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

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Abstract

Freshwater ecosystems are being intensely impacted by anthropogenic inputs of nutrients. The effects of anthropogenic eutrophication on nitrogen (N) and phosphorus (P) stoichiometries in global freshwater ecosystems, however, are unclear. 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 trophic gradients. N and P concentrations were positively correlated and the N:P ratio was negatively correlated with population density in freshwater ecosystems in China. The level of human impact explained much more of the variation in N and P stoichiometries than functional group or climate. Our findings provide empirical support for a faster accumulation of P than N in human-impacted freshwater ecosystems, which should be taken into account in projected scenarios of global nutrient changes.

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 (Elser *et al.* 2009; Vitousek *et al.* 2010), whereas anthropogenic eutrophication in addition to N deposition, may largely affect specific ecosystems (Arbuckle & Downing 2001; Smith & Schindler 2009). For example, freshwater ecosystems, as nutrient sinks, receive P leached from land and anthropogenic P discharges (e.g. excess P-fertilizer 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 *et al.* 1977; Elser *et al.* 2007) may thus be alleviated by these direct P inputs, potentially increasing the risk of water eutrophication. Freshwater ecosystems also receive N inputs, but polluted waterbodies often receive nutrient inputs mainly sewage discharge and excess fertilizers from agricultural lands, with low N:P ratios (Downing & McCauley 1992; Carpenter *et al.* 1998; Arbuckle & Downing 2001). N:P ratios in river and stream loadings in agricultural areas, however, can also tend to increase (Peñuelas *et al.* 2012; Sardans *et al.* 2012), because N is quite mobile, whereas P tends to remain and accumulate in the soil. In contrast, aquatic N:P ratios in some intensely pastured agricultural areas fertilized with animal slurry tend to decrease, because the N:P ratios of animal slurries are low (Peñuelas *et al.* 2012; Sardans *et al.* 2012).

Whatever N:P was in the original external loadings, low oxygen or anaerobic conditions could increase N depletion via denitrification and P enrichment from sediments (i.e. internal P loadings) in heavily impacted waterbodies due to redox reactions or equilibrium P buffering mechanisms, thereby decreasing the water N:P ratios (Saunders & Kalff, 2001; Søndergaard *et al.* 2003; Niemisto *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 the water N:P ratios (Xie *et al.* 2003). Sediment resuspension caused by wind perturbation and water currents decreases the N:P ratios in the overlying water column, because of the lower N:P ratios in the sediments (Søndergaard *et al.* 2003; Niemisto *et al.* 2008; Grantz *et al.* 2014). Interestingly, a recent study showed that denitrification has been stimulated in many lakes by the increased P inputs from human activity (Finlay *et al.* 2013), leading to a higher rate of N removal. N fixation in eutrophic waterbodies may be slightly limited due to self-shading by phytoplankton (Vitousek & Howarth 1991). Moreover, P usually tends to 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). Variations in the rate of nutrient accumulation (i.e. legacies) caused by the synthetic effects of cumulative inputs and removal processes could thus lead to the *in situ* modification of N:P in freshwater ecosystems under anthropogenic impacts.

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). Elemental compositions of aquatic plants often integrate the chemical, biological and spatiotemporal characteristics of their surrounding environments and can reflect differences in the nutrient conditions among 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 the lower solubility of P than N and the ubiquitous N-fixation by autotrophic and heterotrophic bacteria (Schindler *et al.* 1977; Howarth *et al.* 1988), may exert long-term selective pressure on freshwater plants. Plants with the capacity to take up and retain P much more than N can thus be favored in these ecosystems (Güsewell & Koerselman 2002). The hypothesis of the stability of limiting elements (Han *et al.* 2011) states that plant P should be more easily altered by environmental changes than plant N, reflecting their distinguishable homeostatic controls (Güsewell & Koerselman 2002**;** Sterner & Elser *et al.* 2002). A faster accumulation of P than N could then be expected in freshwater macrophytes under the 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 fertilizer use and untreated sewage discharges have increased dramatically with the higher crop demand, urbanization and industrialization (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 fertilizer use and in recent decades have 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 improved in Euro-America in contrast with China, resulting in differences in the N:P stoichiometry of the ecosystems. The recent alleviation of water pollution in Euro-America may also lead to temporal changes in N:P stoichiometry in freshwater ecosystems.

The global patterns of the N and P stoichiometries of freshwater and its macrophytes, and their relationships with large-scale anthropogenic pollution, remain largely unknown. Given the aforementioned concurrent influences of nutrient sources and divergent mechanisms of nutrient cycling, evolutionarily acquired higher efficiency of P use and the existing spatiotemporal disparities, we hypothesize that freshwater ecosystems under anthropogenic impacts may accumulate P at disproportionally higher rates relative to N over long time scales, leading to lower N:P ratios in plants and waterbodies. These shifts in stoichiometric characteristics of N and P in the ecosystems may occur across spatial and temporal scales. We tested the above hypothesis by synthesizing two N and P data sets for global bodies of freshwater and their macrophytes and evaluating the N:P stoichiometries across human-impact levels, regions and periods.

MATERIAL AND METHODS

Data compilation

We compiled data from 157 publications and our field samplings of N and P concentrations and N:P ratios in 433 species of freshwater macrophytes, with 1234 observations from 332 sites worldwide (Fig. S1a; Data source 1). For each site, we recorded geographic location (latitude/longitude), climatic variables (mean annual temperature, MAT, ^{o}C; mean annual precipitation, MAP, mm), type of freshwater ecosystem (heavily vs lightly impacted by humans; see below), sampling period, family/species/functional group of macrophytes and the N and P concentrations and N:P ratios of the macrophytes. We used the coordinates of the geographic centers 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 from 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 fertilizer, sewage effluents and intense pastoral activities and manure use) and defined “lightly human-impacted” sites as areas with oligotrophic waterbodies or that without direct human disturbances (e.g. natural preserves and mountain areas and river valley far from human impacts). Based on the descriptions of environmental conditions from the synthesized 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 nutrient sources.

The macrophytes (*stricto sensu*, 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 the 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 their 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 the waterborne chemistry data associated with the sampling sites of the macrophytes, so we compared the consistency of the patterns of total N (TN) and total P (TP) stoichiometries of the water with macrophytic 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 for which 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 (Data source 2). Site-related information, including latitude, longitude, climatic variables (MAT and MAP), sampling period and type of freshwater ecosystem (heavily vs lightly impacted by humans), was also recorded.

To explore the direct impacts of human activity on N:P stoichiometry in freshwater ecosystems, we extracted the data of environmental factors 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 environmental variables, including population density, gross production, sewage discharge, total N discharge in sewage, total P discharge in sewage, percentage of crop area, N fertilizer use, P fertilizer use, meat production and N deposition. These data, except for N deposition, were documented in the *China Statistical Yearbook* (1991-2014). The data for N deposition were derived from Lü & Tian (2007). We used meat production to estimate the impact of manure use.

We adopted three criteria to assure greater 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 (Fernandez-Alaez *et al.* 1999; Demars *et al.* 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 and environmental factors 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 analyzed the overall statistical characteristics and variations in N and P concentrations and N:P ratios in freshwater macrophytes across different functional groups at the species level, because the influence of species identity is emphasized (Han *et al.* 2005). Reduced major axis (RMA) regression was used to characterize 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 and TP concentrations and TN:TP ratios) at the individual level (i.e. all raw data were pooled), because plant N and P concentrations from each sample are pairwise 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 (e.g. averaged within the same site) for water nutrients, because the influences of site environment and species identity were both highlighted (Reich & Olekysn 2004; Han *et al.* 2005 & 2011, Chen *et al.* 2013). We explored the effects of human-induced 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; trophic levels determined by the OECD classification, 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 the level of human impact, functional group and climate on plant N and P stoichiometry. The level of human impact, functional group and climate were treated as fixed factors, and 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 highly overlapped each other. If more than one functional group was significant, the 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 the level of human impact 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 synthetic anthropogenic influences), N deposition and latitude. Given the significant multi-collinearities among the ten environmental factors, 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 N:P 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), indicating that P concentrations increased more rapidly than N concentrations in the freshwater ecosystems.

Human impacts significantly ($p<0.05$) increased N and P concentrations in the macrophytes and water but decreased their N:P ratios (Fig. 2a and 2c), indicating a faster accumulation of P than N in the freshwater ecosystems. 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 in heavily than lightly human-impacted environments but a lower TN:TP mass ratio (15.9 *vs.* 46.9), respectively (Fig. 2c). The stoichiometric comparisons of different levels of anthropogenic impact in China and Euro-America had the same pattern (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 the plants and water compared to those in lightly impacted areas (Fig. S4). Areas dominated by the impact of sewage, however, had higher N and P concentrations but similar N:P ratios in the plants and water compared to areas dominated by agricultural impacts (Fig. S4).

Human-induced eutrophication had impacts on macrophytic and water N and P stoichiometries across the regions and periods (Fig. 2b, d; Fig. S5). Macrophytic 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. 32). 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 lower in China than Euro-America, respectively (Fig. 2d). Euro-American macrophytic 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 level of human impact explained much more of the variation in macrophytic N and P stoichiometry than functional group or climate (Table 1), despite the divergent influences of the functional groups (Tables S3 and S4, see detailed assessments in the supplementary information). The level of human impact, functional group and site, as predictors, explained 10.2, 4.2 and 46.8% of the variance in plant N, respectively (Table 1). The level of human impact, functional group, MAP and site, as predictors, explained 21.8, 6.2, 0.9 and 38% of the variance in plant P, respectively (Table 1). The level of human impact, 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, respectively (Table 1). The comparisons of macrophytic N and P stoichiometry for specific functional groups across levels of human impact, 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. The GLM analyses thus found that the level of human impact explained much more of the variation in water TN and TP stoichiometry than climate (Table S5).

Water Chl a concentration was correlated positively with water TN and TP concentrations and negatively with water TN:TP ratio (Fig 3). Quantitative assessments of the anthropogenic impacts based on the thresholds of TN, TP and Chl a concentrations indicated that the increased trophic levels would increase water TN and TP concentrations but decrease the TN:TP ratio in freshwater ecosystems. N and P stoichiometry in freshwater and its macrophytes varied slightly with latitude (Fig. S8, see detailed assessment in the supplementary information). Tissue P concentrations in freshwater macrophytes decreased and the N:P ratio increased with absolute latitude, while the latitudinal pattern of N concentrations was not statistically significant (p=0.828; Fig. S8). TN and TP concentrations also decreased in freshwater bodies, and TN:TP increased with increasing absolute latitude (Fig. S8).

DISCUSSION

Our results show an obviously 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, Fernandez-Alaez *et al.* 1999; 2.3 mg g^{-1} and 13.6, Demars & Edwards 2007). Conversely, the P concentration and N:P ratio (2.93 mg g^{-1} and 9.9, respectively) 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 in 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). The average N:P ratios in these anthropogenic nutrient sources were considerably lower than those in natural undisturbed watersheds, lightly impacted lakes and global lakes (Table S6). These comparisons provide further evidence that human impacts have changed the N:P stoichiometry of freshwater macrophytes due to faster 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 application of N and P fertilizers (222, 64 and 98 kg N ha^{-1} y^{-1}; 80, 23 and 31 kg P_2O_5 ha^{-1} y^{-1} for China, America and Europe, respectively) and untreated municipal sewage (12.32, 6.39 and 0.85 t ha^{-1} y^{-1} for China, America and Europe, respectively) are higher in China than Euro-America (Fig 4; Table S3). 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 consumption of fertilizers in China, however, is still increasing, with a decrease in the ratio of N to P fertilizers (Figs. 5 and S9; see more assessments in the supplementary information), and discharges of untreated sewage are also increasing because of China's continuous economic growth, urbanization, industrialization and the demand of ensuring food security (Jin *et al.* 2005; Liu *et al.* 2016; Powers *et al.* 2016), which may unfortunately aggravate the situation.

Our results showed that macrophytic and water N and P concentrations were positively correlated and N:P ratios in China were negatively correlated with the corresponding population density (Fig. 4), indicating synthetic 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 S8). In contrast, these variables jointly explained 11, 21.8 and 16.4% of the total variances for water TN concentration, TP concentration and TN:TP ratio in China, respectively (Table S8). Considerable variance, however, remained unexplained, which may be attributed to the unmatched spatial scales between the nutrient and anthropogenic variables and to 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 Euro-American regions. Intriguingly, the year around 1990 as a break-point via the temporal analysis had also been reported by previous studies about the long-term records of N concentrations in mosses in Europe (Harmens *et al.* 2015), and the temporal trends of N and P fertilizer use in Europe and America (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 diffuse nutrient pollution generated from agriculture and point pollution originated by 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 in detergents, reductions in fertilizer 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 showed that N loading has also generally declined or stabilized in Europe and 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). Indeed, policy effectiveness and implementation in reducing N loads differed regionally among the European countries. In this study, European sites mostly consisted of western and northern Europe with better sewage input controls and watershed management than those countries located at the eastern and southern Europe (Sutton *et al.* 2011). Therefore, our results indicated that water quality might be improved in Euro-America due to recent environmental controls, resulting in the increase of N:P ratios in freshwater ecosystems (Finlay *et al.* 2013; Dove & Chapra, 2015).

The N:P stoichiometric imbalance caused by the trophic levels in the freshwater ecosystems was further supported by the 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 trophic levels due to anthropogenic nutrient discharges (Fig. 5). The water N and P concentrations in these four lakes (except the water N concentration in Lake Okeechobee) increased with year over the survey periods, but the rates of P accumulation (log-transformed, c. 0.012-0.061 mg P L^{-1} y^{-1}) were higher than those of N accumulation (log-transformed, c. -0.003-0.023 mg N L^{-1} y^{-1}), resulting in lower water N:P ratios (Fig. 5).

The influence of N deposition on nutrient cycles in freshwater ecosystems is small relative to other anthropogenic nutrient inputs under intensified human activity. Our results showed that N deposition was negatively correlated with the N:P ratios in freshwater and its macrophytes (Fig. S10), contrary to the increasing N:P ratios reported by Bergstrom *et al.* (2005) and Elser *et al.* (2009). 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 freshwater ecosystems. In our study, however, the rate of N deposition was strongly associated with other anthropogenic factors, and the independent role of N deposition was hard to detect (Table S9). Our stepwise multiple regression of macrophytic and water nutrients against ten anthropogenic variables indicated that N deposition explained a lower proportion of the variability in N:P stoichiometry in freshwater ecosystems than the other variables (Table S9). Some case studies of the N budget support the lower contribution of N deposition to the total N inputs to freshwater ecosystems under anthropogenic impacts (Cook *et al*. 2010; Cui *et al*. 2013; Grantz *et al*. 2014).

Contrary to previous proposals that most of the variability in N:P stoichiometry in terrestrial plants could be explained by species composition and climate (Reich & Oleksyn 2004; Han et al. 2005 & 2011; Chen et al. 2013), freshwater plants can be highly susceptible to changes in the characteristics of the surrounding environment induced by anthropogenic activity (Lacoul & Freedman 2006; Demars & Edwards 2007; Xing et al. 2013). The true roles of functional group and climate could thus be obscured by excess nutrient availabilities from the surrounding environment under human impacts. This study, however, has some limitations. First, the considerable site-related variances indicated that large variances were not well captured by the dichotomous levels of human impact, which may require more detailed and quantitative assessments. Second, much of the variance in N and P stoichiometry in freshwater ecosystems remains unexplained, which may be due to various sources, such as unquantified micro-environments, 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 S9) hinders the detection of the independent role of each factor (e.g. agricultural land use) in shaping the observed pattern. Fourth, little is known about the relative contributions of external and internal nutrient loadings in determining the patterns of nutrient stoichiometry in freshwater ecosystems. A much more detailed regional-scale survey and experimental sampling will be required to quantitatively assess and minimize these uncertainties.

CONCLUSION

We provide data that eutrophication increases the imbalance of N and P cycling (in the sense of decreasing N:P ratios) in global freshwater ecosystems. Our study reveals that human impacts on biogeochemical fluxes have altered N and P stoichiometries in freshwater macrophytes and water at a global scale, due to a faster accumulation of P than N in ecosystems. This pattern was apparent in multiple comparisons of N and P stoichiometries in freshwater ecosystems across human-impact levels, regions and periods, which is in contrast with the general N:P increase in terrestrial, coastal and some local freshwater ecosystems caused by human-induced changes (Peñuelas *et al.* 2012; Sardans *et al.* 2012; Peñuelas *et al.* 2013; Yuan & Chen *et al.* 2015).

Anthropogenic water pollution may thus shift aquatic ecosystems from a state of predominant P limitation to being potentially limited by N or 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 large ecological ramifications for biogeochemical cycling and biological diversity in freshwater ecosystems (Sterner & Elser, 2002). This disturbance thus threatens to involve more and more freshwater systems and can have large implications to the necessary continued focus on P abatement in efforts of ecosystemic conservation and management in the coming decades. Global eutrophication in freshwater ecosystems induced by anthropogenic activity may presumably have potentially large impacts on the trophic webs and biogeochemical cycles of estuaries and coastal areas by freshwater loading and highlight the importance of rehabilitating these ecosystems. Our findings can also help to better parameterize complex N and P biogeochemical models that should be developed for projecting various scenarios of global change (Elser *et al.* 2007; Kroeze *et al.* 2012; Peñuelas *et al.* 2013).

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SUPPORTING INFORMATION

Additional Supporting Information is available in the online version of the paper.

Supplementary discussion

Table S1 Summary of reduced major axis regression results between P and N of freshwater macrophytes for all raw data pooled.

Table S2 Summary of reduced major axis regression results between TP and TN of freshwater for all raw data pooled.

Table S3 Tissue N and P concentrations, and N:P mass ratio in freshwater macrophytes among different functional group.

Table S4 General linear models for plant N and P concentrations, and N:P mass ratio in freshwater ecosystems.

Table S5 Summary of general linear models for water TN and TP concentrations, and TN:TP mass ratio in freshwater ecosystems.

Table S6 Comparisons of average N:P mass ratios in lakes and potential nutrient sources to surface water.

Table S7 Fertilizer use and wastewater discharge among the three regions.

Table S8 Model summary for the stepwise multiple regression of macrophytic and water nutrients on ten anthropogenic variables.

Table S9 Correlations among anthropogenic variables involved in this study.

Figure S1 Spatial and frequency distribution of tissue N and P in freshwater macrophytes.

Figure S2 Spatial and frequency distribution of water TN and TP in freshwater ecosystems.

Figure S3 N and P concentrations and N:P mass ratios in freshwater ecosystems across human-impact levels (lightly / heavily) in each region (Euro-America / China after 1990).

Figure S4 N and P concentrations and N:P mass ratios in freshwater ecosystems across human-impact levels (lightly/agricultural dominated/sewage dominated/both impacted).

Figure S5 N and P concentrations and N:P mass ratios in freshwater ecosystems across periods for Euro-America.

Figure S6 N and P concentrations and N:P mass ratios in freshwater macrophytes across human-impact levels for the specific functional group.

Figure S7 N and P concentrations and N:P mass ratios in freshwater macrophytes across regions and periods for the specific functional group.

Figure S8 N and P concentrations and N:P mass ratios in freshwater ecosystems along the latitudinal patterns.

Figure S9 Temporal trends of N and P fertilizer use in China during several decades.

Figure S10 (a) & (b) Relationship between freshwater N:P mass ratio (plant N:P or water TN:TP) and nitrogen deposition.

Appendix Data sources

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Table 1 Summary of general linear models for the 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

Note: F values in bold denote p<0.05. 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; AP, 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 gave the values calculated from the final model here.

FIGURE LEGENDS

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. Numbers in square brackets are the lower and upper 95% confident intervals of the RMA slopes.

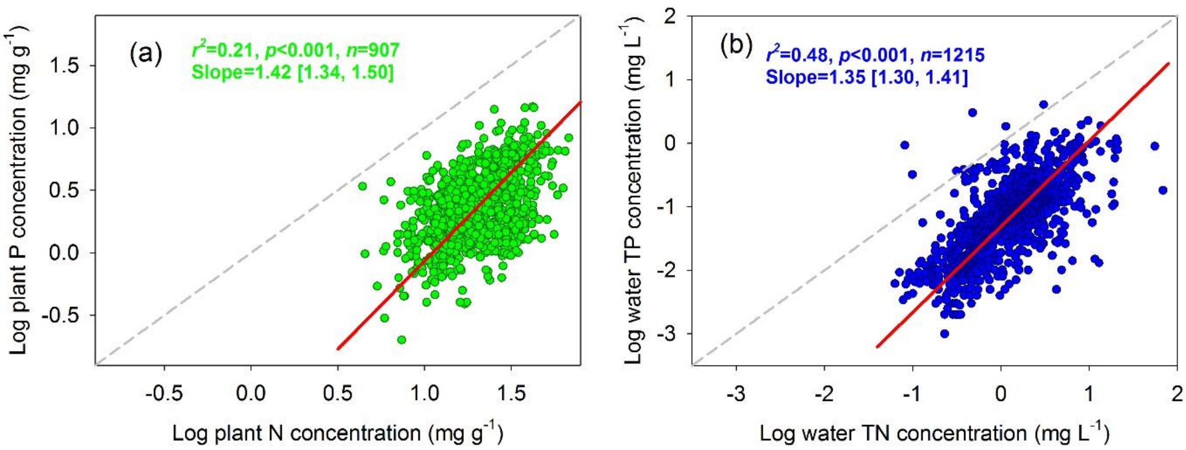
Figure 2 N and P concentrations and N:P mass ratios in freshwater ecosystems across human-impacted levels (lightly / heavily), regions (Euro-America / China after 1990) and periods (before/after 1990 in Euro-America). (a) & (b) Macrophytic N and P concentrations and N:P mass ratios; (c) & (d) water TN 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.

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 trophic 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.

Figure 4 (a-c) N and P fertilizer use among regions (i.e. Europe, the USA and China) during several decades, and **(d-i)** relationship between freshwater nutrients and population density in China. (a) N fertilizer use, (b) P fertilizer use and (c) N:P_2O_5 mass ratios; (d) & (e) & (f) macrophytic plant nutrients vs population density; (g) & (h) & (i) water nutrients vs population density. Fertilizer 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) are fit to the data. Shaded area indicate 95% confidence interval of the regression line.

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 trophic levels due to anthropogenic nutrient discharges. Significant ordinary least squares (OLS) regression lines are fit to the data. Statistical significance is indicated by *, p<0.05; **, p<0.01; ***, p<0.001. The data were derived from previous publications (Qing 2002; Havens *et al.* 2003; Huang *et al.* 2006; Chen 2012; Li *et al.* 2014; Xie *et al.* 2014). 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 by watershed management (Chen 2012; Li *et al.* 2014).**Figure 1**

Figure 2

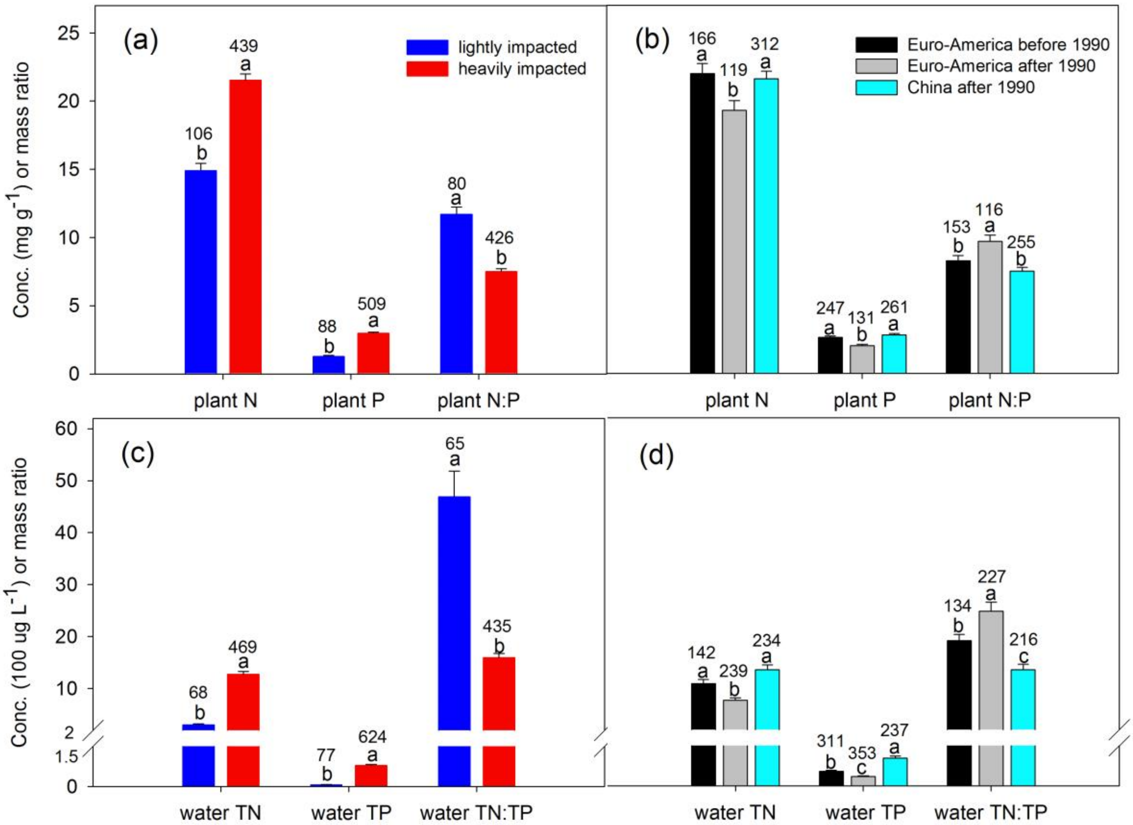

(a)
lightly impacted
heavily impacted
Conc. (mg g-1) or mass ratio
106
b
439
a
88
b
509
a
80
a
426
b
plant N
plant P
plant N:P
(b)
Euro-America before 1990
Euro-America after 1990
China after 1990
166
a
119
b
312
a
247
a
131
b
261
a
153
b
116
a
255
b
(c)
Conc. (100 ug L-1) or mass ratio
68
b
469
a
77
b
624
a
65
a
435
b
water TN
water TP
water TN:TP
(d)
142
a
239
b
234
a
311
b
353
c
237
a
134
b
227
a
216
c

Figure 3

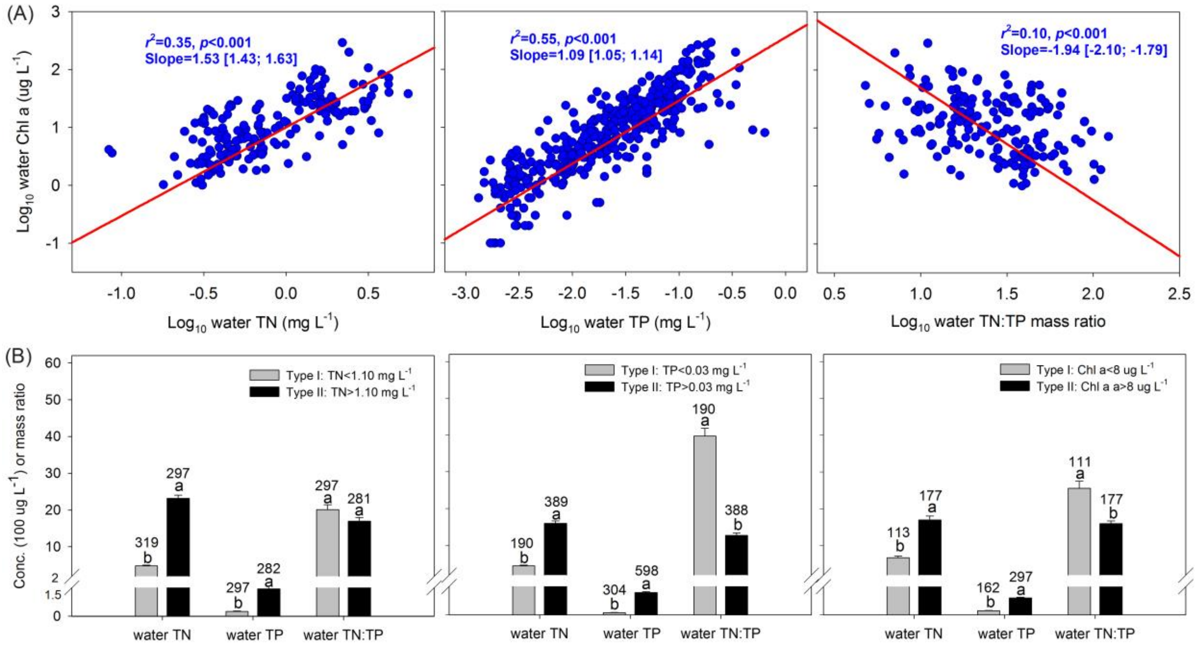

(A)
r²=0.35, p<0.001
Slope=1.53 [1.43; 1.63]
r²=0.55, p<0.001
Slope=1.09 [1.05; 1.14]
r²=0.10, p<0.001
Slope=-1.94 [-2.10; -1.79]
Log10 water Chl a (ug L-1)
Log10 water TN (mg L-1)
Log10 water TP (mg L-1)
Log10 water TN:TP mass ratio
(B)
Conc. (100 ug L-1) or mass ratio
Type I: TN<1.10 mg L-1
Type II: TN>1.10 mg L-1
Type I: TP<0.03 mg L-1
Type II: TP>0.03 mg L-1
Type I: Chl a<8 ug L-1
Type II: Chl a a>8 ug L-1
water TN
water TP
water TN:TP

Figure 5