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Contribution of alkaline phosphatase to phosphorus cycling in natural riparian zones in the Wangyu River running into Lake Taihu

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ABSTRACT

Understanding phosphorus (P) cycling is essential to reducing algal blooms and other negative effects in water. However, few studies have investigated the contributions of alkaline phosphatase (APase) or alkaline phosphatase activity (APA) to P cycling in natural riparian zones. Here, seasonal and spatial development of plankton, P fractions, APA fractions, and fluorescence labeled enzyme activity were investigated in a natural riparian zone in the main diversion channel for water transfer into Lake Taihu. Plankton greatly benefited from APase, and APA production was their important advantage for P acquisition and proliferation. Fluorescence labeling varied greatly between species and seasons, indicating different adaptive strategies for dissolved organic P acquisition. Phytoplankton experienced severe P stress when they were the main APA producers in spring and summer, but bacteria were superior P competitors. During autumn and winter, free APA dominated, phytoplankton APA (phyt-APA) deceased because of low biomass or high dissolved reactive P (DRP) and bacterial APA (bact-APA) proportions were high. Bulk APA and DRP were negatively correlated for the entire data-set and during the productive seasons. Significant negative correlation was observed between phyt-APA and DRP in the lacustrine and wetland zones, but the situation was complicated for bact-APA, probably owing to organic carbon limitation.

Keywords: Alkaline phosphatase (APase); Alkaline phosphatase activity (APA); Phosphorus; Phosphorus cycling; Riparian zones

1. Introduction

Phosphorus (P) is an essential element for primary producers that play an important role in algal blooms and eutrophication [1,2]. Among various P fractions, dissolved reactive phosphorus (DRP) (mainly as inorganic phosphate) is the most biologically available form and can be directly utilized by phytoplankton and bacteria; however, it is often present in levels insufficient to satisfy P demand in productive freshwater ecosystems [3–5]. Nevertheless, plankton species may utilize several mechanisms to overcome P starvation, including high-affinity uptake, luxury uptake, and reducing P requirements by lowing energetic metabolism [6,7]. The importance of dissolved organic

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phosphorus (DOP) is well known, and phosphatase production is considered a crucial planktonic response to P status in water [8,9]. Additionally, alkaline phosphatase (APase) and its activity (APA) have received increasing attention, and high APA is often indicative of P limitation or P stress in algal cultures or natural phytoplankton populations [10,11].

There is evidence that phytoplankton can benefit from DOP hydrolysis and that APA is an important driver of its production, especially during growth seasons [12–14]. For example, Wang et al. [15] reported that bloom-causing phytoplankton species could endure P limitation and enhance DOP utilization, resulting in competitive advantages. Moreover, Kwon et al. [16] found that APA and APase hydrolyzed P (APHP) could efficiently supplement available P and thus contribute to the dominance of bloom species. Bacterioplankton are also important producers of APA that can exert a large influence on P cycling in water [17,18]. In a study in a Mediterranean river, Artigas et al. [19] normalized APA to planktonic biomass and suggested that bacteria were more efficient at utilizing DOP during dry periods.

Most studies conducted to explain the ecological effects of APase on primary productivity in aquatic ecosystems [16,20,21] have considered marine systems, while there have been few such investigations of freshwater systems. Moreover, some studies have only provided information on bulk APA, despite increasing attempts to elucidate its complex sources and heterogeneity among taxa [22,23]. Recently, the fluorescence-labeled enzyme activity (FLEA) technique (formerly known as ELF technique) has allowed APA labeling of individual cells, and this method is increasingly being used to specify different taxa in mixed plankton populations [24–26]. However, few studies employing this technique have been performed in natural riparian zones in water channels, which have important environmental influences and imply significant differences in P cycling to natural lakes [27]. Moreover, few studies of the APA kinetics of different fractions are conducted to date.

Lake Taihu is a typical shallow freshwater lake in China that plays an important role in social development. In the present study, we investigated Chlorophyll *a* (Chl *a*) concentration and bacterial abundance, P fractions and APA (bulk and specific APA) throughout the year in the last natural riparian zone in Wangyu River (Jiangsu, China), which runs into Lake Taihu (Fig. 1). APA kinetics and FLEA characteristics of dominant species were also studied. The riparian zone is unique because of its structure and interactions with surrounding environments. Few nutrients can be removed permanently, and P may only be trapped and accumulated in this area, resulting in its being remobilized to other forms and utilized by organisms when environmental conditions change. It is desirable to study the P status and P supplementary mechanisms employed by plankton in this area.

The specific goals of this study were to: (1) determine P status and responses of plankton in the study area; (2) explain the heterogeneities of APA and its important role in P cycling; (3) examine the regulation patterns of APA by DRP and other factors. The results of this study will provide a better understanding of how plankton responds to P stress and the mechanisms through which APase contributes to P cycling during different seasons in natural riparian zones.

2. Materials and methods

2.1. Study area

Wangyu River originates from the Yangtze River and joins Lake Taihu after flowing through several natural riparian zones. The river is the main diversion channel in the water transfer project from the Yangtze River to Lake Taihu and serves as an important barrier to pollutants entering the lake [28]. The effects of the transfer project are often the subject of debate and there has been a great deal of emphasis on quantification of P in running waters [29–31]. Recently, studies have focused on the ecological functions of riparian buffer areas, which have played an important role as terrestrial–aquatic ecotones and corridors between water bodies [32].

Our study was located in Shengtandang, which is the last riparian area in the Wangyu River running into Lake Taihu (Fig. 1). It is situated along the river and its entrance and exit are positioned in it, with a surface area of 0.6 km^2 and an average depth of ~2.0 m. Because of shallow depth and local winds, stratification is absent or obscure at most times. There are small plots of natural wetland inside and they played an important role in water purification and environmental protection. For the morphometry and processes including horizontal transport and sedimentation, it can be considered as a river-type shallow lake and behave differently from natural lakes [27]. With Wangyu River flowing through it, velocity fluctuates and becomes steady along the mainstream line. However, near the small plots of wetland, Shengtandang functions more like a shallow lake.

2.2. Sampling procedures

Four seasonal samplings were carried out (May, August, October, and February) at five sites: site 1

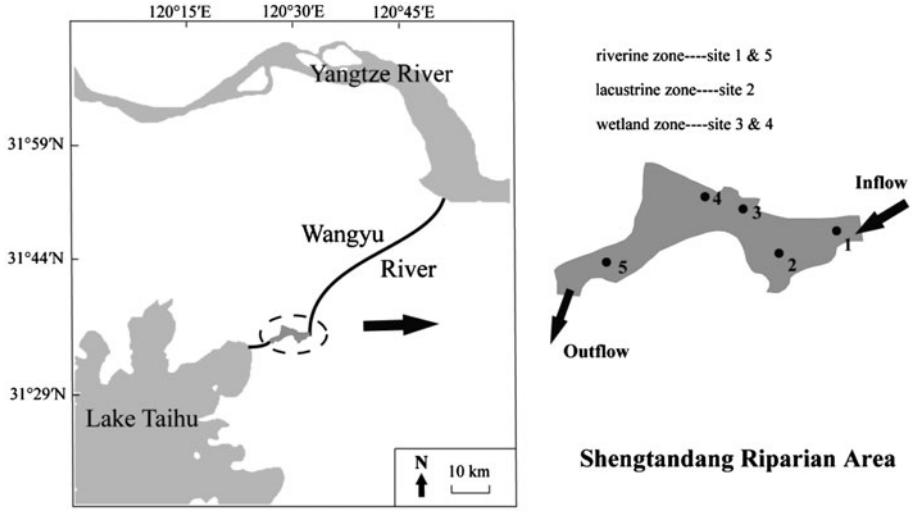

Fig. 1. Research area and sampling sites.

(31°28′9″N, 120°31′41″E), site 2 (31°28′6″N, 120°31′27″E), site 3 (31°28′14″N, 120°31′22″E), site 4 (31°28′16″N, 120°31′16″E), and site 5 (31°28′6″N, 120°30′58″E). The different environmental conditions enabled us to distinguish three distinct regions, a riverine zone (sites 1 and 5), lacustrine zone (site 2), and wetland zone (sites 3 and 4) (Fig. 1). At each site, discrete water samples were collected at three depths from the bottom (0.5, 1.0, and 1.5 m) using a Friedinger water sampler, then diverted into 5-L polyethylene darkened carboys. To decrease the grazing effect by zooplankton, water was immediately filtered through a 200-μm Nitex® mesh, then placed in the dark at 4°C and transported to the laboratory for chemical and enzyme analysis. Since phytoplankton species were representative with larger biomass in the wetland zone [33], phytoplankton analyses and FLEA analysis experiments were carried out at site 3. Supplementary water samples (1 L) were fixed with Lugol's solution and inspected after one week of sedimentation using an inverted microscope for phytoplankton speciation [34]. To prevent contamination, all sample containers were rinsed with 10% hydrochloric acid (HCl) followed by deionized water and sample water prior to collection.

2.3. Analytical methods

2.3.1. Nutrient analysis

Water samples were brought to laboratory station and immediately filtered through GF/F filters (0.45-μm pore size, Millipore) under low vacuum pressure (<15 cm Hg), to minimize cell lysis and physical damage of fragile organisms. Five milliliters $HgCl_2$ (100 ppm final concentration) was added to prevent microbial enzymatic activity [16]. Unfiltered samples and filtrates were collected in acid-washed polypropylene bottles, acidified to pH 1, and then refrigerated until analysis. DRP was determined using the molybdenum blue method [35]. Total phosphorus (TP) and total dissolved phosphorus (TDP) were determined using a combined persulfate digestion for 60 min under high temperature and pressure, followed by spectrophotometric analysis as for DRP [36].

Commercial APase extracted from bovine intestinal mucosa (P7640, Sigma) was used to determine APHP [37]. With triplicate, APase (1 U mL^{-1} final concentration) and Tris/HCl buffer (0.1 mmol L^{-1} final concentration, adjusted at *in situ* pH) were added to 10 mL of filtered water samples, with subsequent incubation for 2 h in darkness at *in situ* temperature. APHP was estimated from the difference in DRP in a sample before and after incubation with APase.

2.3.2. APA analysis

2.3.2.1. Size-fractionated APA. Water samples were processed for APA detection within 2 h. The APA of unfiltered sample (APA_T) and that of 3- and 0.22-μm membrane filtrates ($APA_{<3}$ and $APA_{<0.22}$, respectively) were estimated using *p*-nitrophenylphosphate (*p*NPP, Sigma-Aldrich) as the artificial substrate and APA associated with different size fractions was determined [38,39]. Polycarbonate membrane filters (Millipore) were used and vacuum pressure did not exceed 20 cm Hg.

Triplicate 4.5 mL water samples, supplemented with 0.5 mL of Tris/HCl buffer (adjusted at *in situ* pH) and *p*NPP (close to saturation, 0.3 mmol L^{-1} final concentration) were mixed and incubated at *in situ*

water temperature for 30 min. NaOH (2 μmol L^{-1} final concentration) was then added to terminate the incubation, and blanks with buffer were processed in parallel. The samples were subsequently stirred intensively, after which *p*NPP enzymatic hydrolysis was determined by spectrophotometry in 1-cm cuvettes at 410 nm. Calibration curves of *p*-nitrophenol (*p*NP) were carried out each season to correct for possible background values. The coarse fraction ($APA_{>3}$: APA_T—$APA_{<3}$), which consisted mainly of algae, was defined as phyt-APA, while the finer fraction ($APA_{0.22–3}$: $APA_{<3}$—$APA_{<0.22}$), which was mainly derived from bacteria and picoplankton was defined as bact-APA. The dissolved fraction ($APA_{<0.22}$) was denoted as free APA.

2.3.2.2. Microscopic APA detection. The FLEA characteristics of the dominant species were detected according to the protocol described by Štrojsová et al. [24] and Nedoma et al. [40]. After collection, fresh phytoplankton samples (4.5 mL) were incubated with ELF®97 phosphate (ELFP, 25 μmol L^{-1} final concentration; molecular probes) at *in situ* temperature and samples were buffered with Tris/HCl solution at pH 7.5 to ensure ELFA precipitation [41]. The soluble substrate is cleaved in the presence of phosphatases into ELF®97 alcohol (ELFA), which forms a fluorescent precipitate [42]. Mild centrifugation (300 g) was used to concentrate fresh samples when necessary. The 3-h incubation was terminated by filtering through a membrane filter (0.45-μm pore size, millipore) over mild vacuum (<20 kPa). The filters with retained plankton were stored at −20°C until they were mounted with Citiflor AF1 for inspection with an epifluorescence microscope (Zeiss Axioskop 40, Germany). Phytoplankton cells with a clear ELFA precipitate were defined as positively labeled. At least 30 cells of each dominant phytoplankton species were inspected and the ratios of labeled cells were calculated.

2.3.2.3. Kinetic calculations of APA. The kinetic parameters of APA were estimated using a set of *p*NPP concentrations (0.001, 0.005, 0.01, 0.05, 0.1, 0.2, and 0.3 mmol L^{-1}) and enabling nonlinear analysis. Two models were used for analysis as described by Nedoma et al. [43].

$$v = \frac{V_m \times S}{K_m + S} \quad (1)$$

$$v = \frac{V_{mH} \times S}{K_{mH} + S} + \frac{V_{mL} \times S}{K_{mL} + S} \quad (2)$$

Model 1 was the simple Michaelis–Menten kinetics, where v is the hydrolysis velocity, S is the *pNPP* concentration, V_m is the maximum hydrolysis velocity, and K_m is the half-saturation constant. Model 2 assumed the action of two enzymes (independent and preexisting), where V_{mH} and V_{mL} are the maximum velocities for the high-affinity and low-affinity component, respectively, and K_{mH} and K_{mL} are the corresponding half-saturation constants.

Two equations were fitted to the experimental data by nonlinear regression (Prism 5.0 Graphpad) and analyzed using the *F*-test. Appropriate models were accepted based on a better fit ($p < 0.05$). The kinetics of *p*NPP hydrolysis were also determined based on size-fractionated samples (> and <3 μm) collected during summer.

2.3.3. Physical and chemical analyses

Parameters including water temperature, transparency, and pH were measured using a YSI-multiparameters probe. Flow velocity was measured by Acoustic Doppler Current Profile (Sontek, USA). The euphotic depth was determined as the depth of 1% surface light, which was estimated with Li-cor 192SA spherical sensor. Dissolved organic carbon (DOC) was measured using a total organic carbon analyzer (Malvern liquid TOC, UK). Chl *a* was measured by ethanol extraction [44]: water samples (0.1–0.5 L) were filtered through GF/F filters (0.45-μm pore size, Millipore) and the absorbance was measured at 665 and 750 nm with a spectrophotometer after overnight extraction in ethanol (90% v/v). Bacteria were counted directly after staining formalin-fixed bacterial cells (2% final concentration) with DAPI [45] using an epifluorescence microscope (Zeiss Axioskop 40, Germany).

2.4. Statistical analysis

Water samples were analyzed in triplicate and the results were expressed as the average value. For the whole, the results were analyzed by two-way repeated measures ANOVA (RM-ANOVA), with region as the main factor and season as the repeated measures. Data among groups were compared with one-way ANOVA and the *post hoc* Student Newman-Keuls test (SNK) was used to identify difference between groups. The nonparametric Spearman rank correlation test was applied to analyze the relationships between APA and P fractions. A 2-tailed value of $p < 0.05$ was regarded as statistically significant. All statistical analyses were conducted using the SPSS 17.0 statistical software (SPSS Inc, Chicago, IL) [46].

3. Results

3.1. Environmental conditions in Shengtandang

At each sampling site, water was continuously mixed from different depths, with hydrological and limnological conditions varying significantly among regions (RM-ANOVA, $p < 0.05$; Table 1). Water temperature was lowest during winter and gradually increased in summer. The transparency varied from 30 to 52 cm and pH ranged from 7.38 to 8.78. In the wetland zone, water velocity slowed substantially in response to plots of wetlands, and higher water transparency was observed (SNK test, $p < 0.05$). The euphotic depth was also higher in this region, probably contributing to high photosynthetic activity. In the riverine zone, the water velocity was high and euphotic depth was significantly lower (SNK test, $p < 0.05$). In this region, particulate materials transported by the river were exchanged with the epilimnetic water in different proportions, which led to high turbidity.

3.2. Chlorophyll a and bacterial abundance

The Chl *a* concentrations differed significantly among seasons (ANOVA, $p < 0.05$; Fig. 2), with the lowest value occurring in winter (<10 μg L^{-1}) and significantly higher values being observed during spring and summer (12.15–20.5 μg L^{-1} and 17.3–30.15 μg L^{-1}, respectively; SNK test, $p < 0.05$). These values likely reflected the recovery and bloom of phytoplankton. Chl *a* levels were usually lower at the riverine and lacustrine zones, while they were significantly higher in the wetland zone (SNK test, $p < 0.05$). Chl *a* levels did not differ significantly among sites during winter (ANOVA, $p > 0.05$). Similarly, bacterial abundance was significantly higher in the wetland zone than in other sites (SNK test, $p < 0.05$; Fig. 3), regardless of the season. The riverine zone accounted for the lowest bacterial abundance and increased at site 2.

3.3. FLEA of dominant species

Although fewer algal cells were observed in winter, more than 30 taxa were identified during the study period. Chlorophyceae (*Chlorella vulgaris*) and Cyanobacteria (*Microcystis aeruginosa*) were the dominant species during spring. The later bloom in summer was dominated by Bacillariophyta (*Melosira granulate* (*Ehr.*) *Ralfs*) accompanied by Cyanobacteria (*M. aeruginosa*) and Chlorophyceae (*Ulothrix zonata*). In autumn, Cyanobacteria (*M. aeruginosa*) usually comprised the

Table 1
The main physical and hydrological parameters of surface water

Season	Site	Trans (cm)	Turb (NTU)	pH	DOC (mg L^{-1})	Flow rate (cm s^{-1})	Euphotic depth (m)
Spring (22.1–24.0℃)	1	35	32.5	8.07	3.76	17.5	0.84
	2	38	29.1	8.38	3.55	11.3	1.20
	3	41	25.4	8.21	5.12	5.6	1.56
	4	43	23.4	8.78	5.34	3.8	1.42
	5	37	30.2	7.96	4.06	16.9	0.75
Summer (27.2–30.8℃)	1	37	54.1	8.02	5.84	23.6	0.68
	2	41	52.6	8.17	6.15	13.6	0.96
	3	48	45.5	8.24	7.21	4.3	1.38
	4	46	46.2	8.44	7.52	5.1	1.35
	5	35	53.6	8.15	6.06	21.6	0.79
Autumn (17.1–21.4℃)	1	30	42.6	7.55	1.21	14.2	0.88
	2	34	37.5	7.38	1.15	9.4	0.92
	3	35	30.4	7.48	1.55	3.1	1.24
	4	43	34.5	7.69	1.68	3.6	1.32
	5	33	40.3	7.44	1.48	12.8	0.95
Winter (7.6–8.3℃)	1	42	34.6	7.84	1.18	8.9	1.15
	2	52	29.5	7.75	1.68	7.4	1.20
	3	48	31.2	7.81	1.64	1.2	1.45
	4	51	28.4	7.83	1.26	1.5	1.52
	5	47	32.6	7.79	1.05	8.6	1.13

Notes: Trans, transparency; Turb, turbidity; DOC, dissolved organic carbon.

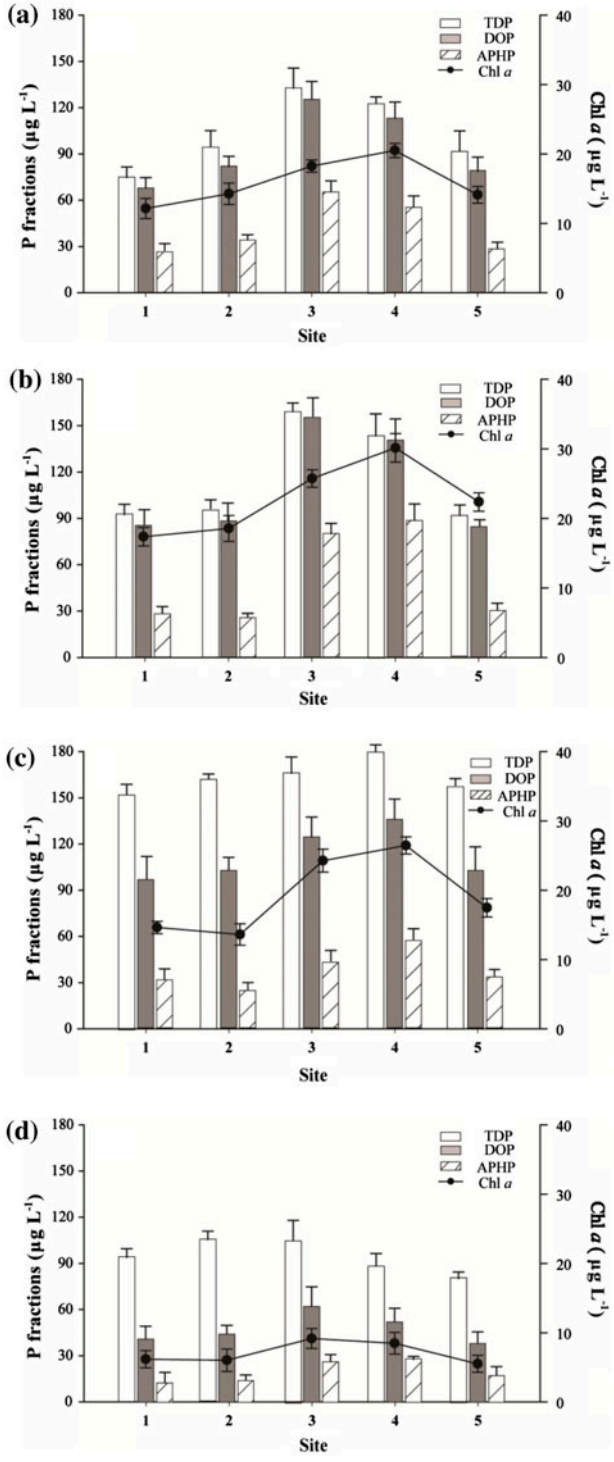

Fig. 2. Seasonal development of P fractions and Chlorophyll *a* concentrations in Shengtandang: (a) spring, (b) summer, (c) autumn, and (d) winter.

majority of the biomass. As expected, fewer species were observed in winter, and Chlorophyceae (*C. vulgaris*) was detected at relatively higher levels.

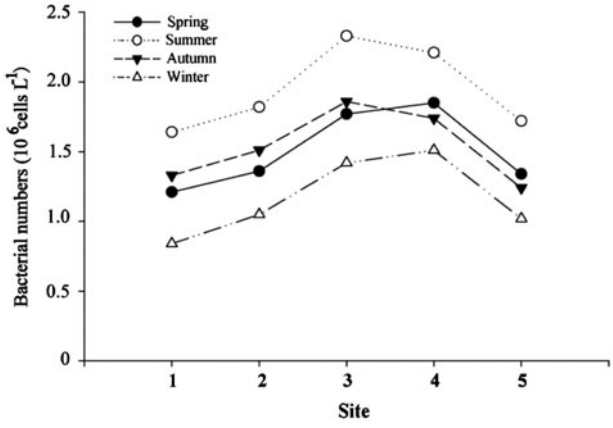

Fig. 3. Seasonal development of bacterial abundance in Shengtandang.

FLEA technique was used to determine the APA of dominant species at the single cell level (Table 2). In spring and summer, dominant algal cells were commonly labeled, most likely originating from algae themselves, as indicated by the even distribution of ELFA fluorescence on the surface. The labeling patterns differed among species, with flake-like, linear structures and large dots for *C. vulgaris*, *M. granulate*, and *M. aeruginosa*, respectively (Fig. 4), and Chlorophyceae having the highest labeling ratio (40.0% for *Chlorella* spp., 35.3% for *U. zonata*). In comparison, the labeling associated with bacteria as small free dots was not clearly detected at that moment. During autumn and winter, the labeling ratio of algal cells decreased significantly (SNK test, $p < 0.05$), and ELFA-labeled bacteria were often detected (Fig. 4(c)). Almost no labeling was detected for *M. aeruginosa*, but the labeling of *C. vulgaris* was still observed during winter.

3.4. Variations of phosphorus fractions

P fractions exhibited a similar seasonal pattern, and TP ranged from 119.94 μg L^{-1} at site 1 in spring to 359.61 μg L^{-1} at site 4 in summer, with an average of 205.11 μg L^{-1} (Fig. 5). The highest TP was observed in the wetland zone in autumn, while lower values were observed during spring and winter (SNK test, $p < 0.05$). TDP was the primary form at most times and was significantly higher in autumn (ANOVA SNK test, $p < 0.05$; Fig. 2), when it ranged from 75.24 to 185.67 μg L^{-1}.

DRP ranged from 3.54 to 61.21 μg L^{-1} and varied significantly across seasons, with the highest concentrations being observed in autumn and winter

Table 2
Seasonal ELF-labeling characteristic of the dominant phytoplankton species in Shengtandang. Indicators (a-d) represent results of one-way ANOVA test using ranking method. Mean values with one certain same letter are not significantly different ($p > 0.05$) and values with entirely different letters are significantly different ($p < 0.05$)[1]

Season	Dominant taxa	Cells inspected	Positive	Negative	Ratio (%)
Spring	*Chlorella vulgaris*	60	24	36	40.0[a]
	Microcystis aeruginosa	40	4	36	10.0[b]
Summer	*Melosira granulate (Ehr.) Ralfs*	36	3	33	8.3[ab]
	Microcystis aeruginosa	30	3	27	10.0[b]
	Ulothrix zonata	34	12	22	35.3[a]
Autumn	*Microcystis aeruginosa*	30	0	30	0.0[d]
Winter	*Chlorella vulgaris*	32	2	30	6.3[c]

[1]Different letters were determined to be statistically different with SNK test, $p < 0.05$.

(RM-ANOVA SNK test; $p < 0.05$; Fig. 6). Typical spring depletion of DRP and the subsequent low values were apparent in summer. DRP was lowest in the wetland zone, and increased in other regions. DOP was the major dissolved P fraction (38.36–155.35 μg L^{-1}), accounting for an average of 75.96% of the TDP in the four seasons (Fig. 2). APHP accounted for 23.80–63.02% of the DOP, with values of 42.00, 50.64, 37.66, and 19.79 μg L^{-1} being observed in spring, summer, autumn and winter, respectively (Fig. 2). It highlights the quantitative importance in terms of P bioavailability. APHP was highest in the wetland zone in summer, when P demand is presumably greatest. Large fluctuations were observed in spring and summer, whereas spatial fluctuations of APHP often showed a plateau in autumn and winter.

Owing to phytoplankton development, DRP exhibited a good inverse power function relationship with Chl *a* ($R^2 = 0.59$) during spring and summer, but the relationship was poor in autumn and winter. In comparison, a significant linear relationship was observed between TP and Chl *a* ($n = 20$, $R^2 = 0.66$, $p < 0.05$), and the relationship between APHP and Chl *a* was also significant ($n = 20$, $R^2 = 0.68$; $p < 0.05$) during all four seasons.

3.5. Fractionation and kinetics of APA

Bulk APA exhibited spatial variability and were significantly higher in the wetland zone than in the other regions at most times (ANOVA SNK test, $p < 0.05$; Fig. 6). The highest bulk APA value (>25 μmol L^{-1} h^{-1}; SNK test, $p < 0.05$) was observed in summer, when phytoplankton blooms are common and the demand for bioavailable P is high. In the other three seasons, especially autumn and winter, when there is sufficient DRP supply, bulk APA decreased to lower values (<10 μmol L^{-1} h^{-1}). The negative power relationship between DRP and bulk APA was significant for the entire data-set ($R^2 = 0.77$, $p < 0.01$, $n = 20$), and this inverse relationship was better during spring and summer ($R^2 = 0.78$, $p < 0.01$, $n = 10$). However, no significant relationship was observed in autumn and winter (Fig. 8(a)).

Size-fractionation revealed that APA was mainly located within phyt-APA (45–87%) during spring and summer, which followed a similar trend as bulk APA. However, APA was mainly associated with bacterial and dissolved fractions in autumn and winter, and free APA dominated at most times. Compared to the results during spring, bact-APA, and free APA were not suppressed with high DRP concentration and their proportions to bulk APA reached the maximum. A significant inverse hyperbolic relationship was observed between phyt-AP and DRP ($R^2 = 0.77$, $p < 0.01$, $n = 12$) in the lacustrine and wetland zones (Fig. 8(b)).

APA is commonly fit to the simple Michaelis–Menten model, but in this study it better fit model 2 ($p < 0.05$, *F*-test; Fig. 7(a)), especially during summer and autumn, which was confirmed statistically in 14 of 20 cases (Table 3). K_{mH} ranged from 0.02 to 10.60 μmol L^{-1} and K_{mL} ranged from 18.9 to 249.0 μmol L^{-1}. During summer, the activity of the >3 μm fraction (phyt-APA) primarily corresponded to the low-affinity component, while the activity of the <3 μm fraction (bact- and free APA) often corresponded to the high-affinity component or the mixture of both components (Table 4, e.g. Fig. 7(b)). The turnover time for DOP was estimated and it was significantly different between sites and seasons (RM-ANOVA, $p < 0.05$; Table 3). During summer, the

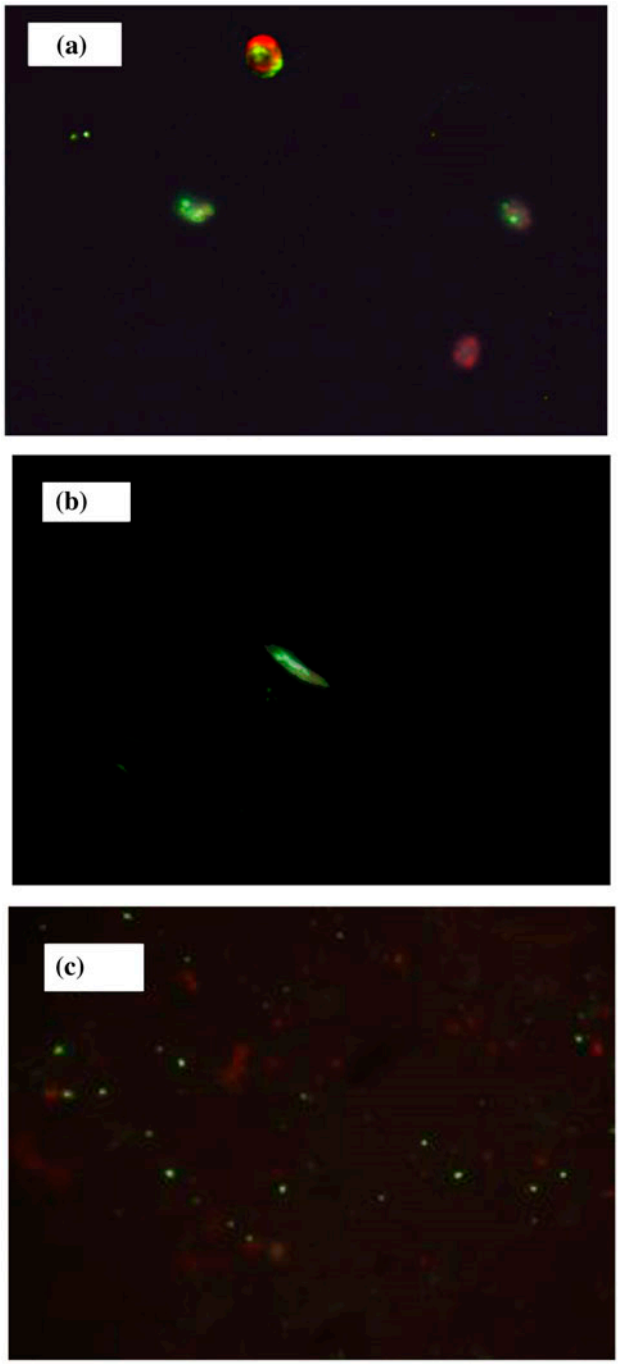

Fig. 4. Micrographs of the ELFA-labeled planktons in Shengtandang. Digital images of phytoplankton taxa taken using the image analysis systems: green and red colors indicate fluorescence of ELFA precipitates and Chlorophyll *a* autofluorescence, respectively: (a) *C. vulgaris* in spring, (b) *M. granulate* (*Ehr.*) *Ralfs* in summer, and (c) Labeling of bacteria in autumn.

turnover rate was high and DOP turnover was fastest at sites 1 and 2 (SNK test, $p < 0.05$). In the other three seasons, the turnover time was shortest in the wetland zone, and the DOP turnover was reduced in the riverine and lacustrine zones.

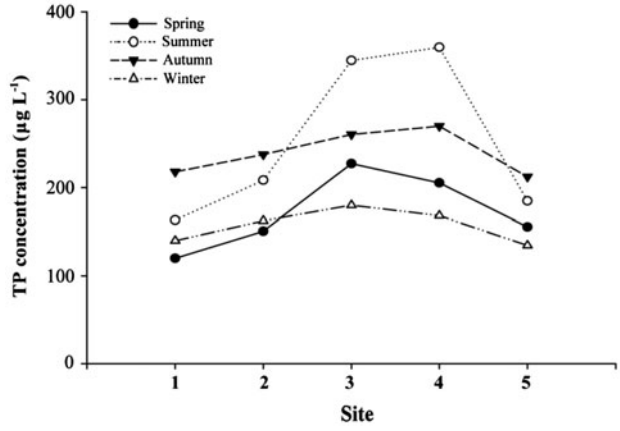

Fig. 5. Seasonal TP concentrations in Shengtandang.

4. Discussion

4.1. P status and responses of plankton

Phosphorus is a key element for assessing water quality or eutrophication risk in aquatic ecosystems, but conventional chemical approaches have focused on DRP and the importance of bioavailable DOP is often ignored [47,48]. It has also been suggested that APA and other bioreporters could be used as important indexes of P status [30,49]. In Shengtandang, DRP was significantly lower (SNK test, $p < 0.05$) during the productive seasons (spring and summer), at which time P deficiency seemed severe. However, high Chl *a* concentration and bacterial abundance were always present, except during winter, and high bulk APA was observed. Given the significant relationships between APHP, TP, and Chl *a* ($p < 0.05$), these findings indicate that APHP was an important supplementary P nutrient and bioavailable P for plankton was underestimated. Overall, classical nutrient criteria may not accurately portray nutrient limitation or nutrient status of plankton. This situation could impede regulatory agencies to evaluate the effect of water transfer project and detect imminent ecosystem-degrading phenomena.

More than 30 phytoplankton taxa were identified and extracellular APA of dominant plankton was clearly detected by FLEA, which confirmed that APA production and DOP hydrolysis were important advantages for nutrient acquisition and proliferation. Results are consistent with those from other freshwater lakes [24,50], with most labeled cells being

Table 3
Kinetic analysis of bulk APA in Shengtandang. The better of the two models was chosen on the basis of *F*-test and model 2 was accepted if it improved the fit at the probability level of $p < 0.05$ Indicators (a-b) represent results of one-way ANOVA test using ranking method at each season. Values with the same letter are not significantly different ($p > 0.05$) and values with different letter are significantly different ($p < 0.05$)[1]

				Model 1		Model 2				
Season	Site	Model	*F*-test (*p*-value)	V_m (μmol L^{-1} h^{-1})	K_m (μmol L^{-1})	V_{mH} (μmol L^{-1} h^{-1})	K_{mH} (μmol L^{-1})	V_{mL} (μmol L^{-1} h^{-1})	K_{mL} (μmol L^{-1})	Turnover time (h)
Spring	1	1	n.s.	8.744	18.6					2.13[a]
	2	2	<0.05			3.657	10.6	14.311	122.1	2.16[a]
	3	2	<0.05			8.013	5.5	15.77	68.8	0.59[b]
	4	2	<0.05			5.137	2.3	8.956	249.0	0.44[b]
	5	1	n.s.	8.889	17.2					1.93[a]
Summer	1	2	<0.05			4.286	0.02	22.5	18.9	0.005[b]
	2	2	<0.01			16.82	0.31	27.83	28.5	0.018[b]
	3	2	<0.05			39.13	4.24	88.76	132.6	0.101[a]
	4	2	<0.05			10.61	2.48	18.54	42.4	0.212[a]
	5	2	<0.01			20.23	7.90	30.31	29.4	0.278[a]
Autumn	1	1	n.s.	4.658	17.1					3.67[a]
	2	2	<0.05			3.324	0.65	9.571	96.0	0.19[b]
	3	2	<0.05			5.933	1.8	22.35	129.4	0.29[b]
	4	2	<0.05			7.756	3.2	23.26	59.3	0.35[b]
	5	2	<0.05			4.528	5.8	15.277	72.6	1.01[a]
Winter	1	1	n.s.	4.27	7.3					1.71[b]
	2	1	n.s.	4.457	13.6					3.05[a]
	3	2	<0.05			3.505	6.6	8.724	170.3	1.72[b]
	4	2	<0.05			2.879	5.0	7.17	186.8	1.63[b]
	5	1	n.s.	4.252	8.7					2.05[b]

[1]Different letters were determined to be statistically different with SNK test, $p < 0.05$.

Table 4
Kinetic analysis of APA fractions (> and < 3 μm) in summer in Shengtandang

Site	APA_T K_m (μmol L^{-1})	>3 μm APA			<3 μm APA		
		Model	R^2	K_m (μmol L^{-1})	Model	R^2	K_m (μmol L^{-1})
1	K_{mH} = 0.02 K_{mL} = 18.9	1	0.993	K_m = 13.86	2	0.996	K_{mH} = 1.16 K_{mL} = 20.56
2	K_{mH} = 0.31 K_{mL} = 28.5	1	0.989	K_m = 59.12	2	0.989	K_{mH} = 1.12 K_{mL} = 25.46
3	K_{mH} = 4.24 K_{mL} = 132.6	1	0.997	K_m = 72.47	1	0.986	K_m =16.12
4	K_{mH} = 2.48 K_{mL} = 42.4	1	0.994	K_m = 87.39	2	0.994	K_{mH} = 0.92 K_{mL} = 18.36
5	K_{mH} = 7.90 K_{mL} = 29.4	1	0.989	K_m = 82.45	2	0.991	K_{mH} = 6.35 K_{mL} = 81.42

observed in spring and summer and the majority of species being Chlorophyceae. This utilization was an endogenous ability that could occur with high DRP (autumn and winter), just with different intensity depending on seasons and competing strengths. These findings were in accordance with the results of APA fractionation. When labeled taxa were examined, the activity was only associated with certain cells and does not indicate the entire population. It is suggested that these cells might be exposed to P-deficient microenvironment, while others were not [24].

When previous studies reported that dominant species were rarely labeled [41,50], the labeling ratio of dominant phytoplankton ranged from 0 to 40.0%. It is indicated that ELFA-labeling of planktons could differ greatly among studies due to many factors, including internal P content, zooplankton grazing, etc. However, *M. aeruginosa* has rarely been labeled as reported [24] and it is possible that they do not undergo severe P deficiency owing to their small size and large surface. During autumn and winter, the labeling ratio of algal cells decreased and the bacteria labeling was often observed, indicating their different adaptive strategies for DOP in water.

Understanding nutrient status is important for controlling algal blooms, and freshwater systems are often defined as P stress or P limitation, which are actually two different concepts. Specifically, P stress indicates a physiological response to DRP supply and potential for DOP hydrolysis, while P limitation refers to a limitation of growth rate or biomass [23,25]. Based on these definitions, P stress is occurring in Shengtandang, especially during productive seasons.

4.2. P cycling and role of APase

During the study period, environmental conditions varied significantly in different zones (RM-ANOVA, $p < 0.05$), and the P cycling differed in response to variations in APHP or APA. In summer (especially in wetland zone), higher APHP and bulk APA were observed (Figs. 2(b) and 6(b)), and DOP turnover was rapid (Table 1). High levels of Chl *a* and bacterial abundance were also observed here with low DRP. The hydrological situation combined with a high euphotic depth here tended to produce high photosynthetic rate and significant P depletion. In comparison, bulk APA was lower in the other regions and DOP turnover was reduced. It was in accordance with Zhang et al. [51] that APA was affected by the hydrological conditions and a high APA could be observed with low flow rate. Considering lower Chl *a* levels and bacterial abundance in the riverine region, rapid velocity and short water residence time probably constrained the growth of or P utilization by plankton. In winter, APHP did not decrease sharply, which can be explained in part by luxury uptake: some phytoplankton species may store available P to sustain growth for the remainder of the period [52].

APA is often used to indicate short term P status, or at least P starvation in freshwater plankton communities [53,54]. However, the interpretation of bulk APA as a whole cannot be indiscriminately applied and it is useful to separate it. The kinetic heterogeneity of bulk APA (Table 3) could partially explain the presence of different APA fractions in this study. Following a similar variation pattern, phyt-APA was high and comprised the largest proportion in spring and

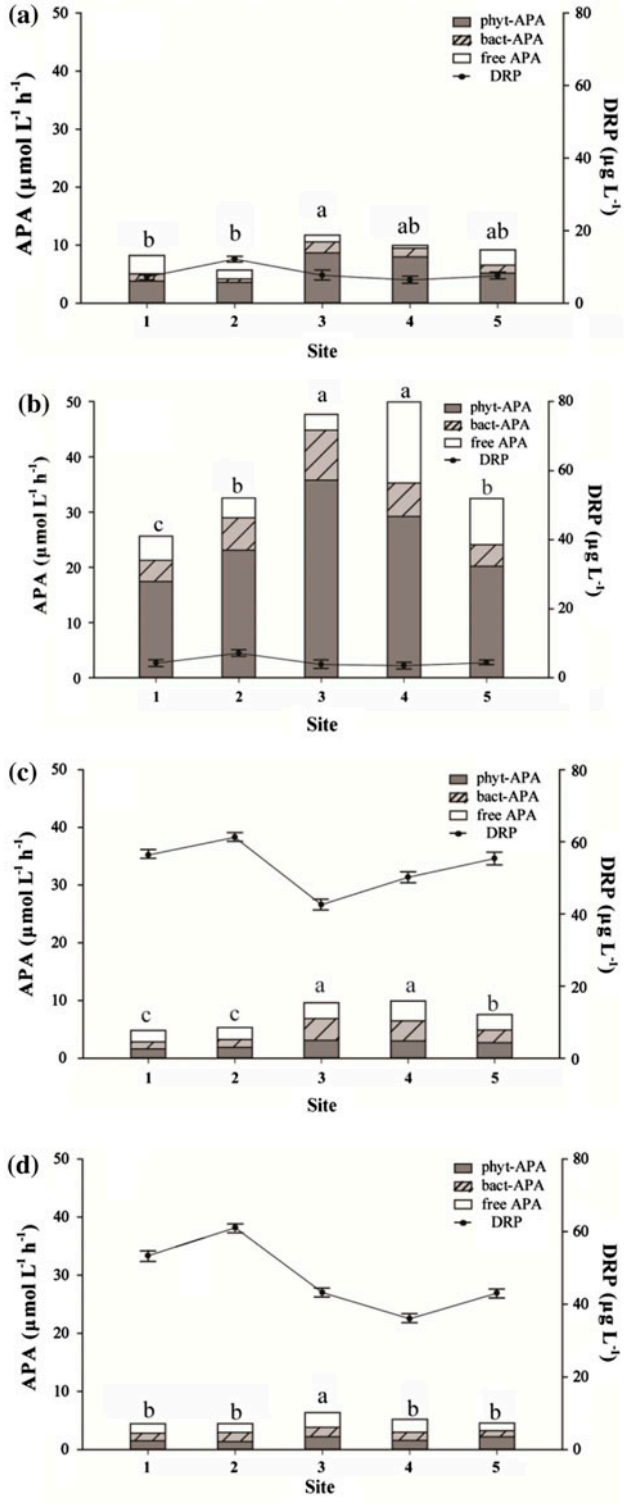

Fig. 6. Seasonal development of APA fractions and DRP in Shengtandang: (a) spring, (b) summer, (c) autumn, and (d) winter. (Different letters on bulk APA columns were determined to be statistically different with SNK test, $p < 0.05$.)

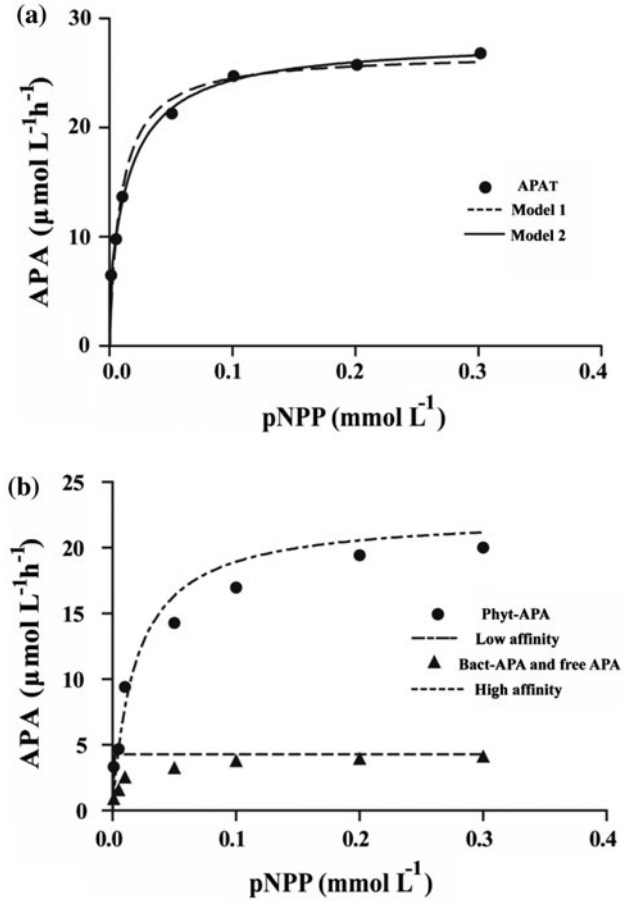

Fig. 7. Kinetic analysis of APA (e.g. site 3 in summer): (a) A comparison of fits of Eqs. (1) and (2) to bulk APA, (b) the two kinetic components (high- and low-affinity components) detected for APA fractions (< and >3 μm) using Eq. (2).

summer, when bact-APA proportion was lower (Fig. 6). Based on the FLEA results, phytoplankton was more likely to suffer P stress and contribute to DOP utilization in the productive seasons. Based on APA kinetic analysis in summer (Table 4), phyt-APA was usually associated with the low-affinity enzyme, and the <3 μm fraction (bact- and free APA) often corresponded to the high-affinity or a mixture of both components. These findings suggest that, although phytoplankton mainly contributed to DOP utilization, bacteria likely utilized DOP more easily owing to their greater affinity for it, which made them superior P competitors in water at this time [55]. In autumn and winter, phyt-APA decreased significantly (SNK test, $p < 0.05$) because of high DRP or low biomass, and free APA dominated at most times. As suggested by

Zhou et al. [56], wastewater effluents and allochthonous activity may have contributed to this result.

The kinetic heterogeneity of DOP hydrolysis has often been overlooked when the complex features of DRP uptake by plankton were discussed [43]. In this study, components representing independent groups of kinetically similar APA were observed (Table 3), most of which were likely localized in different organisms (phytoplankton and bacteria). It is suggested that Shengtandang riparian area is a complex mix of biochemical processes with different characteristic regions. This was also a further evidence of the complex interactions between micro-organisms and P cycling in water.

4.3. Regulation patterns of APA

Robust inverse correlations have commonly been observed between bulk APA and DRP in water, suggesting that it is at least partially suppressed in response to elevated P availability [16,39]. However, despite the significant power relationship between DRP and bulk APA for the entire data-set ($p < 0.01$), no significant relationship ($p > 0.05$) was observed during autumn and winter (Fig. 8(a)). These findings were consistent with Cao et al. [57], who reported that the so-called induction–inhibition mechanism held true with low DRP and could be swamped or even reversed with high DRP concentrations. Bulk APA was clearly detected in the wetland zone (>10 and >4.8 μmol L^{-1} h^{-1} in autumn and winter, respectively), and sufficient DRP failed to fully inhibit APA; therefore, P-limitation cannot explain this phenomenon. Considering the complex sources of APA, it can be assumed that the inhibition of APA is species specific, therefore, field investigations could demonstrate a wide range of variability [58]. For example, the constitutive or basal phosphatase activities of plankton are relatively independent of P status, even when net APA is inhibited by high ambient DRP [49,59]. The availability of organic carbon for bacteria during autumn and winter could also have contributed to this result [60].

As previously reported [57], DOP hydrolysis and uptake of liberated DRP for phytoplankton are metabolically coupled, and phyt-APA was produced specially to compensate for P. Consequently, phyt-APA typically increases with decreasing DRP, and this relationship was particularly evident when compared with other APA fractions [27]. This correlation is significant when there is no other limiting factor and available P plays a key role, but it is less clear where other factors intervene. In Shengtandang, regulation of

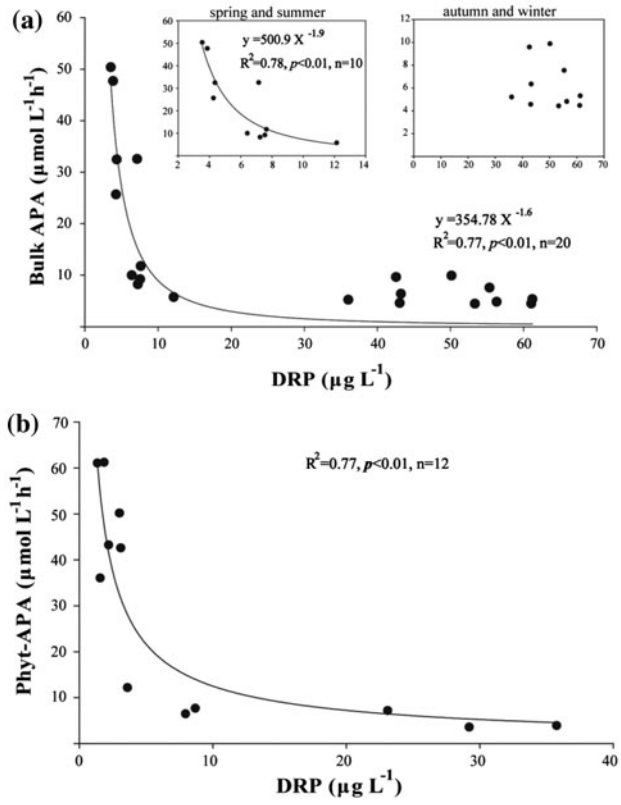

Fig. 8. Relationships between APA and DRP: (a) Bulk APA and DRP for the entire data-set. Inset: data during two periods and (b) Phyt-APA and DRP in the lacustrine and wetland zones.

phyt-APA was demonstrated by an inverse hyperbolic relationship with DRP ($p < 0.05$) in the lacustrine and wetland zones (Fig. 8(b)). However, in the riverine zone in spring, phyt-APA did not increase significantly ($p > 0.05$), despite the lower DRP concentrations. These findings probably reflect the importance of light intensity for phytoplankton growth and APase production in water [6,61]. Specifically, low light availability may impair P acquisition or reduce P demand by phytoplankton.

As previously suggested, the situation was complicated for bact-APA and its production could respond to organic carbon limitation rather than P limitation [60]. As is usually assumed, bacteria are better competitors for P in water, and bact-APA proportions were low in the productive seasons. However, the co-occurence of high bact-APA proportions and DRP was observed in autumn and winter, during which time stimulation by P stress is unlikely. These findings suggest that bacteria may upregulate enzymes to facilitate carbon compensation.

Consequently, the organic moiety of DOP was utilized and DRP accumulated after hydrolysis. Even though DOC content was more than 1 mg L^{-1} in the study area, it should be noted that only part of the DOC (e.g. glucose) was biologically degradable, while a large portion could be refractory [62]. It was also possible that extracellular organic carbon released by algae was very low when phytoplankton began to decline and fall.

5. Conclusions

Based on the results of this study, the following conclusions can be drawn:

(1) APHP was an important supplementary P nutrient, and bioavailable P for plankton was underestimated in Shengtandang riparian area. Confirmed by FLEA technique, plankton in the study area can benefit greatly from APase, and APA production was their important advantage for P acquisition and proliferation, especially during productive seasons with P stress.
(2) The nature and intensity of ELFA-labeling varied greatly between species and seasons. During spring and summer, ELFA-labeled algal cells were often observed, with species mainly belonging Chlorophyceae and few labeled bacteria detected. In autumn and winter, fewer algal cells were labeled and ELFA-labeled bacteria were often observed, indicating their different adaptive strategies for DOP.
(3) Shengtandang is a complex mix of biochemical processes, and heterogeneity of APA kinetics was statistically observed. In spring and summer, phytoplankton was the major producer of APA and bacteria were the superior competitors for P at this time. In autumn and winter, free APA dominated and phyt-APA deceased because of low biomass or high DRP; however, the maximum proportions of bact-APA were attained, probably owing to organic carbon limitation rather than P limitation.
(4) Negative power correlation was significant between bulk APA and DRP for the entire data-set and during the productive seasons, but the relationship was not significant during autumn and winter. The relationship between phyt-APA and DRP was significant in the lacustrine and wetland zones, but not in the riverine zone, which probably reflected the importance of light availability.

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Abbreviations

P	— phosphorus
TP	— total phosphorus
TDP	— total dissolved phosphorus
DRP	— dissolved reactive phosphorus
DOP	— dissolved organic phosphorus
Chl *a*	— Chlorophyll *a*
APase	— alkaline phosphatase
APA	— alkaline phosphatase activity
Phyt-APA	— phytoplankton APA
Bact-APA	— bacterial APA
APHP	— alkaline phosphatase hydrolyzed phosphorus
FLEA	— fluorescence-labeled enzyme activity

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