results

3.1. Isotope Signature

The $\delta^{13}C$ of the potential carbon sources ranged from −29.9‰ to −15.0‰, with phytoplankton and the submersed plant *Ceratophyllum demersum* having the lowest and highest values, respectively. Bulk lake POC and POC of the inflowing stream had similar $\delta^{13}C$ values of −25.6‰ and −24.5‰, respectively. The dominant zooplankton species, the calanoid copepod *Arctodiaptomus altissimus pectinatus*, had $\delta^{13}C$ levels within the range of these sources (Table 2).

Table 2. The $\delta^{13}C$ and $\Delta^{14}C$ of the zooplankton *Arctodiaptomus altissimus pectinatus* and its potential carbon sources in Lake Nam Co and its watershed. $\delta^{13}C$ and $\Delta^{14}C$ are given as ‰ deviation from Vienna Pee Dee Belemnite (V-PDB) and oxalic acid II (OX-II), respectively.

Sources	$\delta^{13}C$	$\Delta^{14}C$
Zooplankton	−26.3 ± 0.9	−45.3
Phytoplankton	−29.9	−36.1
Submersed plant	−15.0 ± 0.3	−44.1
POC $_{bulk}$	−25.6 ± 0.6	−247.3
DOC $_{bulk}$ [1]	−22.9	−70.2
POC $_{allo}$	−24.5 ± 1.0	−120.6
DOC $_{allo}$ [1]	−23.3	−158.3
DIC	0.6	−36.1

Notes: [1] Based on the $\delta^{13}C$ and $\Delta^{14}C$ values of DOC from an inflowing stream to Lake Nam Co [15]. POC $_{bulk}$: lake bulk POC; DOC $_{bulk}$: lake bulk DOC; POC $_{allo}$: allochthonous POC; DOC $_{allo}$: allochthonous DOC; DIC: dissolved inorganic carbon.

The submersed plant, with a $\Delta^{14}C$ value of −44.1‰, was consistent with the fixation of ^{14}C depleted dissolved inorganic carbon (DIC) (−36.1‰) in the lake. Lake bulk POC and allochthonous riverine POC had strongly depleted $\Delta^{14}C$ values of −247.3‰ and −120.6‰, respectively. Zooplankton had a negative $\Delta^{14}C$ value of −45.3‰ (Table 2). $\delta^{13}C$ and $\Delta^{14}C$ values of allochthonous riverine DOC of −23.3‰ and −158.3‰, respectively, have previously been reported for Lake Nam Co [15].

3.2. Contribution of Potential Carbon Sources to Zooplankton

The results of the IsoSource mixing modeling based on $\delta^{13}C$ and $\Delta^{14}C$ values are shown in Table 3. In Lake Nam Co, recently synthesized in situ but ^{14}C-depleted primary producers (phytoplankton and submersed plant) emerged as the most important carbon source, constituting 92.2% of the zooplankton biomass. Allochthonous POC made up 4.7% and DOC made up 3.1% of the carbon source.

Table 3. IsoSource modeling results, based on $\Delta^{14}C$ and $\delta^{13}C$ values, showing the proportional % contribution of the four carbon sources to the Lake Nam Co zooplankton given as mean and (1–99) percentiles.

Sources	Proportion (%)
Phytoplankton	73.9 (67–81)
Submersed plant	18.3 (11–25)
POC $_{allo}$	4.7 (0–10)
DOC $_{allo}$	3.1 (0–7)

4. Discussion

The dominant zooplankton in Lake Nam Co, the calanoid copepod *Arctodiaptomus altissimus pectinatus*, had $\delta^{13}C$ and $\Delta^{14}C$ levels indicating its use of multiple-carbon sources (Figure 2). Modeling results suggested that its food sources originated mainly from aquatic primary producers (phytoplankton and submersed plants) supplemented with consumption of a fraction of t-OC originating from the glaciated watershed.

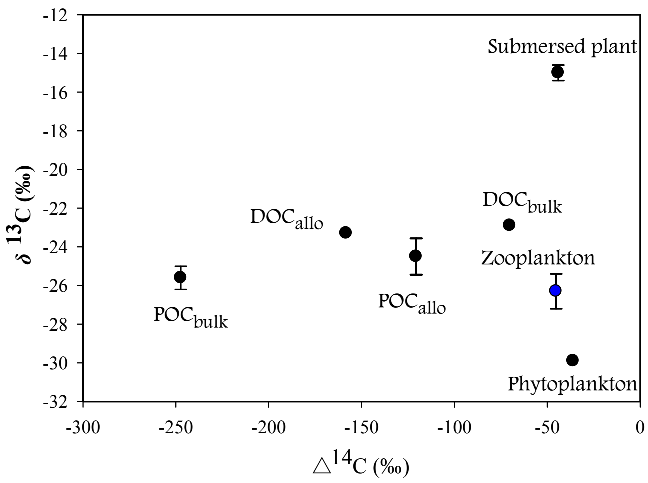

Figure 2. Isotope bi-plots of zooplankton and its potential food sources in Lake Nam Co.

Allochthonous OC, including POC and DOC, contributed to ~7.8% of the carbon in the zooplankton biomass. Allochthonous OC becomes available to zooplankton through direct ingestion of detrital particles or through consumption of micro-heterotrophic organisms that have consumed OC [4,6]. Allochthonous OC (in the inflowing stream) was depleted in Δ^{14}C, indicating that ancient OC was likely released from the glaciated watershed, and zooplankton may become depleted in ^{14}C through the use of ancient OC [25].

Our modeling results also suggested that ~73.9% of the carbon sources incorporated into zooplankton in Lake Nam Co is derived from phytoplankton, supporting the prevailing view that phytoplankton is the preferred food item. It is also known that submersed plants may support zooplankton production via consumption by bacterioplankton and the microbial food web as well as the release of macrophytes detritus [3]. Our isotope mixing calculations estimated that recently synthesized but ^{14}C-depleted *Ceratophyllum demersum* contributed a considerable amount (~18.3%) of the carbon in the diet of zooplankton. Zooplankton might exhibit a ^{14}C-depleted signature via autotrophic carbon pathways because of reservoir effects of ^{14}C-depleted DIC in the watershed: i.e., the presence of radiocarbon-depleted DIC serving as a carbon source for the aquatic primary producers.

Both phytoplankton and submerged vegetation in Lake Nam Co demonstrated a depleted ^{14}C signature. The glacially influenced Lake Nam Co is characterized by a significant radiocarbon reservoir effect as reflected by the ^{14}C-depleted DIC value. The negative Δ^{14}C combined with the δ^{13}C-DIC of ~0‰ might reflect a contribution of limestone bedrock weathering within the lake watershed [32]. DIC from carbonate rock weathering can then be incorporated into the carbon cycling of the lake food webs via primary production and trophic transfer [33].

5. Conclusions

Though the data reported in this study are limited, our findings showed that zooplankton in the pelagic zone of Lake Nam Co incorporated a fraction of glaciated watershed-derived OC. This suggests that in addition to primary production, OC from glaciated watershesd may be capable of stimulating aquatic productivity and deriving energy in lacustrine pelagic food webs, which has been previously demonstrated for other aquatic ecosystems as well. Future changes of the cryosphere triggered by enhanced warming may be pronounced. A continuing recession of glaciers and degradation of permafrost soil horizon in TiP are expected to transfer OC to the downstream aquatic environment.

Therefore, we speculate that the importance of terrestrial organic materials in aquatic ecosystems will become stronger. In the long term, it is important to explore the role of glaciated watersheds as carbon pools for aquatic food web dynamics, a topic not yet well studied in TiP and other similar environments.

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Author Contributions: En Hu, Erik Jeppesen and Zhengwen Liu conceived and designed the experiment. En Hu, Yaling Su and Hu He performed the field sampling. En Hu performed the experiments and wrote the manuscript. All authors revised the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Jansson, M.; Persson, L.; de Roos, A.M.; Jones, R.I.; Tranvik, L.J. Terrestrial carbon and intraspecific size-variation shape lake ecosystems. *Trends Ecol. Evol.* **2007**, *22*, 316–322. [CrossRef] [PubMed]
2. Taipale, S.; Kankaala, P.; Tiirola, M.; Jones, R.I. Whole-lake ^{13}C additions reveal seasonal shifts between multiple food source contributions to zooplankton diet. *Ecology* **2008**, *89*, 463–474. [CrossRef] [PubMed]
3. De Kluijver, A.; Ning, J.; Liu, Z.; Jeppesen, E.; Gulati, R.D.; Middelburg, J.J. Macrophytes and periphyton carbon subsidies to bacterioplankton and zooplankton in a shallow eutrophic lake in tropical China. *Limnol. Oceanogr.* **2015**, *60*, 375–385. [CrossRef]
4. Cole, J.J.; Carpenter, S.R.; Pace, M.L.; van de Bogert, M.C.; Kitchell, J.L.; Hodgson, J.R. Differential support of lake food webs by three types of terrestrial organic carbon. *Ecol. Lett.* **2006**, *9*, 558–568. [CrossRef] [PubMed]
5. Carpenter, S.R.; Cole, J.J.; Pace, M.L.; van de Bogert, M.; Bade, D.L.; Bastviken, D. Ecosystem subsidies: terrestrial support of aquatic food webs from ^{13}C addition to contrasting lakes. *Ecology* **2005**, *86*, 2737–2750. [CrossRef]
6. Cole, J.J.; Carpenter, S.R.; Kitchell, J.L.; Pace, M.L.; Solomon, C.T.; Weidel, B. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 1975–1980. [CrossRef] [PubMed]
7. Karlsson, J.; Berggren, M.; Ask, J.; Bystrom, P.; Jonsson, A.; Laudon, H. Terrestrial organic matter support of lake food webs: Evidence from lake metabolism and stable hydrogen isotopes of consumers. *Limnol. Oceanogr.* **2012**, *57*, 1042–1048. [CrossRef]
8. Pace, M.L.; Cole, J.J.; Carpenter, S.R.; Kitchell, J.F.; Hodgson, J.R.; van de Bogert, M.C. Whole lake carbon-13 additions reveal terrestrial support of aquatic food webs. *Nature* **2004**, *427*, 240–243. [CrossRef] [PubMed]
9. Pace, M.L.; Carpenter, S.R.; Cole, J.J.; Coloso, J.J.; Kitchell, J.F.; Hodgson, J.R. Does terrestrial organic carbon subsidize the planktonic food web in a clear water lake? *Limnol. Oceanogr.* **2007**, *52*, 2177–2189. [CrossRef]
10. Hood, E.; Battin, T.J.; Fellman, J.; Neel, S.O.; Spencer, R.G. Storage and release of organic carbon from glaciers and ice sheets. *Nat. Geosci.* **2015**, *8*, 91–96. [CrossRef]
11. Schuur, E.A.; Vogel, J.G.; Crummer, K.G.; Lee, H.; Sickman, J.O.; Osterkamp, T.E. The effect of permafrost thaw on old carbon release and net carbon exchange from tundra. *Nature* **2009**, *459*, 556–559. [CrossRef] [PubMed]
12. Mann, P.J.; Eglinton, T.I.; McIntyre, C.P.; Zimov, N.; Davydova, A.; Vonk, J.E. Utilization of ancient permafrost carbon in headwaters of Arctic fluvial networks. *Nat. Commun.* **2015**, *6*, 7856. [CrossRef] [PubMed]
13. Hood, E.; Fellman, J.; Spencer, R.G.M.; Hernes, P.J.; Edward, R.; D'Amore, D. Glaciers as a source of ancient and labile organic matter to the marine environment. *Nature* **2009**, *462*, 1044–1047. [CrossRef] [PubMed]
14. Bhatia, M.P.; Das, S.B.; Xu, L.; Charette, M.A.; Wadham, J.L.; Kujawinski, E.B. Organic carbon export from the Greenland ice sheet. *Geochim. Cosmochim. Acta* **2013**, *109*, 329–344. [CrossRef]
15. Spencer, R.G.; Guo, W.; Raymond, P.A.; Dittmar, T.; Hood, E.; Fellman, J. Source and biolability of ancient dissolved organic matter in glacier and lake ecosystems on the Tibetan Plateau. *Geochim. Cosmochim. Acta* **2014**, *142*, 64–74. [CrossRef]

16. Bardgett, R.D.; Richter, A.; Bol, R.; Garnett, M.H.; Baumler, R.; Xu, X. Heterotrophic microbial communities use ancient carbon following glacial retreat. *Biol. Lett.* **2007**, *3*, 487–490. [CrossRef] [PubMed]
17. Singer, G.A.; Fasching, C.; Wilhelm, L.; Niggemann, J.; Steier, P.; Dittmar, T. Biogeochemically diverse organic matter in Alpine glaciers and its downstream fate. *Nat. Geosci.* **2012**, *5*, 710–714. [CrossRef]
18. Hågvar, S.; Ohlson, M. Ancient carbon from a melting glacier gives high ^{14}C age in living pioneer invertebrates. *Sci. Rep.* **2013**, *3*, 2820. [CrossRef] [PubMed]
19. Fellman, J.B.; Hood, E.; Raymond, P.A.; Hudson, J.; Bozeman, M.; Arimitsu, M. Evidence for the assimilation of ancient glacier organic carbon in a proglacial stream food web. *Limnol. Oceanogr.* **2015**, *60*, 1118–1128. [CrossRef]
20. Schell, D.M. Carbon-13 and carbon-14 abundances in Alaskan aquatic organisms: Delayed production from peat in arctic food webs. *Science* **1983**, *219*, 1068–1071. [CrossRef] [PubMed]
21. Xu, B.; Cao, J.; Hansen, J.; Yao, T.; Joswia, D.; Wang, N. Black soot and the survival of Tibetan glaciers. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 22114–22118. [CrossRef] [PubMed]
22. Kang, S.; Xu, Y.; You, Q.; Flügel, W.A.; Pepin, N.; Yao, T. Review of climate and cryospheric change in the Tibetan Plateau. *Environ. Res. Lett.* **2010**, *5*, 015101. [CrossRef]
23. Liu, Y.; Yao, T.; Jiao, N.; Liu, X.; Kang, S.; Luo, T. Seasonal dynamics of the bacterial community in Lake Namco, the largest Tibetan lake. *Geomicrobiol. J.* **2013**, *30*, 17–28. [CrossRef]
24. Middelburg, J.J. Stable isotopes dissect aquatic food webs from the top to the bottom. *Biogeosciences* **2014**, *11*, 2357–2371. [CrossRef]
25. Caraco, N.; Bauer, J.E.; Cole, J.J.; Petsch, S.; Raymond, P. Millennial-aged organic carbon subsidies to a modern river food web. *Ecology* **2010**, *91*, 2385–2393. [CrossRef] [PubMed]
26. Dickson, L.; Bull, I.D.; Gates, P.J.; Evershed, R.P. A simple modification of a silicic acid lipid fractionation protocol to eliminate free fatty acids from glycolipid and phospholipid fractions. *J. Microbiol. Meth.* **2009**, *78*, 249–254. [CrossRef] [PubMed]
27. Boschker, H.T.S.; Kromkamp, J.C.; Middelburg, J.J. Biomarker and carbon isotopic constraints on bacterial and algal community structure and functioning in a turbid, tidal estuary. *Limnol. Oceanogr.* **2005**, *50*, 70–80. [CrossRef]
28. Phillips, D.L.; Gregg, J.W. Source partitioning using stable isotopes: Coping with too many sources. *Oecologia* **2003**, *136*, 261–269. [CrossRef] [PubMed]
29. Mayorga, E.; Aufdenkampe, A.K.; Masiello, C.A.; Krusche, A.V.; Hedges, J.I.; Quay, P.D. Young organic matter as a source of carbon dioxide outgassing from Amazonian rivers. *Nature* **2005**, *436*, 538–541. [CrossRef] [PubMed]
30. McNichol, A.P.; Aluwihare, L.I. The power of radiocarbon in biogeochemical studies of the marine carbon cycle: Insights from studies of dissolved and particulate organic carbon (DOC and POC). *Chem. Rev.* **2007**, *107*, 443–466. [CrossRef] [PubMed]
31. Zigah, P.K.; Minor, E.C.; Werne, J.P.; Leigh McCallister, S. An isotopic ($\Delta^{14}C$, $\delta^{13}C$, and $\delta^{15}N$) investigation of the composition of particulate organic matter and zooplankton food sources in Lake Superior and across a size-gradient of aquatic systems. *Biogeosciences* **2012**, *9*, 3663–3678. [CrossRef]
32. Gao, T.; Kang, S.; Zhang, Q.; Zhou, S.; Xu, Y. Major Ionic Features and Their Sources in the Nam Co Basin over the Tibetan Plateau. *Environ. Sci.* **2008**, *29*, 3009–3016. (In Chinese)
33. Ishikawa, N.F.; Uchida, M.; Shibata, Y.; Tayasu, I. Carbon storage reservoirs in watersheds support stream food webs via periphyton production. *Ecology* **2014**, *95*, 1264–1271. [CrossRef] [PubMed]