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Eutrophication and nutrient limitation in the aquatic zones around Huainan coal mine subsidence areas, Anhui, China

Qitao Yi, Xiaomeng Wang, Tingting Wang, Xijie Qu and Kai Xie

ABSTRACT

The eutrophication of three small lakes in the aquatic zones at the Huainan coal mine subsidence areas, designated as east site (ES), central site (CS), and west site (WS), were studied. Nutrient content, species, and nitrogen (N) to phosphorus (P) ratios were obtained through water quality analyses. Nutrient limitation was evaluated by nutrient enrichment bioassays (NEBs) in the autumn of 2012 and spring of 2013. Average annual concentrations of total phosphorus (TP) were 0.05, 0.08, and 0.10 mg/L, and total nitrogen (TN) concentrations were 0.77, 1.95, and 2.06 mg/L in the water column at CS, ES, and WS, respectively. All of the three lakes exhibited 'meso-eutrophic' states and the TN:TP ratio ranged from 25:1 to 74:1 with variability between seasons and sites. NEBs verified that primary productivity in the lakes at ES and WS were mainly limited by P, while N limitation or N and P co-limitation was present in the aquatic zones at CS due to unavailable dissolved inorganic nitrogen. In the studied lakes, the blue-green algae, which comprised 70% of all identified species, was the predominant taxa, while the micro-zooplankton taxa was dominant, indicating a typical trophic structure of eutrophic lakes.

Key words | coal mines, eutrophication, nutrient limitation, subsided lakes

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INTRODUCTION

Huainan coal mines in Anhui Province are located in the flood plain of the Huaihe River watershed, which is one of the seven biggest watersheds in China. Due to the unique local hydrology and geology, characterized by thick Quaternary deposits, low topography, high groundwater levels, and numerous river flows, underground excavations of coal resources has led to extensive land subsidence and submergence. At present, 150 km^2 pockets of land subsidence have formed, of which around 100 km^2 are permanently flooded, and this area consists of ponds, reservoirs, wetlands, small lakes, etc. Several projects have been carried out by local mining companies or the government in recent years (HMGCL 2010), aimed at investigating the potential use of water in these subsidence areas. These evaluations have been produced using hydrological models based on surveys of local hydrology of surface water and groundwater. They also account for the possibility of further land subsidence along with the continuation of mining activity. These areas were evaluated to have a total water volume capacity of around 700 million tons to one billion tons by 2020, which would produce a vast water resource potential and ecological benefits for the local economy and environment. Nowadays, utilization of water resources and landscape design of some subsidence waters are well planned, and some ecological engineering measures for ecological rehabilitation and restoration also have been put into practice (Xie *et al.* 2013).

Eutrophication is an important issue for ecological rehabilitation and conservation at these aquatic zones, which are generally impacted by surrounding agricultural areas. Previous research has mainly focused on investigating water quality (He *et al.* 2005). It found that the water bodies presented differential degrees of pollution and nutrient states depending on location, wherein natural and well-protected waters exhibited better water quality and lower nutrient level than those heavily impacted by human activities. Communities of

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phytoplankton and zooplankton were also observed to have site-specific characteristics due to the differences in nutrient content, water chemistry, and fishery activities (Deng et al. 2010a, b). However, these studies are too unfocused to understand the eutrophication processes in these types of aquatic zones from a general perspective. Moreover, nutrient limitation and its potential impact on the aquatic ecosystem, was not a major focus of earlier work as a basic but important aspect to understand the eutrophication issues within these waters.

For the last three decades, nutrient balance, mainly concerning nitrogen to phosphorus ratio (N:P) in lentic systems, has been widely used as an indicator of nutrient balance and a limiting factor for phytoplankton growth and community compositions since Redfield (1958) proposed an average element composition of phytoplankton of 106C:16N:1P from his ocean research. However, other research indicates that the N:P ratio rule for nutrient limitation could significantly vary between aquatic habitats, such as oceans, coastal areas, and inland water bodies (Smith 1983; Sterner et al. 2008; Ptacnik et al. 2010) due to differences in available nutrient forms, the environment and algae communities. Some research suggests that a ratio of dissolved inorganic nitrogen (DIN) or nitrate to total phosphorus (TP) could be a better predictor of nutrient limitation (Ptacnik et al. 2010; Bergstrom 2010). Therefore, nutrient enrichment bioassays (NEBs) are usually used as a tool to aid in the judgment of specific limitations in addition to nutrient concentrations and their ratios in different aquatic ecosystems (Kim et al. 2007; Quigg et al. 2011; Symons et al. 2012).

Therefore, in this research, eutrophication due to mining activities in this type of aquatic zone was characterized through the method of water quality investigation, combined with NEBs to address nutrient level, ratio, limitation and balance. First, eutrophication and nutrient balance in the aquatic zones will be characterized to discover whether more nitrogen (N) or phosphorus (P) was loading to the water column. Furthermore, the impact of nutrient level and balance on trophic structures, concerning phytoplankton growth, community, and zooplankton response will be discussed. The research results will have important implications on principles and strategies concerning local ecological rehabilitation, resource utilization, and nutrient management in these aquatic zones, which are also common landscapes around the central and east plain coal mining areas of China.

MATERIALS AND METHODS

Site description

The Panxie coal mine area, which is the largest sub-coal mine area in Huainan coal mine areas, Anhui, China, was selected as the research site. It covers an area of 865 km^2 with a maximum width of 60 km and length of 25 km (Figure 1(a)). Three representative lakes were selected in the eastern, central, and western parts of these mined areas and designated as east site (ES), central site (CS), and west site (WS).

The ES lake is 20 years old with an area of 3.5 km^2 (Table 1). This water body is connected with the Ni River, which receives mine drainage, rural nonpoint source pollution, and domestic sewage from surrounding villages. As a result, the external pollution load is heavy. The CS lake was formed within the last 5 years with an area of 2.5 km^2, and has kept its natural state since its formation. Finally, the WS lake (4 km^2) has existed for at least 10 years, and a main agricultural channel (Xiezhan River) empties into the lake. A control gate is set to drain stormwater during the rainy season from the southern Ji River into this lake.

Water depths of the three lakes range from 3.0 to 6.0 m, akin to shallow lakes. The hydraulic retention time (HRT) of the lakes at ES and WS were evaluated to vary between 1.5 years and 2.5 years according to the average annual inflow flowrate from the Ni River at ES and the Ji River at WS, respectively (Table 1). The lake at CS has a very long HRT because it is actually enclosed without outlets. All three lakes are employed by local farmers for aquaculture, albeit without the addition of fish food or fertilizer. Prior to subsidence, the lands were used as cropland for crops or rice.

Investigation into water quality

Investigations on water quality parameters were conducted in the summer (August), autumn (November), and winter (January) of 2012, and spring (April) of 2013. Surface water samples were taken at 0.5–1.0 m depth underwater using a 5 L Plexiglas water sampler in seven (ES, Figure 1(b)) to nine (CS and WS, Figures 1(c) and 1(d)) sampling sites across each lake based on the specific lake shape. Temperatures, pH, and dissolved oxygen (DO) were read on site using portable YSI electrodes (models pH100 and DO200, Xylem Company New York, USA). The water samples were packed into an ice box, brought to the laboratory and analyzed immediately. Water

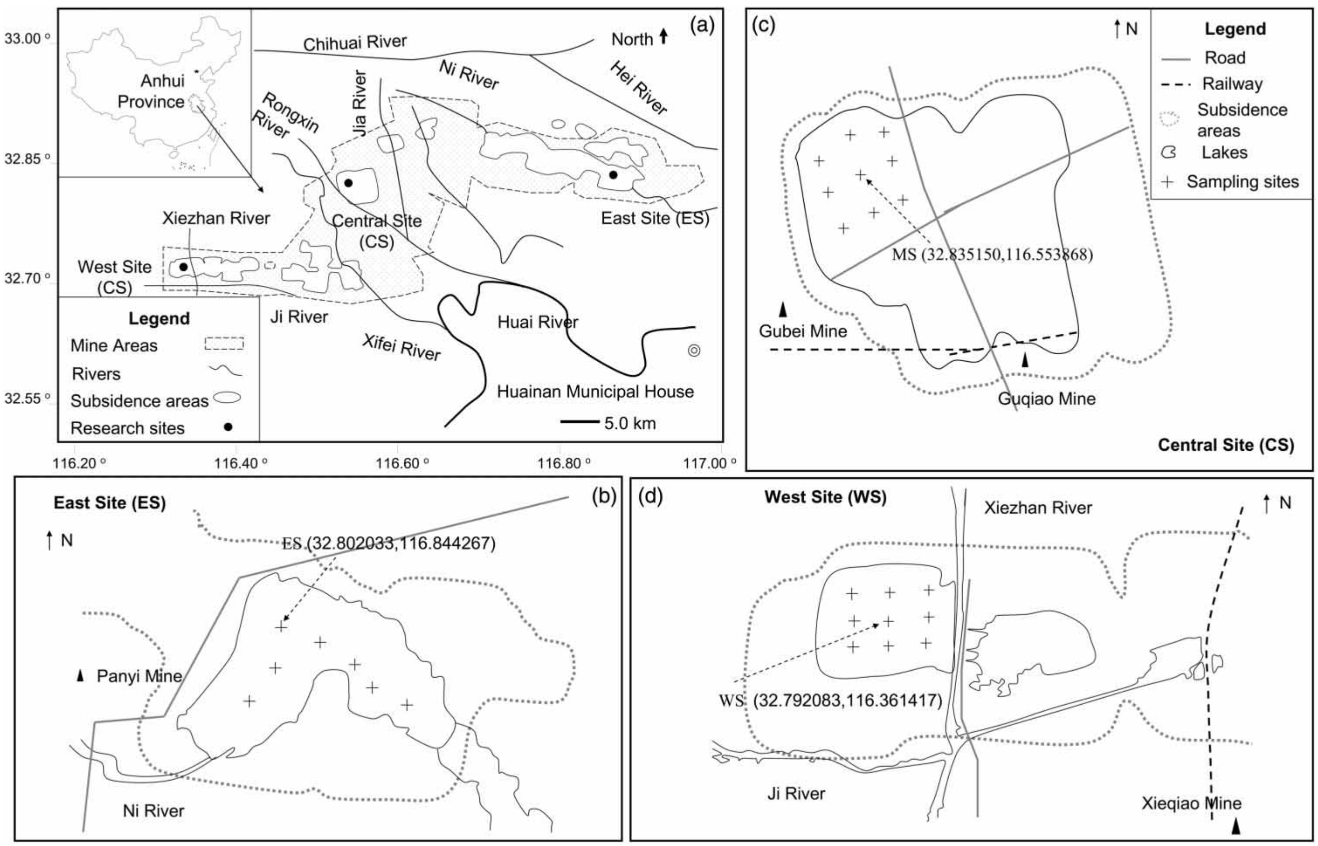

Figure 1 | Location of the Panxie coal mine area (a) and distribution of sampling sites in three selected lakes at the ES, CS, and WS ((b), (c), (d), respectively) around the Huainan coal mine areas.

Table 1 | Dimensions and hydraulics of the three studied lakes in the Panxie coal mine area around the Huainan coal mine areas, Anhui, China

Site	Lake area (km^2)	Water depth (m)	Lake volume (10^6 m^3)	Lake age (years)	HRT (years)
ES	3.5	4.4	15.4	20	1.5
CS	2.5	4.7	11.8	5	–
WS	4.0	5.3	21.2	15	2.5

parameters, including TP, soluble reactive phosphate (SRP), total nitrogen (TN), and individual nitrogen species of nitrate (NO_3-N), nitrite (NO_2-N), and ammonium (NH_4-N), were analyzed according to *Standard Methods* (APHA *et al.* 1998). Dissolved forms of nutrient (SRP, NO_3-N, NO_2-N, and NH_4-N) were filtered through pre-rinsed 0.45-μm cellulose ester filters. The filtrate was placed in acid-washed (10% HCl) 250 mL polyethylene Nalgene bottles, and rinsed twice with filtrate from the sample. TP and SRP analyses were conducted using an acid–molybdenum-blue colorimetric method with persulfate digestion for TP. TN and NO_3-N analyses were examined using a UV colorimetric method after alkaline persulfate digestion for TN. NH_4-N and NO_2-N were determined using phenol–hypochlorite and diazotization with sulfanilamide dihydrochloride, respectively. TN:TP ratio, DIN:TP ratio, DIN:SRP ratio, and trophic state index (TSI) (Carlson 1977) in each lake were calculated to analyze the eutrophic states and potential nutrient limitation (Ptacnik *et al.* 2010).

Nutrient enrichment bioassays

The NEBs were conducted in the autumn of 2012 and spring of 2013 at the three lakes. For each lake, 25 L surface water samples (0.5–1.0 m) were collected into carboys at the central sampling site of each lake (indicated by dashed arrows at each site in Figure 1, respectively). The sampled lake water was shipped to the laboratory and prepared for NEBs immediately. The lake water was first filtered through 80-μm mesh, which removed most rotifers and all crustacean zooplankton while allowing phytoplankton to pass through. Every 1 L filtrated water sample from each lake was then distributed to a series of 2 L conical flasks washed by 10% HCl and rinsed by filtrates. Each flask received one of the following treatments of nutrient: P additions (+P), N additions (+N), N and P additions (+N + P), with filtered lake water as a control (no additions). Each treatment set had three replicates. Solutions of potassium nitrate (KNO_3) and monopotassium phosphate (KH_2PO_4) were made and distributed to flasks containing 1 L of lake water.

Nutrient concentrations were chosen to saturate uptake rates and provide temporary relief from nutrient limitation in order to stimulate primary productivity of plankton. Nitrate concentration in lake water was high at ES and WS while SRP was very low, so P additions were designed to approximately match the Redfield ratio of NO_3-N:SRP in the laboratory. The final attained SRP concentrations were 0.12 mg/L P at these two sites in the autumn of 2012, which are comparable to the TP levels in lake water. Both nitrate and SRP concentrations at CS were very low, where 0.06 mg/L P were added into the incubation flasks as a reference, and 0.3 mg/L nitrate additions were designed to match the Redfield ratio of NO_3-N:SRP in the incubation system (Table 2).

In order to make further efforts to identify the impact of limited nutrients on the algae growth in the spring of 2013, two differential P treatments with additions of low P concentration (+LP of 0.06 mg/L P) and high concentration P (+HP, 0.12 mg/L P) were undertaken for the collected lake water at ES and WS. Two differential N treatments with additions of low concentration N (+LN of 0.5 mg/L N) and high concentration N (+HN of 0.8 mg/L N) were conducted at CS, where nitrate concentration was low and potential N limitation could occur. Initial concentrations of nitrate, SRP and their ratio +N + P treatments at the three sites are summarized in Table 2.

The lake water, in a series of flasks was then incubated in a light incubator where the light intensity was set on a gradient of 100, 80, 60, 40, and 20% with maximum values of 10,000 l× with 12:12 h intervals at day and night. Temperature was set to match the temperature in the water column when collected, ranging from 20° C to 25° C at the three sites during the autumn of 2012 and spring of 2013. Each incubation period lasted approximately 10 days until a complete growth phase of phytoplankton was observed. Flasks were shaken daily to re-suspend the phytoplankton cells. Furthermore, 25 mL water was filtered using 0.45-μm cellulose ester filters to determine chlorophyll-a (Chl-a) concentration through a method of fluorescence spectrophotometer after 24 h methanol extraction at a dark and −20 °C frozen condition. Calibration was performed using a Sigma Chl-a standard. The reproducibility of the measurements was better than 5%, and the detection limit was 0.01 μg/L.

The limiting nutrient was assigned according to a hierarchical logic sequence developed in Maberly *et al.* (2002). Phosphorus limitation was inferred when +P stimulated phytoplankton growth and did not respond to +N. Nitrogen limitation was inferred when +N stimulated phytoplankton growth without a response to +P. Nitrogen and phosphorus

Table 2 | Initial nitrate and SRP concentrations after +N + P treatments for NEBs at three sites in the autumn of 2012 and spring of 2013

Sites	Time	Initial N&P concentrations (mg/L) and ratio	Control	+N	+P	+N+P
ES	Autumn 2012	NO_3-N	0.74	1.04	0.74	1.04
		SRP	0.004	0.004	0.12	0.12
		NO_3-N:SRP	–[a]	–	14	19
	Spring 2013	NO_3-N	1.05	1.30	1.05	1.30
		SRP	0.004	0.004	0.06(+LP)/0.12(+HP)	0.12(+HP)
		NO_3-N:SRP	–	–	38(+LP)/19(+HP)	23
CS	Autumn 2012	NO_3-N	0.16	0.46	0.16	0.46
		SRP	0.001	0.001	0.06	0.06
		NO_3-N:SRP	–	–	6	16
	Spring 2013	NO_3-N	0.20	0.5(+LN)/0.8(+HN)	0.20	0.5(+LN)
		SRP	0.003	0.003	0.06	0.06
		NO_3-N:SRP	–	–	7	18
WS	Autumn 2012	NO_3-N	0.90	1.20	0.90	1.20
		SRP	0.002	0.002	0.12	0.06
		NO_3-N:SRP	–	–	16	42
	Spring 2013	NO_3-N	0.60	0.90	0.60	0.90
		SRP	0.003	0.003	0.06(+LP)/0.12(+HP)	0.12(+HP)
		NO_3-N:SRP	–	–	21(+LP)/11(+HP)	16(+HP)

[a]'–' Indicates no calculation of NO_3–N:SRP ratio due to very low concentration of SRP.

co-limitation was assigned if +N + P significantly accelerated phytoplankton growth without a response to individual +N or +P treatments.

Investigation of phytoplankton and zooplankton community

In order to find out the relationship between eutrophication and trophic structure in the aquatic zones, the investigation for phytoplankton and zooplankton communities was further conducted and complemented in the summer and autumn of 2013. The sampling sites were set on the center of each lake as NEBs. Samples for phytoplankton analysis were preserved without delay in acid Lugol's solution (1% final concentration) for identification of algae while samples for zooplankton analysis were preserved by 4% solution of formaldehyde. Random transects were counted across the settling chamber at ×400 magnification. A minimum of 300 individuals (IND) (single cell or colonies) were counted with a maximum of 100 individuals of the most common taxa being counted. The species were identified to the highest resolution possible. Zooplankton from the study lakes were identified and enumerated using a dissecting microscope.

RESULTS

Characteristics of nutrient contents, species, and ratio

Table 3 lists the annual average concentrations of Chl-a, TN, TP, Secchi disk depth (SD), and TSI in the aquatic zones within the three study sites. Mean concentrations were in the range of 29.3–34.8 mg/m^3, 0.05–0.10, and 0.77–2.06 mg/L for Chl-a, TP, and TN, respectively. Mean TSI values were 59, 54, and 62 at ES, CS, and WS, respectively. All three lakes exhibited 'meso-eutrophic states'. It is noteworthy that TSI formulas were interrelated by linear regression models and should produce the same TSI value for a given combination of variable values (Brown & Simpson 2001). Judged by this principle, the obtained TSI (Chl-a) in the three lakes almost equaled TSI (SD), which indicated that algae has dominated light attenuation in the lakes. There is no significant difference among TSI (Chl-a), TSI (SD), and TSI (TP), suggesting P limitation potential in this type of aquatic zone in accordance with very low SRP concentration in each lake.

Furthermore, seasonal concentrations of N, P, and their species were acquired and then the TN:TP ratio, and DIN:TP ratio for the nutrient structures were calculated and summarized in Table 4. The ranges of TN:TP ratio and DIN:TP ratio were 26–64 and 19–47 at ES; 25–46 and 5–12 at CS; and 42–74 and 25–52 at WS, respectively. CS was very different to ES and WS. Due to lower nutrient levels and concentrations of available inorganic nitrogen (mainly nitrate), the DIN:TP ratio was significantly lower at CS. According to the thresholds for N limitation (<22) and P limitation (>48) proposed by Guildford & Hecky (2000), there is N limiting potential at CS.

Nutrient enrichment bioassays

As shown in Figures 2(a) and 3(a), in the autumn of 2012, P additions both at ES and WS significantly stimulated phytoplankton growth, and Chl-a concentration increased from 21.5 mg/m^3 to 75.8 mg/m^3 at ES (Figure 2(a)), and 15.1 to 125.2 mg/m^3 at WS (Figure 3(a)). Neither site responded to N additions. After Chl-a peaked, phytoplankton decayed rapidly due to nutrient depletion.

In the spring of 2013, the incubation experiments presented different results at ES (Figure 2(b)) and WS (Figure 3(b)). At ES, the +LP treatment stimulated phytoplankton growth and showed obvious P limitation. In particular, +HP treatment greatly enhanced phytoplankton growth with Chl-a concentrations from 36.3 to 56.8 mg/m^3 peaks, which is almost double the Chl-a peaks with +LP treatment. However, the overall growth of phytoplankton presented a decaying trend without P stimulation during the incubation period, probably due to unfavorable laboratory-scale incubation conditions. P limitation did not

Table 3 | Statistics for annual nutrient contents and TSI at ES, CS, and WS

	Chl-a		TP		SD		TN
Sites	Concentration (mg/m^3)	TSI	Concentration (mg/L)	TSI	Concentration (mg/L)	TSI	Concentration (mg/L)
ES	34.7 ± 20.3	64 ± 7	0.10 ± 0.02	70 ± 3	0.9 ± 0.4	63 ± 7	1.95 ± 0.76
CS	29.3 ± 14.9	63 ± 5	0.05 ± 0.02	61 ± 5	0.7 ± 0.2	66 ± 4	0.77 ± 0.09
WS	42.8 ± 20.5	67 ± 4	0.08 ± 0.03	67 ± 5	0.6 ± 0.1	69 ± 1	2.06 ± 0.82

Table 4 | Seasonal variation of nutrient content, species, and ratio in the three studied sites

		TP (mg/L)		SRP (mg/L)		TN (mg/L)		NH_4-N (mg/L)		NO_2-N (mg/L)		NO_3-N (mg/L)			
Sites	**Time**	**Mean[a]**	**Std[b]**	**Mean**	**Std**	**Mean**	**Std**	**Mean**	**Std**	**Mean**	**Std**	**Mean**	**Std**	**TN:TP**	**DIN:TP**
ES	Summer 2012	0.10	0.01	0.008	0.004	1.19	0.12	0.06	0.02	0.01	0.01	0.90	0.02	26	19
	Autumn 2012	0.11	0.02	0.033	0.023	1.59	0.18	0.18	0.06	0.13	0.06	0.85	0.21	34	24
	Winter 2012	0.12	0.02	0.105	0.006	2.95	0.23	0.31	0.006	0.04	0.00	2.24	0.06	53	47
	Spring 2013	0.07	0.01	0.001	0.001	2.04	0.38	0.01	0.00	0.03	0.01	1.19	0.21	64	38
CS	Summer 2012	0.06	0.01	0.003	0.002	0.80	0.03	0.03	0.02	0.00	0.00	0.14	0.01	29	6
	Autumn 2012	0.06	0.03	0.001	0.001	0.72	0.03	0.02	0.01	0.00	0.00	0.17	0.01	25	7
	Winter 2012	0.03	0.00	0.001	0.002	0.67	0.02	0.01	0.01	0.00	0.00	0.16	0.00	46	12
	Spring 2013	0.06	0.01	0.003	0.001	0.88	0.05	0.02	0.01	0.00	0.00	0.13	0.00	32	5
WS	Summer 2012	0.07	0.01	0.002	0.001	1.36	0.08	0.05	0.02	0.02	0.01	0.91	0.03	42	30
	Autumn 2012	0.13	0.02	0.017	0.002	3.17	0.26	0.89	0.07	0.00	0.00	0.79	0.02	55	29
	Winter 2012	0.07	0.01	0.002	0.001	2.19	0.04	0.55	0.01	0.09	0.00	0.91	0.01	74	52
	Spring 2013	0.06	0.00	0.004	0.000	1.51	0.05	0.08	0.01	0.03	0.00	0.57	0.02	55	25

[a]Indicates mean values of all sampling sites.
[b]Indicates standard deviations of all sampling sites.

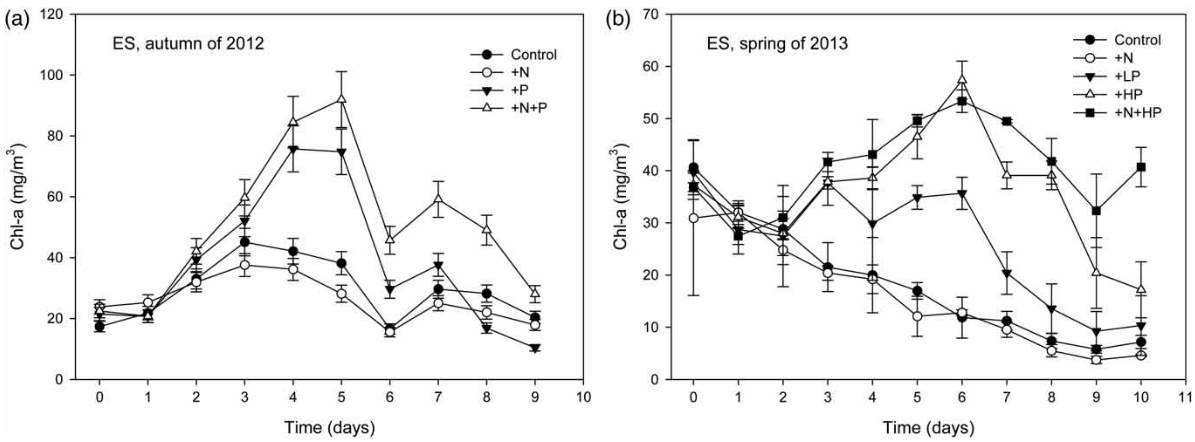

Figure 2 | Phytoplankton growth responses to nutrient enrichments conducted at the ES lake in the autumn of 2012 (a) and spring of 2013 (b). ('Control' indicates no additions of nutrient, ' + N' indicates N additions, ' + P' indicates P additions, and ' + N + P' indicates N&P additions, respectively; ' + LP' and ' + HP' in the spring of 2013 indicate low P additions (0.06 mg/L SRP) and high P additions (final 0.12 mg/L SRP) for NEBs, and nitrate concentration of nitrogen additions (+N) is 0.3 mg/L, the same as the autumn of 2012.)

show a large difference between both +LP and +HP treatments at WS (Figure 3(b)), probably because the +LP treatment could meet the demand of phytoplankton at lower nitrate concentrations (0.6 mg/L, see Table 2) while +HP treatment probably resulted in insufficient N for phytoplankton growth. Furthermore, phytoplankton had higher growth rates with +N + HP because the N supplied at WS was sufficient.

Nitrogen limitation occurred at CS in the autumn of 2012, and N additions made Chl-a concentration increase from 22.0 to 39.9 mg/m^3 (Figure 4(a)), whereas Chl-a had no response to P additions. However, N and P co-limitation took place at CS, where +N + P treatment significantly accelerated phytoplankton growth with Chl-a concentration increasing from 9.0 to 18.1 mg/m^3 while individual +N or +P treatments had no effect on phytoplankton growth. The lake at CS is enclosed with less external nutrient loading compared with that at ES and WS. In addition, nitrogen could be lost through sediment denitrification, leading to low available inorganic nitrogen in the water column.

Phytoplankton community

More than one hundred plankton species were identified in the three lakes, presenting a rather abundant diversity with a Shannon-Wiener index ranging from 2.8 to 4.2 (data not shown). As in other eutrophic lakes, the taxa of Cyanophyta

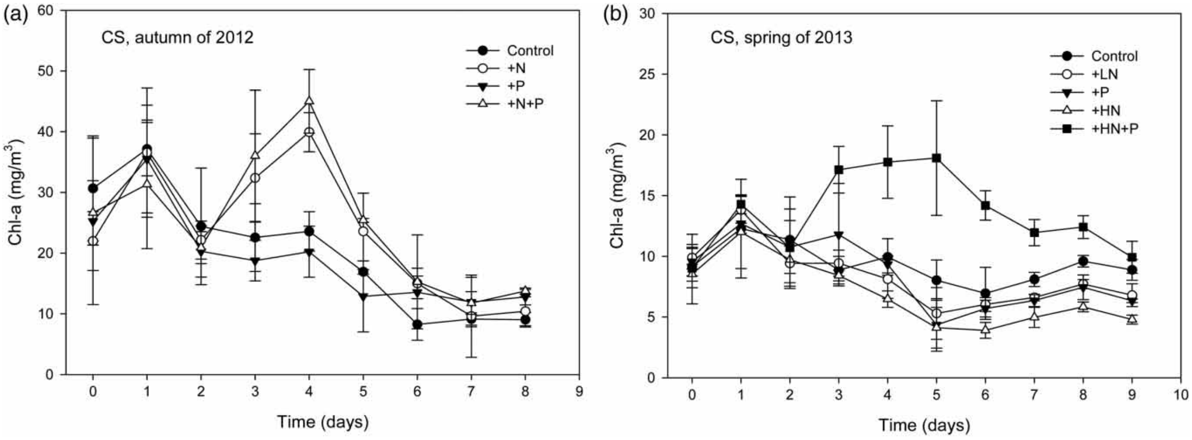

Figure 3 | Phytoplankton growth responses to nutrient enrichments conducted at the WS lake in the autumn of 2012 (a) and spring of 2013 (b). ('Control' indicates no additions of nutrient, ' + N' indicates N additions, ' + P' indicates P additions, and ' + N + P' indicates N&P additions, respectively; ' + LP' and ' + HP' in the spring of 2013 indicate low P additions (final 0.06 mg/L SRP) and high P additions (final 0.12 mg/L SRP) for NEBs, and nitrate concentration of nitrogen additions is (+N) is 0.3 mg/L, the same as the autumn of 2012.)

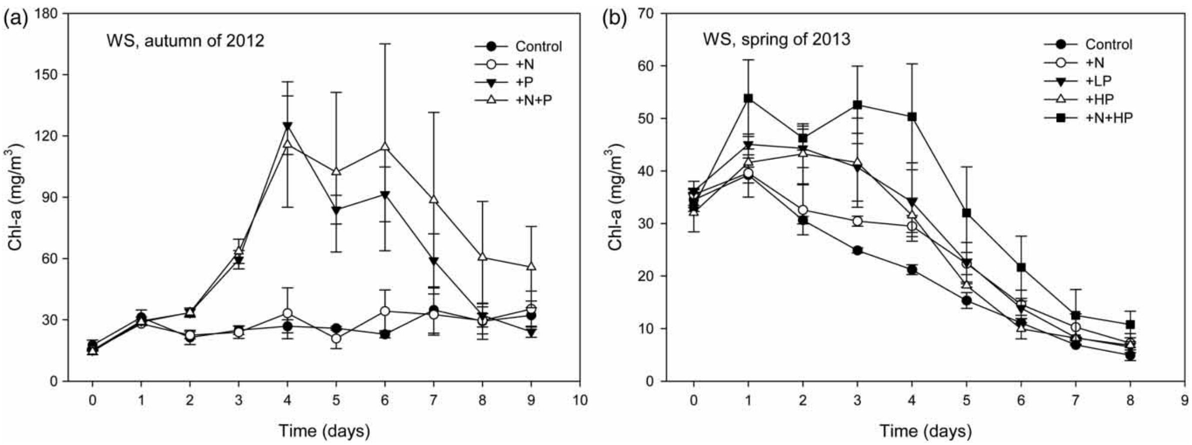

Figure 4 | Phytoplankton growth responses to nutrient enrichments conducted at the CS lake in the autumn of 2012 and spring of 2013. ('Control' indicates no additions of nutrient, ' + N' indicates single N additions, ' + P' indicates single P additions, and ' + N + P' indicates N&P additions, respectively, in the autumn of 2012; ' + LN' and ' + HN' in the spring of 2013 indicate low N additions (final 0.5 mg/L nitrate) and high N additions (0.8 mg/L nitrate) for NEBs, and concentration of phosphorus additions (+P) is 0.06 mg/L, the same as the autumn of 2012.)

and Chlorophyta have become dominant in the phytoplankton community with a 66.0 to 78.0% ratio to the total identified taxa. The most abundant phytoplankton species were *Cyclotella catenata*, *Cryptomonas ovata*, *Chlamydomonas globosa*, *Chroomonas acuta, and Microcystis aeruginos* at ES; *Pseudanabaena* sp., *Arthrospira platensis, Synedra acus, Microcystis aeruginosa, Cyclotella catenata, Anabaena azotica,* at CS; and *Pseudanabaena* sp., *Raphidiopsis curvata, Cryptomonas ovata,* and *Arthrospira platensis* at WS (Table 5). Phytoplankton taxa between the three sites shared a similarity of 21–46% species. It is worth noting that cyanobacteria did dominate at CS, which could be a response to the nitrogen limitation. However, the phytoplankton community was rather different between P-limit lakes at ES and WS. Species of Bacillariophyta, Cryptophyta, and Chlorophyta were dominant at ES while *Pseudanabaena* sp. and *Arthrospira platensis* of Cyanophyta comprised more than 60% of the plankton individuals.

DISCUSSION

Many development plans have been proposed due to a vast water resource potential in the Huainan coal mine subsidence areas, such as flooding buffer zones, establishing aquatic ecological reservation zones, and creating water source reserves for agriculture or industry (Xie *et al.* 2013). However, these proposed functions are only beneficial in

Table 5 | Phytoplankton abundance and predominant taxa in the summer and autumn of 2013 at the three lakes

Sites	Time	Cyanophyta species number	Chlorophyta species number	Bacillariophyta species number	Cryptophyta species number	Euglenophyta species number	Pyrrophyta species number
ES	Summer 2013	9	29	9	3	3	4
		Cyclotella catenata (0.19[a]*), Cryptomonas ovata (0.13), Chlamydomonas globosa (0.09), Chroomonas acuta (0.09), Microcystis aeruginosa (0.08), Chroococcus* Nägeli *(0.05)*; 57 species identified with 8.6×10^6 IND/L in the water column					
	Autumn 2013	11	20	7	3	6	
		Cyclotella catenata (0.19), Chlamydomonas globosa (0.16), Cryptomonas ovata (0.14), Chroomonas acuta (0.07), Microcystis aeruginosa (0.07); 47 taxa identified with 9.8×10^6 cells/L in the water column					
CS	Summer 2013	13	35	8	3	5	2
		Pseudanabaena sp. *(0.38), Arthrospira platensis (0.06), Synedra acus (0.06), Microcystis aeruginosa (0.05), Cyclotella catenata (0.05), Anabaena azotica (0.05)*; 66 taxa identified with 12.7×10^6 IND/L cell density in the water column					
	Autumn 2013	11	30	5	3	4	5
		Pseudanabaena sp. *(0.24), Cryptomonas ovata (0.11), Arthrospira platensis (0.11), Synedra acus (0.09), Microcystis aeruginosa (0.05), Chroomonas acuta (0.05)*; 59 taxa identified with 11.8×10^6 IND/L cell density in the water column					
WS	Summer 2013	14	29	4	3	3	2
		Pseudanabaena sp. *(0.58), Raphidiopsis curvata (0.08), Cryptomonas ovata (0.07)*; 55 taxa identified with 18.9×10^6 IND/L cell density in the water column					
	Autumn 2013	12	25	6	3	5	2
		Pseudanabaena sp. *(0.44), Arthrospira platensis (0.16), Raphidiopsis curvata (0.09)*; 53 taxa identified with 18.9×10^6 IND /L cell density in the water column					

[a]Indicates the values in the brackets are McNaughton dominance index, which are listed in order in species of more than 5% dominance degree.

healthy aquatic ecosystems. This study attempts to characterize the eutrophication of three small lakes in a mining area to provide information on a larger scale. The project area is greatly impacted by agriculture, which probably began the eutrophication at a 'meso-eutrophic state'.

The water column N:P ratio is mainly dependent on external input, internal loading, and biological activity. High N:P ratios indicate that P is insufficient to N from the perspective of nutrient structure and balance. In the agricultural areas, higher N fertilizers have been generally loaded on croplands and transported into subsided waters due to their mobility characteristics, while P belongs to sedimentary elements. Many monitoring results regarding non-point source pollution have demonstrated that there is a higher N:P ratio in the runoff from agricultural lands (Omernik 1977). The lakes at ES and WS have received effluent from local rivers, which contains 2.0 to 6.0 mg/L N and 0.1 to 0.2 mg/L P with rather high N:P ratios. In addition to this, more P could be removed through sedimentation from the water column, while less nitrogen could be lost through denitrification in the oxic waters, resulting in high nitrate concentrations in ES and WS. However, nitrogen concentration at CS only approached half of ES and WS due to lower nutrient load in an enclosed environment, where N losses through denitrification and P sedimentation could lead to lower available nitrogen and phosphorus, which caused responses of algae growth potential to N limitation or N and P co-limitation in the aquatic zones.

As a compressive result, bioassays verified that P was the main limiting nutrient in these aquatic zones. It is worth noting that P is still the most important nutrient in determining phytoplankton biomass or primary productivity in this ecosystem as in other inland freshwater ecosystems. In this research, P additions for NEBs were set at 0.06 and 0.12 mg/L, a comparable level to TP in the water columns, and made the Chl-a concentrations increase two to eight times. Hence, '+HP' treatment would almost double the phytoplankton biomass of '+LP' treatment if there were sufficient available N.

In another aspect, biological activities could have important implications to nutrient content and limitation (Carpenter *et al.* 1995; Hansson *et al.* 1998). There are two important theories to explain the control mechanisms on the trophic structures of ecosystems known as 'bottom-up' and 'top-down' effects on food-web structures (Carpenter *et al.* 1995; Hansson *et al.* 1998; Jeppesen *et al.* 2003). However, recent knowledge indicates that 'bottom-up' control is more important in determining trophic levels, phytoplankton biomass, and food-web structures and functions in eutrophic aquatic ecosystems when the phytoplankton is too large to be grazed by small predators (Kalff 2002).

The phytoplankton community in the research lakes could be mainly controlled by 'bottom-up' mechanisms. These lakes have been found to have dominant species of Cyanophytes and Chlorophytes, which are very common communities in inland eutrophic lakes. By responding to the phytoplankton community, the taxa of micro-zooplankton at the three lakes were found to be dominant since large-size cyanobacteria were inedible to them. Cladocerans displayed great abundance at ES with the mainly identified species of *Ceriodaphnia pulchella, Bosmina longirostris, Diaphanosoma brachyurum, Sida crystallina,* etc. Predominant *Mesocyclops leuckarti* of Copepods was identified at CS and WS, besides also sharing very similar species of Cladocerans as ES.

Deng *et al.* (2010a, b) have published the results of phytoplankton and zooplankton communities in two eutrophic lakes around the Huaibei coal mine areas. They also observed great abundance of green-blue algae, small taxa of Cladocerans and Copepods. However, a great variety of species was identified compared with this research area, which could be caused by the difference of local climate and water chemistry. The Huaibei coal mine areas have a colder and more alkaline water chemistry, most probably affecting the plankton community. Phytoplankton nutrient limitation has also been shown to be related to zooplankton communities. Cladocerans, particularly *Daphnia*, have high P requirements and recycle N/P at a higher ratio than other zooplankton, while Copepods usually contain a higher proportion of nitrogen (Elser *et al.* 2000, 2001); an abundance of Cladocerans in the three research lakes could lead to cascaded P-limitation to phytoplankton in the water column.

Although phytoplankton density and cyanobacteria individuals presented high values in these types of aquatic zones, algae blooms rarely occur, which could be a product of local fishery activities. Local fisheries have cultured two common kinds of Asian carp (bighead and silver) every year under natural conditions using the primary nutrients in the water column. The stress of predators could control the algae bloom, which has been verified to be very effective at other Chinese eutrophic shallow lakes as a non-classical 'top-down' control mechanism (Guo *et al.* 2009). Biological roles on nutrient structure and food-webs could be important issues for future research. There is a need to balance local fishery activities, water utilization and regionally ecological rehabilitation and reservation.

CONCLUSION

Overall, there was a low tolerance to added nutrients in the aquatic zones around the Huainan coal mine subsidence areas due to their single small volumes and low capacity to pollutants, which averaged around 0.05 mg/L TP and 1.0 mg/L TN at virgin states. Strong human impacts can progress the area to a nutrient level of 0.10 mg/L TP and 2.0 mg/L TN in 10–20 years. Phosphorus will be more important than N in managing the eutrophication of aquatic areas in the future. Preventing nutrients from entering the water column by blocking drainages to control point source pollution and establishing ecological buffers to control non-point pollution should be considered. Furthermore, research regarding controlling mechanisms for food-webs, trophic structures, and functions should be enhanced to keep a balance between resource utilization and conservation of aquatic zones.

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