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

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33 W.H. and J.F. designed the research, Z.Y. and W.H. performed the research and
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42 **Abstract**

43 Freshwater ecosystems are being intensely impacted by anthropogenic inputs of nutrients. The
44 effects of anthropogenic eutrophication on nitrogen (N) and phosphorus (P) stoichiometries in
45 global freshwater ecosystems, however, are unclear. Here we evaluated the characteristics of
46 N and P stoichiometries in bodies of freshwater and their herbaceous macrophytes across
47 human-impact levels, regions and periods. Freshwater and its macrophytes had higher N and P
48 concentrations and lower N:P ratios in heavily than lightly human-impacted environments,
49 further evidenced by spatiotemporal comparisons across trophic gradients. N and P
50 concentrations were positively correlated and the N:P ratio was negatively correlated with
51 population density in freshwater ecosystems in China. The level of human impact explained
52 much more of the variation in N and P stoichiometries than functional group or climate. Our
53 findings provide empirical support for a faster accumulation of P than N in human-impacted
54 freshwater ecosystems, which should be taken into account in projected scenarios of global
55 nutrient changes.

56 INTRODUCTION

57 Human activities have drastically accelerated Earth's major biogeochemical cycles, altering the
58 balance of biogeochemical nitrogen (N) and phosphorus (P) cycles (Falkowski et al. 2000;
59 Galloway *et al.* 2008; Vitousek *et al.* 2010; Elser & Bennett 2011; Liu *et al.* 2013; Peñuelas *et*
60 *al.* 2013). Recent studies indicate that enhanced N deposition increases the limitation of P or
61 other nutrients in many ecosystems (Elser *et al.* 2009; Vitousek *et al.* 2010), whereas
62 anthropogenic eutrophication in addition to N deposition, may largely affect specific
63 ecosystems (Arbuckle & Downing 2001; Smith & Schindler 2009). For example, freshwater
64 ecosystems, as nutrient sinks, receive P leached from land and anthropogenic P discharges (e.g.
65 excess P-fertilizer use, P-containing pesticides, P-containing detergents and domestic and
66 industrial sewage) in surface runoff (Carpenter *et al.* 1998; Arbuckle & Downing 2001; Smith
67 & Schindler 2009). The prevailing P limitation in freshwater ecosystems (Schindler *et al.* 1977;
68 Elser *et al.* 2007) may thus be alleviated by these direct P inputs, potentially increasing the risk
69 of water eutrophication. Freshwater ecosystems also receive N inputs, but polluted waterbodies
70 often receive nutrient inputs mainly sewage discharge and excess fertilizers from agricultural
71 lands, with low N:P ratios (Downing & McCauley 1992; Carpenter *et al.* 1998; Arbuckle &
72 Downing 2001). N:P ratios in river and stream loadings in agricultural areas, however, can also
73 tend to increase (Peñuelas *et al.* 2012; Sardans *et al.* 2012), because N is quite mobile, whereas
74 P tends to remain and accumulate in the soil. In contrast, aquatic N:P ratios in some intensely
75 pastured agricultural areas fertilized with animal slurry tend to decrease, because the N:P ratios
76 of animal slurries are low (Peñuelas *et al.* 2012; Sardans *et al.* 2012).

77

78 Whatever N:P was in the original external loadings, low oxygen or anaerobic conditions could
79 increase N depletion via denitrification and P enrichment from sediments (i.e. internal P
80 loadings) in heavily impacted waterbodies due to redox reactions or equilibrium P buffering

81 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
82 enhanced by anthropogenic impacts, such as *Microcystis* blooms, can also selectively drive P
83 (but not N) release from sediments by elevated pH and thus decrease the water N:P ratios (Xie
84 *et al.* 2003). Sediment resuspension caused by wind perturbation and water currents decreases
85 the N:P ratios in the overlying water column, because of the lower N:P ratios in the sediments
86 (Søndergaard *et al.* 2003; Niemisto *et al.* 2008; Grantz *et al.* 2014). Interestingly, a recent study
87 showed that denitrification has been stimulated in many lakes by the increased P inputs from
88 human activity (Finlay *et al.* 2013), leading to a higher rate of N removal. N fixation in
89 eutrophic waterbodies may be slightly limited due to self-shading by phytoplankton (Vitousek
90 & Howarth 1991). Moreover, P usually tends to have a longer residence time and higher
91 retention efficiency than N in freshwater ecosystems (Saunders & Kalff 2001; Jeppesen *et al.*
92 2005; Cook *et al.* 2010; Grantz *et al.* 2014). Variations in the rate of nutrient accumulation (i.e.
93 legacies) caused by the synthetic effects of cumulative inputs and removal processes could thus
94 lead to the *in situ* modification of N:P in freshwater ecosystems under anthropogenic impacts.
95
96
97 Freshwater macrophytes play vital roles in regulating the structure and function of freshwater
98 ecosystems and in restoring water quality (Lacoul & Freedman 2006; Bornette & Puijalon
99 2011). Elemental compositions of aquatic plants often integrate the chemical, biological and
100 spatiotemporal characteristics of their surrounding environments and can reflect differences in
101 the nutrient conditions among freshwater ecosystems under anthropogenic impacts (Lacoul &
102 Freedman 2006; Demars & Edwards 2007; Bornette & Puijalon 2011; Xing *et al.* 2013).
103 Oligotrophic pre-industrial freshwater ecosystems, generally considered to be P-limited
104 because of the lower solubility of P than N and the ubiquitous N-fixation by autotrophic and
105 heterotrophic bacteria (Schindler *et al.* 1977; Howarth *et al.* 1988), may exert long-term

106 selective pressure on freshwater plants. Plants with the capacity to take up and retain P much
107 more than N can thus be favored in these ecosystems (Güsewell & Koerselman 2002). The
108 hypothesis of the stability of limiting elements (Han *et al.* 2011) states that plant P should be
109 more easily altered by environmental changes than plant N, reflecting their distinguishable
110 homeostatic controls (Güsewell & Koerselman 2002; Sterner & Elser *et al.* 2002). A faster
111 accumulation of P than N could then be expected in freshwater macrophytes under the
112 increasing ambient nutrient inputs.

113

114 Freshwater ecosystems across regions and periods vary in their nutrient levels, depending on
115 the stages of industrial and agricultural development and on the policies of environmental
116 protection (Havens *et al.* 2001; Van Drecht *et al.* 2009; Potter *et al.* 2010; Powers *et al.* 2016).
117 For example, China is experiencing rapid economic growth without effective environmental
118 protection (Jin *et al.* 2005). Excessive fertilizer use and untreated sewage discharges have
119 increased dramatically with the higher crop demand, urbanization and industrialization (Havens
120 *et al.* 2001; Jin *et al.* 2005; Haygarth *et al.*, 2014; Liu *et al.*, 2016), resulting in significant
121 degradation of water quality in freshwater ecosystems. In contrast, most European areas and
122 the United States of America (from now on Euro-America) have largely controlled sewage
123 discharges and fertilizer use and in recent decades have increased their technical measures to
124 restore deteriorated waterbodies (Van Drecht *et al.* 2009; Potter *et al.* 2010; Haygarth *et al.*,
125 2014; Powers *et al.* 2016). The environments of freshwater ecosystems may thus be improved
126 in Euro-America in contrast with China, resulting in differences in the N:P stoichiometry of
127 the ecosystems. The recent alleviation of water pollution in Euro-America may also lead to
128 temporal changes in N:P stoichiometry in freshwater ecosystems.

129

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

141

142 **MATERIAL AND METHODS**

143 **Data compilation**

144 We compiled data from 157 publications and our field samplings of N and P concentrations
145 and N:P ratios in 433 species of freshwater macrophytes, with 1234 observations from 332 sites
146 worldwide (Fig. S1a; Data source 1). For each site, we recorded geographic location
147 (latitude/longitude), climatic variables (mean annual temperature, MAT, °C; mean annual
148 precipitation, MAP, mm), type of freshwater ecosystem (heavily vs lightly impacted by humans;
149 see below), sampling period, family/species/functional group of macrophytes and the N and P
150 concentrations and N:P ratios of the macrophytes. We used the coordinates of the geographic
151 centers of the sampling areas when specific geographic coordinates were not provided.
152 Estimates of MAT/MAP at sites without records in the original publications were extracted
153 from a global climatic data set from <http://www.worldclim.org/>. We here defined “heavily
154 human-impacted” sites as areas with eutrophic waterbodies (excluding naturally eutrophic

155 sites) or that received anthropogenic nutrient inputs (e.g. agricultural fertilizer, sewage
156 effluents and intense pastoral activities and manure use) and defined “lightly human-impacted”
157 sites as areas with oligotrophic waterbodies or that without direct human disturbances (e.g.
158 natural preserves and mountain areas and river valley far from human impacts). Based on the
159 descriptions of environmental conditions from the synthesized publications, “heavily
160 impacted” sites were further divided into three groups: sites dominated by agricultural impacts,
161 sites dominated by urban-sewage impacts and sites impacted by both agricultural and urban
162 nutrient sources.

163
164 The macrophytes (*stricto sensu*, herbaceous macrophytes) in this study consisted of aquatic
165 herbs, with mosses, macroalgae, freshwater woody plants and gymnosperms excluded due to
166 the paucity of data. To illustrate the effects of the functional groups on the variations in plant
167 N and P stoichiometry, we have primarily grouped all species in the data set into ferns and seed
168 plants based on phylogenetic information and classified them into emergent, floating-leaved,
169 freely floating and submerged plants based on their life forms. We also grouped all species into
170 graminoids (Cyperaceae and Gramineae families) and forbs (all others). Seed plants were
171 further divided into monocotyledons and dicotyledons.

172
173 Our data set did not contain much information about the waterborne chemistry data associated
174 with the sampling sites of the macrophytes, so we compared the consistency of the patterns of
175 total N (TN) and total P (TP) stoichiometries of the water with macrophytic N and P
176 stoichiometries at regional or larger geographic scales. We compiled and compared data on
177 water TN and TP concentrations, TN:TP ratios and chlorophyll-a (Chl a) concentrations for
178 freshwater ecosystems in Europe, the USA and China, the areas for which most N and P data
179 for freshwater macrophytes were available. The data set contained a total of 1867 records for

180 940 sites from 195 publications (Data source 2). Site-related information, including latitude,
181 longitude, climatic variables (MAT and MAP), sampling period and type of freshwater
182 ecosystem (heavily vs lightly impacted by humans), was also recorded.

183

184 To explore the direct impacts of human activity on N:P stoichiometry in freshwater ecosystems,
185 we extracted the data of environmental factors around those sampling sites in China, excluding
186 the Euro-American sites due to the paucity of coordinated environmental data. The sampling
187 sites were grouped into the corresponding county, district or province based on the published
188 description. We then extracted the corresponding environmental variables, including
189 population density, gross production, sewage discharge, total N discharge in sewage, total P
190 discharge in sewage, percentage of crop area, N fertilizer use, P fertilizer use, meat production
191 and N deposition. These data, except for N deposition, were documented in the *China*
192 *Statistical Yearbook* (1991-2014). The data for N deposition were derived from Lü & Tian
193 (2007). We used meat production to estimate the impact of manure use.

194

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

204

205 **Statistical analysis**

206 All nutrient data and environmental factors were \log_{10} -transformed before analysis to improve
207 data normality because of their highly skewed frequency distribution (Figs. S1 and S2). The
208 data for plant nutrients were averaged at the species (i.e. averaged by the same species) or site-
209 species (i.e. averaged by the same species at each site) level as described by Han *et al.* (2005).
210 We analyzed the overall statistical characteristics and variations in N and P concentrations and
211 N:P ratios in freshwater macrophytes across different functional groups at the species level,
212 because the influence of species identity is emphasized (Han *et al.* 2005). Reduced major axis
213 (RMA) regression was used to characterize the scaling relationships between plant P and N
214 concentrations, between water TP and TN concentrations and between Chl a concentrations
215 and water nutrients (i.e. water TN and TP concentrations and TN:TP ratios) at the individual
216 level (i.e. all raw data were pooled), because plant N and P concentrations from each sample
217 are pairwise and correlative (Reich *et al.* 2010).

218

219 In addition to the aforementioned analyses, we performed the following analyses at the site-
220 species level for plant nutrients and at the site level (e.g. averaged within the same site) for
221 water nutrients, because the influences of site environment and species identity were both
222 highlighted (Reich & Olekysn 2004; Han *et al.* 2005 & 2011, Chen *et al.* 2013). We explored
223 the effects of human-induced eutrophication on N and P stoichiometries in the macrophytes
224 with three comparisons: lightly vs. heavily human-impacted waterbodies (on impact level),
225 Euro-America vs. China (spatially) after 1990 (including 1990), and Euro-America along the
226 temporal gradient (temporally). We extracted data for Euro-America after 1990 to compare
227 with China because most nutrient data for China were from years after 1990. We also conducted
228 several comparisons across various groups based on other two category criteria (types of
229 anthropogenic impact; trophic levels determined by the OECD classification, Vollenweider

230 1968). Statistical differences among groups were identified by *t*-tests with Bonferroni
231 corrections.

232

233 A general linear model (GLM) was used to quantify the effects of the level of human impact,
234 functional group and climate on plant N and P stoichiometry. The level of human impact,
235 functional group and climate were treated as fixed factors, and site was denoted as a random
236 factor to explore the non-independence of plant N and P stoichiometry at a site. Only one of
237 the functional groups was included in each main-effect model, because all functional groups
238 highly overlapped each other. If more than one functional group was significant, the Akaike
239 information criterion (AIC) was used to determine the final model: the model with the lowest
240 AIC value was selected as the final model. Similarly, GLM analyses were also performed to
241 determine the relative contributions of the level of human impact and climate on water TN and
242 TP stoichiometry. Ordinary least squares (OLS) regression was used to explore the
243 relationships between nutrient data and population density (reflecting the synthetic
244 anthropogenic influences), N deposition and latitude. Given the significant multi-collinearities
245 among the ten environmental factors, stepwise multiple regressions were used to discriminate
246 among the effects of potential drivers on N and P stoichiometries in freshwater ecosystems in
247 China. All statistical analyses were conducted using R 2.15.2 (R Development Core Team,
248 2012).

249

250 **RESULTS**

251 N and P concentrations and N:P ratios in freshwater macrophytes varied widely: 6-63 mg N g⁻¹,
252 0-10 mg P g⁻¹, and 2-44 for N:P mass ratios (Fig. S1). Geometric means for N and P
253 concentrations and the N:P mass ratio for all species were 19.7 and 2.45 mg g⁻¹ and 8.5,
254 respectively; the corresponding coefficients of variation (CVs) were 44, 59 and 64% (Fig. S1).

255 Water column TN and TP concentrations and TN:TP ratios also varied widely among
256 freshwater ecosystems: 0-56 mg TN L⁻¹, 0-4 mg TP L⁻¹ and 0-237 for N:P mass ratios (Fig. S2).
257 Geometric means for water TN and TP concentrations and the TN:TP mass ratio for all
258 waterbodies were 1.03 and 0.055 mg L⁻¹ and 18.6, respectively; the corresponding CVs were
259 171, 209 and 168% (Fig. S2). Plant P and N concentrations, as well as water TP and TN
260 concentrations, were positively correlated, with scaling exponents >1 (P against N; Tables S1
261 and S2, and Fig. 1), indicating that P concentrations increased more rapidly than N
262 concentrations in the freshwater ecosystems.

263

264 Human impacts significantly ($p<0.05$) increased N and P concentrations in the macrophytes
265 and water but decreased their N:P ratios (Fig. 2a and 2c), indicating a faster accumulation of P
266 than N in the freshwater ecosystems. The macrophytes had significantly ($p<0.05$) higher N
267 (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
268 (7.5 vs. 11.7) in heavily than lightly human-impacted environments, respectively (Fig. 2a). The
269 ecosystems accordingly had significantly ($p<0.05$) higher water TN (1.27 vs. 0.30 mg L⁻¹) and
270 TP (0.079 vs. 0.007 mg L⁻¹) concentrations in heavily than lightly human-impacted
271 environments but a lower TN:TP mass ratio (15.9 vs. 46.9), respectively (Fig. 2c). The
272 stoichiometric comparisons of different levels of anthropogenic impact in China and Euro-
273 America had the same pattern (Fig. S3). Areas highly impacted by either agricultural inputs or
274 urban sewage, or both nutrient sources, had higher N and P concentrations but lower N:P ratios
275 in the plants and water compared to those in lightly impacted areas (Fig. S4). Areas dominated
276 by the impact of sewage, however, had higher N and P concentrations but similar N:P ratios in
277 the plants and water compared to areas dominated by agricultural impacts (Fig. S4).

278

279 Human-induced eutrophication had impacts on macrophytic and water N and P stoichiometries
280 across the regions and periods (Fig. 2b, d; Fig. S5). Macrophytic N (21.6 vs. 19.3 mg g⁻¹) and
281 P (2.82 vs. 2.03 mg g⁻¹) concentrations were higher but the N:P mass ratio was lower (7.5 vs.
282 9.7) in China than Euro-America, respectively, for the same period (Fig. 32). Water TN (1.33
283 vs. 0.77 mg L⁻¹) and TP (0.104 vs. 0.035 mg L⁻¹) concentrations were accordingly higher but
284 the TN:TP mass ratio (13.5 vs. 24.7) was lower in China than Euro-America, respectively (Fig.
285 2d). Euro-American macrophytic N (22.0 vs. 19.3 mg g⁻¹) and P (2.64 vs. 2.03 mg g⁻¹)
286 concentrations were higher but the N:P mass ratio (8.3 vs. 9.7) was lower before than after 1990
287 (Figs. 2b and S5), respectively. Euro-American water TN (1.08 vs. 0.77 mg L⁻¹) and TP (0.054
288 vs. 0.035 mg L⁻¹) concentrations were accordingly higher but the TN:TP mass ratio (19.1 vs.
289 24.7) was lower before than after 1990, respectively (Figs. 2d and S5).

290

291 The GLM analyses indicated that the level of human impact explained much more of the
292 variation in macrophytic N and P stoichiometry than functional group or climate (Table 1),
293 despite the divergent influences of the functional groups (Tables S3 and S4, see detailed
294 assessments in the supplementary information). The level of human impact, functional group
295 and site, as predictors, explained 10.2, 4.2 and 46.8% of the variance in plant N, respectively
296 (Table 1). The level of human impact, functional group, MAP and site, as predictors, explained
297 21.8, 6.2, 0.9 and 38% of the variance in plant P, respectively (Table 1). The level of human
298 impact, functional group, MAT, MAP and site, as predictors, explained 7.4, 0.4, 0.7, 1.4 and
299 57.9% of the variance in plant N:P, respectively (Table 1). The comparisons of macrophytic N
300 and P stoichiometry for specific functional groups across levels of human impact, regions and
301 periods had similar trends as those for the overall pooled plants (Figs. S6 and S7), further
302 supporting the modest role of functional group in shaping the global N and P stoichiometry in
303 freshwater macrophytes. The GLM analyses thus found that the level of human impact

304 explained much more of the variation in water TN and TP stoichiometry than climate (Table
305 S5).

306

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

317

318 **DISCUSSION**

319 Our results show an obviously higher P concentration and lower N:P ratio (arithmetic means
320 of 2.93 mg g⁻¹ and 9.9, respectively) in freshwater macrophytes than those previously reported
321 in freshwater angiosperms or aquatic vascular plants collected in regions with limited human
322 disturbance (e.g. 1.30 mg g⁻¹ and 11.7, Fernandez-Alaez *et al.* 1999; 2.3 mg g⁻¹ and 13.6,
323 Demars & Edwards 2007). Conversely, the P concentration and N:P ratio (2.93 mg g⁻¹ and 9.9,
324 respectively) in the macrophytes in this study were lower and higher, respectively, than those
325 from 213 sites in eastern China in highly polluted water (3.28 mg g⁻¹ and 7.7, Xia *et al.* 2014)
326 and in 24 highly eutrophic lakes along the middle and lower reaches of the Yangtze River (4.0
327 mg g⁻¹ and 4.2, Xing *et al.* 2013). The average N:P ratios in these anthropogenic nutrient
328 sources were considerably lower than those in natural undisturbed watersheds, lightly impacted

329 lakes and global lakes (Table S6). These comparisons provide further evidence that human
330 impacts have changed the N:P stoichiometry of freshwater macrophytes due to faster
331 accumulation of P than N.

332

333 The pattern of a faster accumulation of P than N in freshwater ecosystems was apparent in
334 regional comparisons. These differences probably reflect the fact that rates of application of N
335 and P fertilizers (222, 64 and 98 kg N ha⁻¹ y⁻¹; 80, 23 and 31 kg P₂O₅ ha⁻¹ y⁻¹ for China, America
336 and Europe, respectively) and untreated municipal sewage (12.32, 6.39 and 0.85 t ha⁻¹ y⁻¹ for
337 China, America and Europe, respectively) are higher in China than Euro-America (Fig 4; Table
338 S3). Residual P concentrations are much higher in cropland soils in China than in other areas
339 around the world (Vitousek *et al.*, 2009; Sattari *et al.* 2012), so we generally expect that the
340 streams in China would have an especially low N:P ratio. The consumption of fertilizers in
341 China, however, is still increasing, with a decrease in the ratio of N to P fertilizers (Figs. 5 and
342 S9; see more assessments in the supplementary information), and discharges of untreated
343 sewage are also increasing because of China's continuous economic growth, urbanization,
344 industrialization and the demand of ensuring food security (Jin *et al.* 2005; Liu *et al.* 2016;
345 Powers *et al.* 2016), which may unfortunately aggravate the situation.

346

347 Our results showed that macrophytic and water N and P concentrations were positively
348 correlated and N:P ratios in China were negatively correlated with the corresponding
349 population density (Fig. 4), indicating synthetic anthropogenic impacts on nutrient cycling in
350 freshwater ecosystems. Multiple anthropogenic variables jointly explained 38, 31 and 46% of
351 the total variances for plant N concentration, P concentration and N:P ratio in China,
352 respectively (Table S8). In contrast, these variables jointly explained 11, 21.8 and 16.4% of the
353 total variances for water TN concentration, TP concentration and TN:TP ratio in China,

354 respectively (Table S8). Considerable variance, however, remained unexplained, which may
355 be attributed to the unmatched spatial scales between the nutrient and anthropogenic variables
356 and to other potential drivers (e.g. geographic properties and hydrological processes) not
357 included in this analysis.

358

359 The pattern of a faster accumulation of P than N in freshwater ecosystems was also apparent in
360 temporal comparisons in Euro-American regions. Intriguingly, the year around 1990 as a break-
361 point via the temporal analysis had also been reported by previous studies about the long-term
362 records of N concentrations in mosses in Europe (Harmens *et al.* 2015), and the temporal trends
363 of N and P fertilizer use in Europe and America (Fig. 4; Sattari *et al.* 2012). In addition, with
364 the Nitrates Directive and the Urban Waste Water Treatment Directive, the European
365 Commission enforced several regulations to control the diffuse nutrient pollution generated
366 from agriculture and point pollution originated by urban sewage discharges at the beginning of
367 1990s (Sutton *et al.* 2011).

368

369 In Euro-American regions, many freshwater ecosystems have undergone steep decreases in P
370 availability in response to phosphate banning policies in detergents, reductions in fertilizer use,
371 sewage input controls and watershed management (Fig. 4; Jeppesen *et al.* 2005; Van Drecht *et*
372 *al.* 2009; Potter *et al.* 2010; Sattari *et al.* 2012; Finlay *et al.* 2013; Dove & Chapra 2015; Powers
373 *et al.* 2016). Previous studies have showed that N loading has also generally declined or
374 stabilized in Europe and American regions (Jeppesen *et al.* 2005; Gerdeaux *et al.* 2006). In
375 some areas, water nitrate concentration in freshwater ecosystems may still remain constant
376 despite reducing N inputs due to the diffuse nature of nitrogenous sources, the storage capacity
377 of nitrate in the aquifers and the decreased denitrification induced by management-driven
378 reductions in water P availability (Sutton *et al.* 2011; Finlay *et al.* 2013). Indeed, policy

379 effectiveness and implementation in reducing N loads differed regionally among the European
380 countries. In this study, European sites mostly consisted of western and northern Europe with
381 better sewage input controls and watershed management than those countries located at the
382 eastern and southern Europe (Sutton *et al.* 2011). Therefore, our results indicated that water
383 quality might be improved in Euro-America due to recent environmental controls, resulting in
384 the increase of N:P ratios in freshwater ecosystems (Finlay *et al.* 2013; Dove & Chapra, 2015).

385

386 The N:P stoichiometric imbalance caused by the trophic levels in the freshwater ecosystems
387 was further supported by the long-term water-nutrient monitoring data for three Chinese lakes
388 (Lake Taihu, Lake Dianchi (Waihai) and Lake Bosten) and one American lake (Lake
389 Okeechobee) with continuously aggravated trophic levels due to anthropogenic nutrient
390 discharges (Fig. 5). The water N and P concentrations in these four lakes (except the water N
391 concentration in Lake Okeechobee) increased with year over the survey periods, but the rates
392 of P accumulation (log-transformed, c. 0.012-0.061 mg P L⁻¹ y⁻¹) were higher than those of N
393 accumulation (log-transformed, c. -0.003-0.023 mg N L⁻¹ y⁻¹), resulting in lower water N:P
394 ratios (Fig. 5).

395

396 The influence of N deposition on nutrient cycles in freshwater ecosystems is small relative to
397 other anthropogenic nutrient inputs under intensified human activity. Our results showed that
398 N deposition was negatively correlated with the N:P ratios in freshwater and its macrophytes
399 (Fig. S10), contrary to the increasing N:P ratios reported by Bergstrom *et al.* (2005) and Elser
400 *et al.* (2009). Previous studies chose natural ecosystems along gradients of N deposition
401 without or far from direct anthropogenic nutrient inputs and then explored the impacts of N
402 deposition on nutrient cycles in freshwater ecosystems. In our study, however, the rate of N
403 deposition was strongly associated with other anthropogenic factors, and the independent role

404 of N deposition was hard to detect (Table S9). Our stepwise multiple regression of macrophytic
405 and water nutrients against ten anthropogenic variables indicated that N deposition explained
406 a lower proportion of the variability in N:P stoichiometry in freshwater ecosystems than the
407 other variables (Table S9). Some case studies of the N budget support the lower contribution
408 of N deposition to the total N inputs to freshwater ecosystems under anthropogenic impacts
409 (Cook *et al.* 2010; Cui *et al.* 2013; Grantz *et al.* 2014).

410

411 Contrary to previous proposals that most of the variability in N:P stoichiometry in terrestrial
412 plants could be explained by species composition and climate (Reich & Oleksyn 2004; Han et
413 al. 2005 & 2011; Chen et al. 2013), freshwater plants can be highly susceptible to changes in
414 the characteristics of the surrounding environment induced by anthropogenic activity (Lacoul
415 & Freedman 2006; Demars & Edwards 2007; Xing et al. 2013). The true roles of functional
416 group and climate could thus be obscured by excess nutrient availabilities from the surrounding
417 environment under human impacts. This study, however, has some limitations. First, the
418 considerable site-related variances indicated that large variances were not well captured by the
419 dichotomous levels of human impact, which may require more detailed and quantitative
420 assessments. Second, much of the variance in N and P stoichiometry in freshwater ecosystems
421 remains unexplained, which may be due to various sources, such as unquantified micro-
422 environments, water depth, salinity, pH and flow velocity (Grimm *et al.* 2003; Lacoul
423 & Freedman 2006; Bornette & Puijalon 2011). Third, the multi-collinearities among the various
424 anthropogenic variables (Table S9) hinders the detection of the independent role of each factor
425 (e.g. agricultural land use) in shaping the observed pattern. Fourth, little is known about the
426 relative contributions of external and internal nutrient loadings in determining the patterns of
427 nutrient stoichiometry in freshwater ecosystems. A much more detailed regional-scale survey

428 and experimental sampling will be required to quantitatively assess and minimize these
429 uncertainties.

430

431 CONCLUSION

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

441

442 Anthropogenic water pollution may thus shift aquatic ecosystems from a state of predominant
443 P limitation to being potentially limited by N or other factors such as light, especially in rapidly
444 developing regions such as China. Continued anthropogenic amplification of the stoichiometric
445 imbalance of global N and P cycles will have further large ecological ramifications for
446 biogeochemical cycling and biological diversity in freshwater ecosystems (Sterner & Elser,
447 2002). This disturbance thus threatens to involve more and more freshwater systems and can
448 have large implications to the necessary continued focus on P abatement in efforts of
449 ecosystemic conservation and management in the coming decades. Global eutrophication in
450 freshwater ecosystems induced by anthropogenic activity may presumably have potentially
451 large impacts on the trophic webs and biogeochemical cycles of estuaries and coastal areas by
452 freshwater loading and highlight the importance of rehabilitating these ecosystems. Our

453 findings can also help to better parameterize complex N and P biogeochemical models that
454 should be developed for projecting various scenarios of global change (Elser *et al.* 2007;
455 Kroeze *et al.* 2012; Peñuelas *et al.* 2013).

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618 **SUPPORTING INFORMATION**

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

620 **Supplementary discussion**

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

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

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

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

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

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

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

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

636 **Table S9** Correlations among anthropogenic variables involved in this study.

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

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

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

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

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

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

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

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

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

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

654 **Appendix Data sources**

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663 2014-274.

664 **Table 1** Summary of general linear models for the N and P concentrations, and N:P mass ratio in freshwater macrophytes.

Factor	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
	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
<i>Functional groups</i>												
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	
<i>Climatic variables</i>												
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
<i>Random factor</i>												
Sites*	106	0.09	4.46	46.8	121	0.13	4.16	38.0	107	0.17	6.14	57.9

665 Note: F values in bold denote $p < 0.05$. Lifeform: emergent, floating-leaved, freely floating, submerged; Phylogeny 1: seed plant, fern; Phylogeny
666 2: forb, grass; Phylogeny 3: monocotyledon, dicotyledon. Abbreviations: MAT, mean annual temperature; AP, annual precipitation; DF, degrees
667 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
668 in the four main-effect models, we gave the values calculated from the final model here.

669 **FIGURE LEGENDS**

670

671 **Figure 1** Relationships between (a) plant P and N concentrations and (b) water TP and TN
672 concentrations for all individual data pooled. Reduced major axis (RMA) regression was used
673 to determine the regression lines. Numbers in square brackets are the lower and upper 95%
674 confident intervals of the RMA slopes.

675

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

683

684

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

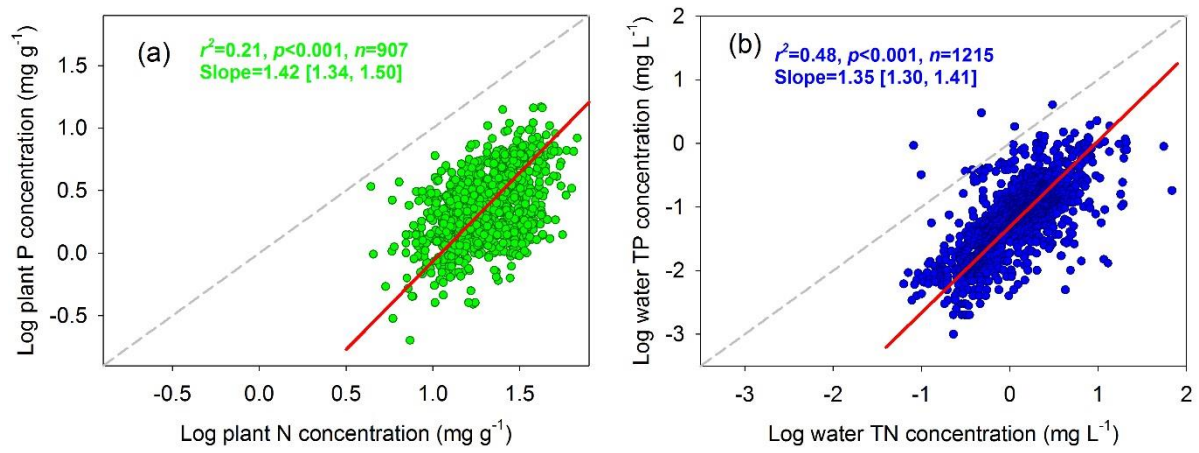
693

694 **Figure 4 (a-c)** N and P fertilizer use among regions (i.e. Europe, the USA and China) during
695 several decades, and **(d-i)** relationship between freshwater nutrients and population density in
696 China. (a) N fertilizer use, (b) P fertilizer use and (c) N:P₂O₅ mass ratios; (d) & (e) & (f)
697 macrophytic plant nutrients vs population density; (g) & (h) & (i) water nutrients vs population
698 density. Fertilizer data were from a statistical database available from FAO (see
699 <http://faostat.fao.org/>; last accessed on April 30, 2015). Europe consisted of Finland, France,
700 Germany, Hungary, Italy, Netherlands, Poland, Spain, Sweden, Switzerland and England.
701 Significant ordinary least squares (OLS) regression lines ($p < 0.05$) are fit to the data. Shaded
702 area indicate 95% confidence interval of the regression line.

703

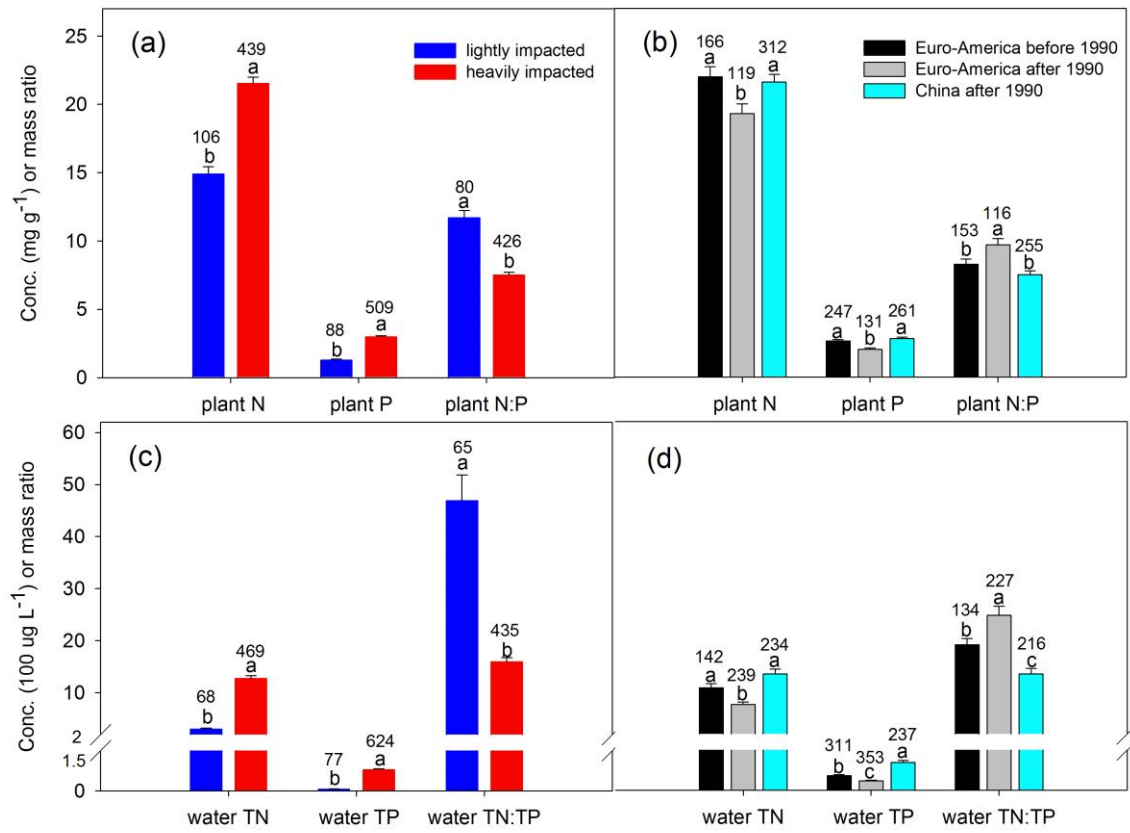
704 **Figure 5** Temporal patterns of yearly average water TN and TP concentrations and TN:TP
705 ratios in three Chinese Lakes (Lake Taihu, Lake Dianchi (Waihai) and Lake Bosten) and one
706 American Lake (Lake Okeechobee) with continuously aggravated trophic levels due to
707 anthropogenic nutrient discharges. Significant ordinary least squares (OLS) regression lines
708 are fit to the data. Statistical significance is indicated by *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.
709 The data were derived from previous publications (Qing 2002; Havens *et al.* 2003; Huang *et*
710 *al.* 2006; Chen 2012; Li *et al.* 2014; Xie *et al.* 2014). Note that nutrient data after 1997 for Lake

711 Taihu and after 2000 for Dianchi were excluded from our analyses due to the reductions in



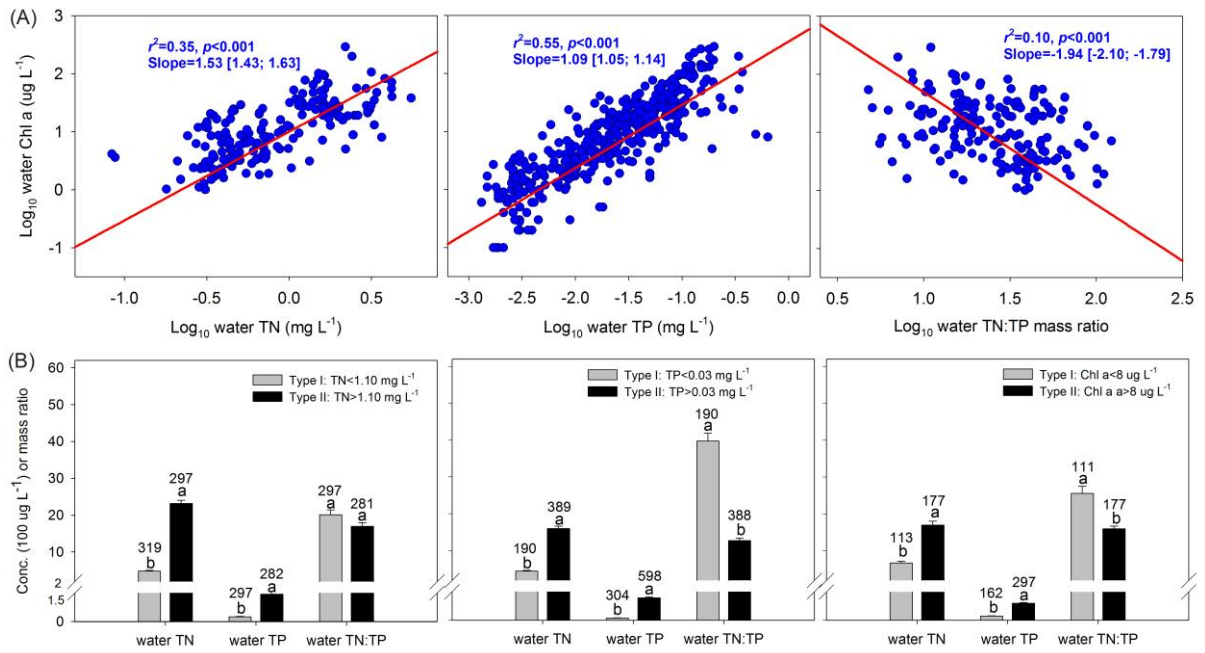
712 nutrient loadings by watershed management (Chen 2012; Li *et al.* 2014). **Figure 1**

713 **Figure 2**



714

715 **Figure 3**



716

717 **Figure 5**

718