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LETTER

Phosphorus accumulates faster than nitrogen globally in freshwater ecosystems under anthropogenic impacts

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Abstract

Combined effects of cumulative nutrient inputs and biogeochemical processes that occur in freshwater under anthropogenic eutrophication could lead to myriad shifts in nitrogen (N):phosphorus (P) stoichiometry in global freshwater ecosystems, but this is not yet well-assessed. Here we evaluated the characteristics of N and P stoichiometries in bodies of freshwater and their herbaceous macrophytes across human-impact levels, regions and periods. Freshwater and its macrophytes had higher N and P concentrations and lower N : P ratios in heavily than lightly human-impacted environments, further evidenced by spatiotemporal comparisons across eutrophication gradients. N and P concentrations in freshwater ecosystems were positively correlated and N : P was negatively correlated with population density in China. These results indicate a faster accumulation of P than N in human-impacted freshwater ecosystems, which could have large effects on the trophic webs and biogeochemical cycles of estuaries and coastal areas by freshwater loadings, and reinforce the importance of rehabilitating these ecosystems.

Keywords

Accumulation, anthropogenic impacts, biogeochemistry, decoupling of nitrogen and phosphorus cycles, freshwater ecosystems, global patterns, imbalance, macrophytes, stoichiometry, waterbodies.

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INTRODUCTION

Human activities have drastically accelerated Earth's major biogeochemical cycles, altering the balance of biogeochemical nitrogen (N) and phosphorus (P) cycles (Falkowski *et al.* 2000; Galloway *et al.* 2008; Vitousek *et al.* 2010; Elser & Bennett 2011; Liu *et al.* 2013; Peñuelas *et al.* 2013). Recent studies indicate that enhanced N deposition increases the limitation of P or other nutrients in many ecosystems (Bergstrom *et al.* 2005; Elser *et al.* 2009; Vitousek *et al.* 2010), whereas anthropogenic eutrophication in addition to N deposition, has large impacts on specific ecosystems (Downing & McCauley 1992; Arbuckle & Downing 2001; Smith & Schindler 2009). For example, freshwater ecosystems, as nutrient sinks, receive P leached from land and anthropogenic P discharge (e.g. excess P-fertiliser use, P-containing pesticides, P-containing detergents and domestic and industrial sewage) in surface runoff (Carpenter *et al.* 1998; Arbuckle & Downing 2001; Smith & Schindler 2009). The prevailing P limitation in freshwater ecosystems (Schindler 1977; Elser *et al.* 2007) may thus be alleviated by these direct P inputs, increasing the risk of eutrophication. Freshwater ecosystems receive both N and P inputs, but polluted waterbodies often receive substantial nutrient inputs from urban sewage, agricultural discharges and animal slurries typically with low N : P ratios (Downing & McCauley 1992; Carpenter *et al.* 1998; Arbuckle & Downing 2001; Peñuelas *et al.* 2012; Sardans *et al.* 2012). However, given that N is quite mobile whereas P tends to remain and accumulate in the soil, N : P ratios in river and stream loadings were also observed to increase in some agricultural areas (Peñuelas *et al.* 2012; Sardans *et al.* 2012). Clearly complex nutrient loadings to water bodies can lead to myriad shifts in freshwater stoichiometry.

Biogeochemical processes can further influence freshwater stoichiometry. Regardless of what the N : P ratio is in the input sources, low oxygen or anaerobic conditions can sometimes increase N depletion via denitrification and P enrichment from sediments in heavily impacted waterbodies, thereby decreasing the water column N : P ratios (Saunders & Kalff 2001; Søndergaard *et al.* 2003; Niemistö *et al.* 2008; Moss *et al.* 2013; Grantz *et al.* 2014). Biological activities enhanced by anthropogenic impacts, such as *Microcystis* blooms, can also selectively drive P (but not N) release from sediments by elevated pH and thus decrease water column N : P ratios (Xie *et al.* 2003). Interestingly, a recent study showed that denitrification had been stimulated in many lakes by increased P inputs from human activity (Finlay *et al.* 2013), leading to a higher rate of N removal. Furthermore, P usually tends to

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have a longer residence time and higher retention efficiency than N in freshwater ecosystems (Saunders & Kalff 2001; Jeppesen *et al.* 2005; Cook *et al.* 2010; Grantz *et al.* 2014). These combined effects of cumulative nutrient inputs and biogeochemical processes could thus lead to *in situ* modification of N : P ratio in freshwater ecosystems under anthropogenic impacts but this is not yet well-studied.

Freshwater macrophytes play vital roles in regulating the structure and function of freshwater ecosystems, and in restoring water quality (Lacoul & Freedman 2006; Bornette & Puijalon 2011). The elemental composition of aquatic plants integrates the chemical, biological and spatiotemporal characteristics of their surrounding environments and can reflect differences in the nutrient supplies of freshwater ecosystems under anthropogenic impacts (Lacoul & Freedman 2006; Demars & Edwards 2007; Bornette & Puijalon 2011; Xing *et al.* 2013). Oligotrophic pre-industrial freshwater ecosystems, generally considered to be P-limited because of lower solubility of P than N and because of N-fixation by autotrophic and heterotrophic bacteria (Schindler 1977; Howarth *et al.* 1988), may have exerted long-term selective pressure on freshwater plants to acquire and sequester nutrients. Plants with the capacity to take up and retain P much more than N can thus be favored in oligotrophic ecosystems (Güsewell & Koerselman 2002). The Stability of Limiting Elements Hypothesis (Han *et al.* 2011) states that plant P should be more easily altered by environmental changes than plant N, reflecting a degree of homeostatic control (Güsewell & Koerselman 2002; Sterner & Elser 2002). A faster accumulation of P than N in ecosystems could then be expected to be reflected in freshwater macrophytes under increasing ambient nutrient inputs.

Freshwater ecosystems across regions and periods vary in their nutrient levels, depending on the stages of industrial and agricultural development and on the policies of environmental protection (Havens *et al.* 2001; Van Drecht *et al.* 2009; Potter *et al.* 2010; Powers *et al.* 2016). For example, China is experiencing rapid economic growth without effective environmental protection (Jin *et al.* 2005). Excessive fertiliser use and untreated sewage discharges have increased dramatically with higher crop demand, urbanisation and industrialisation (Havens *et al.* 2001; Jin *et al.* 2005; Haygarth *et al.* 2014; Liu *et al.* 2016), resulting in significant degradation of water quality in freshwater ecosystems. In contrast, most European areas and the United States of America (from now on Euro-America) have largely controlled sewage discharges and acted to constrain fertiliser run-off in recent decades, and increased their technical measures to restore deteriorated waterbodies (Van Drecht *et al.* 2009; Potter *et al.* 2010; Haygarth *et al.* 2014; Powers *et al.* 2016). The environments of freshwater ecosystems may thus be less severely impacted in Euro-America compared to China, resulting in differences in N : P stoichiometry of the ecosystems.

Global patterns of N : P stoichiometry of freshwater and its macrophytes, and their relationships with large-scale anthropogenic eutrophication, are largely unknown. Based on the rationale and previous work just described, we hypothesise that freshwater ecosystems under anthropogenic impacts accumulate P at disproportionally higher rates relative to N over long time scales, leading to low N : P ratios in water and plants. We tested the above hypothesis by synthesising two N and P data sets for global bodies of freshwater and their macrophytes and evaluating the N : P stoichiometry across human-impact levels, regions and periods.

MATERIAL AND METHODS

Data compilation

We compiled data from 157 publications and our own field sampling of N and P concentrations and N : P ratios in 433 species of freshwater macrophytes with 1234 observations from 332 sites worldwide (Fig. S1a; see Data set S1 for details). For each site, we recorded geographic location (latitude/longitude), climatic variables (mean annual temperature, MAT, °C; mean annual precipitation, MAP, mm), type of freshwater ecosystem (heavily vs. lightly human-impacted; see below), sampling period, family/species/functional group of macrophytes and N and P concentrations and N : P ratios of the macrophyte tissues (see Data set S1 for details). We used coordinates of the geographic centres of the sampling areas when specific geographic coordinates were not provided. Estimates of MAT/MAP at sites without records in the original publications were extracted from a global climatic data set at http://www.worldclim.org/. We here defined 'heavily human-impacted' sites as areas with eutrophic waterbodies (excluding naturally eutrophic sites) or that received anthropogenic nutrient inputs (e.g. agricultural fertiliser, sewage effluents and intense pastoral activities and manure use) and defined 'lightly human-impacted' sites as areas with oligotrophic waterbodies or without direct human disturbances (e.g. natural preserves and mountain areas and river valleys relatively far from human impacts). Based on the descriptions of environmental conditions from the synthesised publications, 'heavily impacted' sites were further divided into three groups: sites dominated by agricultural impacts, sites dominated by urban sewage impacts and sites impacted by both agricultural and urban sewage discharges.

The macrophytes (*sensu stricto,* herbaceous macrophytes) in this study consisted of aquatic herbs, with mosses, macroalgae, freshwater woody plants and gymnosperms excluded due to the paucity of data. To illustrate the effects of functional groups on the variations in plant N and P stoichiometry, we have primarily grouped all species in the data set into ferns and seed plants based on phylogenetic information, and classified them into emergent, floating leaved, freely floating and submerged plants based on life forms. We also grouped all species into graminoids (Cyperaceae and Gramineae families) and forbs (all others). Seed plants were further divided into monocotyledons and dicotyledons.

Our data set did not contain much information about waterborne chemistry data associated with the sampling sites of macrophytes, so we compared the consistency of patterns of water total N (TN) and total P (TP) stoichiometries with those of macrophyte N and P stoichiometries at regional or larger geographic scales. We compiled and compared data on water TN and TP concentrations, TN : TP ratios and chlorophyll-a (Chl a) concentrations for freshwater ecosystems in Europe, the USA and China, the areas where most N and P data for freshwater macrophytes were available. The data set contained a total of 1867 records for 940 sites from 195 publications (Fig. S2a; see Data set S2 for details). Site-related information, including latitude, longitude, climatic variables (MAT and MAP), sampling period, and type of freshwater ecosystem (heavily vs. lightly human-impacted), was also recorded (see Data set S2 for details).

To explore the direct impacts of human activity on N : P stoichiometry in freshwater ecosystems, we extracted data for anthropogenic variables around those sampling sites in China, excluding the Euro-American sites due to the paucity of coordinated environmental data. The sampling sites were grouped into the corresponding county, district or province based on the published description. We then extracted the corresponding anthropogenic variables, including population density, gross production, sewage discharge, total N discharge in sewage, total P discharge in sewage, percentage of crop area, N fertiliser use, P fertiliser use, meat production, and N deposition (see Data sets S1 and S2 for details). These data, except for N deposition, were obtained from *China Statistical Yearbook* (1991–2014). The data for N deposition were derived from Lü & Tian (2007). We used meat production to approximate the impact of manure use.

We adopted three criteria to assure the consistency in our literature syntheses. First, freshwater macrophytes consisted of plants from lakes, river, streams, ponds, reservoirs and continental wetlands but not coastal wetlands. Second, we focused mainly on N and P data from leaves and shoots, excluding other material, such as stems, roots, rhizomes, inflorescences and litter. Many studies did not separate shoots into specific organs for some macrophytes (e.g. submerged or freely floating plants), probably due to the approximately equal nutrient concentrations in stems and leaves (Fernández-Aláez *et al.* 1999; Demars & Edwards 2007). Third, the samples for the analyses of N, P and Chl a concentrations had been collected during the growing season.

Statistical analysis

All nutrient data were $\log_{10}$-transformed before analysis to improve data normality because of their highly skewed frequency distribution (Figs. S1 and S2). The data for plant nutrients were averaged at the species (i.e. averaged by the same species) or site-species (i.e. averaged by the same species at each site) level as described by Han *et al.* (2005). We analysed overall statistical characteristics and variations in macrophyte N and P stoichiometries across functional groups at the species level, because the influence of species identity was emphasised (Han *et al.* 2005). Reduced major axis (RMA) regression was used to characterise the scaling relationships between plant P and N concentrations, between water TP and TN concentrations, and between Chl a concentrations and water nutrients (i.e. water TN, TP and TN : TP ratios) at the individual level (i.e. all raw data were pooled), because these variables from each sample were in pairs and correlative (Reich *et al.* 2010).

In addition to the aforementioned analyses, we performed the following analyses at the site-species level for plant nutrients and at the site level (i.e. averaged within the same site) for water nutrients, because the influences of site environment and species identity were both highlighted (Reich & Oleksyn 2004; Han *et al.* 2005, 2011; Chen *et al.* 2013). We explored the effects of anthropogenic eutrophication on N and P stoichiometries in the macrophytes with three comparisons: lightly vs. heavily human-impacted waterbodies (on impact level), Euro-America vs. China (spatially) after 1990 (including 1990), and Euro-America along the temporal gradient (temporally). We extracted data for Euro-America after 1990 to compare with China because most nutrient data for China were from years after 1990. We also conducted several comparisons across various groups based on other two category criteria (types of anthropogenic impact; eutrophication levels determined by OECD classification scheme, Vollenweider 1968). Statistical differences among groups were identified by *t*-tests with Bonferroni corrections.

A general linear model (GLM) was used to quantify the effects of human-impact level, functional group and climate on plant N and P stoichiometry. Human-impact level, functional group and climate were treated as fixed factors, and the site was denoted as a random factor to explore the non-independence of plant N and P stoichiometry at a site. Only one of the functional groups was included in each main-effect model, because all functional groups were highly overlapping with each other. If more than one functional group was significant, Akaike information criterion (AIC) was used to determine the final model: the model with the lowest AIC value was selected as the final model. Similarly, GLM analyses were also performed to determine the relative contributions of human-impact level and climate on water TN and TP stoichiometry. Ordinary least squares (OLS) regression was used to explore the relationships between nutrient data and population density (reflecting the integrated anthropogenic influences), N deposition and latitude. Given significant multicollinearities among the ten anthropogenic variables (see Data sets S1 & S2 for details), stepwise multiple regressions were used to discriminate among the effects of potential drivers on N and P stoichiometries in freshwater ecosystems in China. All statistical analyses were conducted using R 2.15.2 (R Development Core Team, 2012).

RESULTS

N and P concentrations and N : P ratios in freshwater macrophytes varied widely: 6–63 mg N g^{-1}, 0–10 mg P g^{-1}, and 2–44 for N : P mass ratios (Fig. S1). Geometric means for N and P concentrations and the N : P mass ratio for all species were 19.7 and 2.45 mg g^{-1} and 8.5 respectively; the corresponding coefficients of variation (CVs) were 44, 59 and 64% (Fig. S1). Water column TN and TP concentrations and TN : TP ratios also varied widely among freshwater ecosystems: 0–56 mg TN L^{-1}, 0–4 mg TP L^{-1} and 0-237 for TN:TP mass ratios (Fig. S2). Geometric means for water TN and TP concentrations and the TN:TP mass ratio for all waterbodies were 1.03 and 0.055 mg L^{-1} and 18.6 respectively; the corresponding CVs were 171, 209 and 168% (Fig. S2). Plant P and N concentrations, as well as water TP and TN concentrations, were positively correlated, with scaling exponents > 1 (P against N; Tables S1 and S2, and Fig. 1).

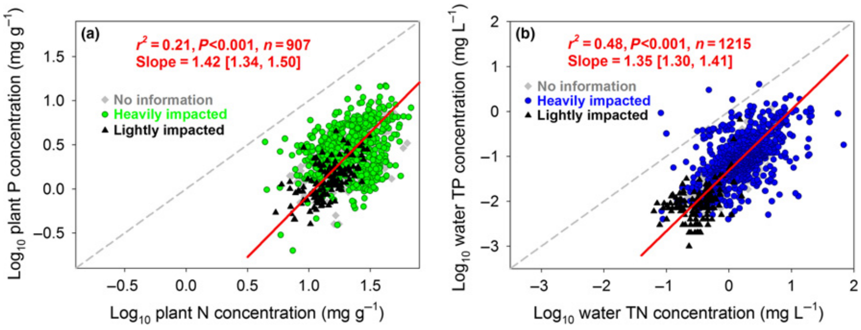

Figure 1 Relationships between (a) plant P and N concentrations and (b) water TP and TN concentrations for all individual data pooled. Reduced major axis (RMA) regression was used to determine the regression lines for all data. Numbers in square brackets are the lower and upper 95% confident intervals of the RMA slopes. The phrase 'no information' represented the data from sites without detailed human-impact information.

Human impacts significantly ($P < 0.05$) increased N and P concentrations in freshwater and its macrophytes but decreased their N : P ratios (Fig. 2a and c). The macrophytes had significantly ($P < 0.05$) higher N (21.5 vs. 14.9 mg g^{-1}) and P (2.99 vs. 1.29 mg g^{-1}) concentrations but a lower N : P mass ratio (7.5 vs. 11.7) in heavily than lightly human-impacted environments respectively (Fig. 2a). The ecosystems accordingly had significantly ($P < 0.05$) higher water TN (1.27 vs. 0.30 mg L^{-1}) and TP (0.079 vs. 0.007 mg L^{-1}) concentrations but a lower TN:TP mass ratio (15.9 vs. 46.9) in heavily than lightly human-impacted environments respectively (Fig. 2c). The stoichiometric comparisons across human-impact levels in China and in Euro-America had similar patterns (Fig. S3). Areas highly impacted by either agricultural inputs or urban sewage, or both nutrient sources had higher N and P concentrations but lower N : P ratios in freshwater and its macrophytes compared to those in lightly impacted areas (Fig. S4). Areas dominated by the sewage impacts, however, had higher N and P concentrations but similar N : P ratios in freshwater and its macrophytes compared to areas dominated by agricultural impacts (Fig. S4).

Human-induced eutrophication had impacts on N and P stoichiometries in freshwater and its macrophytes across regions and periods (Fig. 2b, d; Fig. S5). Macrophyte N (21.6 vs. 19.3 mg g^{-1}) and P (2.82 vs. 2.03 mg g^{-1}) concentrations were higher but the N : P mass ratio was lower (7.5 vs. 9.7) in China than Euro-America respectively, for the same period (Fig. 2b). Water TN (1.33 vs. 0.77 mg L^{-1}) and TP (0.104 vs. 0.035 mg L^{-1}) concentrations were accordingly higher but the TN : TP mass ratio (13.5 vs. 24.7) was again lower in China

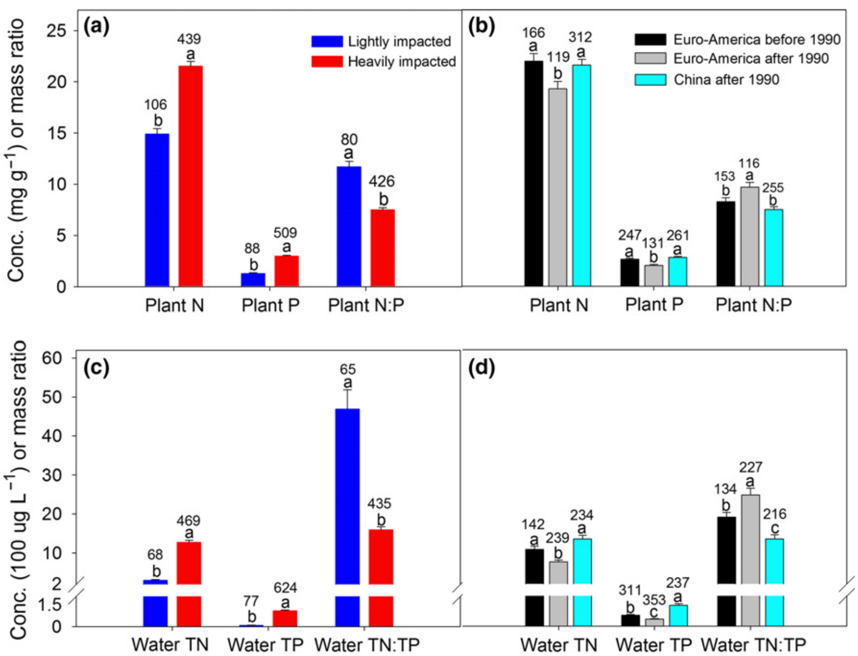

Figure 2 N and P concentrations and N : P mass ratios in freshwater ecosystems across human-impact levels (lightly/heavily), regions (Euro-America/China after 1990) and periods (before/after 1990 in Euro-America). (a) & (b) Macrophyte N and P concentrations and N : P mass ratios; (c) & (d) water TN and TP concentrations and TN:TP mass ratios. Bars indicate geometric means with standard errors. Different letters above the bars indicate significant differences ($P < 0.05$) identified by t-tests with Bonferroni corrections. Numbers above the bars indicate sample sizes.

than Euro-America respectively (Fig. 2d). Euro-American macrophyte N (22.0 vs. 19.3 mg g^{-1}) and P (2.64 vs. 2.03 mg g^{-1}) concentrations were higher, but the N : P mass ratio (8.3 vs. 9.7) was lower before than after 1990 (Figs. 2b and S5) respectively. Euro-American water TN (1.08 vs. 0.77 mg L^{-1}) and TP (0.054 vs. 0.035 mg L^{-1}) concentrations were accordingly higher but the TN:TP mass ratio (19.1 vs. 24.7) was lower before than after 1990 respectively (Figs. 2d and S5).

The GLM analyses indicated that the human-impact level explained much more variation in macrophyte N and P stoichiometry than functional group or climate (Table 1), despite the significant influences of functional groups (Tables S3 and S4, see detailed assessments in supplementary information). The human-impact level, functional group and site, as predictors, explained 10.2, 4.2 and 46.8% of the variance in plant N concentration respectively (Table 1). Human-impact level, functional group, MAP and site, as predictors, explained 21.8, 6.2, 0.9 and 38% of the variance in plant P concentration respectively (Table 1). Human-impact level, functional group, MAT, MAP and site, as predictors, explained 7.4, 0.4, 0.7, 1.4 and 57.9% of the variance in plant N : P ratios respectively (Table 1). The comparisons of macrophyte N and P stoichiometry for specific functional groups across the human-impact levels, regions and periods had similar trends as those for the overall pooled plants (Figs. S6 and S7), further supporting the modest role of functional group in shaping the global N and P stoichiometry in freshwater macrophytes. Accordingly, GLM analyses found that the human-impact level explained much more variation in water TN and TP stoichiometry than climate (Table S5).

Water Chl a concentration was strongly positively correlated with water TN and TP concentrations and weakly negatively with TN:TP ratio (Fig. 3). Quantitative assessments of anthropogenic impacts based on the thresholds of TN, TP and Chl a concentrations indicated that eutrophication increased water TN and especially TP concentrations, leading to a decrease in TN:TP ratio (Fig. 3). Finally, N and P stoichiometry in freshwater and its macrophytes varied slightly with latitude (Tables S6; Fig. S8, see detailed assessment in supplementary information). Tissue P concentrations in freshwater macrophytes decreased and the N : P ratio increased with absolute latitude, while the latitudinal pattern of tissue N concentrations was not statistically significant ($P = 0.828$; Table S6; Fig. S8). TN and TP concentrations in freshwater bodies also decreased, and TN:TP ratios increased with increasing absolute latitude (Table S6; Fig. S8).

DISCUSSION

Our results showed a markedly higher P concentration and lower N : P ratio (arithmetic means of 2.93 mg g^{-1} and 9.9 respectively) in freshwater macrophytes than those previously reported in freshwater angiosperms or aquatic vascular plants collected in regions with limited human disturbance (e.g. 1.30 mg g^{-1} and 11.7, Fernández-Aláez *et al.* 1999; 2.3 mg g^{-1} and 13.6, Demars & Edwards 2007) (data not included in our data set). Conversely, the P concentration and N : P ratio in the macrophytes in this study were lower and higher, respectively, than those from 213 sites in eastern China in highly polluted water (3.28 mg g^{-1} and 7.7, Xia *et al.* 2014) and those from 24 highly eutrophic lakes along the middle and lower reaches of the Yangtze River (4.0 mg g^{-1} and 4.2, Xing *et al.* 2013) (data not included in our data set). N : P ratios in anthropogenic nutrient sources were considerably lower than those in natural undisturbed watersheds, lightly impacted waterbodies and global lakes (Table S7). These comparisons provide further evidence that human impacts have changed N : P stoichiometry of freshwater macrophytes due to a greater accumulation of P than N.

The pattern of a faster accumulation of P than N in freshwater ecosystems was apparent in regional comparisons. These differences probably reflect the fact that rates of

Table 1 Summary of general linear models for tissue N and P concentrations, and N : P mass ratio in freshwater macrophytes

	$\log_{10}$ plant N				$\log_{10}$ plant P				$\log_{10}$ plant N : P			
	Main-effect model			Final model	Main-effect model			Final model	Main-effect model			Final model
Factor	DF	MS	F	SS%	DF	MS	F	SS%	DF	MS	F	SS%
Human-impact level	1	2.05	**102.64**	10.2	1	9.30	**268.36**	21.8	1	2.29	**83.66**	7.4
Functional groups												
Life form	3	0.43	**6.62**		3	0.49	**4.75**		3	0.18	2.16	
Phylogeny1	1	0.25	**11.64**		1	0.25	**7.12**		1	0.01	0.06	
Phylogeny2	1	1.23	**59.52**		1	2.64	**82.00**	6.2	1	0.12	**4.53**	0.4
Phylogeny3	1	0.85	**42.75**	4.2	1	1.66	**49.03**		1	0.12	**4.23**	
Climatic variables												
MAT	1	0.01	0.62		1	0.06	1.97		1	0.23	**8.35**	0.7
MAP	1	0.05	2.38		1	0.38	**11.74**	0.9	1	0.44	**16.10**	1.4
Random factor												
Sites	106	0.09	**4.46**	46.8	121	0.13	**4.16**	38.0	107	0.17	**6.14**	57.9

F values in bold denote $P < 0.05$. Human-impact level: lightly vs. heavily impacted. Lifeform: emergent, floating-leaved, freely floating, submerged; Phylogeny 1: seed plant, fern; Phylogeny 2: forb, grass; Phylogeny 3: monocotyledon, dicotyledon. Abbreviations: MAT, mean annual temperature; MAP, mean annual precipitation; DF, degrees of freedom; MS, mean squares; SS, proportion of variances explained by the variable. Because DF, MS and F values of MAT, MAP and site differ in the four main-effect models, we presented the values calculated from the final model here (Table S4).

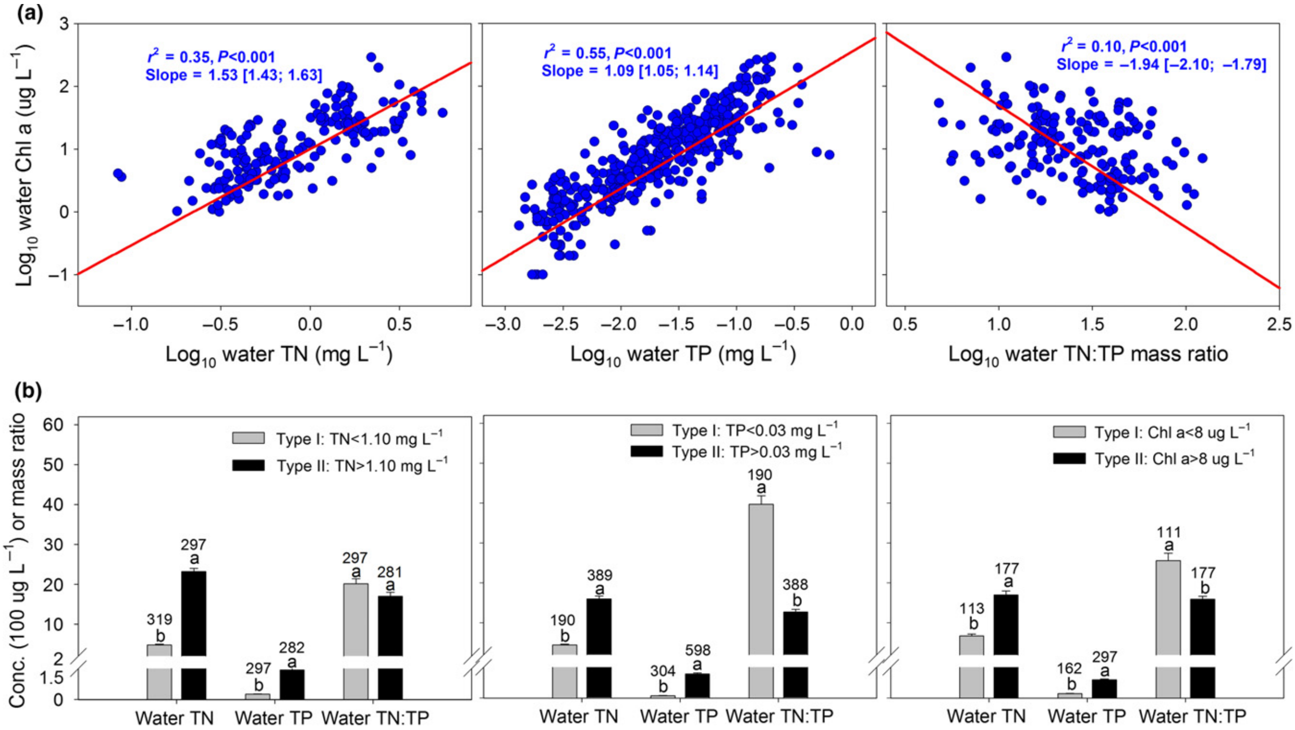

Figure 3 (a) Relationships between concentrations of chlorophyll-a (Chl a) and TN and TP and TN:TP mass ratio for all individual data pooled. Reduced major axis (RMA) regression was used to determine the regression lines. Numbers in square brackets are the lower and upper 95% confident intervals of the RMA slopes. (b) Water TN and TP concentrations and TN:TP mass ratios in freshwater ecosystems across eutrophication levels determined by OECD classification scheme (Vollenweider 1968). Bars indicate geometric means with standard errors. Different letters above the bars indicate significant differences ($P < 0.05$) identified by *t*-tests with Bonferroni corrections. Numbers above the bars indicate sample sizes.

application of N and P fertilisers (222, 64 and 98 kg N ha^{-1} $year^{-1}$; 80, 23 and 31 kg P_2O_5 ha^{-1} $year^{-1}$ for China, the USA and Europe respectively) and untreated municipal sewage (12.32 and 0.85 t ha^{-1} $year^{-1}$ for China and Europe respectively) are higher in China than Euro-America (Table S8; Fig 4). Residual P concentrations are much higher in cropland soils in China than in other areas around the world (Vitousek *et al.* 2009; Sattari *et al.* 2012), so we generally expect that the streams in China would have an especially low N : P ratio. The use of fertilisers in China, however, is still increasing, with a decrease in the ratio of N to P fertilisers (Figs. 4 and S9; see more assessment in supplementary information), and discharge of untreated sewage is also increasing because of China's continued rapid economic growth, urbanisation and industrialisation (Jin *et al.* 2005; Liu *et al.* 2016; Powers *et al.* 2016), which may unfortunately aggravate the situation.

Our results showed that macrophyte and water N and P concentrations were positively correlated and the N : P ratios were negatively correlated with the corresponding population density in China (Fig. 4) which serves as a proxy for the integrated anthropogenic impacts on nutrient cycling in freshwater ecosystems. Multiple anthropogenic variables jointly explained 38, 31 and 46% of the total variances for plant N concentration, P concentration, and N : P ratio in China respectively (Table S9). In contrast, these variables jointly explained a smaller 11, 22 and 16% of the total variances for water TN concentration, TP concentration and TN:TP ratio in China respectively (Table S9). Considerable variance, however, remained unexplained, which may reflect the unmatched spatial scales of nutrient and anthropogenic variables, as well as the impacts of other potential drivers (e.g. geographic properties and hydrological processes) not included in this analysis.

The pattern of a faster accumulation of P than N in freshwater ecosystems was also apparent in temporal comparisons in the Euro-American regions. Intriguingly, the year around 1990 as a break-point via the temporal analysis had also been reported by previous studies concerning the long-term records of N concentrations in mosses in Europe (Harmens *et al.* 2015), and the temporal trends of N and P fertiliser use in Europe and the USA (Fig. 4; Sattari *et al.* 2012). In addition, with 'the Nitrates Directive and the Urban Waste Water Treatment Directive', the European Commission enforced several regulations to control the non-point pollution generated by agricultural inputs and point pollution originated from urban sewage discharges at the beginning of 1990s (Sutton *et al.* 2011).

In Euro-American regions, many freshwater ecosystems have undergone steep decreases in P availability in response to phosphate banning policies for detergents, reductions in fertiliser use, sewage input controls and watershed management (Fig. 4; Jeppesen *et al.* 2005; Van Drecht *et al.* 2009; Potter *et al.* 2010; Sattari *et al.* 2012; Finlay *et al.* 2013; Dove & Chapra 2015; Powers *et al.* 2016). Previous studies have shown that N loading also declined or stabilised in most

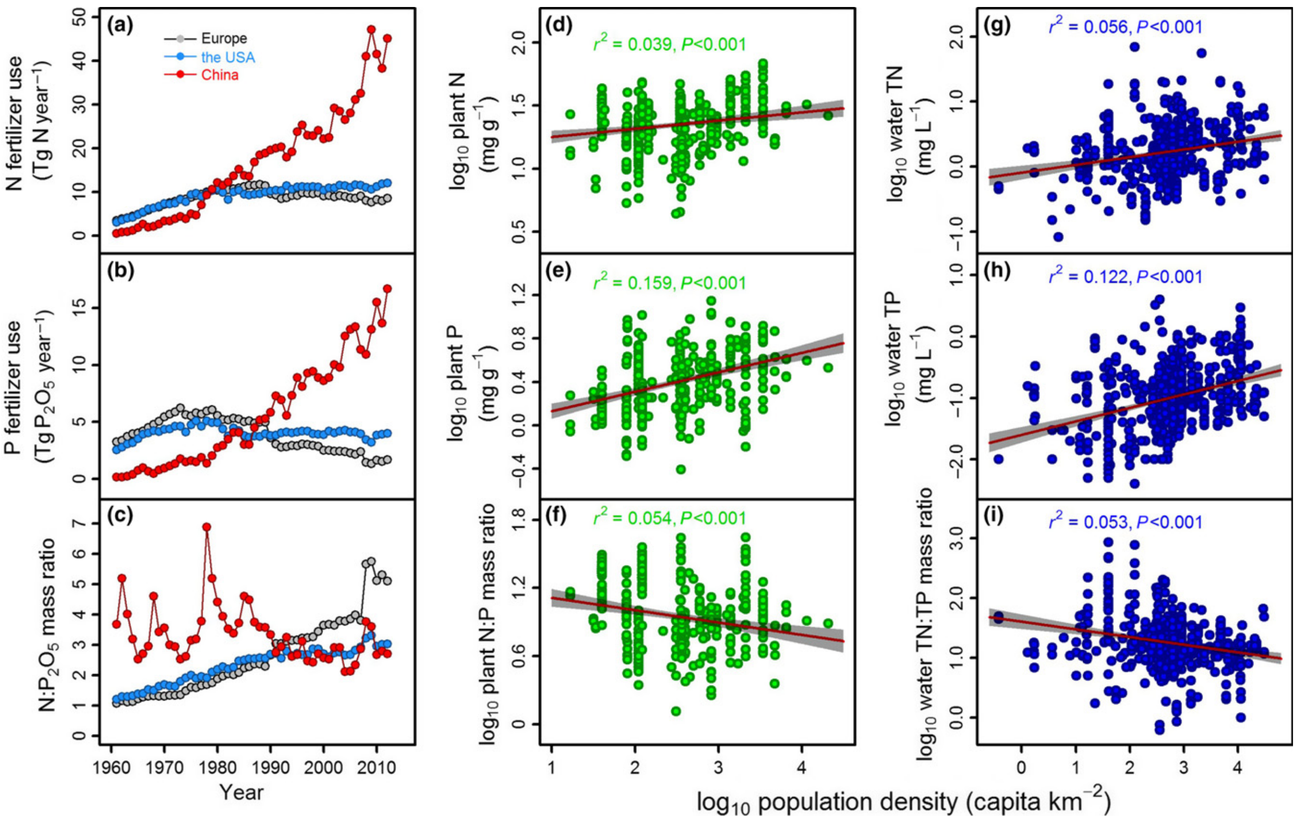

Figure 4 (a-c) N and P fertiliser use among regions (i.e. Europe, the USA and China) during several decades, and **(d-i)** relationships between freshwater nutrients and population density in China. (a) N fertiliser use, (b) P fertiliser use and (c) N : P_2O_5 mass ratios; (d) & (e) & (f) macrophyte plant nutrients *vs.* population density; (g) & (h) & (i) water nutrients *vs.* population density. Fertiliser data were from a statistical database available from FAO (see http://faostat.fao.org/; last accessed on April 30, 2015). Europe consisted of Finland, France, Germany, Hungary, Italy, Netherlands, Poland, Spain, Sweden, Switzerland and England. Significant ordinary least squares (OLS) regression lines ($P < 0.05$) were fit to the data. Shaded area indicates 95% confidence interval of the regression line.

Euro-American regions (Jeppesen *et al.* 2005; Gerdeaux *et al.* 2006). In some areas, water nitrate concentration in freshwater ecosystems may still remain constant despite reducing N inputs due to the diffuse nature of nitrogenous sources, the storage capacity of nitrate in the aquifers and the decreased denitrification induced by management-driven reductions in water P availability (Sutton *et al.* 2011; Finlay *et al.* 2013). However, our results indicated that water quality might be less severely impacted due to recent environmental controls, resulting in the increase of N : P ratios in freshwater ecosystems in most Euro-American regions.

The stoichiometric imbalance of N vs. P (decreasing N : P ratio) caused by eutrophication in freshwater ecosystems is further supported by long-term water nutrient monitoring data for three Chinese lakes (Lake Taihu, Lake Dianchi (Waihai) and Lake Bosten) and one American lake (Lake Okeechobee) with continuously aggravated eutrophication due to anthropogenic nutrient discharges (Fig. 5). The water N and P concentrations in these four lakes (except water N in Lake Okeechobee) increased with year over the survey periods, but the increasing temporal trends of P concentrations (log-transformed, ranging *c.* 0.012–0.061 mg P L^{-1} $year^{-1}$) were higher than those of N concentrations (log-transformed, ranging *c.* − 0.003–0.023 mg N L^{-1} $year^{-1}$), resulting in the declines in water N : P ratios (Fig. 5).

The influence of N deposition on nutrient cycles in freshwater ecosystems in most regions is likely small relative to other anthropogenic nutrient inputs under intensified human activity. Indeed, our results showed that N deposition was negatively correlated with N : P ratios in freshwater and its macrophytes (Fig. S10), contrary to previously reported increases in N : P ratios (Bergstrom *et al.* 2005; Elser *et al.* 2009). These previous studies chose natural ecosystems along gradients of N deposition without or far from direct anthropogenic nutrient inputs and then explored the impacts of N deposition on nutrient cycles. In our study, however, the rate of N deposition was strongly associated with other anthropogenic factors and thus the independent role of N deposition was difficult to detect (Table S10). Our stepwise multiple regression of macrophyte and water nutrients against ten anthropogenic variables indicated that N deposition explained a lower proportion of the variability in ecosystem N : P stoichiometry than the other variables (Table S9). Some case studies of the N budgets support the low contribution of N deposition to total nitrogen inputs to freshwater ecosystems under anthropogenic impacts (Cook *et al.* 2010; Cui *et al.* 2013; Grantz *et al.* 2014).

Contrary to previous reports that most of the variability in N : P stoichiometry in terrestrial plants can be explained by species composition and climate (Reich & Oleksyn 2004; Han *et al.* 2005, 2011; Chen *et al.* 2013), freshwater plants can be

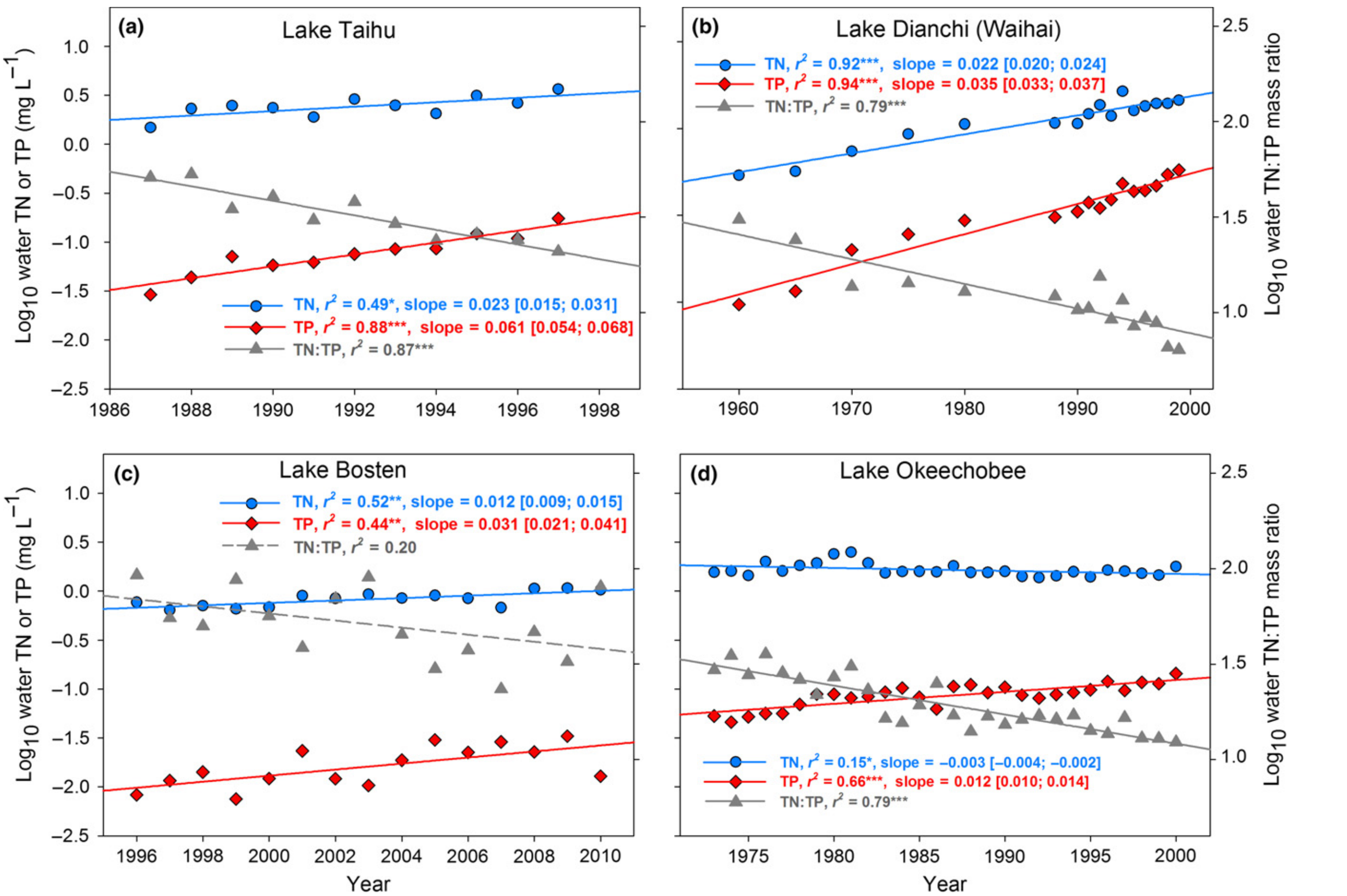

Figure 5 Temporal patterns of yearly average water TN and TP concentrations and TN:TP ratios in three Chinese Lakes (Lake Taihu, Lake Dianchi (Waihai) and Lake Bosten) and one American Lake (Lake Okeechobee) with continuously aggravated eutrophication due to anthropogenic nutrient discharge. Significant ordinary least squares (OLS) regression lines were fit to the data. Numbers in square brackets are the lower and upper 95% confident intervals of the slopes. Statistical significance is indicated by *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. One dashed line denotes the relationship with $P > 0.05$. The data were derived from previous publications (Qin 2002; Havens *et al.* 2003; Huang *et al.* 2006; Chen 2012; Li *et al.* 2014; Xie *et al.* 2011). Note that nutrient data after 1997 for Lake Taihu, and after 2000 for Dianchi were excluded from our analyses due to the reductions in nutrient loadings via watershed management (Chen 2012; Li *et al.* 2014).

highly susceptible to changes in the characteristics of surrounding environments induced by anthropogenic activities (Lacoul & Freedman 2006; Demars & Edwards 2007; Xing *et al.* 2013). The true roles of functional group and climate in N : P stoichiometry in freshwater plants may thus be obscured by excess nutrient availabilities from surrounding environments under human impacts.

It is worth mentioning the limitations of the present study. First, the considerable site-related variance indicates that large amount of variation is not well captured by the dichotomous human-impact level that we used. Second, much of the variance in N and P stoichiometry in freshwater ecosystems remains unexplained, which may be due to impacts of various sources, such as irradiance, water depth, salinity, pH and flow velocity (Grimm *et al.* 2003; Lacoul & Freedman 2006; Bornette & Puijalon 2011). Third, the multi-collinearities among the various anthropogenic variables (Table S10) hinder the detection of the independent role of each factor (e.g. agricultural land use) in shaping the observed pattern. Fourth, less information is available about the degree to which the important trend toward decreasing N : P with increasing human impact can be driven by the stoichiometry of cumulative nutrient inputs relative to the effect of biogeochemical processes that occur in freshwater under highly eutrophic conditions. Much more detailed regional scale surveys and experimental studies will be required to quantitatively assess and minimise these uncertainties.

CONCLUSION

We provide data that eutrophication increases the imbalance between N and P cycling of global freshwater ecosystems, as manifested in decreasing N : P ratio. Anthropogenic impacts on biogeochemical fluxes have altered N and P stoichiometries of freshwater and its macrophytes at the large scale, due to a faster accumulation of P than N in ecosystems. Similar patterns are apparent in multiple comparisons of N and P stoichiometries in freshwater ecosystems across regions and periods. These findings are in contrast with the N : P increase reported for terrestrial, coastal, and some local freshwater ecosystems caused by human-induced changes (Elser *et al.* 2009; Peñuelas *et al.* 2012, 2013; Sardans *et al.* 2012).

Our findings indicate that anthropogenic eutrophication might thus shift aquatic ecosystems from a state of predominant P limitation to being potentially limited or co-limited by N, or by other factors such as light, especially in rapidly developing regions such as China. Continued anthropogenic amplification of the stoichiometric imbalance of global N and P cycles will have further ecological ramifications for ecosystem dynamics, community structure and biological diversity in freshwater ecosystems (Sterner & Elser 2002). These disturbances threaten to involve more and more freshwater systems and highlight the importance of continued focus on improving nutrient management in the coming decades. In addition, shifts in N : P ratios due to global eutrophication in freshwater ecosystems induced by anthropogenic activities may presumably have major impacts on trophic webs and biogeochemical cycles of estuaries and coastal areas by freshwater loading, highlighting the importance of ameliorating nutrient intensification in catchments. Our findings can also help to better parameterise complex N and P biogeochemical models that should be developed for projecting various scenarios of global changes in nutrient cycling (Elser *et al*. 2007; Kroeze *et al*. 2012; Peñuelas *et al*. 2013).

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AUTHORSHIP

W.H. and J.F. designed the research, Z.Y. and W.H. performed the research and analysed the data, and Z.Y., W.H. J.P., J.S., J.E., E.D., P.R. and J.F. wrote the paper.

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