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We investigated water quality, phytoplankton composition and eutrophication status in a typical alpine glacial lake of Tianchi, a UNESCO/MAB (Man and Biosphere) nature reserve in Xinjiang Autonomous Region of China based on a two-year field work in this study. Ammonium was identified as the most discriminant variable followed by total phosphorus, suggesting that they played an important role in phytoplankton growth and succession in Tianchi Lake. Emission sources were identified through the analysis of anthropogenic activities in the region, and the current status of eutrophication was evaluated based on the calculation of integrated trophic level index. It was clear that historical overgrazing and increasing tourism have brought a great amount of nutrient inputs to the waterbody.

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Response of phytoplankton community to water quality in local alpine glacial lake of Xinjiang Tianchi, China: potential drivers and management implications

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Eutrophication has become one of the most serious threat to aquatic ecosystems in the world. With the combined drivers of climate change and human activities, eutrophication has expanded from warm shallow lakes to cold-water lakes in relatively high latitude regions, and has attracted greater concerns over lake aquatic ecosystem health. A two-year field study was carried out to investigate water quality, phytoplankton characteristics and eutrophication status in a typical alpine glacial lake of Tianchi, a scenic area and an important drinking water source in Xinjiang Autonomous Region of China in 2014 and 2015. Clear seasonal and annual variations of nutrients and organic pollutants were found especially during rainy seasons. For the phytoplankton community, Bacillariophyta held the dominant position in terms of both species and biomass throughout the year, suggesting the dominant characteristics of diatoms in phytoplankton structure in such a high-altitude cold-water lake. It was quite different from those plain and warm lakes troubled with cyanobacteria blooming. Moreover, the dominant abundance of *Cyclotella* sp. in Tianchi might suggest a regional warming affected by climate change, which might cause profound effects on local ecosystems and hydrological cycle. Based on water quality parameters, a comprehensive trophic level index *TLI* (*Σ*) was calculated to estimate the current status of eutrophication, and the results inferred emerging eutrophication in Tianchi. Results from Canonical Correspondence Analysis (CCA) and correlation analysis of phytoplankton genera and physico-chemical variables of water indicated that abiotic factors significantly influenced the phytoplankton community and its succession in Tianchi Lake. Those abiotic factors could explain 77.82% of the total variance,and ammonium was identified as the most discriminant variable, which could explain 41% of the total variance followed by TP (29%).An estimation of annual nutrient loadings to Tianchi was made, and the results indicated that about 212.97 t of total nitrogen and 32.14 t of total phosphors were transported into Tianchi Lake annually. Human social-economic activities (runoff caused by historical overgrazing and increasing tourism) were identified as the most important contributors to Tianchi nutrient loadings.

1. Introduction

Over the past decades, global climate change has been considered as one of the most serious threats to ecosystems and has attracted much attention throughout the world. The global average surface temperature has increased by about 0.74°C in last century, with the majority of the increase occurring in the most recent 30 years; ~0.2°C per decade mainly as a result of rising greenhouse gases.[1-3] Abundant research has shown the sensitivity of lakes to climate and that lakes might be sentinels of climate change.[4-10] Furthermore, alpine lakes with special location and fragile environment, face greater challenges as growing evidence has illustrated that global warming is amplified with elevation and so high-mountain regions would be more affected by climate change.[11-13] On the worldwide hydrological map, eutrophication has been identified as the primary water quality issue, posing a critical threat to aquatic systems. [14] The adverse effects brought about by eutrophication could be summarized in two categories: changes in physical-chemical properties and ecological effects. Water transparency reduction, dissolved oxygen decrease, formation of reduced substances, and increase of organic matter in sediments commonly occur in eutrophic waters as well as increased biomass and productivity of phytoplankton and shifts in phytoplankton composition to bloom-forming species.[15, 16]

Cold-water lakes usually refer to alpine lakes or lakes located in high latitudes and remote regions with low water temperature all the year round. Most of these lakes are drinking water sources and rarely disturbed by human activities in the past. Normally in these

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areas, atmosphere is thin and dissolved organic carbon is very low, which makes cold-water lakes exposed to high UV level. Average annual temperature is below 0°C and the water temperature is often below 4°C with 5-9 months ice age every year.[17,18] The joint effect of high UV and low temperature conditions has led to differences in vertical structure of lake ecosystems between cold-water and plain lakes. Cold water lakes are more vulnerable to environmental pollution, and they may be indicators of environmental change.[17, 19] Nowadays, the influence range of eutrophication and nuisance algae-bloom is gradually expanding from warm and densely populated basins to alpine lakes and relatively higher latitude regions due to global warming. A series of studies have been carried out in some world-famous cold-water lakes concentrated in Europe and North America, such as Lake Winnipeg in Canada,[20,21] Lake Baikal in Russia,[22, 23] lakes in the Beartooth Mountain Range and high-elevation lakes in the Sierra Nevada, USA,[24, 25] and the four largest lakes in the UK.[26] Nuisance blooms of heterocystous Cyanobacteria in Lake Winnipeg, an important glacial lake in Canada, have nearly doubled in size since the mid-1990s, as a result of rapid increase in loading and concentration of phosphorus. The increased livestock production and climate-related spring floods are considered to be the primary drivers of nutrients.[20] Long-term monitoring of nutrients enrichment of high-elevation lakes in the Sierra Nevada has revealed measurable eutrophication as response to climate change and atmospheric deposition.[24] A 400-yr sediment record from alpine Beartooth Lake has been examined and a rapid change in the diatom community structure was observed. These taxonomic shifts may be caused by elevated lake trophic status and changes in thermal stratification patterns related to climate change.[25, 27] Associated with the development of tourism, eutrophication and filamentous green algae blooms have become a recent phenomenon in the near shore of Lake Baikal since the summer of 2011.[23] It was reported that climate change was likely to promote changes in land use and shoreline integrity, which could accelerate cultural eutrophication in Lake Baikal.[22] Climate change and excessive external nutrients were considered as a “double whammy”,[20] but investigations into the eutrophication of cold-water lakes are very few in China with major attention paid on Lake Taihu and Dianchi. Due to the ecosystem sensitivity and fragility of cold-water lakes, they are more likely to fall into eutrophication under the pressures of climate change, population expansion, agricultural production, tourism activities and sewage discharge. It is essential that more and more attention should be paid to the trophic state and phytoplankton structure change of cold-water lakes in China.

Lake Tianchi is a typical alpine glacial lake located in the Tianchi scenic area as a well-known scenic site in northwest China which was established in 1980 and has been open for tourism since1982. It is one of the first batch of national key scenic spots and also a UNESCO Bogda Man and Biosphere reserve and world heritage site.[28-30] As the location belongs to typical arid and semi-arid area in the middle part of Asia, the reserve has ecological significance with vulnerable habitats. In recent years, however, extreme climate events, frequent drought, historical overgrazing and increasing tourism have all contributed to deterioration of water quality in Lake Tianchi. Filamentous green algae clusters have been frequently observed in summer, along the southern bank of Lake Tianchi and in Western Tianchi since 2008. As far as we know, no research has been reported on the eutrophication mechanism and phytoplankton structure change of Lake Tianchi. So we chose this typical cold-water lake as our study area, which has received less attention to its current water quality than those warm lakes known for eutrophication in China. Integrated study including water quality sampling and analysis, phytoplankton community identification, eutrophication status evaluation, pollution sources estimation and targeted measures presentation was conducted to provide a detailed profile of the lake eutrophication. Phytoplankton investigation in Xinjiang region was rare, so our study filled in the gap, which could lay a good foundation for ecological restoration in this world heritage site.

The aim of this study was to investigate the dynamic changes in lake water quality and aquatic ecosystem under anthropogenic pressures, explore the possible pollution sources and enhance the understanding of trophic status for improved management of this national nature reserve and scenic spot. A two-year seasonal field sampling campaign was conducted in Lake Tianchi to analyze the spatial and temporal distribution of nutrients. Human disturbances were also analyzed to identify the driving forces for phytoplankton overgrowth and lake eutrophication.

2. Materials and methods

2.1. Study area

The scenic spot is located in the middle of the eastern Tianshan Mountains with average altitude of over 1900m. The area belongs to a temperate continental climate zone and is mainly influenced by upper westerly air with large daily variations of temperature. The detailed geological position is as follows: east longitude: 88°00'-88°20', north latitude: 43°45'-43°59'. It covers a catchment area of about 380 km^2 (38069 ha) including a 60 km^2 core area centered by Tianchi Lake which is situated at 1920 m altitude near Bogeda Peak (Fig. 1). The catchment is endowed with complete vertical natural landscape composed of high mountains and permanent glaciers, alpine meadows, valleys, and forests which is of great value for landscape appreciation and scientific research. The information on the primary chemical and physical features of the Tianchi Lake was provided by the Tianchi Management Committee as shown in Table 1. During the past decades, human activities have become more intensive in this region. The major anthropogenic disturbances were shown in (Fig. 1), including overgrazing, non-native fish species introduction, dam building and tourism. Even though ecological protection measures for resident emigration and grazing-bans have been taken into action in recent years, accumulative negative effects could not be eliminated within a short time, and the water quality in Lake Tianchi deteriorated. There are many controversies over the quantitative effects of human activities on Tianchi eutrophication, but it is essentially necessary to take actions to reduce the anthropogenic loadings, and our study could provide a solid science base to enhance management.

2.2. Sampling and chemical analysis

Water samples were taken in May (late spring and early summer), August (late summer and early autumn) and November (winter before lake became frozen) during 2014-2015. These samples were evenly distributed in downstream Western Tianchi

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and concentrated on the southern bank in Tianchi Lake (Fig. 1). Surface water samples were directly taken in polyethylene bottles, and organic glass water sampler was used for deeper water samples at depths of 2~12 m (about 6 m) and below 12 m. Water samples of 100 ml, 500 ml and 1L were taken for nutrients, chlorophyll *a* (Chl-*a*) and phytoplankton analysis, respectively, and were stored under refrigeration at 4 °C until analysis. Chl-*a* and phytoplankton were only taken for surface water, and 1% $MgCO_3$ solution and 1% Lugol's iodine solution were added, respectively, immediately after sampling.

Water temperature, pH and dissolved oxygen were measured in-situ by aHQd Portable Meter Configurator (HACH Company, USA) and water transparency was measured with a standard Secchi disc. For nutrients, total nitrogen (TN) was determined byalkaline potassium persulfate digestion-UV spectrophotometric method (GB11894-89, China); ammonium nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N) were determined by a continuous flow analyzer (Auto Analyzer 3 System, SEAL Analytical GmbH company); total phosphorus (TP) and soluble reactive phosphorus (SRP) were analyzed spectrophotometrically by the molybdenum-bluemethod based on the Standard Methods for Water and Wastewater Examination 31 with a pre-treatment filtration for SRP samples using 0.45μmmicro-filtration membrane. For organic synthetic pollution indicators, chemical oxygen demand (COD) and permanganate index were measured respectively according to GB11914 and GB11892.

2.3. Phytoplankton identification and counting

For phytoplankton, chlorophyll-a concentration was measured spectrophotometrically by acetone method.[32] Qualitative identification and quantitative counting were done under microscope at 400 x magnification to distinguish different algae, and counting results were calculated in terms of cells/litre. The method for phytoplankton identification and counting was applied according to previous publications.[33] Total microcystins and microcystins-LR were also analyzed to investigate the safety for drinking water sources.

2.4. Statistical analysis

The association between chlorophyll-a and algae cells was determined by Pearson's correlation coefficients, and Pearson's Correlation Analysis was also applied to determine the relationships between physical-chemical parameters and phytoplankton abundance. Furthermore, CCA (canonical correspondence analysis) was performed to elucidate the relationships between phytoplankton genera and environmental variables using the software program CANOCO 4.5. To satisfy the assumption of normal distribution in data, species data and environmental variables were all log (X+1) transformed before the analyses except pH. For statistical analysis of data, only species with more than 1% of total phytoplankton density and having appeared at least 3 times during the study period, were considered. Monte Carlo simulations with 499 unrestricted permutations were conducted to test the significance of environmental variables explaining the variance of phytoplankton. Variables were considered to be significant when $P<0.05$.

3. Results and discussion

3.1. Physical and chemical variables

Water temperature was highest in August and lowest in winter seasons, and water in Western Tianchi was even colder in 2015 than in 2014, though all the temperatures are relatively low in cold-water lakes. The maximum water temperature was not higher than 20°C and 15°C in Tianchi and Western Tianchi, respectively. It was significantly different from temperate lakes in which water temperature could even exceed 30°C in summer seasons.[33-37] DO and Secchi-depth (SD) were higher in winter and might decrease over the summer seasons mainly due to the occurrence of algae blooms and pH did not show significant variations during the period (Fig. S1). For nutrients, nitrogen (ammonium, nitrate, and TN) mainly increased during rainy seasons (June). Ammonium might constantly decrease and TN and nitrate might decrease in August while increase again in winter. However, phosphorus (TP and SRP) might decrease over time, and the disparate trend between nitrogen and phosphorus probably suggested their diverse pollution sources. The annual mean TN and TP concentrations of Tianchi in 2014 and 2015 were 1.147, 0.169, 0.672 and 0.029 mg/L, respectively. All nutrient concentrations were lower in 2015 as a result of dilution effect by more precipitation in June. Nutrient contents in Tianchi were primarily lower than most highly eutrophic warm lakes,[37-40] but were comparable to similar cold-water lakes troubled with emerging eutrophication.[20, 23, 41] Normally, total phosphorus concentration of less than 10 μg/L is considered to be oligotrophic, while 100 μg/L is the threshold of hyper-eutrophication formulated by EPA.[42] There are also many reports presenting that the eutrophication thresholds of N for freshwaters in the Great Lakes of the Chinese Pacific Drainage Basin are from 0.500 to 1.00 mg/L and that of P are from 0.02 to 0.10 mg/L.[40, 43] A region-specific lake eutrophication assessment was done in China, and values considered as lake eutrophication standards for the Mongolia-Xinjiang plateau lakes were 1.42 mg/L and 0.117 mg/L of TN and TP, respectively.[44] Based on this criterion, water trophic status of shallow area of both Tianchi and Western Tianchi had significantly exceeded the threshold values of eutrophic water in 2014. For organic pollutants, higher COD and permanganate index during June and August might be related to tourism boom in summer (Fig. 2). In addition, obvious P limitation was found in 2015, as the Redfield ratio (TN/TP) was greater than 33:1.[42]

3.2. Characteristics and succession of phytoplankton community

During the 2-year study period, a total of 57 phytoplankton species were identified in all water samples mainly including Bacillariophyta (33), Chlorophyta (13) and Cyanobacteria (5) (Table S1). Considerable blooming of harmful filamentous algae was observed in 2014, especially in June, and shallow areas were almost covered by algae at the bottom (Fig. S2). *Spirogyra* sp. was the most abundant filamentous species accounting for 62.5% frequency of all samples according to taxonomic identification results, which may indicate an ongoing process of eutrophication.[23, 45] Few toxin-producing cyanobacteria species were found and this agreed with low concentrations of total algal toxins and microcystins. All this suggested that the algae bloom in this high-altitude scenic area reflected green algae blooming, obviously different from warm lakes with regular cyanobacteria blooming. This green algae blooming might be caused by intense sunlight, warm temperature and

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suitable nutrient concentration during early summer and summer. In 2015, no bloom was apparent and little *Spirogyra* sp. was observed in summer, which might reflect relatively low nutrient concentration caused by dilution effect.[3]

Algae cell density and dominant species were calculated to illustrate detailed features of phytoplankton community structure (Fig. 3). Quantities of algae cells in Tianchi rapidly increased in summer from May ($19.88*10^4$ cells/litre) to August ($1051.94*10^4$ cells/litre) and then sharply decreased at the end of Octorber ($31.24*10^4$ cells/litre). The dominant species in June were *Synedra* sp., *Chlamydomonas* sp., and dominance index of *Cyclotella* sp. reached 0.983 in August, inferring that almost all the phytoplankton was *Cyclotella* sp. and this kind of simplex ecosystem would be vulnerable to external disturbances. The increasing abundance of *Cyclotella* sp. in Tianchi suggested the influence of higher temperature. Previous research had also concluded that *Cyclotella* abundance had increased over the last century in many alpine lakes, and this widespread increase of *Cyclotella* in Northern Hemisphere lakes was due to regional warming as well as the lengthening of the ice-freeperiod.[27, 46] On the contrary, there were very few algae cells in Western Tianchi in 2015, remaining about 10^3 cells/litre, most of which were bacillariophyta. This might be due to rise in water level resulting from heavy rainfalls during the summer season in 2015 and poorer buffer capacity of this small shallow lake. Meanwhile, relatively lower temperatures than 2014 in Western Tianchi were observed, as was discussed above, which might limit the growth of phytoplankton. However, *Fragilaria* sp. dominance (dominance index reached 0.426 in winter) might infer an early stage of anthropogenic eutrophication in accord with similar studies.[23, 47]

To elucidate seasonal and annual succession of the phytoplankton community, Jaccard similarity coefficient was calculated (Table 2) and Shannon-Weiner index was also used to investigate the species diversity of aquatic systems (Table 3).

The results showed low species similarity and fast succession of phytoplankton during the study period especially overwinter, and Jaccard similarity coefficients within the same year were relatively higher. This low species similarity was mainly due to the shift of phytoplankton assemblages with changes in water conditions. The seasonality of plankton in Tianchi reflected an obvious trend that Chrysophyta and Cryptophyceae were first developed in late spring and followed by green algae and diatoms and then diatoms completely dominant, which was primarily in line with PEG (Plankton Ecology Group) model. The model demonstrated that fast-growing algae (Cryptophyceae and small centric diatoms) developed in spring and followed by green algae, diatoms, dinoflagellates and filamentous cyanobacteria in turn during summer with cyanobacteria a dominant in late summer and early autumn, and significance of diatoms increased again with the progress of autumn.[48, 49] Nevertheless, Bacillariophyta held dominant position at both species and biomass throughout the year, while cyanobacteria was rare, which suggested diatoms was the dominant phytoplankton structure in such high-altitude cold-water lakes. Furthermore, low values of species richness were observed in terms of Shannon-Weiner index as previous studies reported on lakes with high nutrient concentrations.[50, 51] It was inferred that phytoplankton biodiversity in Tianchi was relatively low, and this kind of simple system is vulnerable to external disturbances. The seasonal variation of richness demonstrated a downward trend and fell to lowest level (<<1) in August with slight rise in winter except for Western Tianchi. Clearly, the lowest richness values were caused by the absolute dominance of *Cyclotella* sp. and *Fragilaria* sp. in Tianchi and Western Tianchi, respectively. The water stability after transition from dry to rainy season (June-August) may lead to boom of *Cyclotella* sp. in Tianchi and thus contributed to the low richness, while its population collapsed in winter as temperature dropped sharply and richness increased again.[51, 52] In the downstream Western Tianchi, the succession of phytoplankton community might have a delay and finally *Fragilaria* sp. became the dominant species, and richness fell to the lowest point in October/November.

3.3. Eutrophication assessment

Water quality based methods are commonly applied in eutrophication assessment according to biologically useful nutrients including nitrogen and phosphorus in the water body. Carlson Trophic State Index (*TSI*) and modified Trophic State Index (TSI_M) have been successively developed in order to characterize the multi-dimensional nature of eutrophication based on chlorophyll and transparency of lakes as the primary estimators.[36, 42] To quantitatively evaluate and illustrate the eutrophication status of lakes, a comprehensive trophic level index, *TLI* (Σ) (eq. 1), considering influences of SD, TN, TP, Chl-*a* and permanganate oxygen demand (CODMn), was used to estimate lake trophic state on the basis of the Lake (Reservoir) Eutrophication Assessment Methods and Classification Technology Requirements (NO.2001090) by China Environmental Monitoring Station.[53, 54]

$$TLI(\sum)=\sum_{j=1}^{m} W_j * TLI(j) \qquad \text{(Equation 1)}$$

where W*j* is correlative weight for trophic level index of *j*, TLI(*j*) is the trophic level index of *j*, such that

$$W_j=\frac{r_{ij}^2}{\sum_{j=1}^{m} r_{ij}^2} \qquad \text{(Equation 2)}$$

where r_{ij} is the correction coefficient between Chl-a and the parameter *j*; m is the total number of parameters.

Individual trophic state index of Chl-*a*, TN, TP, SD and COD_{Mn} was calculated, respectively, according to the assessment standard values of trophic state (eq. 3):

$$TLI(i)=a*\ln(i)+b \qquad \text{(Equation 3)}$$

where i represents Chl-a, TN, TP, SD or COD_{Mn}, a and b are the coefficients obtained from the field data.

Moreover, regional lake eutrophication assessment standard is considered to be more appropriate to estimate lakes located in different geographical and climatological areas. In this study, correlative weights and coefficients (a and b) of water quality variables were chosen from the research results of region-specific lake eutrophication assessment in Mongolia-Xinjiang region[31] instead of average results in China.[55] Nutritional status was graded with a series of consecutive numbers from zero to 100 and evaluation grades were divided into six levels from Oligotrophic to Hypereutrophic with classification standard as 30, 50, 60,70 and 80.[44] Calculation results were listed in Table 4, and the spatial and temporal distributions of eutrophication levels agreed well with the actual conditions. Light eutrophication (50-60) occurred at the sourthern shallow of Tianchi in June 2015 as a result of dramatic increase of algae and Chl-*a,* while the other districts remained in mesotrophic (30-50) and oligotrophic (0-30) status as the nutrient concentrations were relatively lower than that of 2014 which might

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be caused by dilution effects of rainy seasons.

3.4. Driver identification of phytoplankton growth and eutrophication

3.4.1. Relationships between phytoplankton and water quality factors

As is widely recognized, chlorophyll *a* is a major photosynthetic pigment in algae and macrophytes, and is most often used as an estimator of algal biomass as well as building linkages between nutrients and biological response of algae.[32, 44] Temporal and spatial distribution of chlorophyll *a* is shown in Fig. 4. The mean concentration of Chl-*a* was 3.185 mg/m^3 and the Chl-*a* concentration ranged from 0.12 to 67.53 mg/m^3. The results showed a sudden increase of Chl-*a* concentration in June accompanied by the increase of temperature, particularly in the southern shallow area of Tianchi and reached a peak at 67.53 mg/m^3 which far exceeded common criteria of 10-15 mg/m^3 for natural lakes, indicating an overgrowth of phytoplankton and showing eutrophication trends.[42]

Correlation coefficients between Chl-*a* andalgae cells were calculated to examine whether Chl-*a* could be selected as a representative variable of phytoplankton biomass and biological factors in Tianchi for eutrophication assessment. Significant positive correlations were obtained between Chl-*a* and total phytoplankton biomass with R^2=0.905 as well as each phylum including Bacillariophyta, Chlorophyta, Dinoflagellate and Cryptophyta. Concentrations of Chl-*a* fluctuated in parallel with algae cells, so we chose Chl-*a* as the bio-indicator of algae to investigate the relationship between algae growth and nutrients/water physical-chemical properties. Chl-*a* was positively correlated with ammonium, water temperature, COD_{Mn} and pH, but negatively with nitrate (Table S3). The highest correlation occurred between Chl-*a* and ammonium indicating that ammonium significantly influenced the growth of phytoplankton in Tianchi. The opposite trend of ammonium and nitrate might be caused by the different assimilative ways that algae utilize these two N forms. Ammonium and nitrate are commonly considered as the two most basic reactive forms of inorganic N, and their assimilation by photosynthetic algae dominates the N cycle in aquatic ecosystems. However, ammonium is easier to assimilate because of the same oxidation state in most amino acids, and nitrate must be first reduced to ammonium by means of specialized enzymes and then be assimilated.[56, 57] Depending on the redox potential, inorganic N is either occurring in the form of ammonium or is oxidized to nitrates. Concentrations of these two compounds are strongly negatively correlated in lakes. This also causes their polarized correlations with Chl a. A stepwise multiple regression analysis with Chl-*a* concentration as the dependent variable was carried out(R= 0.742; p < 0.05) and the equation to summarize these relationships is described as follows:

$$C_{Chl\text{-}a}=0.544WT+105.827C_{ammonium}+4.335pH-35.444C_{TP}-3.714C_{DO}-7.313 \quad \text{(Equation 4)}$$

where WT and pH refer to the water temperature and pH value, respectively; $C_{ammonium}$, C_{TP} and C_{DO} represent the concentrations of water ammonium, total phosphorus, and dissolved oxygen, respectively.

Environmental variables responsible for phytoplankton community variability were identified with Canonical Correspondence Analysis (CCA).[58] CCA was only carried out for Tianchi, as algae density was relatively low in Western Tianchi. Forward selection indicated that four of the ten physico-chemical variables (ammonium, TP, pH and DO) made independent and significant contributions to variance in phytoplankton data (P<0.05, Monte Carlo test). The codes of phytoplankton were shown in Table S2 in Supporting Information, and the statistical information of CCA analysis was shown in Table 5. Results clearly illustrated the existing relationships between environmental variables and phytoplankton species. Eigenvalues and the percentage for variance in axis1were much higher than that in axis 2. High species-environment correlation for canonical axis indicated a strong correlation between phytoplankton species distribution and environmental variables. Results from CCA ordination of most abundant phytoplankton genera and physico-chemical variables indicated that abiotic factors significantly influenced the phytoplankton community, accounting for 77.82% of the total variance (Table 5 and Fig. 5). Manual selection results showed that the most discriminant variable was ammonium concentration, which explained 41% of the total variance followed by TP concentration (29%), suggesting that ammonium and TP played an important role in the phytoplankton composition in Tianchi Lake.

CCA sample biplots showed the potential influence of environmental gradients on phytoplankton (Fig. 5). NH_4^+-N, TP and SRP had positive correlations with axis1 while TN, NO_3^--N and COD_{Mn} had negative correlations with axis 1. Several phytoplankton species such as D1 (*Chroomonas acuta*), A2 (*Synedra acus*), A3 (*Asterionella sp.*), and B2 (*Chlamydomonas sp.*), which were distributed at the right side of Axis1, were positively related to the concentrations of nutrients such as ammonium and TP. They were enhanced in late spring and early summer from May to June as nutrients began to increase after winter. The rainy season in June brought nitrogen into the water body, promoting more phytoplankton species such as A1 (*Cyclotella sp.*), A7 (*Navicula sp.*) and D2 (*Cryptomonas sp.*) which were distributed at the left side of axis1 and positively correlated with TN. In winter, phytoplankton abundance decreased as a result of the reduction of light energy associated with cooler temperature.[33] From the CCA biplots, it is clear that the abundances and dynamics of the phytoplankton community were well adapted to variable environmental parameters. The changing nutrient status of lake water had resulted in major shifts in phytoplankton structure and density.

3.4.2. Human disturbances on water nutrients

To provide more accurate guidance for lake eutrophication control, a better understanding of human driving forces that influence water quality is necessary. Tianchi Lake was affected by four anthropogenic pressures: historical grazing, tourism, invasive fish species introduction and dam building. To evaluate contributions of different external nutrient sources and to identify to which extent human activities disturbed the lake, runoff and emissions from tourism, hotel and catering industry were taken into consideration to estimate annual nutrient loadings to Tianchi. Tourism pollution was assessed in terms of faecal wastes, and all generated nutrient loadings were assumed to be directly discharged into Tianchi as there were no centralized or disperse sewage treatment systems. Loadings of TN, TP and ammonia-N were calculated as follows (eqs. 5, 6, 7):

$$F_r = V_s \times D_s \times C_s \quad \text{(Equation 5)}$$

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$$F_t = P_t \times T_t \times C_f \quad \text{(Equation 6)}$$

$$F_{h-c} = \sum_{i=1}^{m} D_{hi} \times B_i \times T_{oi} + \sum_{j=1}^{n} D_{cj} \times S_j \times T_{oj} \quad \text{(Equation 7)}$$

Where, F_r, F_t and $F_{h\text{-}c}$ represent nutrient loadings to the lake from run-offs, tourists and hotel and catering industry, respectively; V_s, D_s and C_s refer to annually transported solids volume by debris flow, average local soil bulk density, and soil nutrients content; P_t, T_t and C_f refer to annual total tourist population, average tourist stays in the scenic spot, and average production of nutrients per person per day; D_{hi} and D_{cj} refer to emission coefficients of the i hotel and the j restaurant, which were obtained from the Chinese Domestic Pollution Emission Handbook (Table S4); B_i and T_{oi} refer to the number of beds and annual open days of the i hotel, and S_j and T_{oj} refer to seats and annual open days of the j restaurant. D_s, C_s P_t, T_t, B_i and S_j values were provided by Xinjiang Tianchi National Natural Reserve Management Commission, while V_s and C_f values were obtained from literature;[28, 59-61] T_{oi} and T_{oj} were applied with 180 d as from May to October was peak travel season.

Based on the estimation, about 212.97 t of total nitrogen and 32.14 t of total phosphorus were probably transported into Tianchi Lake, annually (Table 6). These annual nutrient loadings were much smaller compared to the three most eutrophic freshwater lakes in China like Dianchi Lake, Taihu Lake and Chaohu Lake. Given the different sizes of catchment areas, however, the pollution of these diffuse nutrient sources in Tianchi region was considerable. It was estimated to be 560.45 kg/km^2/y TN and 84.58 kg/km^2/y TP which was exported to the lake. In comparison with the results of Dianchi Lake (3746.58 kg/km^2/y TN and 452.05 kg/km^2/y TP), Taihu Lake (657.53 kg/km^2/y TN and 27.40 kg/km^2/y TP) and Chaohu Lake (576 kg/km^2/y TN and 222.84 kg/km^2/y TP), it could be seen that the phosphorus loading in Tianchi had exceed that in Taihu and the nitrogen loading was already approximate to that of Taihu and Chaohu Lake.[35, 62-64] From a global perspective, annual TN and TP export rates from catchments ranged 1-20630 kg/km^2/y and 0.008-5100 kg/km^2/y, respectively. Moreover, the world average export in pasture for TN and TP was estimated as about 713 and 50 kg/km^2/y, respectively.[65, 66] As an important summer pasture in the past, the annual TP loads for the Tianchi reserve were relatively high compared to the average level and many European lowland areas, such as catchments in northeast Germany (4-25 kg/km^2/y) and the Ireland (17.5-78.5 kg/km^2/y).[67, 68] Although the current eutrophication symptoms were relatively weak, high nutrient loadings would be potential threats to lake ecosystems especially under climate change. Mitigating eutrophication in Tianchi from excessive phosphorus and nitrogen transfers called for more attention from policy makers. Non-point sources contributed the majority of nutrients loading to Tianchi, especially for total phosphorus, followed by tourist wastes, but the contribution of hotel and catering industry was relatively very small. Generally, runoff was considered as an important factor of TP export in catchments.[14, 65] As is well known, vegetation and grassland play an important role in soil and water conservation. Unfortunately, historical overgrazing has also led to serious grassland degradation and laid the basis for soil erosion triggered by more intensive precipitation, which contributed most to the nutrient transfers in runoffs. In addition, relatively high loadings of ammonia-N were contributed by tourist faeces, although human faeces did not account for a large percentage on the whole. Due to the significant role of ammonia-N for algae growth, more importance should be placed on reduction of domestic sewage discharge, and centralized sewage treatment facilities and bio-toilets should be constructed in the future. Tourist impacts were represented by faecal wastes in our estimation to simplify the calculation. Tourist garbage, emissions brought by boating and other tourism related influences were not taken into consideration. Thus, the results would underestimate the effects of traveling activity and needed to be further studied.

It is worth noting that the installation of an artificial dam would change the hydrodynamic conditions, increase water retention time, and decrease amount of annual water exchange volume.[69, 70] Thus, nutrient inputs might be deposited in the shallow area and might add extra uncertainty to our estimation of nutrient loadings. Besides, the introduction of non-native fish species would cause changes in species composition of local lake ecosystems, and promote cascade responses of top-down forces and bottom-up control.[26] These comprehensive response mechanisms of eutrophication effects needed to be further investigated in the future.

4. Conclusion and suggestions

From the present study, it could be concluded that eutrophication trend has already developed in Tianchi Lake, and clear diatoms dominance was observed in the phytoplankton structure in such a high-altitude cold-water lake. Water temperature and nutrient concentrations were relatively lower than highly eutrophic lakes, but inferring the potential for eutrophication. Significant positive correlations were obtained between Chl-a and algae cells. Chl-a was selected as a representative variable of phytoplankton biomass and biological factors in Tianchi for eutrophication assessment. Based on a comprehensive trophic level index *TLI* (Σ), the current status of eutrophication was estimated, showing emerging eutrophication in Tianchi. In CCA analysis, ammonium was identified as the most discriminant variable followed by total phosphorus, suggesting that they played an important role in phytoplankton growth and succession in Tianchi Lake. Moreover, anthropogenic pressures from historical overgrazing and increasing tourism, as major external sources, caused huge nutrient inputs to water body. The calculated high annual nutrient loadings called for more action of policy makers to mitigate eutrophication in Tianchi.

The findings provide for a better understanding of present eutrophication conditions in Lake Tianchi, and the information will be helpful for decision makers to mitigate potential negative effects. From a technical perspective, external nutrient inputs should firstly be reduced by controlling flood run-off from the steep and degraded slopes using dyke structures, bio-toilets should be established to minimize excretal pollution input, and tourist garbage collection and recycling system should be constructed to clean up the lake surroundings. Regular multi-media environmental monitoring mechanism is vital to assess the pollution dynamics, and intensive management and control should be implemented to ensure the environmental quality of this world-famous nature reserve region.

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Table 1. Descriptive table for the primary chemical and physical characteristics of the Tianchi Lake.

Length (m)	width (m)	Area (km^2)	direction	Maximum depth (m)	Average depth (m)	COD (mg/L)	TN (mg/L)	TP (mg/L)
1300	700	2.8	North-northeast	103	60	4.12	0.28	0.041

Table 2. Time series of phytoplankton structure changes (a) Tianchi; (b) Western Tianchi.

(a)

Date	Cyanobacteria	Bacillariophyta	Chlorophyta	Pyrroptata	Cryptophyta	Chrysophyta	In total	Common species	Js
14.06	1	11	3	0	0	0	15	/	/
14.08	2	6	0	0	0	0	8	6	0.35
15.05	0	6	1	1	0	0	8	2	0.14
15.06	0	3	2	3	1	1	10	4	0.29
15.08	0	6	1	3	1	0	11	6	0.4
15.11	0	8	0	1	3	0	12	8	0.53

(b)

Date	Cyanobacteria	Bacillariophyta	Chlorophyta	Pyrroptata	Cryptophyta	Chrysophyta	In total	Common species	Js
14.06	4	22	5	0	0	0	31	/	/
14.08	4	16	3	0	1	0	24	16	0.41
15.05	0	8	1	0	0	0	9	3	0.10
15.06	0	4	1	1	0	0	6	3	0.25
15.08	1	10	1	0	0	0	12	3	0.20
15.11	0	10	0	0	0	0	10	8	0.57

Table 3. Shannon-Weiner index of phytoplankton community.

Date	Shallow area of Tianchi	Center of Tianchi	Western Tianchi
15.05	1.137	1.257	1.787
15.06	1.028	1.124	1.278
15.08	0.138	0.090	1.257
15.11	0.364	0.399	0.250

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Table 4. Temporal changes of trophic state in Tianchi.

Location	Date	*TLI*(∑)	
		Trophic index	Trophic state
Shallow area of Tianchi	15.05	39.307	Mesotrophic
	15.06	50.375	**Light-eutrophic**
	15.08	42.159	Mesotrophic
	15.11	35.807	Mesotrophic
Center of Tianchi	15.05	32.311	Mesotrophic
	15.06	45.293	Mesotrophic
	15.08	42.103	Mesotrophic
	15.11	37.748	Mesotrophic
Drain outlet of Tianchi	15.05	31.697	Mesotrophic
	15.06	36.756	Mesotrophic
	15.08	41.297	Mesotrophic
	15.11	35.063	Mesotrophic
Marina of Tianchi	15.05	29.830	Oligotrophic
	15.06	39.252	Mesotrophic
	15.08	40.415	Mesotrophic
	15.11	38.581	Mesotrophic
Western Tianchi	15.05	33.948	Mesotrophic
	15.06	31.192	Mesotrophic
	15.08	27.026	Oligotrophic
	15.11	24.701	Oligotrophic

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Table 5. Summary of CCA analysis of relations between most abundant phytoplankton species and different variables for Tianchi.

	Physico-chemical factors			
	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.426	0.226	0.095	0.051
Species-environment correlations	0.979	0.951	0.896	0.925
Cumulative percentage variance of species data	37.8	57.9	66.3	70.8
Cumulative percentage variance of species–environment relation	48.6	74.4	85.2	91.0
Sum of all eigenvalues	1.127			
Sum of all canonical eigenvalues	0.877			
Variance explained by the CCA	77.82%			

Table 6. Estimation results of nutrient loadings from different sources.

	Total Nitrogen		Total Phosphorus		Ammonia-N	
	Loadings (t/a)	Percent (%)	Loadings (t/a)	Percent (%)	Loadings (t/a)	Percent (%)
Run-off	189.09	88.79	29.64	92.22	11.02	51.93
Tourist waste	22.49	10.56	2.37	7.37	9.45	44.53
Hotel and catering industry	1.39	0.65	0.13	0.40	0.75	3.53
Total	212.97 t/a		32.14 t/a		21.22 t/a	

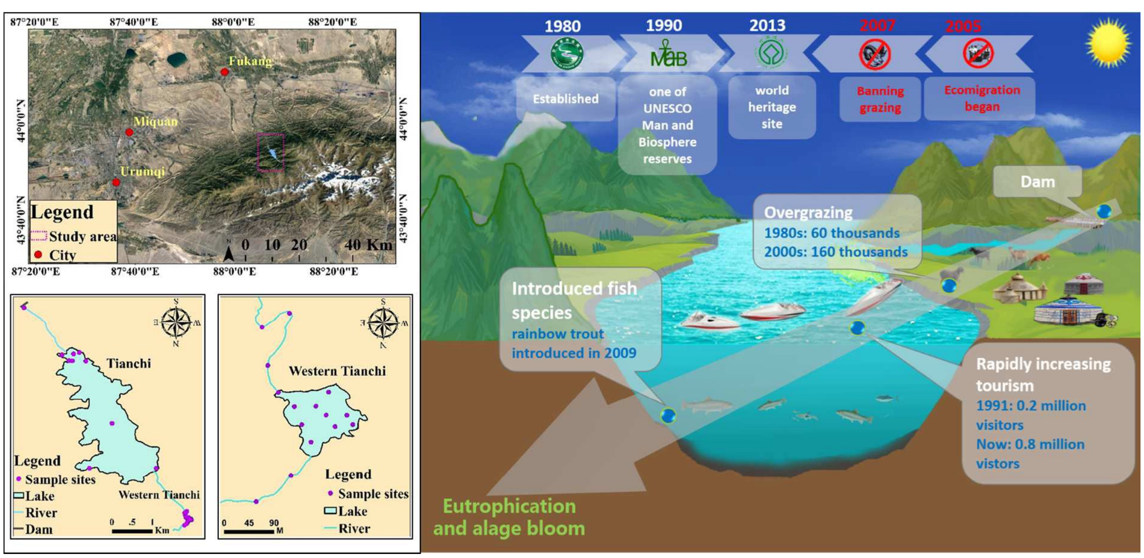

Fig. 1. Location, sampling sites and sketch map of Lake Tianchi

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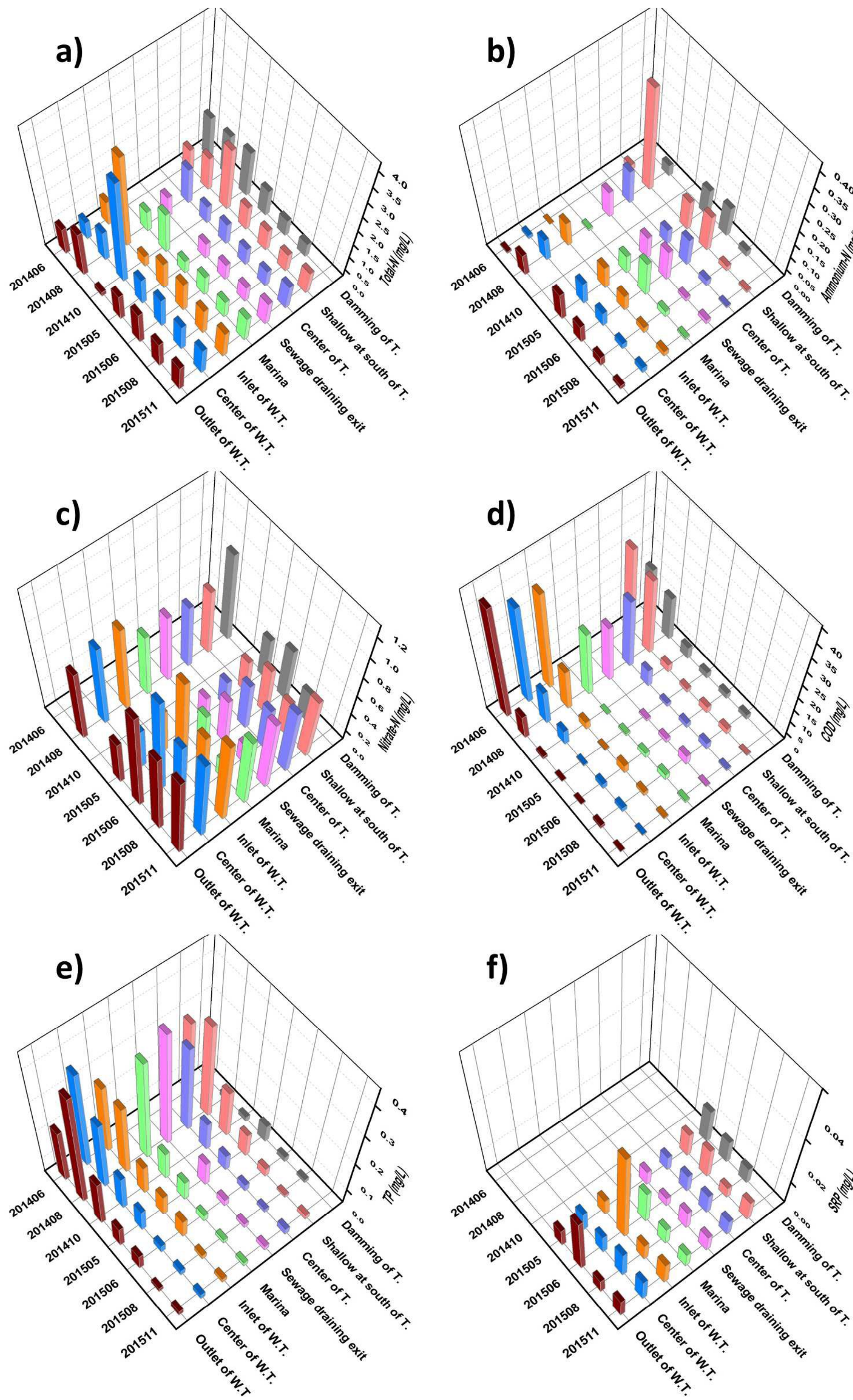

Fig. 2. Variation of physical-chemical properties in surface water samples during the study period

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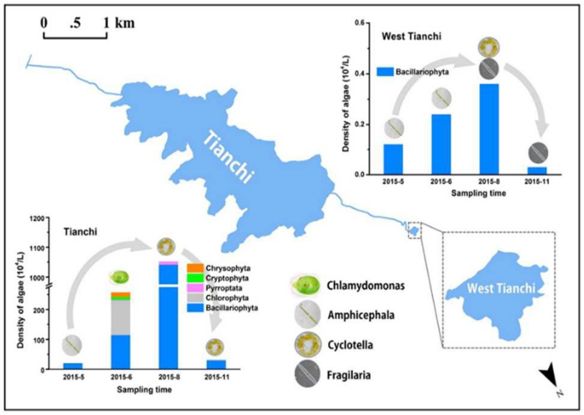

Fig. 3. Phytoplankton community characteristics

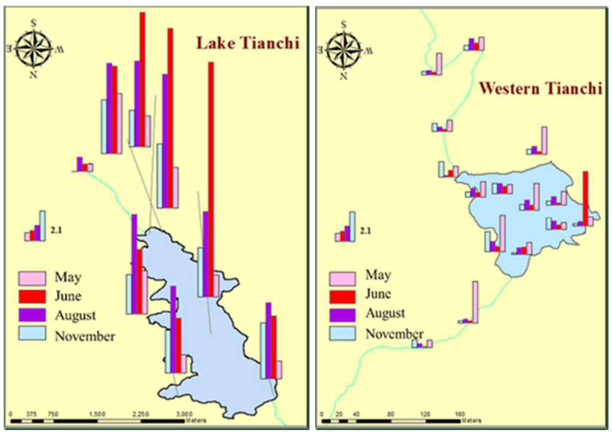

Fig. 4. Temporal and spatial distribution of chlorophyll *a*

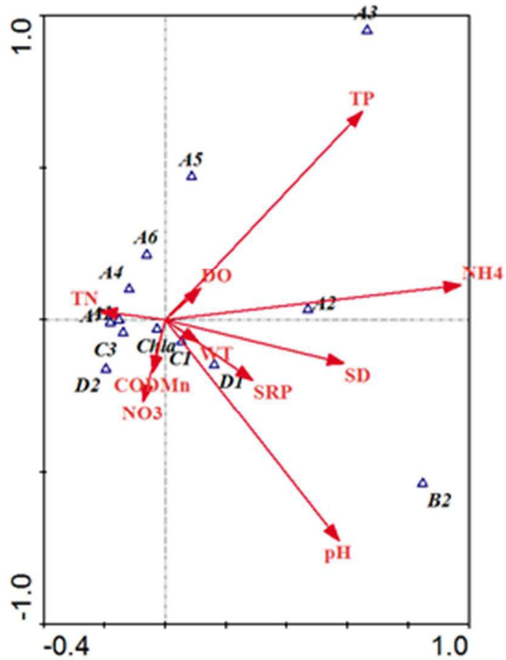

Fig. 5. Spatial ordination result from CCA of most abundant phytoplankton species in relation to physical and chemical parameters. The length of the environmental variable arrows' length represented the relative explanatory of each variable within the ordination in relation to individual sample positions. See Table S2 for phytoplankton genus name abbreviations.

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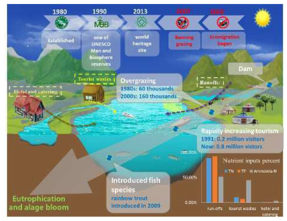

Eutrophication status investigation and anthropogenic activities analysis in a typical alpine glacial lake was carried out in this study.