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Using oxygen isotopes in phosphate to assess biological phosphorus cycling in a small and shallow freshwater lake system

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Abstract

Reducing excess phosphorus (P) loads that cause eutrophication in aquatic systems is essential for meeting water quality standards. The oxygen isotopic composition of phosphate ($\delta^{18}O_p$) is a powerful tool for tracking P sources and cycling in diverse natural ecosystems. Here, we use $\delta^{18}O_p$ distribution in a small freshwater body (a lagoon–lake system) with high biological activity. We report $\delta^{18}O_p$ values seasonally along the water flow path in lagoon–lake system adjacent to Lake Biwa, Japan. The $\delta^{18}O_p$ values of inflowing water originating as agricultural runoff were constant throughout the study period at +16.3‰ ± 0.2‰. The $\delta^{18}O_p$ values in the system were generally offset from temperature-dependent isotopic equilibrium with the surrounding water, ranging from +11.1‰ to +17.8‰. The $\delta^{18}O_p$ values of the lake water approached equilibrium values in July and October, when dissolved inorganic P (DIP) retention rates were high, consistent with extensive biologically mediated phosphate cycling. A $\delta^{18}O_p$ two end-member mixing model, involving inflowing P and biologically recycled P, suggests that P turnover rates in the lagoon–lake system were high during the productive seasons. In contrast, the longer lake water residence time in the non-irrigation season (winter) allowed $\delta^{18}O_p$ values to deviate toward lower values relative to both equilibrium and agricultural source $\delta^{18}O_p$ values, suggesting that P metabolism was dominated by extracellular/ecto-enzymatic hydrolysis of dissolved organic P under low DIP concentrations. This work highlighted the utility of $\delta^{18}O_p$ for understanding P dynamics in shallow lake ecosystems.

Phosphorus (P) is essential to all organisms and is present in the environment as both dissolved and particulate inorganic and organic compounds (Benitez-Nelson 2000). Dissolved inorganic P (DIP) is generally the preferred form of P utilized by organisms in freshwater (Reynolds and Davies 2001); however, P-containing dissolved organic matter (DOP) can also be utilized as a source for P after extracellular/ecto-enzymatic hydrolysis to DIP, especially when ambient DIP levels are low in the water column (Karl and Björkman 2015). In addition, DOP can serve as a source of carbon (C) for heterotrophic consumption when the demand for C is not met, even when DIP is available (Burkhardt et al. 2014). Excess P inputs from anthropogenic sources (e.g., agricultural runoff) can reduce water quality and cause biodiversity loss, ecosystem degradation, and harmful algal blooms (Wetzel 2001; Ishida et al. 2019). Therefore, it is important to understand P sources, fate and transport in aquatic systems and the biogeochemical processes involved in P transformations (Paytan et al. 2002; Karl and Björkman 2015). However, this is not a trivial task, and therefore P dynamics in aquatic systems is less well-documented compared to the more comprehensive investigations of C and nitrogen

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(N) dynamics, even though P dynamics is linked to C and N cycling through biological processes (Davies et al. 2014; Karl and Björkman 2015).

Understanding P cycling in an aquatic system requires that all external sources of P to the system be identified and that P transformation through ecological processes be traced. Cellular/molecular P metabolism has been studied intensively, but the operation of these processes in natural systems is poorly understood because suitable tracer techniques and analytical methods are not readily available (Benitez-Nelson 2000). Several studies using P-flux models with external inputs and internal loading constrained by direct observations have been performed to reveal the status of lake systems, and the results were used to inform management decisions to improve water quality (Carpenter and Lathrop 2008; Matisoff et al. 2016). However, these models can only depict net expressions for the sum of all the reactions involved and do not take P-cycling processes into account. Specifically, using this approach, the different microbially mediated P-cycling processes that are prevalent in shallow lakes that respond rapidly to external loading, are not revealed (Wetzel 2001; Li et al. 2015). Bioassays using added radioactive phosphate have also been used to monitor P movements and assess P cycling (e.g., P uptake and turnover rates) in laboratory settings and in the ocean (Robarts et al. 1998; Benitez-Nelson 2000). However, radioisotopes have limited uses in natural systems because of the short isotope half-lives, environmental perturbations caused by such experiments, and safety and ethical problems (Benitez-Nelson 2000; Davies et al. 2014). Consequently, while radioisotopes have provided quantitative insights into turnover rates in aquatic systems, there is a necessity for an alternative and easily applicable analytical method, such as the use of stable isotope ratios. This approach will advance our insights into long-term trends and processes in global biogeochemical cycles, providing globally representative estimations of P turnover rates (Paytan and McLaughlin 2007).

P has only one stable isotope, ^{31}P, so P stable isotope techniques cannot be used to trace P sources and transformations in the way isotopes are routinely used to trace C, N, oxygen (O), and sulfur (S) sources and transformations. However, P is strongly bound to O in many inorganic and organic P compounds, and O has three stable isotopes that can be used as tracers. The P–O bond in phosphate (Pi) is resistant to inorganic hydrolysis under typical temperature and pressure conditions found within Earth's surface environments (Longinelli and Nuti 1973; Blake et al. 1997). In contrast, most metabolic pathways of P cycling are catalyzed by phosphoenzymes involving cleavage of P–O bonds, promoting O isotope exchange with intracellular or extracellular fluids, and leading to isotope fractionation between Pi and water (Longinelli and Nuti 1973; Blake et al. 2005). Accordingly, the stable isotope ratio of O in Pi ($\delta^{18}O_p$) could be utilized as a natural stable isotope tracer to track P transformations in ecosystems (Blake et al. 1997; Paytan et al. 2002; McLaughlin et al. 2013*b*). The ubiquitous enzyme inorganic pyrophosphatase (PPase) catalyzes intracellular Pi-water exchange, which leads to rapid temperature-dependent equilibrium O isotope fractionation between Pi and intracellular water, and some of the intracellular P can be released into the environment upon cell lysis (Fricke and O'Neil 1996; Blake et al. 2005). If effective biological turnover (Pi uptake, intracellular Pi-water exchange, and cycling of Pi between intracellular and extracellular spaces of living organisms) repeatedly occurs, overwhelming other processes, then a temperature-dependent equilibration of the $\delta^{18}O_p$ in environmental water will be detected (Blake et al. 2005; Paytan and McLaughlin 2012). On the other hand, if the $\delta^{18}O_p$ signature in an ecosystem is not overwritten by biological turnover, it is possible to estimate the relative importance of intracellular biological P cycling vs. external P inputs from the extent to which $\delta^{18}O_P$ values in the environment deviate from input signatures and move toward isotopic equilibrium values (McLaughlin et al. 2006). The prevalent equations for temperature-dependent oxygen isotopic equilibrium value are those formulated by Longinelli and Nuti (1973) and Chang and Blake (2015). For example, in a study on Lake Erie by Elsbury et al. (2009), lake water $\delta^{18}O_p$ values ranged from +10‰ to +17‰, largely deviating from the expected equilibrium of around +14‰. This discrepancy indicated only partial biologically mediated P recycling relative to phosphate input, with values higher than expected equilibrium allowing for identifying additional sources. Specifically, since the riverine weighted average $\delta^{18}O_p$ was +11‰, a potential P source characterized by elevated $\delta^{18}O_p$ values, such as the release of legacy P from sediments, has been suggested.

The above framework based on $\delta^{18}O_p$ values has been used to track non-point and point sources of P and to investigate the geochemical cycling of P in various water bodies (e.g., Young et al. 2009; Paytan and McLaughlin 2012; Wang et al. 2022). In previous freshwater studies, $\delta^{18}O_p$ has been primarily used to investigate large, open water bodies with rapid flows, short residence times, and low biological activities (e.g., such as rivers and streams) (Young et al. 2009; Gooddy et al. 2016; Wang et al. 2023) and large deep lakes with multiple sources (Elsbury et al. 2009; Tonderski et al. 2017). Ishida et al. (2019) developed an isoscape approach to identify non-point P sources from paddy fields and bedrock in a large river watershed, showing that the dominant P source was from upstream tributaries with short travel times and low biological utilization. Tonderski et al. (2017) employed the $\delta^{18}O_p$ tracer to identify diverse P sources in an agricultural stream catchment at different periods of the year; the temporal differences in $\delta^{18}O_p$ related to changes in biological activity and hydrological pathways, highlighting the potential of $\delta^{18}O_p$ end-member mixing models to quantify the proportional contribution of P from multiple sources. Shallow lakes, which have a number of morphometric characteristics differentiating them from running water systems, are generally more productive than deep lakes, and a greater proportion of their total productivity is attributable to nutrient coupling processes at the land–water interface (Wetzel 2001). The influence of human activities in shallow lake ecosystems typically involves increases in the loading of nutrients and nutrient cycling and uptake of autotrophic and heterotrophic organisms are expected to respond rapidly to changes in P inputs in these systems.

In the present study, we conducted synoptic sampling of shallow lake water and potential P sources in tributary lagoons of Lake Biwa in Japan (Fig. 1). These lagoon–lakes serve as vital transitional zones, playing important roles in buffering against excessive nutrient inputs from the terrestrial environment to the main lake (Nakamura 2009). Although conventional hydrochemical analyses, such as the concentrations and composition of different P fractions or stoichiometric nutrient balance of the lagoon–lake ecosystem, have been done in the past, these data do not provide insight into the sources of nutrients that increase eutrophication risk, or the biogeochemical processes that govern the fate of these nutrients within the system. It is therefore important to assess P cycling and retention in the lagoon–lakes to better understand the processes involved in natural recycling/buffering in these systems. Here, we measured $\delta^{18}O_P$ along the water flow path in a lagoon–lake system adjacent to Lake Biwa, during four seasons, to assess the contributions of external and internal P sources and metabolic P turnover pathways within the small and shallow lagoon–lake system.

Materials and methods

Study area

Lake Biwa, the largest lake in Japan, has a surface area of 670.3 km^2 and a maximum depth of 103.6 m (Kawanabe et al. 2020). The lake is surrounded by small, shallow, partly isolated waterbodies (geologically categorized as lagoon–lakes) called “naiko” in Japanese. Reclamation of the lagoon–lakes, mainly for agriculture, has left only 23 naikos remaining, with a total area of 4.25 km^2 (0.6% of the surface area of the main lake) (Kawanabe et al. 2020).

This work focuses on Lakes Yanagihira and Hira, which are small and shallow lagoon–lakes on the southeastern shore of Lake Biwa (Fig. 1). These lakes have surface areas of 0.06 and 0.13 km^2, volumes of 114,000 and 284,000 m^3, and maximum depths of 3.1 and 2.3 m, respectively. The lakes are considered subtropical warm systems that do not freeze, and the water is completely mixed by mild wind perturbations. The lakes featured an average annual water column temperature of 18.5°C and dissolved oxygen (DO) concentration of 8.1 mg L^{-1} (Yi et al. 2020). The main land uses around the lakes are rice paddies (74%) and residential areas (20%) (Okano et al. 2018). Agricultural discharges from rice paddies flow through an

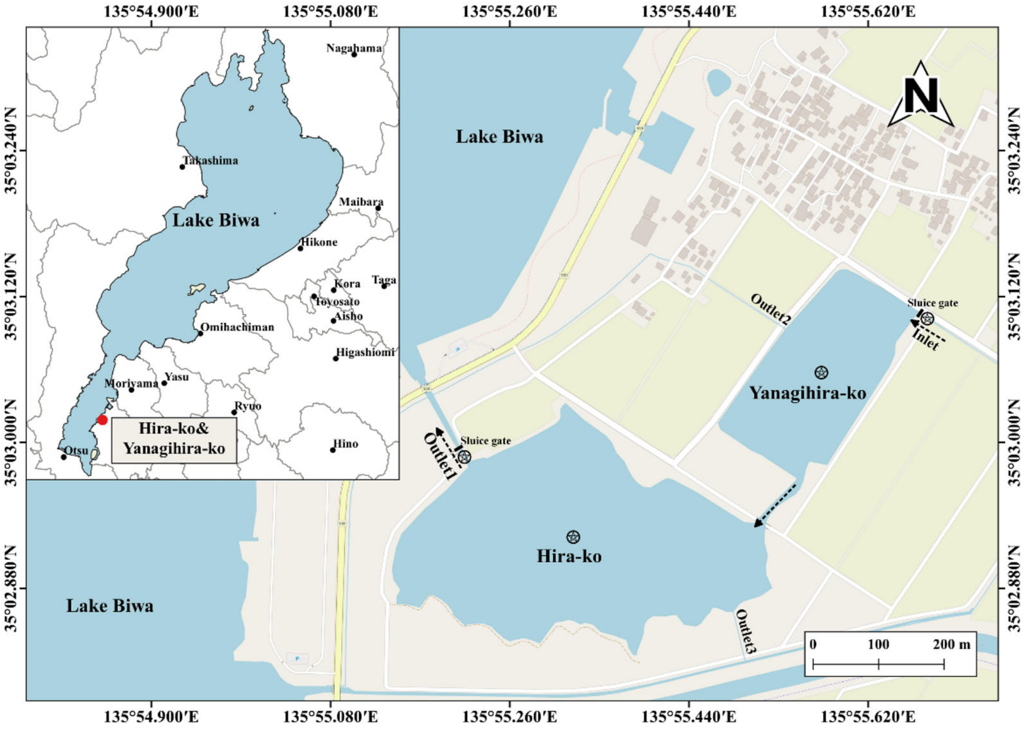

Fig. 1. Locations of Lakes Yanagihira and Hira adjacent to the east coast of the south basin of Lake Biwa (Japan) and the inlet, pelagic, and outlet sampling sites (stars in circles). Gray bars indicate the locations of sluice gates. Arrows indicate the water flow direction.

irrigation system into Lake Yanagihira. The velocity of flow entering Lake Yanagihira changes seasonally, with a high flow rate during the irrigation period (typically from April to September) (Ikeya et al. 2024). Water exiting Lake Yanagihira flows into Lake Hira through a narrow channel, and then the water enters Lake Biwa through a sluice gate at the northwestern end of Lake Hira. Negligible discharges occur through small outlets at the southern end of the lake. We treated the two lagoon–lakes as one single connected system. The agricultural inputs are the main sources of water and dissolved nutrients to Lake Biwa, at least since the 1980s when domestic and industrial effluents in the region were diverted into treatment plants (Nakano et al. 2008; Liu et al. 2020). The topography of the watershed features an elevation difference of less than 10 m and a shallow gradient of 0.13°. The water level in the watershed remains approximately 0.5 m above that of Lake Biwa, implying restricted groundwater seepage into the lagoon–lakes and a prevailing surface water flow pattern within the watershed.

Sampling procedures

Water samples were collected from four sites along the water flow path in the lagoon–lake system from the Lake Yanagihira inlet to the Lake Hira outlet. Sampling was conducted during four seasons, on 11 April, 25 July, and 17 October 2017, and on 11 January 2018. No inflowing water from irrigation channels was observed entering the system on 11 January 2018. Before the sampling commenced, soluble reactive P concentrations were measured to determine the water volume needed for analysis. Typically, 10–80 L were deemed to be enough for duplicate measurement of $\delta^{18}O_P$. Water samples from the inlet and outlet sites were collected using a 10 L plastic bucket or a 4 L plastic pitcher. Water samples from the surface and 0.5 m above the bottom at each pelagic site in both lagoon–lakes were collected using a 6 L Van Dorn sampler. Each water sample was transferred to a 20 L polyethylene container and transported to the laboratory for processing for isotope analysis. A second water sample was collected at each site to determine water oxygen isotope ratios ($\delta^{18}O_{H2O}$), chlorophyll *a* (Chl *a*) and total and dissolved inorganic and organic P concentrations. Water temperature and DO concentrations were determined using a multiparameter water quality sensor (U-50; Horiba). Water flow rates and the cross-sectional areas of the flow channels were determined using an electromagnetic flowmeter (AEM1-D; Advantec) to calculate discharges at the inlet and outlet of the system. To assess the isotopic signature of sedimentary phosphate inputs relative to that of phosphate in lake water, sediment samples were collected from one pelagic site in each lagoon–lake using a Type HR gravity core sampler (RIGO) at each sampling time. Each sediment core was divided into 3 cm slices to a depth of 9 cm and then into 5 cm slices to a depth of 14 cm. This allowed us to assess if sediments are a potential source of phosphate for lake water.

Isotope analyses

Each water sample was kept in cold, dark conditions while in the field and was then filtered within 12 h of the collection, first through a Whatman GF/F glass-fiber filter and then through a 0.45-μm membrane filter. To determine the $\delta^{18}O_p$ values of the sediment, 3.3 g of oven-dried sediment (at 60°C) were shaken in 200 mL of 0.5 mol L^{-1} $NaHCO_3$ solution to extract weakly absorbed Pi, as described by Zohar et al. (2010). Phosphate in water samples or sediment extracts was then separated through a series of dissolution and precipitation steps to form pure Ag_3PO_4 for $\delta^{18}O_p$ analyses using a modified procedure developed by McLaughlin et al. (2004) and described in detail by Ishida et al. (2019).

$\delta^{18}O_p$ of Ag_3PO_4 was measured on a thermal conversion elemental analyzer connected via a ConFlo III system to a Deltaplus XP isotope ratio mass spectrometer (TC/EA-IRMS; Thermo Fisher Scientific) at the Research Institute for Humanity and Nature in Kyoto, Japan. The instrument was calibrated for $\delta^{18}O_p$ analysis using three internal standards with $\delta^{18}O_p$ values of +8.3‰ ± 0.29‰, +14.4‰ ± 0.14‰, and +23.1‰ ± 0.27‰. The $\delta^{18}O_p$ values of the internal standards were determined using two independently calibrated standards (STDL +11.3‰ ± 0.15‰ and STDH +20.0‰ ± 0.25‰), using the traditional fluorination method as described by McLaughlin et al. (2004). The analytical precision (standard deviation) between replicate analyses of the standards was 0.4‰. $\delta^{18}O_{H2O}$ was determined using a water isotope analyzer (L2120-I; Picarro), which had an analytical precision of 0.05‰.

Chemical analyses

Each water sample used for DIP and total dissolved P (TDP) analyses was passed through a Millipore HA membrane with 0.45-μm pores. Each water sample for total P (TP) analysis was passed through a sieve (200-μm mesh) and stored in a 50 mL plastic tube. The DIP concentration was determined as PO_4–P by ion chromatography using a method described in detail by Maruo et al. (2016). The TP and TDP concentrations were determined after autoclaving 10 mL water with potassium peroxydisulfate at 121°C for 30 min to convert all the P into PO_4–P. The PO_4–P concentration of the samples was determined using an AACS II continuous flow nutrient autoanalyzer (Bran + Luebbe). The particulate P (PP) concentration was defined as the difference between the TP and TDP concentrations, and the DOP concentration was defined as the difference between the TDP and DIP concentrations. A 200 mL aliquot of each sample was used for Chl *a* analysis. The water was passed through a Whatman GF/F glass-fiber filter, Chl *a* was extracted from the filter with 10 mL of 90% acetone at 4°C for 24 h in the dark and Chl *a* concentrations in the extracts were determined using an RF-1500 spectrofluorometer (Shimadzu) and an equation published by Parsons et al. (1984).

Isotope model for P turnover in the lagoon–lake system

The offset of $\delta^{18}O_p$ in a water sample from the $\delta^{18}O_p$ expected at isotopic equilibrium with the surrounding water can be used to track isotopically distinct P sources and/or assess the degree of P cycling in the system (Elsbury et al. 2009; Paytan and McLaughlin 2012). In a water body in which the DIP concentration does not limit biological productivity, $\delta^{18}O_p$ can be affected by (1) intracellular temperature-dependent isotope exchange equilibrium and (2) source mixing (Paytan and McLaughlin 2012). The $\delta^{18}O_p$ for an environmental sample ($\delta^{18}O_{\text{p-sample}}$) can be described using the modified stable isotope mixing mass balance model published by Okuda (2015),

$$\delta^{18}O_{\text{p-sample}} = b\,\delta^{18}O_{\text{p-Eq}} + (1-b)\,\Sigma\left(f_n\,\delta^{18}O_{\text{p-source}\,n}\right), \tag{1}$$

where b is the fraction of the DIP that is biologically recycled ($0 \le b \le 1$), $\delta^{18}O_{\text{p-Eq}}$ is the expected equilibrium $\delta^{18}O_p$ that we calculated using the empirical equation of Longinelli and Nuti (1973) (the reasons for selecting this equation are described in Supporting Information Text S1), and $\Sigma(f_n\,\delta^{18}O_{\text{p-source}\,n})$ is the sum of the $\delta^{18}O_p$ for DIP sources n contributing the fractions of the DIP f_n ($f_1 + f_2 + \ldots + f_n = 1$). Therefore, b can be expressed as

$$b = \left\{\Sigma\left(f_n\,\delta^{18}O_{\text{p-source n}}\right) - \delta^{18}O_{\text{p-sample}}\right\} \Big/ \left\{\Sigma\left(f_n\,\delta^{18}O_{\text{p-source n}}\right) - \delta^{18}O_{\text{p-Eq}}\right\}. \tag{2}$$

Metabolic DIP turnover rates in the whole lagoon–lake system can be described using an exponential decay curve,

$$y = e^{-\beta x}, \tag{3}$$

where y is the fraction ($1-b$) of DIP that has not been biologically recycled, x is the water transport time (d) or the time it takes for water to flow from the inlet to the sampling point of interest, and β is a decay constant ($1/\beta$ is the mean DIP turnover time for the system). The water transport time to a sampling point was estimated by multiplying the water exchange (residence) time for the whole lagoon–lake system by the ratio between the distance (km) of the sampling point from the inlet and the total distance between the inlet and outlet. The residence time (s) for the whole system was defined as the sum of the residence times for the two lagoon–lakes, determined from the water discharge rates ($m^3\,s^{-1}$) at the outlet of the lagoon–lakes and the water volumes (m^3) of the lagoon–lakes.

Calculating retention rates for different P fractions

The retention rate (%) for each P fraction in the lagoon–lake system was calculated as a function of the water discharge rate and P concentration of the fraction using the equation,

$$\text{Retention rate}\,(\%) = \left(\left(Q_{\text{in}}C_{\text{in}} - Q_{\text{out}}C_{\text{out}}\right)/Q_{\text{in}}C_{\text{in}}\right) \times 100, \tag{4}$$

where Q_{in} and Q_{out} are the water fluxes ($m^3\,s^{-1}$) at the inlet and outlet, respectively, and C_{in} and C_{out} are the P concentrations ($\mu mol\,L^{-1}$) of a specific fraction at the inlet and outlet, respectively.

Results

Environmental characteristics

The water temperatures during the study period ranged between 4.5°C and 28.5°C (Fig. 2) and were almost the same at all the sampling sites at each sampling time. The mean $\delta^{18}O_{H2O}$ for the water samples was −6.9‰, and the range was from −6.3‰ to −9.4‰ (Supporting Information Table S1). The water residence time for the lagoon–lake system from April to October 2017 ranged from 37 to 18 d, respectively, and the residence time in January 2018 was 83 d, which was approximately three times longer than the residence time at the other sampling times.

The Chl *a* concentration at the inlet was low (<1.62 $\mu g\,L^{-1}$) except during January. Chl *a* concentrations increased along the flow path and were always highest at the Lake Hira pelagic site. The mean Chl *a* concentration at the pelagic sites in the two lagoon–lakes and at the outlet site ranged from 2.17 to 57.36 $\mu g\,L^{-1}$ and were lowest in April and highest in October 2017.

TP concentrations were between 1.86 and 8.48 $\mu mol\,L^{-1}$ and were always higher at the inlet site than at the other sites except in July 2017. PP was the dominant component at most sampling times, contributing between 16% and 94% of TP (the highest contribution was in January 2018). TDP concentration decreased along the flow path in April and October. In October, the TDP concentration at the inlet was high (5.39 $\mu mol\,L^{-1}$) but decreased rapidly to < 0.90 $\mu mol\,L^{-1}$ at the pelagic site in Lake Yanagihira. In contrast, the TDP concentrations throughout the system, even at the inlet, were low (< 0.45 $\mu mol\,L^{-1}$) in January. DOP concentrations ranged from 0.19 to 4.07 $\mu mol\,L^{-1}$, which comprised 42–100% of the TDP concentrations. DIP concentrations ranged from below the detection limit to 1.91 $\mu mol\,L^{-1}$, and, except for January, the concentrations were highest at the inlet and decreased along the flow path toward the outlet. DIP concentrations were >0.36 $\mu mol\,L^{-1}$ throughout the system, even at the outlet, in April and July, but concentrations at the pelagic sites in October were <0.31 $\mu mol\,L^{-1}$. DIP concentrations at all the sites in January were low (~0.15 $\mu mol\,L^{-1}$). The DIP concentration of the water flow exhibited spatiotemporal variation, with a median value of 0.81 $\mu mol\,L^{-1}$ (interquartile range [IQR]: 0.67, 1.17), 0.41 $\mu mol\,L^{-1}$ (IQR: 0.33, 0.60), 0.29 $\mu mol\,L^{-1}$ (IQR: 0.01, 0.82), and 0.06 $\mu mol\,L^{-1}$ (IQR: 0.04, 0.14) observed in April, July, September, and January, respectively. TP and DIP retention rates for the lagoon–lake system were 67–88% and 83–100%, respectively, and the highest retention rates were found in October (Table 1).

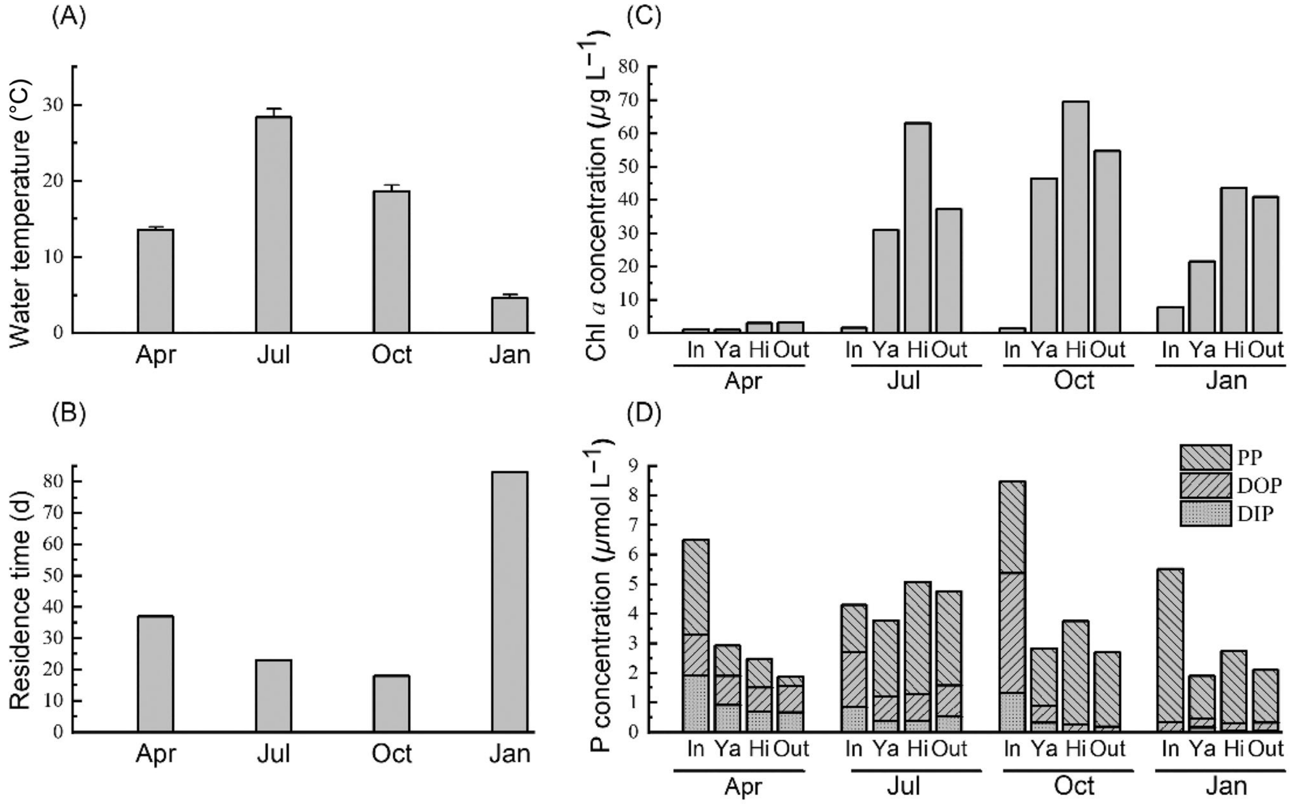

Fig. 2. (**A**) Mean water temperatures at the sampling sites, (**B**) lake water residence times, (**C**) chlorophyll *a* (Chl *a*) concentrations, (**D**) particulate P (PP), dissolved organic P (DOP), and dissolved inorganic P (DIP) concentrations at the inlet (In), outlet (Out), and pelagic sites in Lakes Yanagihira (Ya) and Hira (Hi) on 11 April, 25 July, and 17 October 2017 and 11 January 2018. The vertical bars in A represent the standard deviation among the sampling sites at each sampling event.

$\delta^{18}O_p$ in water and sediment

The $\delta^{18}O_p$ values at the inlet, which represent the phosphate in agricultural runoff, were almost constant (+16.1‰ to +16.5‰) during the study period except in January 2018 (Fig. 3; Supporting Information Table S1). These values were not in isotopic equilibrium with respect to the water oxygen isotopes and temperature, as determined by Longinelli and Nuti (1973) equation. The $\delta^{18}O_p$ values of water samples from the lagoon–lake system varied spatially and temporally (typically decreasing from the inlet to the outlet) and ranged

Table 1. Water discharge rates and total phosphorus (TP) and dissolved inorganic phosphorus (DIP) concentrations, fluxes, retention rates, and retention amount in the lagoon–lake system on 11 April, 25 July, and 17 October 2017 and 11 January 2018. A hyphen means no data available.

			Concentration (μmol L^{-1})		Flux (t P yr^{-1})		Retention rate (%)		Retention amount (g P m^{-2} yr^{-1})	
Month	**Site**	**Water discharge rates (m^3 s^{-1})**	**TP**	**DIP**	**TP**	**DIP**	**TP**	**DIP**	**TP**	**DIP**
April	Inlet	0.189	6.50	1.91	1.20	0.35	85	83	173	49
	Outlet	0.100	1.86	0.66	0.18	0.06				
July	Inlet	0.322	4.31	0.84	1.36	0.26	67	81	154	37
	Outlet	0.096	4.75	0.51	0.45	0.05				
October	Inlet	0.291	8.48	1.32	2.41	0.38	88	100	358	64
	Outlet	0.116	2.69	ND	0.30	0.00				
January	Inlet	-	5.52	0.02	-	-	-	-	-	-
	Outlet	0.045	2.10	0.06	0.08	2.98				

ND, not detected, and the flux was deemed as zero.

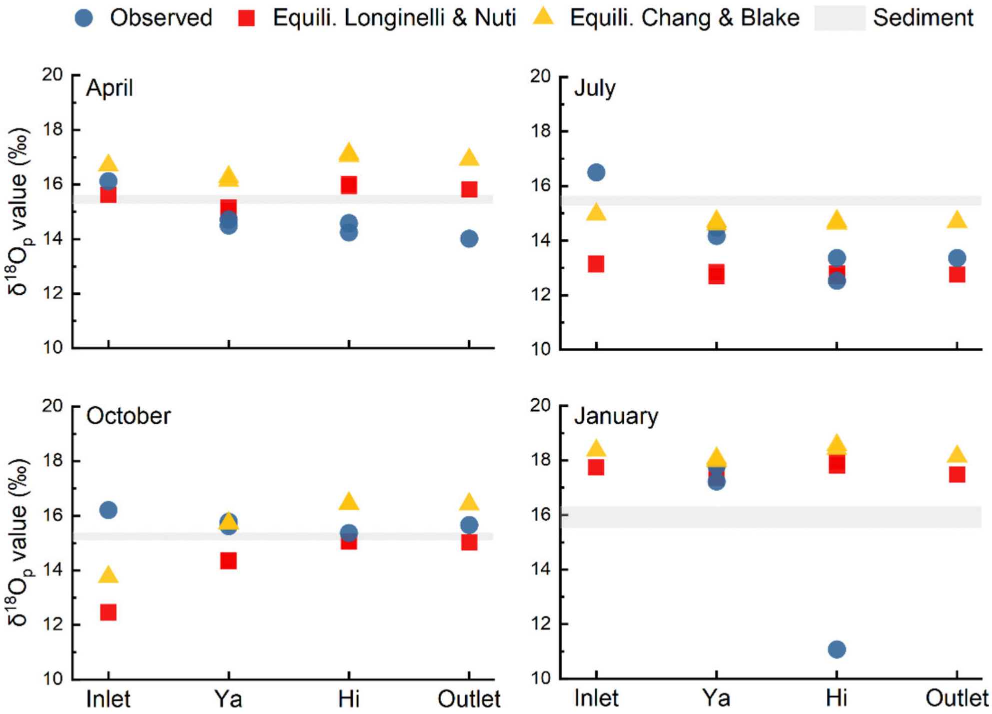

Fig. 3. Oxygen isotope compositions of phosphate ($\delta^{18}O_p$) at the inlet, outlet, and pelagic sites in Lakes Yanagihira (Ya) and Hira (Hi) in April, July, and October 2017 and January 2018. Circles indicate the observed values, squares the expected equilibrium using Longinelli and Nuti (1973) equation, and triangles the expected equilibrium using Chang and Blake (2015) equation. Horizontal gray bars indicate 95% confidence intervals for $\delta^{18}O_p$ of sediment.

between +11.1‰ and +17.8‰, with January lacking inlet isotopic data. The $\delta^{18}O_p$ values at the surface and near the bottom at the pelagic sites were not significantly different from each other at any sampling time (paired *t*-test, $t = -1.7$, df = 5, $p > 0.05$). In April, the $\delta^{18}O_p$ values in the lagoon–lake water decreased along the flow path and were below the expected equilibrium values at the pelagic site in Lake Hira and at the outlet. In contrast, in July and October, the $\delta^{18}O_p$ values gradually approached the expected equilibrium values along the flow path. In January 2018, $\delta^{18}O_p$ could not be determined at the inlet and outlet because the PO_4–P concentrations in the samples were too low, but at the pelagic site in Lake Yanagihira, the $\delta^{18}O_p$ values were close to the equilibrium value (+17.5‰) and they were below the equilibrium value at the pelagic site in Lake Hira (+11.1‰). The $\delta^{18}O_p$ values of sediment extracts obtained from different depths were homogeneous and seasonally stable and ranged between +14.9‰ and +15.9‰ except in February 2018, when the $\delta^{18}O_p$ value was slightly higher (+17.1‰) at the sediment surface and decreased with depth, approaching the values found for the other sediment samples at about 6 cm deep (Supporting Information Fig. S1).

Isotope model of P metabolism in the ecosystem

Potential sources of P for waterways encompass irrigation water, groundwater, and domestic wastewater. However, in the watershed studied, groundwater and domestic wastewater were evaluated to have a negligible effect due to the topography of the area and the wastewater treatment plant (WWTP) that does not dispose of treated water in the lake. There are, therefore, two potential P sources entering the lagoon–lake system, that is, agricultural runoff from the inlet and internal loading from the bottom sediment within the system. Based on our observation, however, just one P source, from external loading, should be taken into consideration because the internal supply flux was negligible, as described below (*see* Discussion). The $\delta^{18}O_{p\text{-sample}}$ values of the lake water could thus be explained by a mixture of two end-members with different isotope signatures, the $\delta^{18}O_p$ values of the inlet and the DIP released from cells which are in isotopic equilibrium (i.e., $\delta^{18}O_{p\text{-external}}$ and $\delta^{18}O_{p\text{-Eq}}$). The fraction of intracellularly recycled P in the DIP pool, b, could therefore be calculated for each sampling site along the water flow path using Eq. 5,

$$b = \left(\delta^{18}O_{p\text{-external}} - \delta^{18}O_{p\text{-sample}}\right) / \left(\delta^{18}O_{p\text{-external}} - \delta^{18}O_{p\text{-Eq}}\right). \quad (5)$$

Fractions of unmetabolized P (DIP entering from the inlet), that is, $1 - b$, gradually decreased along the flow path through the lagoon–lake system in July and October 2017 (Fig. 4); however, in the other two sampling dates, $\delta^{18}O_p$ values were lower than expected equilibrium, suggesting another process affecting the ratios. The estimated decay constants β for the

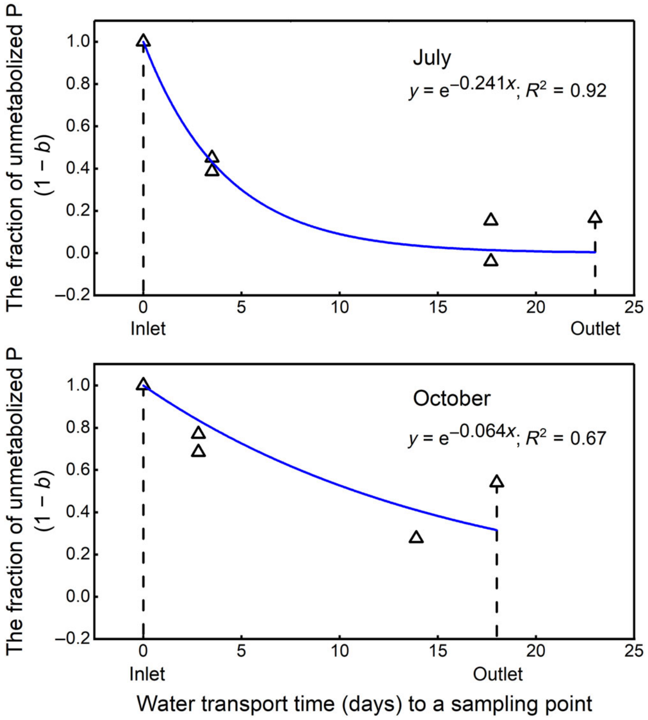

Fig. 4. Exponential decay curves for the unmetabolized P fraction (1 − *b*) plotted against time for water flowing from the inlet to the outlet of the Lakes Yanagihira and Hira system in July and October 2017. Nonlinear regression analysis was performed to fit an exponential decay curve to the data using the Marquardt–Levenberg algorithm in OriginPro 2018 software.

fitting curves shown in Eq. 3 for July and October 2017 were 0.241 and 0.064, respectively, which corresponded to DIP turnover times of 4 and 16 d, respectively.

Discussion

The role of the lagoon–lakes system in P cycling

The Water Framework Directives in Europe stress the necessity of reducing the input of nutrients to the freshwater ecosystem and strive to achieve "good ecological and chemical status" of surface water, thus, there is an urgent need for the sources of P to be identified and for P removal rates to be determined (Koiv et al. 2011). Specifically, it is essential to decrease P agricultural runoff loads in Lake Biwa, which accounts for 16.0% of TP inputs to the lake (Lake Biwa Environmental Research Institue 2018) because agricultural water is not treated to remove P. Constructed wetlands or ponds are suitable for reducing non-point source pollution and have been used to treat agricultural runoff in many countries (Campbell et al. 2004; Nakamura 2009). Artificial or natural lagoon–lakes around Lake Biwa serve as a type of constructed wetlands that function to reduce influent pollutants. Our results demonstrated that a portion of the agricultural P inputs to Lake Biwa is indeed retained in the lagoon–lakes surrounding the main lake, reducing input loads. The TP and DIP retention rates in the natural lagoon–lakes that we studied were 67–88% and 81–100%, in Lakes Yanagihira and Hira, respectively, and were much higher than the TP retention rates of 9–55% found for artificially constructed lagoons adjacent to Lake Kasumigaura (Nakamura 2009) and the mean TP retention rate of 56% for 54 lakes and reservoirs in areas with different climates around the world (Koiv et al. 2011). Much of the P load to lakes is retained largely through sedimentation, which is usually greater than the fraction assimilated by biota, and gross sedimentation is closely linked to the hydrological characteristics and lake morphometry (e.g., relative depth) (Wetzel 2001; Koiv et al. 2011; Land et al. 2016). It has been suggested that the small tributary lagoons of Lake Biwa, which have longer hydraulic residence times, are able to retain more of the external P in sediments (Nakamura 2009), thus resulting in a higher P retention. Koiv et al. (2011) similarly concluded that this trend, while found in the whole data set of water bodies, was much stronger in lakes with areas larger than 25 km^2. It is noted that the retention rate of P was determined through instantaneous sampling (such as flow rate and concentrations), assuming steady-state conditions in spatial and temporal scales. However, the validity of the steady-state assumption may be undermined by hydrological factors like rainfall events affecting lake dynamics, potentially overlooking short-term fluctuation in P loading of inflow water and the resultant microbial dynamics in P cycling. Nonetheless, the significance of these lagoon–lakes in reducing terrestrial P input into Lake Biwa and preventing water body eutrophication remains undisputed. The area of the lagoon–lakes surrounding Lake Biwa has decreased to one-seventh of its original size in the past decades due to reclamation (Kawanabe et al. 2020). This is significant, as the average retention of TP estimated to be around 228 g P m^{-2} yr^{-1} for the studied lakes (Table 1) was likely much greater in the past.

Sources of P input to the lagoon–lake system

The $\delta^{18}O_p$ value of the inflowing water was not in isotopic equilibrium with oxygen isotopes and temperature of the water and this value was distinct from the $\delta^{18}O_p$ of lake water, indicating that $\delta^{18}O_p$ can be used to identify this source of P. In previous studies, $\delta^{18}O_p$ of potential P source samples (e.g., WWTP, sediment, soil, fertilizer, groundwater) across global watersheds, spanned from +4.2‰ to +32.7‰ (Young et al. 2009; Wang et al. 2022), encompassing the full spectrum of source samples (+12.7‰ to +18.5‰) present in the Lake Biwa watershed (Ishida et al. 2019; Ide et al. 2020). The main external source of P to the lagoon–lake system, captured at the inlet, is the runoff of agricultural irrigation (Shiga Prefecture 2019), which had seasonally stable $\delta^{18}O_p$ values of +16.1‰ to +16.5‰. Organic and chemical fertilizers have $\delta^{18}O_p$ values ranging from +15.5‰ to +27‰, reflecting the isotope signatures of phosphate ore from different mines (Gruau et al. 2005; Young et al. 2009). The manufactured chemical fertilizers used in the watershed around the study area have $\delta^{18}O_p$ values of +12‰ to +13‰ (Ishida et al. 2019), a signature different from the $\delta^{18}O_p$ of the water samples at the inflow site. However, the $\delta^{18}O_p$ values in soil samples from several rice paddies in the watershed were +18.0‰ to +18.2‰ (Ishida et al. 2019; Ide et al. 2020), so it is likely that the P supplied to the lagoon–lake system via the irrigation channels originated from a mixture of chemical fertilizers and P released from rice paddy soils.

Bottom lake sediments could also supply P to the water column in lakes (Wetzel 2001; Matisoff et al. 2016). Although the sediment P flux was not measured directly in the present study, the measured $\delta^{18}O_p$ values of $NaHCO_3$ extractable (or labile) Pi of sediment collected at the pelagic sites in both lagoon–lakes were vertically homogenous and seasonally invariable, averaging +15.4‰, except for Lake Hira surface sediments in February. We calculated the equilibrium $\delta^{18}O_p$ value for the sediment extracts from the mean $\delta^{18}O_{H2O}$ (−6.9‰ ± 0.7‰) and mean bottom water lake temperature (16.4 ± 8.9°C) during the study period to be +15.1‰, which was similar to the $\delta^{18}O_p$ values of labile Pi extracted from the sediment in both lagoon–lakes. It has previously been found that $\delta^{18}O_p$ values of $NaHCO_3$ extracts of agricultural soils largely converged on the equilibrium value upon burial, suggesting extensive biological cycling in the sediment (Ishida et al. 2019; Tian et al. 2020). Regardless of the controlling mechanisms of sediment $\delta^{18}O_p$ values, the $\delta^{18}O_p$ values of DIP in lake water differed from those of the sediments, and there was no clear evidence for a flux from the bottom sediment into the overlying water. Specifically, previous studies reported a $\delta^{18}O_p$ gradient between surface and deep water, with $\delta^{18}O_p$ values of deep water and sediment being comparable and different from surface water, suggesting that P was supplied by a benthic flux from the sediment (Elsbury et al. 2009; Paytan et al. 2017). In this study, no difference between $\delta^{18}O_p$ values in surface and near-bottom water was found at either lake at any time, probably due to the frequent mixing of water in these shallow lakes, making it hard to discern sediment sourcing. However, a sufficient supply of oxygen (DO concentration > 4.7 mg L^{-1}) to the sediment surface (Supporting Information Table S1) caused by mixing in the study area, may have the capacity to prevent P from being released from the bottom sediment (Wetzel 2001). Dredging can also decrease the amount of P released from sediments (Yu et al. 2017), and Lakes Yanagihara and Hira were dredged intensively by a public rehabilitation project (between 2001 and 2006) to restore the lake environments. This dredging removed over 95% of N and P effluxes, and lowered the P diffusion fluxes from the sediment for Lakes Yanagihira and Hira to 3 kg yr^{-1} and undetectable, respectively (Shiga Prefecture 2019). These estimates are much lower than the mean calculated external P input flux of 1.66 t yr^{-1} (Table 1). Based on these estimates, we conclude that during our study period, the P contribution to the lakes from internal loading from the sediment was negligible in these lakes and that external P inputs from agricultural runoff are the main source of P to the system.

Biological P cycling in the lagoon–lake system

The $\delta^{18}O_p$ values of water within the lagoon–lake system ranged from +11.1‰ to +17.8‰, with a similar range in $\delta^{18}O_p$ values of +10.3‰ to +17.6‰ ($n = 15$) observed in Yasu River, the largest tributary of Lake Biwa (Ishida et al. 2019). A similar range of values have also been noted in other estuarine, marine, lakes, and catchments, where the sources of P and its metabolic processes are expected to display marked spatiotemporal variability (Paytan and McLaughlin 2012; Davies et al. 2014; Wang et al. 2022). The lake water $\delta^{18}O_p$ values in this study differed from the expected equilibrium values and displayed distinct gradients and spatial changes along the water flow path and between seasons. This suggested that phosphate from agricultural inputs entering the lake is not being fully taken up and intracellularly processed by the living biomass, offering the opportunity to shed light on P sources, different metabolic processes and the cumulative biological utilization effects within the lagoon–lakes spatially and temporally between seasons. The $\delta^{18}O_p$ values of the water approached the expected isotope exchange equilibrium values as the lake water moved through the lagoon–lake system toward the outlet in July and October but were much lower than the expected equilibrium values in January and April. This could be explained using two different isotope mixing models based on the metabolic processes associated with inorganic and organic P utilization within the lagoon–lake system; one of these processes is more prevalent in July and October and the other in January and April, as discussed below.

The mean turnover times of phosphate observed in this study for July and October were 4–16 d, consistent with turnover rates reported in previous studies at multiple sites and seasons using P radioisotope addition experiments, which ranged from less than 1 d to ~ 6 weeks in lakes of low to high productivity (Rigler 1973; Robarts et al. 1998; Wetzel 2001). We did not use radioisotopes to estimate P turnover rates because of environmental regulations; we, however, used the $\delta^{18}O_p$ mixing model to estimate the minimum P turnover rates. The DIP turnover time calculated for each location and time reflects the integrated DIP supply relative to demands by resident biota in the system (Lean and Nalewajko 1979; Fisher et al. 1992; Wetzel 2001). A fast turnover time indicates P deficiency (Zohary and Robarts 1998; Sohm and Capone 2010), hence more oligotrophic lakes will have shorter turnover times (Peters 1979; Prepas 1983). In a study by Robarts et al. (1998), rapid ^{32}Pi turnover times of 4–63 min were found for the summer stratification period in the P-limited area in the north basin of Lake Biwa, which increased to 200 min following a typhoon that mixed the water leading to increased DIP concentrations. Notably, radioactive P turnover times reflect the metabolic state of the plankton within a specific sample, representing a specific time at the location of sample collection and incubation, while those derived from $\delta^{18}O_p$ mixing models take the hydrologic condition of an entire lake into account and reflect integrated ecosystem processes within the whole system. The long turnover times we found indicated that sufficient P was present to support productivity in July and October when P inputs were high because of rice paddy irrigation and rainfall runoff. This is consistent with the $\delta^{18}O_p$ values gradually approaching equilibrium (evidence for cumulative intracellular biological recycling) along the water flow path. Although a potential weak P limitation was suggested by a DIN : DIP ratio of 23 (IQR: 16, 47) (R. Yi unpubl.), in comparison to the N : P ratio thresholds for P limitation based on the Redfield ratio (16) (Redfield 1958) or the ratio of 22 observed in Lake Biwa (Tezuka 1985), utilizing nutrient stoichiometry as indicators to evaluate P limitation conditions is complicated as these rations change based on species composition and ecological conditions (Li et al. 2017). The biological P turnover in this study was higher in July than in October. Indeed, previous studies reported that turnover rates varied widely over time and were typically higher in warmer months (May–August) than in cooler months (Wetzel 2001; Labry et al. 2002; Sylvan et al. 2006). This would be expected because water temperature strongly affects metabolic rates (Price and Sowers 2004), including the biological cycling of P in aquatic ecosystems (McLaughlin et al. 2004; Tonderski et al. 2017). The water temperature was probably one of the most important factors affecting P metabolic rates in our study area because the phytoplankton abundances and lake water residence times were not very different between July and October. Despite the expected negligible isotopic effect of $\delta^{18}O_p$ in abiotic processes (such as adsorption/desorption, precipitation, and transport) (Jaisi et al. 2011), the distinction between mixing effects (physical blending of sources) and processing effects (alterations in composition due to various biogeochemical processes) can pose a challenge. However, this study emphasizes gradients and spatial changes of $\delta^{18}O_p$ along a water flow path and not absolute isotope values, offering insights into the P metabolic rate in lake ecosystems. This perspective provides a useful framework for understanding P metabolism within the lake ecosystems.

The $\delta^{18}O_p$ values in January were close to the oxygen isotope equilibrium value in Lake Yanagihira but lower (+11.1‰) than the equilibrium value (+18.0‰) in Lake Hira. A $\delta^{18}O_p$ value much lower than equilibrium values could not be explained by external phosphate inputs or intracellular biological recycling. Inorganic P sources with such a low $\delta^{18}O_p$ value have never been found in the Lake Biwa catchment. However, under P-limited conditions, microorganisms can break down DOP to release Pi and satisfy their P requirements (McLaughlin et al. 2013*a*; Lin et al. 2016). The typical rice paddy irrigation period in Shiga Prefecture is April–September, so January is outside the irrigation season and little water is discharged. Indeed, no water flow into the lagoon–lake system was observed in January, and this is reflected in the water residence time of 83 d. It is likely that DIP was depleted because of the long residence time, despite the low productivity in January, promoting DOP utilization. Extracellular remineralization of organic P is accompanied by kinetic fractionation, which involves the rapid incorporation of one or more ^{16}O atoms in preference to ^{18}O atoms from ambient water into the regenerated DIP (Blake et al. 2005; Colman et al. 2005). These disequilibrium processes typically result in the $\delta^{18}O_p$ of produced DIP becoming lower than the equilibrium values (Liang and Blake 2006; Paytan and McLaughlin 2012). This could explain the low $\delta^{18}O_p$ in January and suggests that at least a fraction of the DIP was derived from organic P compounds hydrolyzed by extracellular phosphoenzymes. This is further supported by the low DIP concentration and high DOP contribution to the TDP pool.

The fractionation involved in the kinetic isotopic effect differs depending on regeneration pathways unique to specific enzymes and organic substrates. For example, the most common enzymes in the freshwater environment, alkaline phosphatase (APase) and 5′-nucleotidases (5′Nase), induce fractionation to different degrees. Phosphomonoester hydrolysis by APase causes isotopic fractionation of −30‰, but 5′Nase target nucleotides and the fractionation in this process is −10‰ (Liang and Blake 2006). Detailed knowledge of the isotope fractionation associated with different DOP substrate and enzyme combinations at any given time/location will allow the proportion of DIP in a water sample derived from extracellular DOP to be calculated. The O isotope compositions of Pi moiety groups bound to unhydrolyzed DOP ($\delta^{18}O_{Porg}$) can be assumed to be in isotope equilibrium with the ambient water. This is supported by the rapid and extensive turnover of P by aquatic organisms and the similar equilibrium $\delta^{18}O_P$ values for living organisms and sinking cells (Paytan et al. 2002; Blake et al. 2005). We estimated the contribution of organic P to DIP in January using a mixing model similar to that published by McLaughlin et al. (2013*a*) and described in Supporting Information Text S2. The estimated contributions of DOP to the DIP pool in January ranged between 27.5% and 78.6%, depending on assumptions made regarding substrates and enzymes (Supporting Information Table S2). These fractions are similar to the proportions found by McLaughlin et al. (2013*a*) of up to 81.9% in the oligotrophic surface water of the Atlantic Ocean. Our calculations provide conservative estimates of the DIP fractions that may have formed through DOP hydrolysis because any phosphate derived from DOP could be taken up by an organism, undergo further biological cycling, and be released into the environment, erasing the low $\delta^{18}O_p$ value (McLaughlin et al. 2013*a*). However, our calculations can be used as a first-order approximation of DOP contribution to DIP.

In April, the $\delta^{18}O_p$ values of the water samples decreased along the water flow path, like in July and October, but the $\delta^{18}O_p$ values were somewhat lower than expected equilibrium values downstream of the inlet, like in January. The DIP concentrations were high ($\sim 0.8\ \mu mol\ L^{-1}$) in April, but $\delta^{18}O_p$

values were lower than the equilibrium value, indicating that enzyme-mediated DOP regeneration have occurred. High APase activity has been used to indicate DOP utilization (Reynolds and Davies 2001), but no consistent relationship between DIP concentrations and APase production was found because different phytoplankton taxa have very different APase activities and P acquisition quotas (Nicholson et al. 2006; McLaughlin et al. 2013*a*). In addition, DOP remineralization may occur in marine and soil systems to meet the demand for organic C rather than P, even at high DIP concentrations (Colman et al. 2005; Nicholson et al. 2006; Spohn and Kuzyakov 2013). For example, low phytoplankton abundances indicated by low Chl *a* concentrations have been found during the spring when algal blooms generally occur, and this was explained by increases in grazing as suggested by the high *Daphnia* biomass in April (S. Ban unpubl.). If zooplankton graze phytoplankton faster than the phytoplankton growth rate, the phytoplankton biomass would be sustained at low levels (George et al. 2015). Large *Daphnia* are the most likely zooplankton to graze on the phytoplankton biomass in lakes at a relatively wide range of nutrient inputs (Carpenter et al. 1995). We suggest that the high DIP concentration in April could be caused by relatively low utilization of DIP in response to a decrease in phytoplankton abundance caused by extensive grazing by *Daphnia*. Under such conditions, a larger fraction of heterotrophic bacteria is likely to escape zooplankton grazing pressure and the bacterial community demand for C may exceed the amount of readily available DOC in the system. To obtain C to sustain growth, the heterotrophic bacteria would produce extracellular enzymes to hydrolyze bioavailable DOP to access the C in DOP even at a high DIP concentration (Clark et al. 1998; Burkhardt et al. 2014). Macroorganisms, such as benthic invertebrates and/or fish, also have the potential to impact freshwater P cycling both directly, through feeding and excretion, and indirectly, by influencing grazing pressure, thus affecting planktonic P cycling (Wetzel 2001; Griffiths 2006). However, the direct contribution of fish and invertebrates to nutrient recycling is likely small compared to that of microorganisms in the overall quantitative cycling of P in aquatic systems (Griffiths 2006). Extracellular enzymatic DOP hydrolysis would explain the low $\delta^{18}O_P$ values measured. We estimated that the amount of P regenerated through extracellular enzymatic DOP hydrolysis in April was 2.0–21.8% of the contribution of organic P to DIP. Less DOP was utilized in April than in January, when the water residence time was longer, DIP concentrations were lower, and more time was available for hydrolysis to occur.

Conclusions

Owing to increased P loading that contributes to lowering water quality, insight into the dynamics of P metabolism within ecosystems is needed to shed light on P sources, cycling, and mechanisms for P retention. Analyzing $\delta^{18}O_p$ in a small lagoon–lake system of Lake Biwa, the largest lake in Japan, we found both spatial variation with values approaching equilibrium and seasonal variability in the absolute values and their spatial distribution. This indicates that the P dynamics in the lagoon–lake system varied seasonally, depending on biological and hydrological conditions. The $\delta^{18}O_p$ measurements revealed that the dominant source of P in the system is external loading derived from agricultural runoff, but that P was recycled along the pathway from the source toward the system outlet. Specifically, $\delta^{18}O_p$ values of lake water approach temperature-dependent isotope exchange equilibrium with considerably high DIP retention during the productive season. This suggests that the lagoon–lake system plays an important role in reducing the agricultural P loading into the main lake, due to high biological P uptake. During the winter non-irrigation season, characterized by low water discharge and longer residence time of the lake waters, the $\delta^{18}O_p$ values are much lower than the expected equilibrium or the source isotope signatures, suggesting the dominance of extracellular enzymatic hydrolysis of DOP. The utilization of DOP provided access to P at times of low DIP, such as January, or to organic C to support bacterial community growth in April. The seasonal and spatial patterns of $\delta^{18}O_p$ along the flow path can be explained by isotope mixing models that include P source signature and community P metabolism. This is the first time that measurements of P turnover in a small lake ecosystem were performed using $\delta^{18}O_p$ mixing models to allow quantitative assessment of P metabolism. However, the linear mixing model used in this study has its limitations when the number of sources exceeds stable isotopes plus one. Our approach focused solely on two primary end-members P source in the watershed, while other sources were deemed negligible. Nevertheless, we acknowledge that there are inherent limitations and uncertainties, including potential mixing effects from the simultaneous input of sediments and degradation of water column DOP to phosphate, leading to variations in isotopic ratio. By relying on instantaneous samples rather than time-series sampling, there may be limitations in discerning the long-term dynamics of P. Further investigation is needed to trace detailed changes of $\delta^{18}O_p$ through time and process; however, we believe these data demonstrate that $\delta^{18}O_p$ mixing models are a powerful tool for improving our understanding of P metabolism in aquatic ecosystems, which cannot be readily achieved by measuring P concentrations alone.

Data availability statement

Data are available in the article Supporting Information.

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Conflict of Interest

None declared.

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