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RESEARCH ARTICLE



Different pathways of nitrogen and phosphorus regeneration mediated by extracellular enzymes in temperate lakes under various trophic state

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Abstract

Several Italian and Chinese temperate lakes with soluble reactive phosphorus concentrations $< 0.015 \text{ mg L}^{-1}$ were studied to estimate nitrogen and phosphorus regeneration mediated by microbial decomposition and possible different mechanisms driven by prevailing oligo- or eutrophic conditions. Leucine aminopeptidase (LAP), beta-glucosidase (GLU) and alkaline phosphatase (AP), algal, and bacterial biomass were related to trophic and environmental variables. In the eutrophic lakes, high algal and particulate organic carbon concentrations stimulated bacterial respiration ($> 20 \text{ } \mu\text{g C L}^{-1} \text{ h}^{-1}$) and could favor the release of inorganic phosphorus. High extracellular enzyme activities and phosphorus solubilizing bacteria abundance in sediments accelerated nutrient regeneration. In these conditions, the positive GLU-AP relationship suggested the coupling of carbon and phosphorus regeneration; an efficient phosphorus regeneration and high nitrogen levels (up to 0.067 and $0.059 \text{ mg L}^{-1} \text{ NH}_4$ and NO_3 in Italy; 0.631 and $1.496 \text{ mg L}^{-1} \text{ NH}_4$ and NO_3 in China) led to chlorophyll *a* peaks of 14.9 and $258.4 \text{ } \mu\text{g L}^{-1}$ in Italy and China, respectively, and a typical algal composition. Conversely, in the oligo-mesotrophic lakes, very low nitrogen levels (in Italy, 0.001 and $0.005 \text{ mg L}^{-1} \text{ NH}_4$ and NO_3 , respectively, versus 0.053 and 0.371 mg L^{-1} in China) induced high LAP, while low phosphorus (33.6 and $46.3 \text{ } \mu\text{g L}^{-1}$ total P in Italy and China, respectively) led to high AP. In these lakes, nitrogen and phosphorus regeneration were coupled, as shown by positive LAP-AP relationship; however, the nutrient demand could not be completely met without the supply from sediments, due to low enzymatic activity and phosphorus solubilizing bacteria found in this compartment.

Keywords Nutrient regeneration · Extracellular enzyme activity · Phosphorus solubilizing bacteria · Bacterial respiration · Phytoplankton · Lakes · Trophic status

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Introduction

The organic matter present in natural aquatic ecosystems consists of a heterogeneous mixture of both dissolved and particulate polymeric compounds, which are not only from terrestrial origin (Pugnetti et al. 2010) but also from phytoplankton photosynthetic production and exudates from benthic microalgae or macrophytes (Maie et al. 2006). The biogeochemistry of organic matter in aquatic environments is the result of physical, chemical, and biological processes (Amon et al. 2001). The prokaryotic (i.e., both bacteria and picophytoplankton) community is a major driver in nutrient regeneration through the production of extracellular enzymes, which has been considered as a limiting step in organic matter utilization (Munster and Chrost 1990). Through the hydrolytic activity of microbial enzymes, carbon, nitrogen, and phosphorus are recycled, supporting the microbial heterotrophic metabolism (Hoppe et al. 2002; Millar et al. 2015). Previous studies (Hudson et al. 1999; Cotner and Biddanda 2002; Chrost and Siuda 2006) demonstrated that the plankton community contributes to nutrient supply more significantly than external loading or larger organisms such as fish. There are many reports showing that, under nutrient limitation, algae, bacteria, and zooplankton can secrete extracellular enzymes; therefore, extracellular enzyme activity is usually considered an important indicator of nutrient limitation in aquatic ecosystems (Drerup and Vis 2017; Hill et al. 2012; Sala et al. 2001). Under phosphorus deficiency, apart from organic phosphorus hydrolysis mediated by alkaline phosphatase, plankton can acquire inorganic phosphorus through the acceleration of turnover rate of the P transport proteins embedded in the cell's plasma membrane (Pitt et al. 2010), the utilization of phosphorus from polyphosphate body (Jacobson and Halmann 1982), and the regeneration and release from sediments induced by organic detritus decomposition (Solim and Wanganeo 2009). Together with phosphorus, nitrogen is a major nutrient that affects the productivity of aquatic systems; in lakes, the cycle of this element is driven by microbial processes: bacterial oxidation and reduction of nitrogen compounds are coupled with assimilation by algae, photosynthetic bacteria, and aquatic plants (Wetzel 2001). Within the nitrogen cycle, a balance occurs between nitrogen sources and losses, where the first ones include nitrogen fixation and input from precipitations and surface drainage, while nitrogen losses are due to effluent outflow, reduction by bacterial denitrification, and sedimentation of inorganic and organic nitrogen-containing compounds. Under increasing nutrient loading, lakes become more productive; in this condition, their trophogenic zone can become depleted in nitrate and ammonia concentrations due to photosynthetic assimilation. Most of the readily available nitrogen occurs

in soluble form in the water, in the interstitial water of sediments, and in the vegetation of shallow productive lakes.

Nutrient regeneration mediated by microbial extracellular enzymes has widely been investigated (Pusceddu et al. 1999; Manini et al. 2003; Cao et al. 2005; Zhou et al. 2007; Arnosti et al. 2014; Li et al. 2016); however, little attention has been given to compare the relationships among microbial activities, biomass, trophic state, and nutrient coupling in oligomesotrophic and eutrophic lakes (Chrost and Siuda 2006). Extracellular enzyme production is not a general response to nutrient depletion, but a species-specific or cell-specific process (Strojsova and Vrba 2006). The pattern of enzyme production is highly variable with environmental conditions and species (Hoppe et al. 2002). Indeed, in oligotrophic waters, the relative importance of the microbial food web and of heterotrophic metabolism, mostly based on regeneration processes through extracellular enzyme activity, is generally higher than in eutrophic systems (Biddanda et al. 2001; Cotner and Biddanda 2002). On the other hand, internal nutrient regeneration due to microbial decomposition and chemical desorption is usually stronger in eutrophic waters, therefore increasing the whole nutrient pool (Torres et al. 2017). Hence, the relationship among nutrient regeneration and microbial activity depends on species and overall trophic conditions.

Only in recent years, the causative linkage between microbial recycling of nutrients and eutrophication in shallow water bodies has received attention (Caruso et al. 2005, 2010, 2013, 2017; Chrost and Siuda 2006; Li et al. 2016; Tang et al. 2017; Torres et al. 2017). Warmer climatic conditions are also expected to increase lake dependence on nutrient regeneration (Shimoda et al. 2011); in transitional environments, Zacccone et al. (2014) found that the heterotrophic prokaryotic metabolism was stimulated during warm seasons, when prokaryotes were able to degrade organic substrates more rapidly than in other periods. Based on these considerations, studies focusing on the response of microbial metabolism to trophic gradients could improve current understanding of the role played by the microbial loop in natural ecosystems under different trophic state.

The dynamics of microbial processes involved in the carbon and phosphorus cycles in lakes under similar temperate latitude conditions were the main focus of the scientific cooperation research program between the Italian National Research Council and the Chinese Academy of Sciences within the collaborative research project “Understanding expression and regulation of microbial enzymes involved in organic matter decomposition and the interrelationship between alkaline phosphatase and eutrophication in Italian and Chinese water bodies at different trophic levels.” The hypothesis of this research was that nitrogen and phosphorus regeneration mediated by microbial decomposition and their driving mechanisms could vary depending on the prevailing oligo- or eutrophic status. To assess this, in this study, the dynamics of microbial activities (enzymatic decomposition and

heterotrophic production) and biomass, the coupling between microbial activities and nutrient regeneration, and the mechanisms of nutrient regeneration, also including the role of the sediments in such processes, were investigated in oligo-mesotrophic compared to eutrophic lakes. Particularly, the rates of microbial activities involved in carbon, nitrogen, and phosphorus cycles across brackish and freshwater bodies covering from oligo- to hypertrophic conditions were studied in two seasonal periods (autumn 2008 and spring 2009).

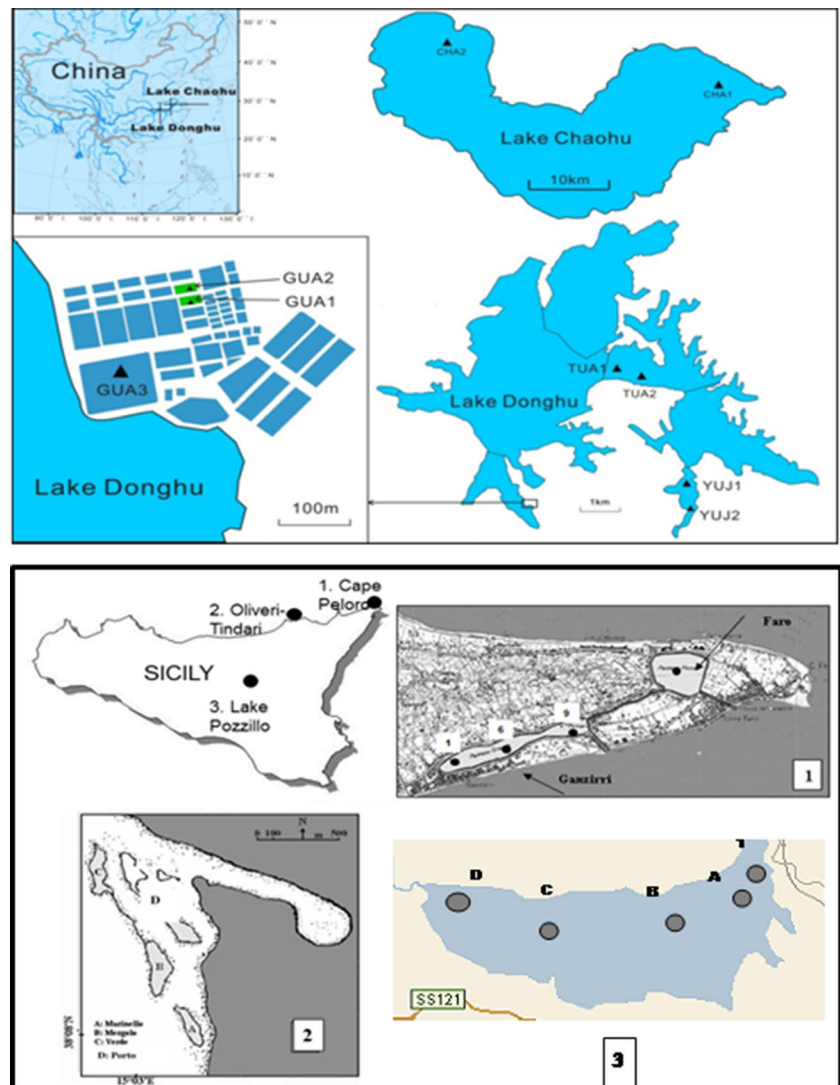
Materials and methods

Study sites description

The ecosystems investigated in Italy were Cape Peloro, Oliveri-Tindari, and Pozzillo, all located in Sicily (Fig. 1). The Cape Peloro ecosystem is a brackish system located in

the north-eastern corner of Sicily, which consists of two neighboring water bodies, lakes Ganzirri and Faro, connected to each other by means of narrow shallow channels. Lake Ganzirri is a shallow water body (maximum depth about 7 m, 0.34 km² surface area), which is colonized by macroalgae covering its sandy bottoms and suffers periodic dystrophic crises in summer. Lake Faro is a small and deep coastal lake (maximum depth 28 m, 0.26 km² surface area), which is characterized by the presence of hydrogen sulfide in the hypolimnion and by a red water layer, located at a 10-m depth, linked to the development of phototrophic sulfur bacteria. The Oliveri-Tindari ecosystem is a small coastal area (approximately 0.69 km²) located along the Tyrrhenian Sea. It currently includes four shallow brackish water bodies: Lakes Marinello, Mergolo, Verde, and Porto, which are separated from the sea by a system of littoral sand bars (Leonardi and Giacobbe 2001). Lakes Marinello, Mergolo, and Verde receive surface runoff inputs of nutrients from the surrounding

Fig. 1 Map showing the sampling sites in Chinese and Italian lakes



lands used for pasture or agriculture (Caruso et al. 2005, 2013; Leonardi et al. 2005). Lake Verde, due to its location under a cliff colonized by seagulls, is particularly rich in nutrients and occasionally suffers algal blooms and anoxia in summer (Leonardi et al. 2006); lake Porto is mainly influenced by seawater inflows. Lake Pozzillo, with a total surface area of 7.7 km² and a maximum depth of 49.5 m, is the largest artificial lake in Sicily, where algal blooms of *Planktotrix rubescens* have developed in recent years. Their detailed characteristics have been reported by Caruso et al. (2010).

In China, lakes Chaohu, Donghu (Yujiahu and Tuanhu), and Guanqiao ponds were investigated, which all suffer severe nutrient loading and algal blooms. Lake Chaohu covers a surface area of 770 km² and has a mean depth of 3.0 m. A great amount of industrial wastewater and domestic sewage were discharged into the lake, causing serious eutrophication and cyanobacteria blooms, mainly composed of *Microcystis* and *Anabaena* (Xu et al. 2005). Lake Donghu is a medium-sized shallow freshwater lake, with a surface area of 27.9 km² and an average depth of 2.2 m. Lakes Yujiahu and Tuanhu are two basins of lake Donghu, which have different characteristics. Lake Yujiahu is more eutrophic, as it is affected by a wastewater inlet which led to high nutrient loading, while lake Tuanhu is far away from the wastewater drainage and has a relatively low trophic status. Guanqiao ponds are located nearby lake Donghu, and are used for aquaculture. Due to large inputs of fish feeds, their water quality has progressively worsened, as evidenced by the occurrence of algal blooms (*Microcystis* and *Anabaena*) in summer.

Sampling procedure

Surface water samples (0.2 m) were collected in autumn 2008 and spring 2009. In Italy, only one station, which was located at the deepest site of each water body, was sampled in lakes Marinello, Mergolo, Verde, and Porto; in lake Faro, one more sampling (4 m) was performed. Due to the larger spatial extension, three and six stations were sampled in lakes Ganzirri and Pozzillo, respectively. In China, three ponds within the Guanqiao ponds and two sampling sites within lakes Tuanhu, Yujiahu, and Chaohu were chosen for this study. Mixed water was taken with a Friedinger sampler. Samples (500 ml or 1 L, depending on the trophic status) for qualitative phytoplankton analysis were concentrated by filtration and resuspension to a final volume of 30–50 mL and preserved with Lugol's solution. Samples for bacterial abundance (BA), biomass (BB), production (BP), and respiration (BR) were also collected. Simultaneously, surface sediments (0–1 cm) were sampled using a Peterson grab sampler to analyze extracellular enzymes and functional bacteria. All the samples were immediately stored in cooling boxes and kept under refrigerated conditions (+ 4 °C) until their arrival at the laboratory. The water

samples were processed the same day, and the sediments were stored at 4 °C in the dark for 2–4 days before analysis.

Physical, chemical, and trophic parameters

Measurements of temperature, dissolved oxygen, and pH were performed using a multi-parametric YSI probe. Water transparency was determined using a Secchi disc. Nutrient concentrations, in terms of ammonia (NH₄), nitrite (NO₂), nitrate (NO₃), total phosphorus (TP), total dissolved phosphates (TDP), and soluble reactive phosphorus (SRP), were measured using a Cary 50 Varian spectrophotometer, according to the analytical procedures reported by Strickland and Parsons (1972), except for ammonia concentrations which were determined according to Aminot and Chaussepied's (1983) method. The concentration of dissolved organic phosphorus (DOP) was calculated as the difference between DTP and SRP. The concentration of particulate phosphorus (PP) was calculated as the difference between TP and TDP. Chlorophyll *a* (Chl. *a*) content was measured using the ethanol extraction method (Lorenzen 1967). Total suspended matter (TSM), particulate organic carbon (POC), and particulate nitrogen (PON) were also determined. TSM was evaluated as the dry weight of the particles collected on pre-combusted (480 °C for 4 h) and pre-weighed Whatman GF/F filters. To estimate POC and PON, 500-mL water samples were concentrated on pre-combusted Whatman GF/F filters and processed at 980 °C in a Perkin-Elmer CHN-Autoanalyzer 2400, using acetanilide as standard (Iseki et al. 1987).

The trophic state index (TSI) of lakes was assessed according to the classical Carlson (1977) TSI. It was calculated using three parameters, including Chl. *a* (μg L⁻¹), Secchi disk transparency (trans, in meters), and TP (μg L⁻¹) according to the following equations:

$$\text{TSI (Chl.}a) = 9.81 \text{ Ln (Chl.}a) + 30.6 \quad (1)$$

$$\text{TSI (trans)} = 60 - 14.41 \text{ Ln (trans)} \quad (2)$$

$$\text{TSI (TP)} = 14.42 \text{ Ln (TP)} + 4.15 \quad (3)$$

The comprehensive TSI (TSI_c) was calculated using the formula

$$\text{TSI}_c = 0.540 \text{ TSI (Chl.}a) + 0.297 \text{ TSI (trans)} + 0.163 \text{ TSI (TP)} \quad (4)$$

The comprehensive TSI_c ranks lakes on a numerical scale of 0 to 100, and the scale values correspond to different trophic state conditions: 0 < TSI ≤ 30 oligotrophic, 30 < TSI ≤ 40 oligo-mesotrophic, 40 < TSI ≤ 50 mesotrophic, 50 < TSI ≤ 60 light eutrophic, 60 < TSI ≤ 70 middle eutrophic, and 70 < TSI ≤ 100 hypereutrophic (Yang et al. 2012).

Biological parameters

The dominant algal species were identified using a Zeiss microscope at $\times 640$ magnification according to Hu et al. (1979).

Samples for BA counts were collected into sterile cryogenic vials (50 mL), fixed in formaldehyde (2% final concentration), and stored at $+4\text{ }^{\circ}\text{C}$ until analysis. Suitable aliquots (1–2 mL) were filtered through polycarbonate black membrane (0.2- μm porosity) and the filters stained with 4',6-diamidino-2-phenylindole (DAPI; Porter and Feig 1980). They were further observed under a Zeiss AXIOPLAN 2 imaging epifluorescence microscope equipped with an AxioCam digital camera (Zeiss). All cells in a minimum of 20 microscopic fields per sample were counted. For BB determinations, the length (L) and width (W) of the cells were measured using Image-Pro Express software, and the volume of the cells was calculated as $(\pi/4)W^2(L-W/3)$ (Bratbak 1985). The formula used to calculate bacterial cellular carbon content, according to Loferer-Kröbber et al. (1998) was $218 \times V^{0.86}$. Total BB ($\mu\text{g C L}^{-1}$) was determined from bacterial abundance multiplied by the cellular carbon content.

BP was measured by [^3H]-leucine incorporation (Kirchman et al. 1985), as modified for the micro-method according to Smith and Azam (1992). Triplicate 1-mL aliquots of unfiltered water samples were incubated with L-[4,5- ^3H] leucine (specific activity 1.0 Ci/mmol) in 2.0-mL polypropylene tubes for 1 h at room temperature. Two samples added with 5% trichloroacetic acid prior to [^3H]-leucine addition served as blanks. Incubations were stopped by the addition of 5% trichloroacetic acid (final concentration). After rinsing of the obtained pellets with 1.5-mL cool 5% trichloroacetic acid and 80% ethanol, 1 mL of scintillation cocktail was added. The radioactivity incorporated into bacterial cells was counted in a liquid scintillation counter (1219 Backbeta, LKB). Leucine incorporated into bacterial cells was converted into carbon production using a factor of 3.1 kg C/mol (Simon and Azam 1989).

For BR measurements, the electron transport system assay according to Packard (1985) was used. This method is based on the conversion of tetrazolium salt [INT: 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride] into formazan (Packard and Williams 1981). Water samples, varying from 500 mL to 2 L, were pre-filtered through a 250- μm mesh size net to remove large particles and concentrated on GF/F Whatman glass fiber membranes under reduced pressure (1/3 atm). Filters were folded into cryovials and immediately stored in liquid nitrogen until analysis in laboratory (< 45 days) to prevent enzymatic decay. The INT reduction reaction was monitored spectrophotometrically at 490 nm. The ETS V_{max} (in $\mu\text{g C L}^{-1} \text{h}^{-1}$) was corrected for in situ temperature with the Arrhenius equation and a value of 15.8 kcal/mol for activation energy (Packard et al. 1975). BR values

were used to calculate BGE, which gives an indication of efficiency in the conversion of the assimilated C into biomass, by applying the formula $\text{BP}/(\text{BP} + \text{BR})$.

Phosphorus solubilizing bacteria (PSB) present in sediments, including inorganic phosphorus solubilizing bacteria (IPB) and organic phosphorus solubilizing bacteria (OPB), were determined using the traditional culture method and reported as the number of colony forming units (CFU) (Nautiyal 1999). Briefly, sediment slurry obtained from 5.0-g sediment and 45-mL sterile water was diluted 1000 times. For IPB, 0.2-mL diluted slurry was spread onto IP agar plates [glucose, 10 g; $\text{Ca}_3(\text{PO}_4)_2$, 5 g; NaCl, 0.3 g; $(\text{NH}_4)_2\text{SO}_4$, 0.5 g; MgSO_4 , 0.3 g; KCl, 0.3 g; MnSO_4 , 0.03 g; FeSO_4 , 0.03 g; agar, 20 g; pH 7.2; distilled water, 1000 mL] containing calcium phosphate as the sole phosphorus source. Bacterial colonies were counted after incubation at $30\text{ }^{\circ}\text{C}$ for 5 days, and colonies with unique morphology were picked up and purified. For OPB, 0.2-mL diluted slurry was inoculated into OP medium, having a composition similar to IP medium but containing 0.025 g lecithin as the sole phosphorus source and 5-g CaCO_3 instead of calcium phosphate. Bacterial colonies were counted after incubation at $30\text{ }^{\circ}\text{C}$ for 4 days.

Total extracellular enzymes activities (EEA) were determined on whole unfiltered 200-mL water samples as the potential rates of LAP, GLU, and AP activities, according to the Hoppe multiconcentration method (1983). Fluorogenic methylumbelliferyl (MUF)-derived compounds, MUF-phosphate, and MUF- β -glucopyranoside (Sigma-Aldrich) were used as substrates for AP and β -GLU activities, respectively. L-leucine-4 methyl-7-coumarinylamide (Leu-MCA, Sigma-Aldrich) was used for LAP activity measurements. Ten milliliters of water samples (five tubes and in triplicate) was added with increased amounts (0.1–20 μM , final concentrations) of each fluorogenic substrate, to reach enzyme saturation (Caruso et al. 2005). The fluorescence released by hydrolysis of the substrates into the highly fluorescent products MUF and MCA was measured using a spectrofluorometer (Mode 960, Shanghai Jingmi company) at time zero, immediately after the substrate addition, and 1–3 h after incubation at in situ temperature. Excitation/emission wavelengths were the following: 380/440 nm for LAP, 365/445 nm for AP, and β -GLU. Sterilized water samples were used as the blanks. Hydrolytic rates were estimated from the increase in fluorescence due to enzymatic hydrolysis of the non-fluorescent substrates. Calibration was performed with known concentrations of the standards MUF and MCA.

For enzymatic measurements in sediments, 2 g of samples were re-suspended in a 1:10 ratio (w/v) into sterile water (obtained from the same study site), vortexed, and left undisturbed for 30 min at room temperature; aliquots of the obtained supernatants were diluted into sterile water (1: 10 v/v ratio) and treated as described above for water samples.

Statistical analysis

Environmental and microbial variables were analyzed by Spearman correlation coefficients and linear regression using SigmaPlot 2000 and 18.0 package for windows. Spearman's test was performed using the SPSS 18.0 package (SPSS, Chicago, IL), choosing a probability value of 0.05 as the threshold level for statistical significance.

Results

Tables 1 and 2 show the averaged values of the main physical and chemical parameters and TSI obtained in each lake. Compared to the Italian lakes, all the Chinese lakes were characterized by significantly ($P < 0.01$) higher TSM, TP, and Chl. *a* concentrations, and consequently lower transparency values which resulted in higher TSI values. According to TSI, all the studied lakes in China were eutrophic, while the Italian ones were under oligo-mesotrophic conditions. The obviously great differences between eutrophic and oligo-mesotrophic lakes were also reflected in different algal composition. In the eutrophic lakes, the dominant algal species belonged to Chlorophytes and Cyanobacteria, while in the oligo-mesotrophic lakes, they belonged to dinoflagellates and Bacillariophyceae (see Tab. 1 SI supplementary materials).

Differences in the community structures were also associated to differences in trophic conditions considering under this term also differences in the nutrient concentration and composition. In the eutrophic lakes, all fractions of nitrogen, PP, POC, and PON exhibited significantly higher ($P < 0.01$) values than those measured in oligo-mesotrophic lakes.

However, no distinct difference was observed for SRP and DOP; this latter showed very low concentrations in two types of lakes, especially in the eutrophic systems (Fig. 2). SRP was found at concentrations lower than 0.015 mg L^{-1} in all studied lakes (Fig. 2d). For each lake, the N:P ratios, as in the DIN:SRP ratios—where DIN was calculated from the sum of NH_4 , NO_2 , and NO_3 —are reported (Tab. 1). N:P ratios lower than the Redfield ratio (16) were found in lakes Mergolo and Porto, indicating N limitation. In lakes Pozzillo and Marinello, P limitation was enhanced, as suggested by N:P ratios over 35. All the Chinese lakes were characterized by P limitation, quantified by N:P ratios by one order of magnitude higher than Italian lakes, with a peak in lake Yujia.

In the eutrophic lakes, significant positive relationships between BR and BB as well as between SRP and TP were observed (Table 2 SI). LAP showed significant positive relationships with PP, Chl. *a*, POC, and PON; also, AP and GLU were linked by a significant positive relationship (Table 2 SI). In oligo-mesotrophic lakes, significant positive relationships were detected among BP, BR, nitrogen compounds, Chl. *a*, and PON (Table 3 SI). Additionally, LAP and GLU showed significant positive relationships with Chl. *a*, POC, and PON; a significant positive relationship was observed between LAP and GLU too (Table 3 SI). Significant positive relationships were found between BGE and BP, consistently both in eutrophic and oligo-mesotrophic lakes (Table 2 SI and 3 SI).

Concerning the secretion of specific extracellular enzymes by the microbial community, in oligo-mesotrophic lakes, the highest measured LAP and AP activity rates (Fig. 3 a and c, respectively) were coupled with lower nitrogen and phosphorus concentrations, indicating nitrogen and phosphorus limitation (Fig. 2). In the eutrophic lakes, a relatively low SRP

Table 1 Mean values \pm standard deviation of temperature, transparency, dissolved oxygen (DO), pH, total suspended matter (TSM), chlorophyll *a* (Chl. *a*), total phosphorus (TP), soluble reactive

Study site	<i>n</i>	Temperature (°C)	Transparency (m)	DO (mg L ⁻¹)	pH	TSM (mg L ⁻¹)	Chl. <i>a</i> (μg L ⁻¹)	TP (μg L ⁻¹)	SRP (μg L ⁻¹)	TSI
Italy: lake Ganzirri	6	16.4 ± 0.4	1.5 ± 0.5	6.7 ± 0.4	7.9 ± 0.2	33.5 ± 11.0	5.1 ± 0.7	46.9 ± 24.2	8.3 ± 3.0	31 ± 4
Italy: lake Pozzillo	12	10.8 ± 0.3	0.4 ± 0.1	8.2 ± 0.8	8.2 ± 0.2	22.4 ± 14.5	10.5 ± 4.8	43.7 ± 16.4	6.2 ± 3.0	41 ± 2
Italy: lake Faro	4	15.3 ± 1.1	5.5 ± 1.4	7.8 ± 1.2	7.8 ± 0.1	23.7 ± 4.2	1.7 ± 0.9	33.6 ± 10.2	9.5 ± 3.0	19 ± 3
Italy: lake Marinello	2	12.2 ± 0.1	1.0 ± 0.1	8.3 ± 1.3	8.2 ± 0.1	14.5 ± 0.8	2.7 ± 0.3	33.9 ± 3.5	3.8 ± 1.3	28 ± 2
Italy: lake Verde	2	14.2 ± 0.5	1.0 ± 0.1	7.9 ± 0.7	7.9 ± 0.3	39.6 ± 12.8	14.9 ± 0.7	44.7 ± 3.3	4.1 ± 5.0	38 ± 1
Italy: lake Mergolo	2	12.5 ± 0.5	1.1 ± 0.5	8.0 ± 1.1	8.1 ± 0.2	42.2 ± 9.2	2.8 ± 0.2	60.3 ± 4.6	10.2 ± 9.8	29 ± 1
Italy: lake Porto	2	12.2 ± 0.9	1.1 ± 0.2	7.8 ± 0.1	7.8 ± 0.1	38.3 ± 3.1	0.3 ± 0.0	35.4 ± 15.2	8.3 ± 5.3	17 ± 3
China: lake Yujia	4	28.4 ± 1.2	0.2 ± 0.0	11.5 ± 1.9	7.7 ± 0.2	273.2 ± 89.2	123.1 ± 20.6	198.0 ± 23.1	13.8 ± 11.0	60 ± 5
China: lake Tuanhu	4	27.8 ± 0.4	0.9 ± 0.3	9.7 ± 0.9	7.8 ± 0.0	330.3 ± 63.6	26.0 ± 15.2	46.3 ± 10.6	9.8 ± 5.0	42 ± 3
China: lake Chaohu	4	18.9 ± 5.5	0.3 ± 0.1	6.9 ± 0.3	7.3 ± 1.4	526.3 ± 128.5	104.1 ± 28.3	258.9 ± 85.2	10.4 ± 6.0	58 ± 5
China: pond Guanqiao	6	27.6 ± 0.7	0.5 ± 0.2	7.3 ± 1.7	8.5 ± 0.2	600.1 ± 184.1	258.4 ± 45.2	778.1 ± 158.2	14.5 ± 8.0	64 ± 8

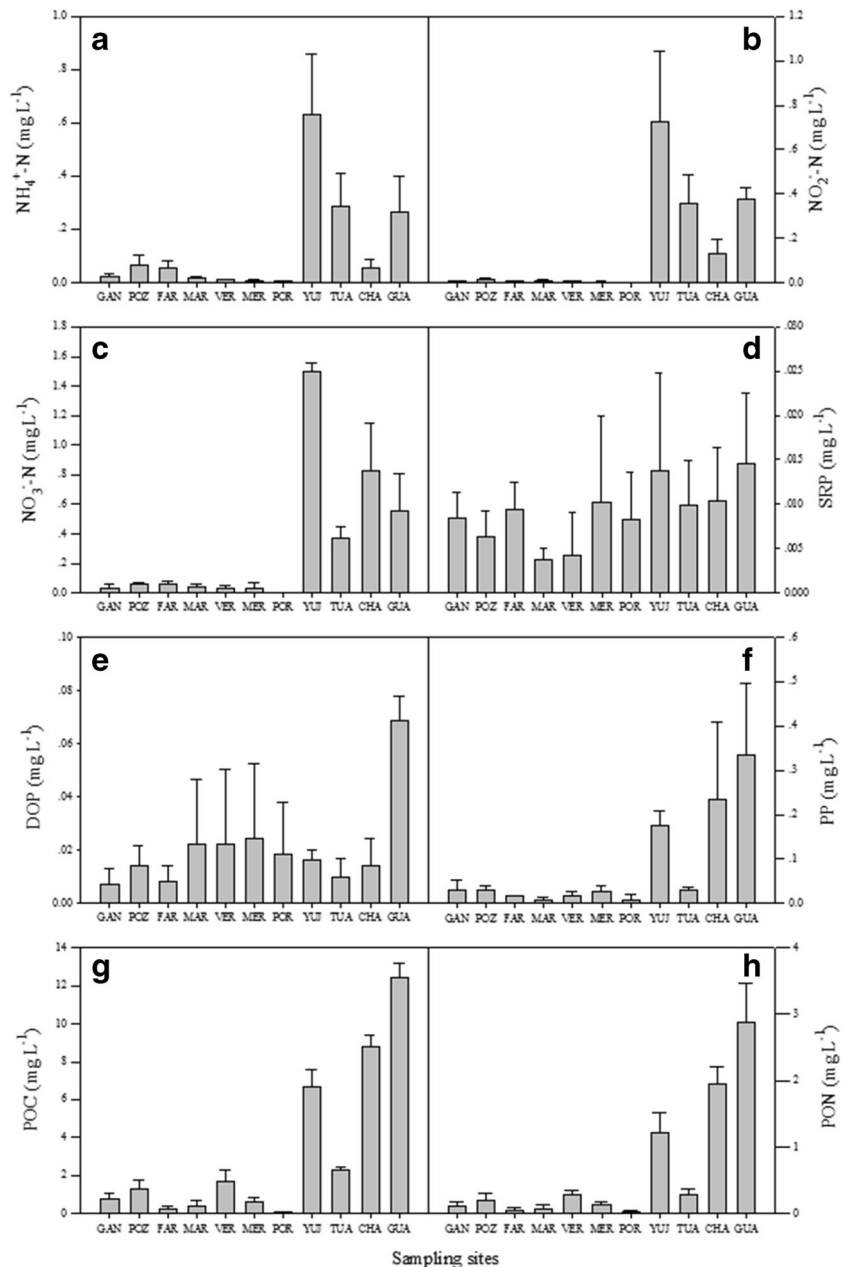
n, number of collected samples

Table 2 Mean values ± standard deviation of ammonia (NH₄), nitrite (NO₂), nitrate (NO₃), and N:P molar ratios (calculated from DIN:SPR ratios where DIN = NH₄ + NO₂ + NO₃). The factors used to convert the micrograms of nitrogen and phosphorus into micromoles were 14 and 31, respectively

Study site	<i>n</i>	NH ₄ ⁺ (mg L ⁻¹)	NO ₂ ⁻ (mg L ⁻¹)	NO ₃ ⁻ (mg L ⁻¹)	N:P (as DIN:SRP)
Italy: lake Ganzirri	6	0.019 ± 0.013	0.005 ± 0.004	0.034 ± 0.032	15.5 ± 17.2
Italy: lake Pozzillo	12	0.067 ± 0.067	0.011 ± 0.011	0.059 ± 0.058	48.8 ± 59.5
Italy: lake Faro	4	0.054 ± 0.046	0.005 ± 0.005	0.059 ± 0.022	27.5 ± 7.3
Italy: lake Marinello	2	0.001 ± 0.000	0.013 ± 0.002	0.057 ± 0.011	37.1 ± 0.9
Italy: lake Verde	2	0.001 ± 0.000	0.008 ± 0.001	0.011 ± 0.002	21.9 ± 0.8
Italy: lake Mergolo	2	0.001 ± 0.000	0.001 ± 0.000	0.005 ± 0.001	9.0 ± 0.2
Italy: lake Porto	2	0.001 ± 0.000	0.001 ± 0.000	0.005 ± 0.001	2.1 ± 0.7
China: lake Yujia	4	0.631 ± 0.409	0.725 ± 0.311	1.496 ± 0.060	457.5 ± 155.7
China: lake Tuanhu	4	0.288 ± 0.127	0.358 ± 0.382	0.371 ± 0.078	229.3 ± 87.4
China: lake Chaohu	4	0.053 ± 0.037	0.130 ± 0.062	0.827 ± 0.513	214.9 ± 65.4
China: pond Guanqiao	6	0.265 ± 0.499	0.373 ± 0.604	0.558 ± 0.338	182.5 ± 10.8

n, number of collected samples

Fig. 2 Mean concentrations ± standard deviation of nitrogen (ammonia, NH₄ (a); nitrite, NO₂ (b); nitrate, NO₃ (c); and particulate organic nitrogen, PON (h)), phosphorus (soluble reactive phosphorus, SRP (d); dissolved organic phosphorus, DOP (e); and particulate phosphorus, PP (f)), and carbon (particulate organic carbon, POC (g)) fractions measured in the water column of Chinese and Italian lakes. GAN, Ganzirri; POZ, Pozzillo; FAR, Faro; MAR, Marinello; VER, Verde; MER, Mergolo; POR, Porto; YUJ, Yujiahu; TUA, Tuanhu; CHA, Chaohu; GUA, Guanqiao



concentration was measured in correspondence with low AP activity rates (Figs. 2d and 3c). No distinct differences between oligo-mesotrophic and eutrophic lakes were found for BA, BB, and BP (Fig. 3d, e, and f, respectively), while compared to oligo-mesotrophic lakes, a significantly higher BR value measured in eutrophic lakes (Fig. 3g) resulted in a lower BGE value (Fig. 3h). In sediments, significantly higher AP, LAP, and GLU activities were recorded in eutrophic lakes (Fig. 4b, c, and d, respectively). Also, in eutrophic lakes, a great abundance of PSB was observed on plate cultures, while no PSB were found in oligo-mesotrophic lakes (Fig. 4a).

Discussion

This study analyzed for the first time a wide range of temperate region ecosystems covering similar geographical latitudes, focusing on nitrogen and phosphorus regeneration through the organic polymers decomposition mediated by the extracellular enzymatic activities, taking into consideration the trophic status as the main discriminating factor. This was the main objective of this research, although water bodies under different haline conditions were compared (i.e., Chinese freshwater lakes versus Italian fresh and brackish water lakes). Salinity has been reported to affect significantly the composition of microbial communities (Tang et al. 2012) and consequently influence the patterns of microbial activity. However, controversial results for this parameter have been reported, with higher protein decomposition rates sometimes found at higher salinity levels (Cunha et al. 2010) or higher hydrolytic activities measured in diluted waters (Caruso 2010; Monticelli et al. 2014), also in wetlands (Morrissey et al. 2014). Nevertheless, in this study, lakes comprised within the same regional territory and showing a quite narrow salinity range (i.e., Oliveri-Tindari, from 28.05 to 31.12 and from 24.34 to 29.85 in November and in March, respectively; data not shown) were found to be discriminated according to their trophic status, ranging from oligo- to eutrophic status. This observation reinforced our idea that within the considered areas, the trophic status, rather than salinity, was the main driver of ecosystem functioning, with particular reference to microbial processes. Similarly, comparing the microbial loop functioning in the benthic compartment of three different coastal lagoons, Manini et al. (2003) suggested the source and quality of the organic inputs as well as the benthic bacterial ability of mobilizing organic matter pool and converting the mobilized carbon into bacterial biomass as the main factors in regulating the efficiency of the lagoons in channeling detrital carbon towards higher food web levels. Further studies also underlined that lake microbial communities are significantly affected by the trophic status (Kostrzewska-Szlakowska 2005; Dai et al. 2016) especially in sediments (Shao et al. 2013; Huang et al. 2017). To our knowledge,

however, the relationships linking microbial processes involved in organic matter turnover in aquatic ecosystems with their trophic status have rarely been deepened (Manini et al. 2003; Caruso et al. 2005, 2010, 2017; Chrost and Siuda 2006; Shao et al. 2013; Li et al. 2016; Huang et al. 2017; Torres et al. 2017).

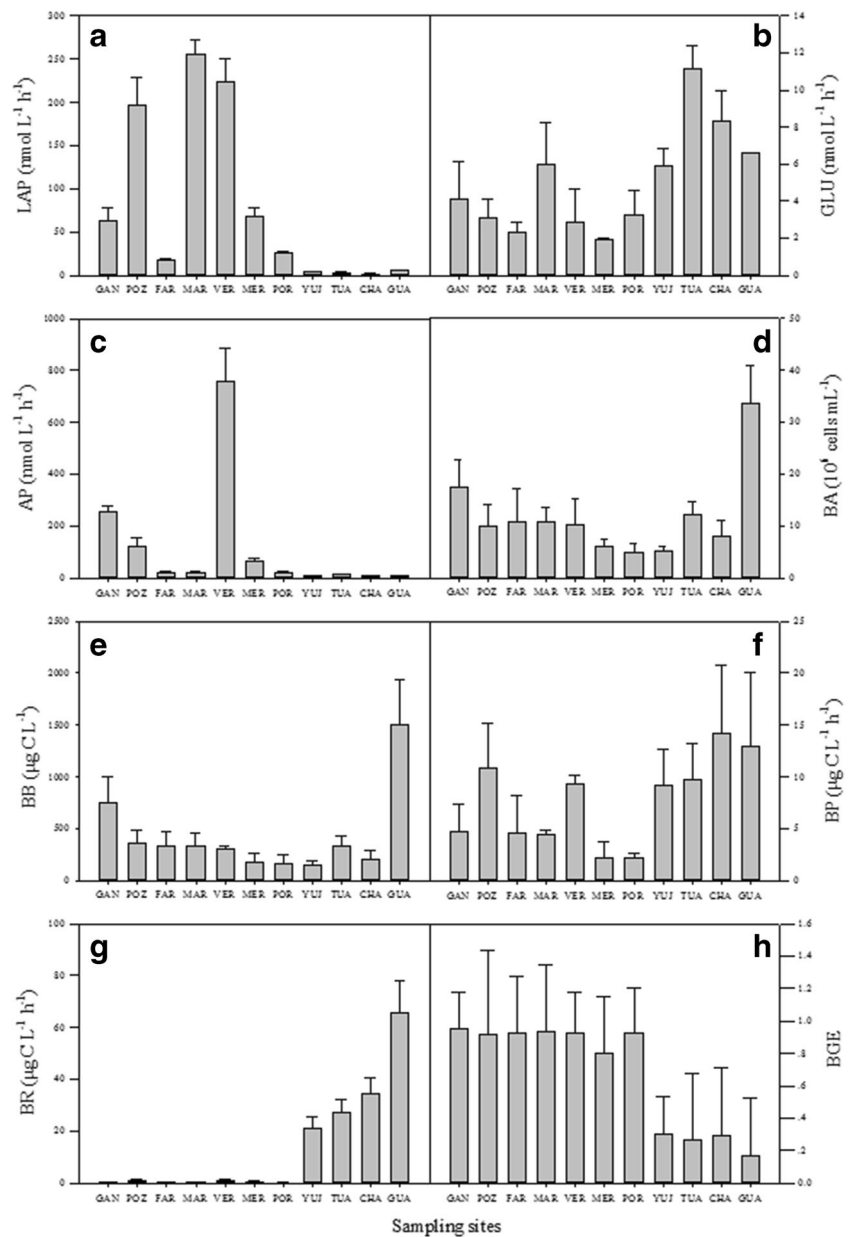
Microbial dynamics in eutrophic versus oligo-mesotrophic lakes

The microbial patterns observed in the present investigation pointed out some main differences between eutrophic and oligo-mesotrophic lakes. Firstly, compared to the oligo-mesotrophic lakes, the algal community structure of the eutrophic lakes showed differences that should be partially attributed to those in nutrient concentrations and composition. In addition, in the eutrophic lakes, no correlations between Chl. *a* and BP, BR, and BB were found (Table 2 SI), suggesting that algae and bacteria were in a reciprocal competitive relationship. In such environments, some dominant algal species (i.e., *Microcystis*) could not be able to produce extracellular enzymes (Cao et al. 2005), while extracellular enzymes should originate mainly from bacteria, as indicated by the strong microbial activity of algal attached bacteria (Cao et al. 2016). The significant direct correlations of bacterial biomass and respiration with SRP and TP found in the studied eutrophic lakes (Table 2 SI) suggested that in such environments, phosphorus should be the key limiting factor for primary production; at the same time, in the oligo-mesotrophic lakes, the relationships between bacterial production and respiration, Chl. *a* and nitrogen, suggested that, besides phosphorus, also nitrogen was a limiting factor. Furthermore, in the eutrophic lakes, carbon and phosphorus cycling were coupled, as suggested by the significant positive relationship between AP and GLU (Table 2 SI). However, the detection of high GLU activity rates (Fig. 3b) in association with low SRP and DOP contents (Fig. 2d and e, respectively) led us to suppose that in the eutrophic lakes, the microbial community, in spite of an active hydrolytic process on polysaccharides, could not be fully able to overcome both the carbon and phosphorus deficiency.

Possible mechanisms of nutrient regeneration and the role of sediments

The low AP activity rates measured in the eutrophic lakes in correspondence with relatively low SRP values suggested that, besides phosphorus regeneration from the hydrolysis of organic phosphates commonly mediated by AP, another regeneration pathway was possible to satisfy the plankton needs. Given that a great amount of algal detritus, as indicated by high PP, POC, and PON (Fig. 2f, g, and h, respectively), was accumulating in such

Fig. 3 Mean values \pm standard deviation of microbial activities (leucine aminopeptidase, LAP (a); beta-glucosidase, GLU (b); alkaline phosphatase, AP (c); bacterial production, BP (f); bacterial respiration, BR (g); and bacterial growth efficiency, BGE (h)) and biomass (bacterial abundance, BA (d); bacterial biomass, BB (e)) measured in the water column of Chinese and Italian lakes. GAN, Ganzirri; POZ, Pozzillo; FAR, Faro; MAR, Marinello; VER, Verde; MER, Mergolo; POR, Porto; YUJ, Yujiahu; TUA, Tuanhu; CHA, Chaohu; GUA, Guanqiao



conditions, it could be inferred that phosphorus could be recycled and released from sediments. In the eutrophic lakes, the sedimentation and decomposition of higher PP, PON, and POC could be hypothesized to make an important contribution to regeneration and release of nutrients from the sediments, especially phosphorus. This hypothesis could further be corroborated by the significantly higher EEA rates and PSB abundance found in the benthic compartment compared to those found in the oligo-mesotrophic lakes (Fig. 4). Even though higher EEA rates and PSB abundance could be partially attributed to higher temperature, it is likely that in the examined eutrophic lakes, the kind of available polymeric substrates was the main factor accounting for the high PSB and EEA values

measured in this study. Particularly, among microbial enzymes, AP is involved in the regeneration of P from organic phosphates; the amount of SRP potentially released by AP in the sediments reached peak values of 140 $\mu\text{g g}^{-1}$ in Verde and 380 $\mu\text{g g}^{-1}$ in Guanqiao (Fig. 4). PSB have been recognized to play an important role in P mineralization in freshwater ecosystems (Singh and Tejo 2012), a process which could accelerate SRP production (Qu et al. 2013; Maitra et al. 2015). OPB and IPB have been found to facilitate the SRP release through the enzymatic hydrolysis of organic P and unbinding inorganically bound P by organic acids, respectively (Jana 2007; Zhou et al. 2011). Finally, even though BA and BB showed comparable values in all the studied environments (Fig. 3d and e,

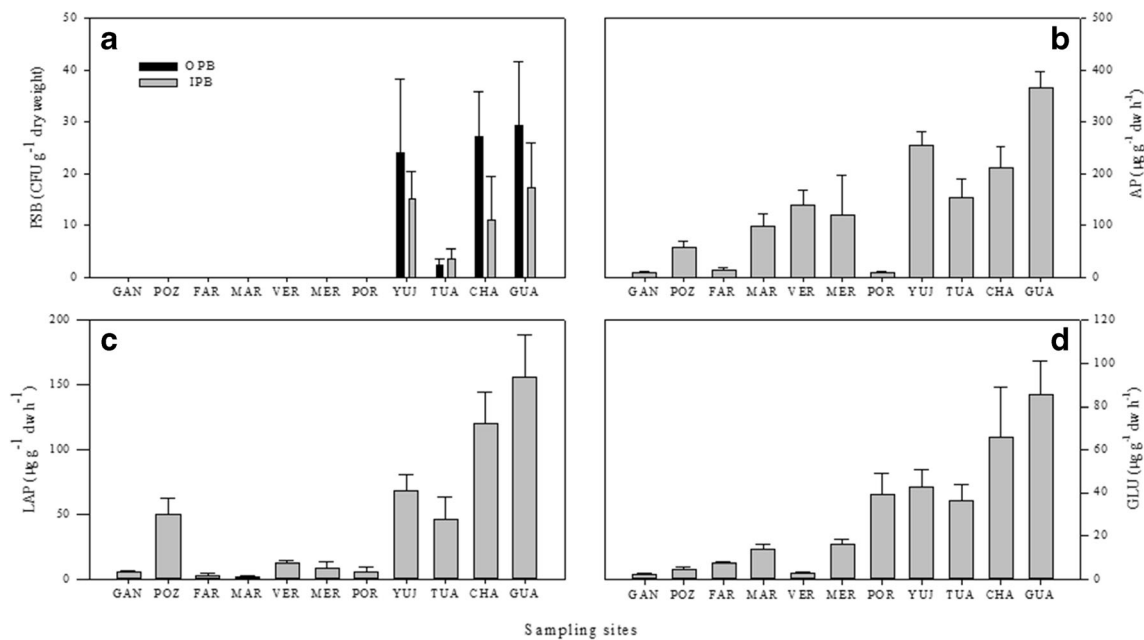


Fig. 4 Mean values \pm standard deviation of phosphorus solubilizing bacteria (a) (PSB, in colony forming units per gram of sediment, dry weight of sediment) and extracellular enzyme activity rates (alkaline phosphatase, AP (b); leucine aminopeptidase, LAP (c); beta-glucosidase, GLU (d), in micrograms per gram and per hour, dry

weight of sediment) found in the sediments of Chinese and Italian lakes. GAN, Ganzirri; POZ, Pozzillo; FAR, Faro; MAR, Marinello; VER, Verde; MER, Mergolo; POR, Porto; YUJ, Yujiahu; TUA, Tuanhu; CHA, Chaohu; GUA, Guanqiao

respectively), the significantly higher BR ($P < 0.01$) recorded in the eutrophic lakes compared to those found in the oligo-mesotrophic ones (Fig. 3g) resulted in a significantly lower BGE value ($P < 0.01$, Fig. 3h). This result was in contrast with previous research showing that BGE increased in eutrophic conditions under carbon sufficiency (del Giorgio and Cole 1998). A possible explanation for our results was the limitation of available organic carbon and a bacterial respiratory pattern regulated by the dissolved oxygen in the eutrophic lakes. In Polesie Lubelskie peatlands, nutrient availability stimulated the outbreak of algal bloom, which covering the surface water reduced the amount of dissolved oxygen available, further changing bacterial respiratory pattern from aerobic to anaerobic one (Szafranek-Nakonieczna and Stępniewska 2014). In lake Erie, harmful algal bloom was associated with the occurrence of anoxia and hypoxia (Watson et al. 2016). In some ecosystems, the consumption of dissolved oxygen was mainly attributed to bacterial respiration and algal detritus decomposition (Harrison et al. 2017; Huang and Chen 2013). Thus, under carbon limitation, bacteria consumed more organic carbon for respiration than for production, further reducing the oxygen content. Furthermore, the oxygen consumption induced by bacterial respiration and decomposition of organic carbon could fuel the release of phosphorus from the sediment of eutrophic lakes, made available by the reduction of iron-bound phosphorus complex under anoxic conditions

(Golterman 2001). Thus, another mechanism of phosphorus release could be related to the need of bacteria to mineralize a larger part of the sedimentary organic matter in order to obtain the same amount of energy; this could directly led to phosphorus regeneration and indirectly reduce the oxygen available (Golterman 2001; Torres et al. 2017). Hence, the regeneration and release of phosphorus from the sediments through physical-chemical (anoxic desorption and release) and biological (PSB and AP hydrolysis) pathways jointly compensated the phosphorus required for plankton growth in the eutrophic lakes, whose deficiency should be closely related with algal coverage, organic detritus (carbon) accumulation, and decomposition. In other words, in the eutrophic lakes, plankton could acquire more phosphorus mainly through its “pumping” from the sediments induced by organic detritus.

Compared to the eutrophic lakes, in the oligo-mesotrophic lakes, the significantly lower nitrogen and phosphorus concentrations (Fig. 2) coupled with higher LAP and AP (Fig. 3a, c) indicated the importance of extracellular enzymes in nutrient cycling. Also, in the sediments of such lakes, low EEA rates and PSB were found (Fig. 4), pointing out that nutrient regeneration mainly occurred in water column through the activity of extracellular enzymes. Moreover, the algal composition and especially the low BR activity (Fig. 3g) found in the oligo-mesotrophic lakes suggested that extracellular enzymes should be mainly produced by algae. On the other hand, in these

environments, the relationship between algae and bacteria should be mutualistic, as suggested by the significant positive relationships between Chl. *a* and BP and BR (Table 3 SI). In addition, the significant positive relationship between GLU and LAP (Table 3 SI) indicated the close coupling between carbon and nitrogen cycling.

Conclusion

In the studied lakes, nutrient concentration and fractionation affected the algal species composition and biomass, which in turn influenced the excretion of organic carbon and dissolved oxygen content. Two different pathways of nutrient regeneration were observed according to the trophic conditions. In the eutrophic lakes, characterized by carbon and phosphorus limitation, high algal coverage on surface water and strong decomposition of a great amount of algal-derived organic carbon (mostly by bacteria) resulted in oxygen consumption. This occurred in association with high AP activity rates and high abundance of PSB in sediments, which jointly accelerated the regeneration and release from the sediments of phosphorus, potentially available to satisfy plankton needs. In these conditions, carbon and phosphorus cycling were coupled. In the oligo-mesotrophic lakes, characterized by nitrogen and phosphorus limitation, algae and bacteria could gain nitrogen and phosphorus through producing their own LAP and AP enzymes, respectively. In these lakes, the coupling between carbon and nitrogen cycling was indicated by the relationship between GLU and LAP. In summary, different algal and bacterial species exhibited their specific nutrient regeneration patterns through either production of extracellular enzymes or “pumping” from sediments, driving the shift from one to another pathway according to the different trophic condition.

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