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PNAS. Volume 117 (21), 11566-11572, ISSN 0048-9697.

Improvement in Municipal Wastewater Treatment Alters Lake Nitrogen to Phosphorus Ratios in Populated Regions

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Abstract

Large-scale and rapid improvement in wastewater treatment is common practice in developing countries, yet this influence on nutrient regimes in receiving waterbodies is rarely examined at broad spatial and temporal scales. Here, we present a study linking decadal nutrient monitoring data in lakes with the corresponding estimates of 5 major anthropogenic nutrient discharges in their surrounding watersheds over time. Within a continuous monitoring dataset covering the period 2008–2017, we find that due to different rates of change in TN and TP concentrations, 24 lakes out of 46 lakes, mostly located in China's populated regions, showed increasing TN/TP mass ratios; only 3 lakes showed a decrease. Quantitative relationships between in-lake nutrient concentrations (and their ratios) and anthropogenic nutrient discharges in the surrounding watersheds indicate that increase of lake TN/TP ratios is associated with the rapid improvement in municipal wastewater treatment. Due to the higher removal efficiency of TP than TN, TN/TP mass ratios in total municipal wastewater discharge have continued to increase from 10.7 (7.6–15.1) (median and 95% confidence interval) in 2008 to 17.7 (13.2–27.2) in 2017. Improving the municipal wastewater collection and treatment worldwide is an important target within the 17 sustainable development goals set by the United Nations. Given potential ecological impacts on biodiversity and ecosystem function of altered nutrient ratios in wastewater discharge, our results suggest that long-term strategies for domestic wastewater management should focus not merely on total reductions of nutrient discharges but should also consider their stoichiometric balance.

Keywords

nutrient balance | water quality change | anthropogenic source | wastewater treatment | aquatic ecosystem

Significance Statement

Due to different rates of change in TN and TP concentrations in lakes, increases in TN/TP mass ratios were observed in many China's freshwater lakes during 2008–2017. This growing imbalance has important implications for aquatic ecology that remain poorly considered and understood. Here, we show that changes in municipal wastewater treatment are a major driver for increases in lake TN/TP mass ratios, as phosphorus is more effectively removed than nitrogen from wastewater. Our findings highlight the need to more efficient nitrogen reduction besides phosphorus reduction in wastewater treatment to reduce risk of phytoplankton blooms and toxin production, and maintain ecosystem biodiversity in downstream waterbodies.

Introduction

"Clean water and sanitation" are targeted by the 17 sustainable development goals (SDGs) recommended by the United Nations (UN) (1). Due to this comprehensive goal, municipal wastewater management is receiving increasing attention globally (2-4). Most human activities produce wastewater. The annual production of municipal wastewater worldwide now exceeds 300 × 10^9 m^3 (5). This volume of wastewater is continuing to grow with the urbanization and associated population increase (4). Increasing wastewater production together with inadequate collection and treatment facilities have led to negative impacts on water quality, biodiversity, and overall functioning of receiving aquatic ecosystem (6-8). For instance, nitrogen (N) produced by human excreta accounts for ~15–20% of total anthropogenic production of reactive N per year (4) while phosphorus (P) produced by human excreta represents ~25% of worldwide P demand (4, 9). Many developing countries are tackling the problem of excess nutrients by increasing municipal wastewater treatment capacity (10-13), which is often more achievable than reducing non-point nutrient sources through reducing fertilizer application rates and watershed conservation practice (7, 8, 14). China, with an annual municipal wastewater discharge of ~60 × 10^9 m^3, is a case in point (15). Since 2005, the construction of wastewater treatment plants (WWTPs) has grown rapidly in China. In 2005, the percentage of municipal wastewater being treated on the national scale was only ~40%, while it had reached over 90% in 2017 (15). However, neither the results of these measures in relation to changing lake nutrient concentrations nor their long-term impacts on relative balance of nutrient availability in lakes are known.

Imbalance in N and P supplies can have a variety of ecological impacts in aquatic ecosystems (16-19). First, N enrichment relative to P could favor plankton species with strong competitive abilities for using P, such as toxin-producing *Microcystis* spp. and *Planktothrix* spp. (20-23). Second, the per-cell production of N-rich toxins (e.g., microcystins) can be enhanced by N enrichment under oligotrophic conditions (19, 21). Third, increasing N/P ratios could favor a lower number of slow-growing phytoplankton species with high optimal N/P ratios at the expense of species with lower optimal N/P ratios, imposing stoichiometric constraints on filter-feeding zooplankton (18, 19). By decreasing rates of energy transfer through food webs, shorter trophic webs with the fewer predators could further develop (24). These scenarios have been documented in diverse lakes such as Lake Zurich in Switzerland (23), Lake Okeechobee in the USA (25), Lake Erie in the USA-Canada (26), as well as in Lake Taihu (27) and other eutrophic lakes in China (28).

To quantify the overall impacts of anthropogenic activities on TN and TP concentrations and their ratios in lakes, we compiled a paired TN and TP monitoring dataset from freshwater lakes widely distributed over China. We collected the nutrient monitoring data from 111 freshwater lakes in 2017 and further investigated temporal trends of water quality in 46 of these lakes for which continuous monthly data were available during 2008–2017. Comprehensive nutrient discharge inventories were developed for each of the 46 lake catchments. Five major anthropogenic discharge sources were quantified: municipal wastewater, rural wastewater, crop farming, livestock operations, and aquaculture. Quantitative relationships between lake nutrient concentrations and anthropogenic discharges were assessed using generalized linear models. This study addressed the central hypothesis that the recently increasing trend of TN/TP mass ratio in freshwater lakes in the populated regions of China is primarily induced by the rapid adoption of improved wastewater treatment during 2008–2017.

Results and Discussion

Nutrient Concentrations and Ratios in China’s Lakes

In the surveyed lakes in 2017, mean TN concentration was 996 (380–2723) μg/L (median and 95% confidence interval (CI), n=111) and mean TP concentration was 32 (7–114) μg/L (n=111) (Figure 1 and SI Appendix Figure S1; SI Appendix Dataset S1). Based on the Chinese surface water quality standards (SI Appendix Table S1), half of all the surveyed lakes in 2017 (55 out of 111) had TN concentrations higher than the

Grade III limit (1000 μg/L), above which lakes are considered to be polluted in China (Figure 1A). In contrast, only 27 lakes had TP concentrations higher than the Grade III limit (50 μg/L) (Figure 1B). The mean TP concentration (~30 μg/L) across all the lakes in this dataset is similar to the levels in Europe (~25 μg/L) (29) and the USA (20–40 μg/L) (30). However, the TN concentration (~1000 μg/L) is much higher than those in Europe (~650 μg/L) (29) and the USA (~600 μg/L) (31). TN/TP mass ratio was 31.6 (12.5–128.2) in the surveyed lakes in 2017 (Figure 1C). In freshwater ecosystems, when algae is competing for limited nutrients, limitation of algal growth is mainly determined by N when water TN/TP mass ratio lower than 9, while P is main limiting factor when this value is higher than 23 (32). Based on these criteria, over 2/3 of the 111 surveyed lakes had TN/TP mass ratios higher than 23 in 2017 (Figure 1C), indicating widespread TN enrichment relative to TP. The mean lake TN/TP mass ratio in 2017 in China approaches the level for European lakes (31 (5–96)) (29) but is much higher than that in the USA (21 (4–98)) (31).

Available monthly monitoring data for 46 lakes during 2008–2017 further indicate that TN and TP concentrations are declining at different rates in lakes (SI Appendix Figure S2) and that increases of TN/TP mass ratios are widespread among Chinese lakes (Figure 2, SI Appendix Table S2–S3). During 2008–2017, TP concentrations decreased in 22 out of the 46 lakes. The decreasing rate is indicated by slope of the regression line of concentration (logarithm scale) versus time (see Methods). The decreasing rate for TP concentration is 0.003–0.026 resulting half-life of 2.22–20.6 years. Only 8 lakes, however, showed increases in TP concentrations (SI Appendix Figure S2). In contrast, TN concentrations increased in 22 lakes, with a rate of 0.001–

0.034, while TN decreased in only 12 lakes, with a rate of 0.003–0.028 (SI Appendix Table S2). As a consequence, 24 of 46 lakes showed increases in TN/TP mass ratios, with a rate of 0.0022–0.0453 per month and doubling time of 1.3–26.3 years; while only 3 lakes showed decreases in TN/TP mass ratios (Figure 2).

Response of Lake Nutrient Regime to Shifting Anthropogenic Discharges

Shifts in anthropogenic nutrient discharges from the surrounding watershed are usually responsible for alterations of lake nutrient conditions (8, 33, 34). We therefore characterized temporal changes in N and P discharges of the five anthropogenic sources listed above with a spatial resolution of 1 km^2 during 2008–2017 in China (SI Appendix Supplementary Text S1). Results show that anthropogenic nutrient discharges are much larger in more populated Eastern China (the east of the so-called 'Hu Huanyong Line', with a population density of ~300 persons per km^2) (35) than in less-populated Western China (~20 persons per km^2) (SI Appendix Figure S3). However, dominant anthropogenic sources in different sub-regions varied distinctly due to spatial variability in types of human activities (SI Appendix Figure S4). Due to disproportionate changes in N and P discharges, TN/TP mass ratios in the total anthropogenic discharge have significantly increased in eastern China during 2008–2017 (Figure 2C). In particular, in the lower reaches of the Yangtze River and Pearl River Basins, TN/TP mass ratios of the total anthropogenic discharge were as much as 30% higher in 2017 relative to 2008 (Figure 2C). However, small increases or even decreases in the ratios were observed in the large yet less populated regions of western China.

We took a closer look at 46 lakes with continuous nutrient monitoring data and found that lakes located in more densely populated regions usually had significant increases in their TN/TP mass ratios during 2008–2017 (Figure 3A). 24 lakes with significant increases in TN/TP mass ratios had larger population densities ($P < 0.01$, *t*-test) in their surrounding watersheds, with a level of 299 (92–743) (median and 95% CI) people per km^2. By comparison, 22 lakes with no changes or decreases in TN/TP mass ratios had population density of 51 (1–477) people per km^2 in their watersheds. Municipal wastewater discharge in the watersheds where lakes experienced increases in TN/TP mass ratios was much higher than that for the other lakes ($P < 0.01$, *t*-test). Consequently, N and P discharges contributed by municipal wastewater in the total local nutrient discharge were also higher in the watersheds where lakes had the increasing TN/TP ratios (Figure 3A).

Linear correlation analysis (36) was performed to examine the direct relationships between lake nutrients (e.g., TN, TP concentrations, TN/TP mass ratios) and the explanatory variables (e.g., discharge from different sources, climate variables in lake catchments) (Table 1). Among all the variables, the strongest correlations for lake nutrient concentrations and TN/TP mass ratios were with the municipal wastewater discharge in the surrounding watershed (for TN, $R^2 = 0.44$, $P < 0.01$, $n = 46$; for TP, $R^2 = 0.29$, $P < 0.01$, $n = 46$; for TN/TP, $R^2 = 0.17$, $P < 0.01$, $n = 46$) (Figure 3B). A generalized linear model (GLM) was further performed to quantify the relative importance of each explanatory variable in variations of lake nutrients. GLM results further confirmed that the dominant factor responsible for variations of TN/TP mass ratio in lakes was municipal wastewater discharge in the watersheds, accounting for ~39% of the variations (Table 1). Apart from municipal wastewater discharge, we found other sources could also influence lake nutrient concentrations. For instance, rural wastewater discharge was also an important factor in explaining variations of TN and TP concentrations in lakes (Table 1)

Impacts of Wastewater Treatment on Lake Nutrients and Their Implications

The apparent importance of municipal wastewater discharge in influencing in-lake nutrient dynamics motivated us to investigate the nutrient discharge records collected in influents and effluents of 4,960 WWTPs during 2008–2017 in China (details in SI Appendix Dataset S2). To mitigate serious water pollution, treatment of municipal wastewater has advanced rapidly in China over the past decade. The number of WWTPs had increased rapidly from 1,535 units in 2008, with a volume of treated wastewater of 22×10^9 m^3, to 4,960 units in 2017, with a volume of treated wastewater of 57×10^9 m^3 (SI Appendix Figure S5). Average effluent TN and TP concentrations decreased from 16.4 and 1.0 mg/L in 2008 to 10.7 and 0.42 mg/L in 2017, respectively (Figure 4A). By comparing nutrient concentrations in influent and effluent, it is apparent that existing wastewater treatment technologies in China have differential removal efficiencies for TN and TP from the municipal wastewater (Figure 4A). Wastewater ammonium is microbially converted to N_2 by nitrification and denitrification, a process that requires careful “tuning” of facility operations (37). However, removing P from municipal wastewater is a simpler and more effective process involving coagulation, flocculation and sedimentation (38). Thus, average removal efficiency from municipal wastewater by WWTPs is as high as 90% for TP, but less so (60–70%) for TN, resulting in the WWTP effluent with much higher TN/TP mass ratios (23.2 (14.7–37.0), median and 95% CI) that the influent (8.9 (7.0–

11.1)) ($P < 0.01$, *t*-test) (SI Appendix Figure S6). As the percentage of treated municipal wastewater in the total wastewater discharge increases, more P than N is effectively removed from municipal wastewater discharge. Consequently, TN/TP mass ratio in the total municipal wastewater discharge has increased sharply from 10.7 (7.6–15.1) in 2008 to 17.7 (13.2–27.2) (median and 95% CI) in 2017 (Figure 4B and 4C).

To evaluate how changes may be responsible for observed increases on lake TN/TP mass ratios, we created a set of simple null models for TN and TP concentrations and TN/TP mass ratios based on quantitative relationship model revealed in GLM analysis (SI Appendix Table S4; see details in Methods and SI Appendix Supplementary Text S2). Two typical scenarios were simulated: null scenario (NUS) and real situation scenario (REA). Under NUS, we assumed that no new WWTPs were constructed after 2008, and the percentage of wastewater being treated remained unchanged during 2008–2017. Under REA, the development of WWTPs followed the observed trajectory (SI Appendix Dataset S2). The results showed that estimated nutrient concentrations under NUS were statistically different from the results under REA (SI Appendix Table S5-S6). Under NUS, the estimated TN and TP concentrations in lakes would be 2.4 and 1.4 times, respectively, higher than those under REA. However, average TN/TP mass ratio in lakes under NUS was ~25% lower than under REA (SI Appendix Figure S7). These impacts on TN/TP mass ratios in lakes might be alleviated by increasing N removal efficiency by WWTPs (SI Appendix Supplementary Text S3). A simple prediction based on the quantitative relationship in SI Appendix Table S4 indicates that, if TP removal efficiency remained at the 2017 level and TN removal efficiency had a 3% increase per year, lake TN/TP mass ratios would return to 2008 levels within 10 years (SI Appendix Figure S8). This suggests that TN removal efficiency by WWTPs should be improved by 27% over 10 years.

In 2015, the United Nations Development Programme set a target to halve the proportion of untreated wastewater in the world by 2030 (1). Encouraged by this target, China and many other developing countries worldwide (e.g., India, Indonesia and Nigeria) have built or are constructing numerous WWTPs (39). Therefore, the challenge of imbalanced lake TN/TP ratios caused by improved sanitation is not confined to China but also likely occurs in other countries. To achieve coherent and sustainable wastewater management, potential ecological consequences induced by N over-enrichment relative to P in aquatic ecology will require consideration in future sanitation approaches in which N and P removals are considered in a holistic way. Possible short-term strategies could include refining operations of existing facilities (40), developing more efficient N-removal technologies (37), and introducing new standards setting TN/TP ratio targets for effluent discharge (38). In the longer term, increasing nutrient recovery from municipal wastewater along with source separation of human excrement may also be promising (2-4, 41).

In summary, consistent with recent changes in anthropogenic nutrient discharges, numerous Chinese lakes in highly populated regions are showing increasing TN/TP mass ratios, which can bring some unexpected consequences for the functioning of aquatic food webs, such as the increasing prevalence of non-N_2 fixing cyanobacteria (*Microcystis*, *Planktothrix*) in the eutrophic lakes (23-28). Promoting a better balance between N and P supplies will allow a greater diversity of taxa as well as improved food web control of phytoplankton biomass. These modern perspectives on nutrient stoichiometry in lakes imply that more effort should be placed on N removal from municipal wastewater in the future to achieve desired water quality outcomes as human society seeks to achieve SDGs for sanitation, water, and aquatic ecosystem health. Our findings document an unappreciated impact of wastewater treatment on aquatic ecosystems and also provide a science-based argument for more effectively managing water quality by balancing N and P removals from wastewater.

Methods

Lake TN and TP Monitoring Data

We obtained an extensive lake nutrient monitoring dataset during 2008–2017 from the water quality monitoring program performed by the Ministry of Ecology and Environment, China. As in (42) and (43), we did not differentiate natural lakes or artificial reservoirs, since both waterbody types can be influenced by anthropogenic nutrient discharges in surrounding watersheds. The dataset consists of two parts: I. nutrient data revealing the condition of lakes in 2017; II. nutrient data revealing the temporal trend. In dataset I, a total of 111 freshwater lakes distributed in China's 30 provinces were included. Measured TN and TP concentrations, locations, surface areas and water volumes for these lakes were provided in SI Appendix Dataset S1. In the dataset II, 46 out of the 111 lakes with continuous monitoring data (generally with > 40 monthly and paired TN and TP monitoring data during 2008–2017) were selected to assess temporal trends in nutrient concentrations and ratios. These 46 lakes were widely distributed in China, and had differences in lake characteristics (e.g., area, water volume) and human activity types in their watersheds (SI Appendix Table S3).

Changes in lake nutrient concentrations are a first-order process (43-44). Therefore, we used ln-transformed TN (or TP concentrations or TN/TP mass ratio) in regression analysis between monthly nutrient data and corresponding sampling time. If $P < 0.05$, then the slope (k) in the regression was defined as the monthly increase rate (if k is > 0, month^{-1}) or decrease rate (if k is < 0, month^{-1}) during the study period; if $P > 0.05$, it was defined as no significant change in nutrient concentration or ratio during 2008–2017. Half-life ($T_{1/2}$) or doubling time (T_2) of nutrient concentrations or ratios in lakes was estimated as follows: $T_{2 \text{ or } 1/2} = \ln(2) / k$. TN or TP concentration, TN/TP mass ratio, increase or decrease rate, hydrological information and location for each lake is provided in SI Appendix Table S3. Information about surface areas and water volumes in the surveyed lakes were derived from the HydroLAKES database by Global HydroLab (45), and Wang and Dou (46).

Procedures for selecting sampling sites in lakes and collecting water samples were generally consistent throughout the study period and were based on the “Technical Specifications Requirements for Monitoring of Surface Water and Waste Water in China” (47). A depth-integrated sample was collected at each sampling site. For lakes with a depth greater than 5 m, water samples at 0.5 m below surface were collected. For lakes with depths between 5 and 10 m, water was collected at 0.5 m below the surface and 0.5 m above the bottom and then mixed to make a composite sample. The sampling site was usually set at the center of the lake. However, for the large lakes, multiple sampling sites were established at the center of the lake, the deep zone and the shore zones, respectively. Standard methods (shown below) were used for measuring TN and TP concentrations. An unfiltered aliquot of water was prepared from each bulk sample. TN concentration was determined by persulfate digestion, followed by automated colorimetric analysis (N-(1-naphyl) ethylene diamine dihydrochloride spectrophotometry), with a method detection limit (MDL) of 50 μg/L-N (47). TP concentration was determined by persulfate digestion, followed by automated colorimetric analysis (ammonium molybdate and antimony potassium tartrate under acidic conditions), with an MDL of 10 μg/L-P (47). All the TN and TP concentrations lower than the MDL were set to 1/2 of the MDL in the subsequent data analyses.

Anthropogenic N and P Discharges

To identify the driving forces for alterations of nutrient concentrations and their ratios in lakes, we analyzed the temporal dynamics of major anthropogenic sources for N and P discharges during 2008–2017 in China by applying a hierarchical model of national nutrient cycling updated from Cui et al. (48) and Liu et al. (49). In China, human activities have contributed to the bulk of N and P discharges into water since the national economic reforms were implemented in 1978 (48, 49). Thus, in this study, five major and independent human-induced N and P discharge sectors were selected, including municipal wastewater, rural wastewater, livestock operation, crop farming, and aquaculture. Data on anthropogenic activity was collected from a number of national statistical databases, literature and government reports, whilst most parameters were derived primarily from studies based on the field experiments (see SI Appendix Supplementary Text S1). By emphasizing impacts of increasing adoption of WWTPs, estimates of N and P discharges from municipal wastewater were based on a national-scale investigation during 2008–2017. Total N and P discharges from

WWTPs were further aggregated from each of the 4,960 WWTPs. A detailed description of the temporal changes of number of WWTPs, volumes of treated and untreated wastewater, TN and TP concentrations in influent and effluent, N and P discharges through wastewater during 2008–2017 in China's 31 provinces was provided in SI Appendix Dataset S2.

Analysis of Drivers of Lake Nutrient Regimes

46 lakes with the continuous nutrient monitoring data during 2008–2017 were selected to identify the specific relationships between lake nutrients concentrations (or ratios), and temporal anthropogenic discharges in surrounding watersheds. To address spatial variabilities in human activities in China, a raster data set based on the population distribution (35), livestock distribution (50), and land use distribution (51) was developed. The result was a high-resolution map (1 km × 1 km grid) of annual anthropogenic nutrient discharges incorporating all the five major sources during 2008–2017. By using the HydroSHEDS database, watershed boundaries for the 46 lakes were delineated by using the ArcMap 10.2.2 software (52-53).

We conducted a general linear model (GLM) analysis (54) to quantify the relative associations of each explanatory variable with variations in TN and TP concentrations and TN/TP mass ratios in lakes (SI Appendix Supplementary Text S2). Explanatory variables included the 5 anthropogenic discharge variables (discharges from 5 different sources) and 2 climate variables (precipitation and temperature in watersheds) (36). Based on the Bayesian information criterion (36, 55), the best models revealing the quantitative relationship between lake nutrients and anthropogenic discharges were established (results in SI Appendix Table S4). These models were further applied in the simple scenario analysis. Scenario analysis was divided into two parts in this study. First, to quantify the impacts of WWTPs on temporal change of water quality during 2008–2017, we created simple null models for TN and TP concentrations, and TN/TP mass ratios in lakes individually. The null hypothesis is that the construction of WWTPs had caused no significant changes of lakes nutrients during 2008–2017. Two typical scenarios were compared for the null hypothesis test: Null scenario (NUS) representing the temporal trends of nutrient concentrations and their ratio if wastewater treatment capacity and efficiency remained at the level of 2008 throughout the study period; Real situation scenario (REA), representing the temporal trends of nutrient concentrations and their ratios in which the development of WWTPs in watersheds followed the observed trajectory (see details in SI Appendix Supplementary Text S2). Single factor ANOVA test was used to verify the null hypothesis. In addition to the null test, we also estimated the potential response of TN/TP mass ratio in lakes under different future scenarios with improvement of TN removal efficiency by WWTPs, while TP removal efficiency remained at the same level in 2017 (see details in SI Appendix Supplementary Text S3). Three typical scenarios were simulated where TN removal efficiency by WWTPs was improved by 1%, 2% and 3% per year, respectively, in the next decade. The statistical analyses and data processing were carried out by using Excel 2010 (Microsoft Corporation, Washington, USA), SPSS 16 (International Business Machines Corporation, New York, USA), lm and relaimpo functions (56) in R software (R Core Team) and ArcMap 10.2.2 (Environmental Systems Research Institute, Redlands, USA).

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Acknowledgments

We greatly thank Miao Qi (Tianijn University), Shengjie Guo (Tsinghua University) and Dazhen Zhang (Tsinghua University) for the work on the initial data processing on lake water quality data, investigation data about WWTPs and delineation of watershed boundaries. We thank Shengli Tao (EDB, Toulouse) for providing original R source code on GLM analysis. We appreciate helpful comments and suggestions in pre-reviews by Yongzhen Peng (BUT), Jiuhui Qu (THU), Jinnan Wang (CAEP), Yong Liu (PKU), Yi Zheng (SUST), Hongtao Duan and Jiacong Huang (NIGL, CAS). Y. Tong is supported by National Key Research and Development Program (NO. 2018YFA0903000) and National Natural Science Foundation of China (NO.

41977324). H.W. Paerl is supported by the US National Science Foundation (NO. 1831096). A.B.G. Janssen is funded by the KNAW project SURE+ (NO. PSA-SA-E-01). X. Kong is supported by a postdoctoral fellowship from the Alexander von Humboldt Foundation in Germany. J. Peñuelas and J. Sardans are funded by an ERC-SyG-2013-610028 IMBALANCE-P grant. Y. Tong is also thankful to his little son "Koala" since he was always sleeping when his daddy was busy typing.

Author contributions

T.Y., L.Y., D.X. and L.X. conceived the research. W.M., T.Y., D.X., Z.W., W.X., H.W., Z.W., and Z.Y. collected the data and performed the calculations. T.Y., P.J., S.J., E.J., R.C., P.H.W. and L.Y. wrote the paper. All the authors contributed to the interpretation of the data and commented on the manuscript.

Competing financial interests

The authors declare no competing financial interests.

Additional information

Supplementary text S1-S3

Figures S1 to S8

Tables S1 to S6

Datasets S1 to S5

SI References

Figure captions

Figure 1. Annual average TN, TP concentrations and TN/TP mass ratios in 111 freshwater lakes in mainland China in 2017. [1]TN concentration of 500 μg/L and TP concentration of 25 μg/L represent the Grade II limits in the Chinese water quality standards (SI Appendix Table S1). [2]TN concentration of 1,000 μg/L and TP concentration of 50 μg/L represent the Grade III limits. [3]Based on the Guildford and Hecky's definition (32), P limitation for algal growth occurs when TN/TP mass ratio is > 23, while N limitation occurs when this ratio is < 9. [4]The details for each lake are provided in SI Appendix Dataset S1.

Figure 2. Temporal trends of nutrient concentrations and their mass ratios in 46 lakes with continuous monitoring data during 2008–2017, and relative changes on TN/TP mass ratios in aggregated anthropogenic discharges (with a spatial resolution of 1 km^2 grid). [1]Red dots in part B represent the average values of monthly TN/TP mass ratios, and black error lines represent the standard deviations. [2]Definitions of "increasing", "no change" and "decreasing" are provided in the methods. [3]Change of TN/TP ratio in discharge refers to the change of TN/TP mass ratio in the grid in 2017 compared with the value in 2008. [4]The "Hu Huanyong" Line is used as a geographic boundary between the high-developed and densely-populated Eastern region and the less-developed and sparsely-populated Western region in China. [5]The details about the nutrient concentrations, locations and hydrological information for 46 lakes are provided in SI Appendix Table S2 and S3.

Figure 3. Comparison of municipal wastewater discharges in the surrounding watersheds for different categories of lakes (A) and quantitative relationships between lake TN (or TP concentrations, or TN/TP mass ratios), and corresponding municipal wastewater discharges in the surrounding watersheds (B). [1]Group I: lakes with significant increases in TN/TP mass ratios during 2008–2017; group II: lakes with no change or decreases in TN/TP mass ratios during 2008–2017. Results by T-tests showed significant differences ($P < 0.05$) between Group I and Group II for all the four selected variables. Details in each lake were provided in SI Appendix Table S3. [2]Correlation analysis is performed by a linear model. Dots represent the observed data for each lake. The average values (dot) and standard deviations (error bars) of lakes nutrients during 2008–2017 were applied in the analysis. The black line represents the fitted results for TN, TP and TN/TP mass ratio. The gray line represents the 95% confidence interval. Nutrient discharges in watersheds were normalized by the volume of lakes.

Figure 4. Temporal changes of TN, TP and TN/TP ratios in the municipal wastewater in China during 2008–2017. A. Monthly changes of TN and TP concentrations (mean value) in influent and effluent, and their removal efficiencies by WWTPs during

2008–2017. B. Temporal changes of TN/TP mass ratios in municipal wastewater discharge (including total and untreated discharge) on the national scale during 2008–2017. C. Change of TN/TP mass ratios in municipal wastewater discharge (including total untreated discharge) on the regional scale during 2008–2017. The red and blue lines represent the regression lines between N and P discharges in municipal wastewater in 2008 and 2017, respectively. Their slopes represent the TN/TP values in municipal wastewater discharge.

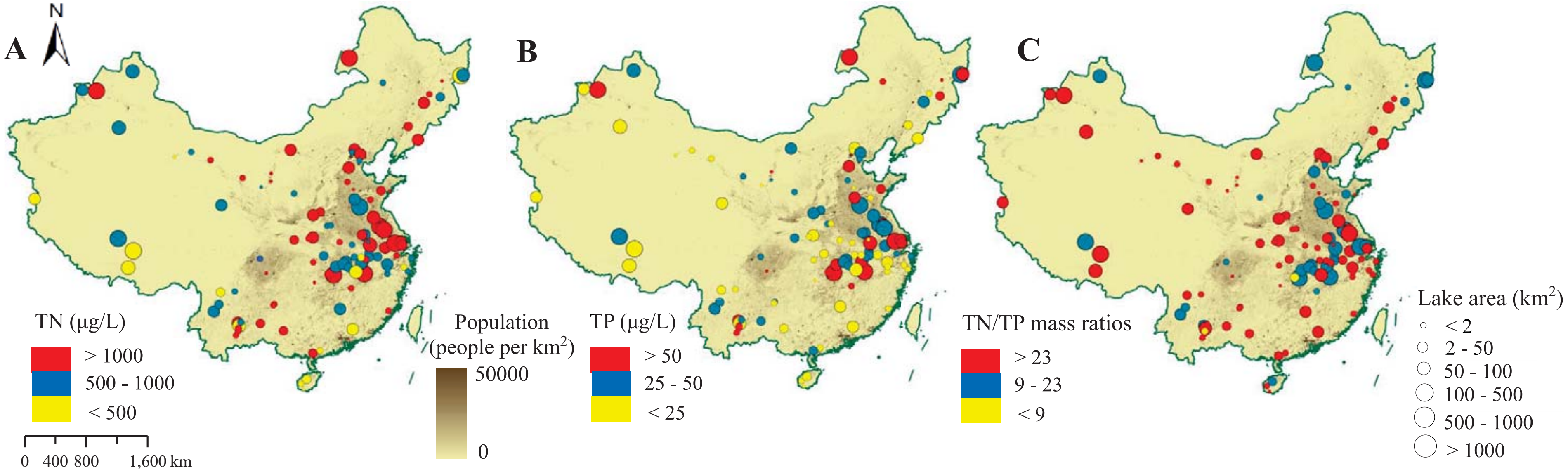
Data availability

The authors declare that all the data supporting the findings of this study are available within the article and its supporting information.

Table 1. Correlation and GLM analyses on the relationships between lake nutrient concentration and ratios and corresponding explanatory variables (n=46)

Variables	**TN concentration**		**TP concentration**		**TN/TP mass ratio**	
Correlation analysis	***r***	***P***	***r***	***P***	***r***	***P***
Municipal wastewater	0.667	0.000*	0.540	0.000*	0.409	0.006*
Rural wastewater	0.585	0.000*	0.718	0.000*	0.152	0.325
Crop farming	0.345	0.023*	0.556	0.000*	0.065	0.677
Livestock farming	0.371	0.012*	0.613	0.000*	-0.092	0.052
Aquaculture	0.209	0.184	0.210	0.182	-0.221	0.154
Precipitation	-0.068	0.655	-0.105	0.486	0.277	0.062
Temperature	0.009	0.951	-0.087	0.567	0.206	0.169
GLM analysis	**SE×10^{-3}**	**RI, %**	**SE×10^{-3}**	**RI, %**	**SE**	**RI, %**
Municipal wastewater	2.1	47.7	1.5	13.3	2.2	39.0
Rural wastewater	0.4	7.6	0.2	23.3	2.4	15.4
Crop farming	0.2	7.6	0.3	13.5	2.5	2.5
Livestock farming	0.5	26.3	0.3	35.4	6.7	14.2
Aquaculture	5.2	5.0	1.2	5.3	6.9	9.6
Precipitation	0.2	2.2	0.0	1.8	1.64	4.9
Temperature	20.1	3.4	1.0	7.2	0.0	14.3

*$P < 0.05$; SE, standard error; RI, relative importance explained by the variable.



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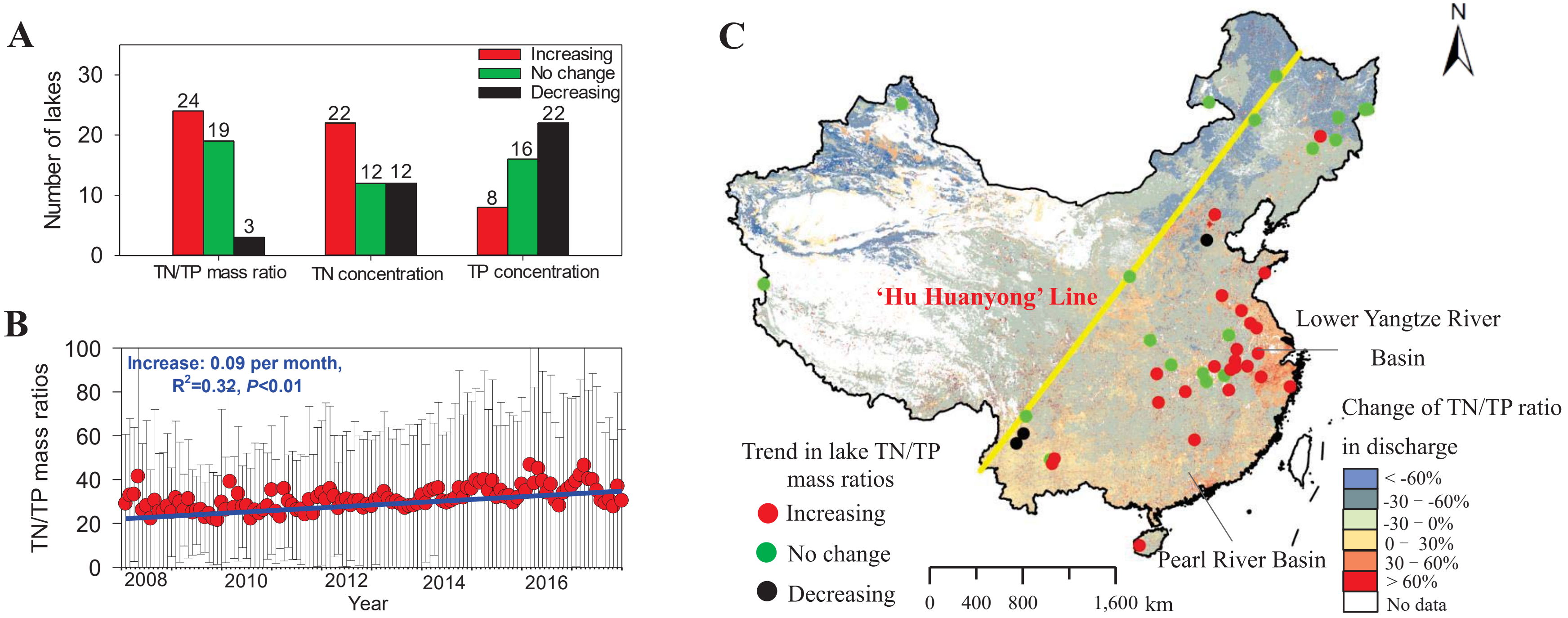

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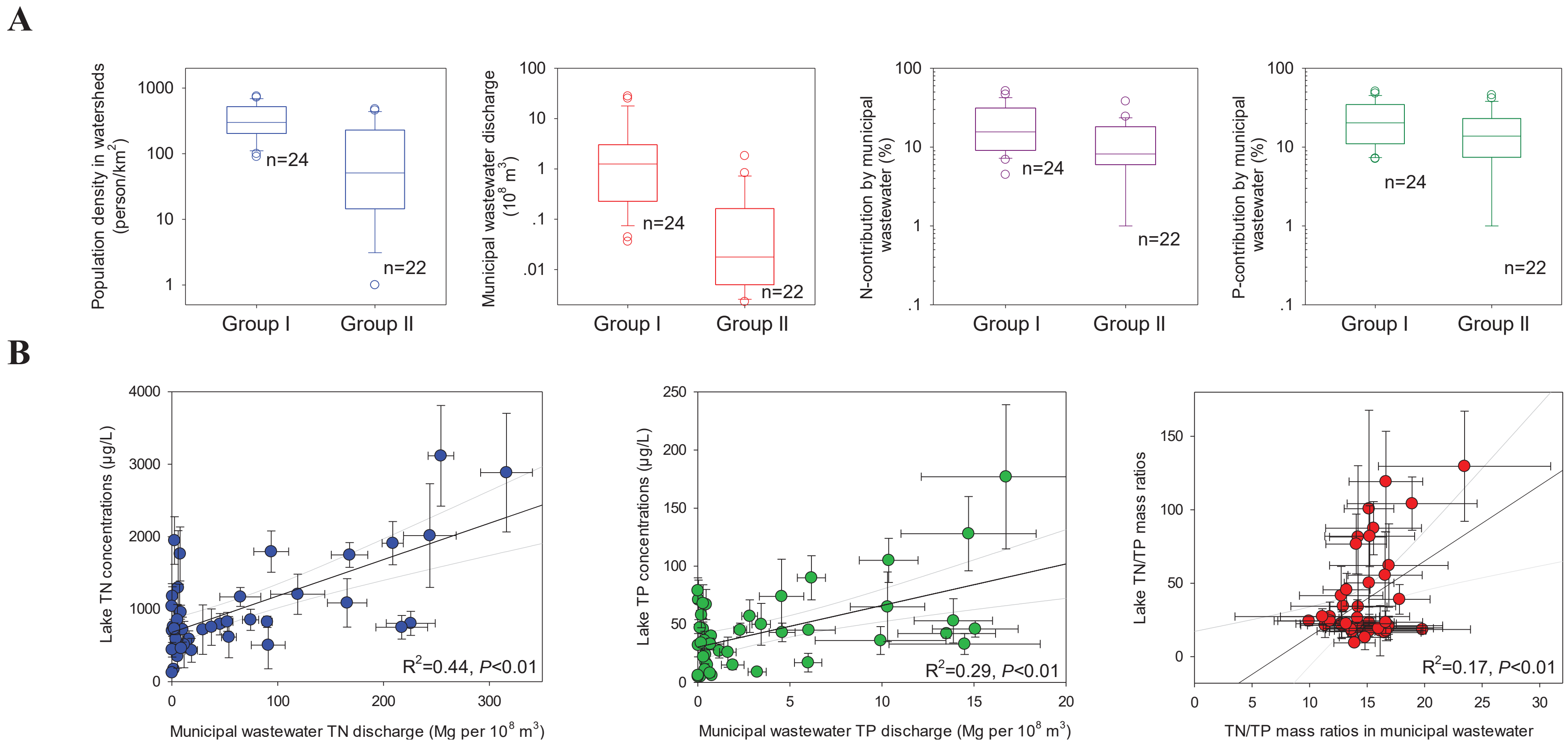

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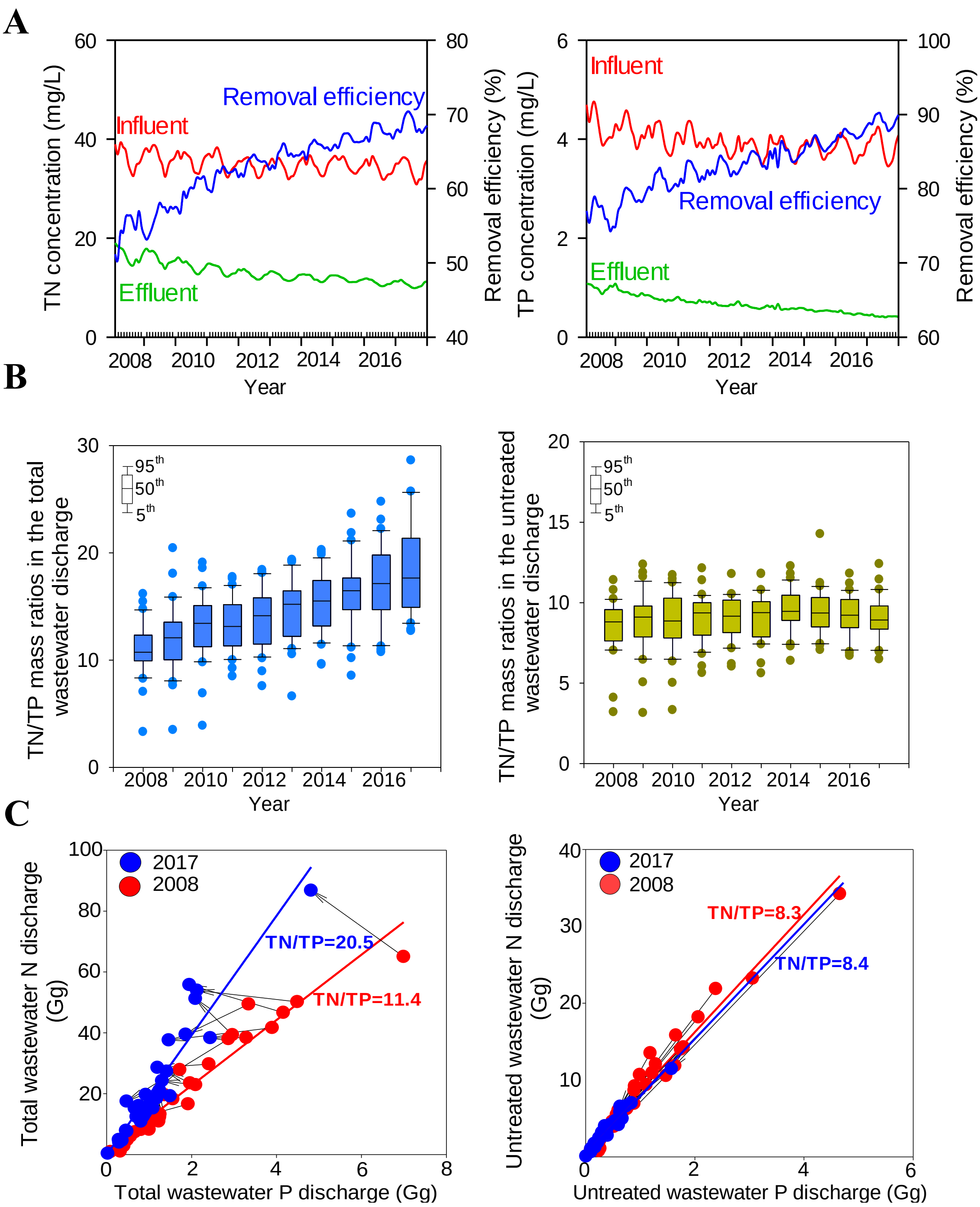

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