Nutrient stoichiometry of linked catchment-lake systems along a gradient of land use

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SUMMARY

1. Catchments export nutrients to aquatic ecosystems at rates and ratios that are strongly influenced by land use practices, and within aquatic ecosystems nutrients can be processed, retained, lost to the atmosphere, or exported downstream. The stoichiometry of carbon and nutrients can influence ecosystem services such as water quality, nutrient limitation, biodiversity, eutrophication and the sequestration of nutrients and carbon in sediments. However, we know little about how nutrient stoichiometry varies along the pathway from terrestrial landscapes through aquatic systems.

2. We studied the stoichiometry of nitrogen and phosphorus exported by three catchments of contrasting land use (forest versus agriculture) and in the water column and sediments of downstream reservoirs. We also related stoichiometry to phytoplankton nutrient limitation and the abundance of heterocystous cyanobacteria.

3. The total N : P of stream exports varied greatly among catchments and was 18, 54 and 140 (molar) in the forested, mixed-use and agricultural catchment, respectively. Total N : P in the mixed layers of the lakes was less variable but ordered similarly: 35, 52 132 in the forested, mixed-use and agricultural lake, respectively. In contrast, there was little variation among systems in the C : N and C : P ratios of catchment exports or in reservoir seston.

4. Phytoplankton in the forested lake were consistently N limited, those in the agricultural lake were consistently P limited, and those in the mixed-use lake shifted seasonally from P- to N limitation, reflecting N : P supply ratios. Total phytoplankton and cyanobacteria biomass were highest in the agricultural lake, but heterocystous (potentially N fixing) cyanobacteria were most abundant in the forested lake, corresponding to low N : P ratios.

5. Despite large differences in catchment export and water column N : P ratios, the N : P of sediment burial (integrated over several decades) was very low and remarkably similar (4.3–7.3) across reservoirs. N and P budgets constructed for the agricultural reservoir suggested that denitrification could be a major loss of N, and may help explain the relatively low N : P of buried sediment.

6. Our results show congruence between the catchment export N : P, reservoir N : P, phytoplankton N versus P limitation and the dominance of heterocystous cyanobacteria. However, the N : P stoichiometry of sediments retained in the lakes was relatively insensitive to catchment stoichiometry, suggesting that a common set of biogeochemical processes constrains sediment N : P across lakes of contrasting catchment land use.

Keywords: carbon, cyanobacteria, nitrogen, phosphorus, reservoirs
Introduction

Understanding the stoichiometry of nutrients as they flow and cycle through catchments can provide insights into processes operating at ecological levels of organisation from populations to landscapes (Sterner & Elser, 2002). Nitrogen (N) and phosphorus (P) are the nutrients most likely to limit growth of autotrophs (Elser et al., 2007), and the ratio at which N and P are supplied can determine which nutrient is limiting and mediate competitive interactions between taxa (Redfield, 1958; Tilman 1982; Sterner & Elser, 2002; Hessen et al., 2004). Carbon (C)/nutrient ratios reflect the severity of autotroph nutrient limitation and the degree to which heterotrophs are energy- versus nutrient limited (Sterner & Elser, 2002; Hessen et al., 2004) and mediate the efficiency of energy transfer in food chains (Malzahn et al., 2007; Dickman et al., 2008). The stoichiometry of C, N and P can also influence ecosystem services such as water quality and eutrophication (Smith, 1983; Schindler et al., 2008) and the sequestration of nutrients and carbon in sediments (Falkowski, Barber & Smetacek, 1998; Hessen et al., 2004; Cole et al., 2007). Thus, understanding the coupled cycling and stoichiometry of these elements is crucial.

As elements travel through catchments, they may be transported downstream, sequestered or lost to the atmosphere at different rates and efficiencies. A critical mediator of catchment-scale element transport is how biota respond to various stoichiometric ratios and how they in turn mediate stoichiometry. For example, the N : P ratio can determine the relative abundance of N-fixing cyanobacteria (Smith, 1983; Watson, McCauley & Downing, 1997; Downing, Watson & McCauley, 2001). In turn, N fixation can mediate the flux of N into water columns, potentially exerting a strong influence on whole-ecosystem fluxes and cycling of N and P (Schindler et al., 2008; Sterner, 2008). Given the well-recognised importance of coupled biogeochemical cycles, it is surprising that relatively few studies have examined how C : N : P stoichiometry varies along catchment flowpaths, although several studies have examined fluxes of single elements (e.g. Seitzinger et al., 2002; Cole et al., 2007; Downing et al., 2008; Harrison et al., 2009). Most studies examining multiple elements address one particular aspect of transport through catchments, such as exports from land to water (Carpenter et al., 1998; Allan, 2004). Others focus on a single ecosystem type, for example the relationship between nutrient ratios and autotroph taxonomic composition in lakes (Smith, 1983; Watson et al., 1997; Downing et al., 2001).

Land use is an important factor controlling the export of carbon and nutrients from catchments (Howarth et al., 1996; Carpenter et al., 1998; Allan, 2004). High nutrient export rates from agricultural catchments can cause eutrophication and degrade water quality (Carpenter et al., 1998), but the N : P ratio of export from agricultural catchments is highly variable and depends on the type of agriculture. For example, agricultural landscapes dominated by row crops tend to export nutrients at high N : P, while those with extensive areas of pasture or animal operations export at low N : P (Downing & McCauley, 1992; Arduke & Downing, 2001; Alexander et al., 2009). Very little is known about the C : N or C : P ratio of export from catchments.

Lakes can integrate and regulate catchment exports (Kling et al., 2000; Williamson et al., 2008). Lake nutrient concentrations, phytoplankton biomass and composition, and sediment accumulation rates may reflect catchment land use (Arduke & Downing, 2001; Knoll, Vanni & Renwick, 2003; Jones et al., 2004; Dodson, Lillie & Will-Wolf, 2005), and lakes can modulate the downstream transport of C, N and P because of relatively high rates of sedimentation (Cole et al., 2007; Downing et al., 2008) and denitrification (David et al., 2006; Harrison et al., 2009). Among lake types, reservoirs may be particularly important in integrating and modulating catchment exports because they receive relatively large amounts of materials from landscapes (owing to large catchment/lake area ratios; Thornton, 1990). In particular, reservoir sediments represent potentially important sinks for C and N at regional and global scales (Dean & Gorham, 1998; Renwick et al., 2005b; Downing et al., 2008; Harrison et al., 2009).

Despite the recognised importance of lakes and reservoirs as integrators and modulators of catchment exports, it is not clear how C, N and P stoichiometry changes as elements flow from landscapes through streams and lakes. In this article, we examine C, N and P stoichiometry in three catchment-reservoir systems of contrasting land use (forest versus agriculture). Specifically, we relate the stoichiometry of C, N and P in catchment exports, the water columns of the reservoirs and the sediments buried in the reservoirs. We also related N : P stoichiometry to...
phytoplankton nutrient limitation status and the abundance of heterocystous cyanobacteria. We know of no other studies that have examined all of these stoichiometric components and how they vary with catchment land use and hope that this information will provide insights into the transport and retention of multiple elements through catchments.

Study sites

Our three study sites were Acton, Pleasant Hill and Burr Oak Lakes, all of which are reservoirs in Ohio, U.S.A. (Table 1; see also Knoll et al., 2003; Vanni et al., 2006b; Bremigan et al. 2008; and Hale et al. 2008). Catchments of these reservoirs fall along a gradient of agricultural versus forested land; developed (urban/suburban) areas represent <10% of the land (Table 1). Acton Lake (39°34′N, 84°44.5′W) is a highly eutrophic reservoir in southwestern Ohio (Vanni et al., 2001, 2005; Renwick et al., 2008). Greater than 80% of its catchment (sometimes referred to as the Upper Four Mile Creek catchment; Renwick et al., 2008), is agricultural land, and forest constitutes <15% (Table 1). The percentage of catchment land in agriculture has been relatively stable since the lake was constructed, although row crops (mostly corn and soy) have increased from ~55% of agricultural land in 1954 to ~95% in 2001, with a corresponding decrease in pasture and hay (Medley et al., 1995; Renwick et al., 2008). Since the early 1990s, there has been a pronounced increase in the use of conservation tillage in the Acton catchment (Renwick et al., 2008). Within Acton’s catchment, we measured nutrient fluxes in three streams: Four Mile Creek, Little Four Mile Creek and Marshall’s Branch, at locations just upstream of where they enter Acton Lake (Vanni et al., 2001; Renwick et al., 2008). Together, these three streams drain ~86% of the lake’s catchment.

Pleasant Hill’s catchment, referred to as the ‘mixed’ catchment, is located in central Ohio (Table 1). It is composed of 46% forest and 41% agricultural land (~66% of which is row crops and ~34% pasture). Based on county-level data, there has been a moderate increase in the extent of forested and developed lands in the catchment since the lake was constructed (Birch & Wharton, 1982). We monitored one major stream, Clear Fork, just upstream of where it enters Pleasant Hill Lake (40°38.5′N, 82°19.7′W) a eutrophic reservoir (Knoll et al., 2003; Vanni et al., 2005). Clear Fork drains 88% of the lake’s catchment.

Burr Oak’s catchment, which we refer to as the ‘forested catchment’, is located in southeastern Ohio. It is composed of 83% forest and 6% agricultural land (Table 1); row crops and pasture constitute ~6 and 94% of the agricultural land, respectively. Based on county-level data, the percentage of catchment land that is categorised as forested has more than doubled since the lake was constructed in 1955 (Birch & Wharton, 1982; Table 1). This is the result of regrowth of previously cleared forest, due largely to the establishment of Wayne National Forest in 1934, within which much of Burr Oak’s catchment lies. We monitored one major stream, the East Branch of Sunday Creek, just upstream of where it enters Burr Oak Lake (39°31.7′N, 82°2.6′W), a mesotrophic reservoir (Knoll et al., 2003; Vanni et al., 2005). This stream drains approximately 70% of the lake’s catchment.

Methods

Stream nutrient export from catchments to lakes

We quantified stream discharge and C, N and P concentrations using a high-resolution sampling programme during two water years (1 October 1999–30 September 2001), following methods of Vanni et al. (2001). Stage was recorded every 10 min and converted to discharge using standard rating curve techniques. Nutrient concentrations were quantified from water samples taken by ISCO programmable

<table>
<thead>
<tr>
<th>Site</th>
<th>Lake area (ha)</th>
<th>Year of construction</th>
<th>Lake mean depth (m)</th>
<th>Catchment area (ha)</th>
<th>Catchment land use (% of area)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burr Oak</td>
<td>278</td>
<td>1950</td>
<td>4.3</td>
<td>8580</td>
<td>83.2 6.2 6.4</td>
</tr>
<tr>
<td>Pleasant Hill</td>
<td>317</td>
<td>1933</td>
<td>4.8</td>
<td>51670</td>
<td>46.2 41.3 9.6</td>
</tr>
<tr>
<td>Acton</td>
<td>240</td>
<td>1955</td>
<td>3.9</td>
<td>25934</td>
<td>12.4 80.8 5.4</td>
</tr>
</tbody>
</table>

samplers. Concentrations vary markedly with discharge (Vanni et al., 2001), so we used a storm-dependent sampling programme. Samplers took a water sample every 6 h from the three agricultural streams over the whole 2-year period (Vanni et al., 2001; Renwick et al. (2008), and samples were picked up weekly. The streams from the mixed and forested catchments are located several hours from our laboratory, so samples were taken every 8 h from April until September and every 24 h from October through March. Samples were picked up weekly April–September and every 3 weeks otherwise. For all streams, we quantified nutrients on one to five samples per week during base flow conditions. During storm events, we analysed all the samples during, and two to three samples immediately before and after, the event. The delay in processing of some samples had relatively minor effects on loading estimates; specifically, it overestimates annual nitrate and soluble reactive P loading by 11.8 and 17.6%, respectively (M. J. Vanni, unpubl. data). In this article, we use the uncorrected data.

Reservoir nutrients and phytoplankton

Reservoirs were sampled for water column total P (TP) and total N (TN), phytoplankton biomass and nutrient limitation status, and the abundance of N-fixing and non-fixing cyanobacteria. Samples were taken between April and October in 2000 and 2001 from the euphotic zone at the deepest area of the reservoir (the ‘outflow’ site, close to the dam; Vanni et al., 2006a) with a tygon tube and stored in 4-L Nalgene bottles until returning to the laboratory. A common water sample was used for nutrient analyses and for all phytoplankton parameters. Sampling frequency varied among lakes and parameters. In Acton, phytoplankton biomass and nutrients were sampled at least weekly, whereas the other lakes were sampled once or twice per month for these parameters. In all three lakes, nutrient limitation status was assayed once or twice per month, and cyanobacteria were quantified three or four times in each sampling year.

Phytoplankton biomass was quantified as chlorophyll a concentration. Samples were filtered onto A/E filters, frozen, extracted in acetone and analysed using a Turner TD-700 fluorometer. We enumerated cyanobacteria and their heterocysts during the period of summer cyanobacteria blooms (July–September) to evaluate the potential importance of N fixation and related this to phytoplankton N limitation. Water from the integrated samples was preserved with Lugol’s solution, and all cyanobacteria filaments and all heterocysts on these filaments were enumerated, after settling, at 400× magnification using inverted microscopy. In 19 of 21 samples, fields were examined until at least 1000 filaments had been counted. In two samples, cyanobacteria filaments were much less common and either 4.5 or 9.0 mL of lake water was completely examined (386 or 601 filaments were examined in these two samples).

Phytoplankton nutrient limitation was quantified using bioassays with N and/or P additions, following methods identical to those in Vanni et al. (2006b). For visual purposes, we express the severity of nutrient limitation for each treatment (+N, +P, +N & P) as \[ \Delta r_{\text{treatment}} = \ln(\text{chl}_{\text{treatment}}/\text{chl}_{\text{control}})/t, \] where chl_treatment and chl_control are final chlorophyll concentrations in the particular treatment and control flasks, respectively, and t is the duration of the experiment (2 days). To statistically test for nutrient limitation effects, we conducted one-way ANOVAs on each experiment, followed by the Tukey HSD test to assess specific treatment differences. If the mean final log-transformed chlorophyll concentration in a single-nutrient treatment was significantly greater than the control, we considered phytoplankton to be limited by that nutrient. Furthermore, if chlorophyll in the +N & P treatment was significantly greater than both of the single-nutrient treatments, we considered this to be evidence of colimitation.

Reservoir carbon, nitrogen and phosphorus burial

To determine burial rates of C, N and P in reservoir sediments, cores were taken with a 5.1-cm-diameter gravity corer at all three reservoirs during September and October 2000. In each lake, we took a total of nine cores, three each near the lake’s outflow site, a site near stream inflows (‘inflow’) and a site midway between these two (‘middle’). Cores were extruded and sliced into 4-cm sections that were frozen until analysis. Cores were analysed for C (organic and total), N and P concentrations, and dry bulk density (g dry mass cm⁻³). Net burial rates of C, N and P, over the lifespans of the reservoirs, were determined by multiplying sediment concentration (g C, N or P g
sediment$^{-1}$) by sedimentation rate (g yr$^{-1}$). Burial ratios were calculated as burial rate of one element divided by burial rate of the other element and are expressed here in molar units.

Previous surveys had quantified sedimentation rates (cm year$^{-1}$) through 2001 for Acton (Renwick, Carlson & Hayes-Bohanan, 2005a) and 1998 for Pleasant Hill (Renwick & Andereck, 2006). We used those rates here. Several sediment surveys have been carried out in Acton Lake since it was constructed in 1957 (Renwick et al., 2005a). Thus, we also used the sediment rate over the last interval (1987–2001) as a measure of more recent burial. For Burr Oak, we determined sediment thickness in 2000 using a probe inserted into lake sediments until it reached hard bottom (Downing et al., 2008). This was performed along the length of the lake, in a series of 10 transects, for a total of 35 measurements. Mean sediment thickness was then converted to volumetric sedimentation rate and then sediment accumulation rate (g dry mass) using bulk density data. It was not possible to measure sedimentation rates over shorter intervals in any of these lakes; attempts to age the core slices using $^{210}$Pb and $^{137}$Cs were not successful (D. R. Engstrom, University of Minnesota, pers. comm.).

**Nutrient analyses**

Stream nutrient samples were filtered through pre-combusted, pre-weighed A/E filters. All filters were analysed for suspended sediment (SS) concentrations; only a subset of filters were analysed for particulate C (PC), N (PN) and P (PP) to minimise costs and because of the strong relationship between SS and concentrations of these particulates (Vanni et al., 2001). Filtrate samples were preserved in Nalgene bottles with concentrated sulphuric acid to a pH of <2 and refrigerated at 4 °C until analysed for soluble reactive phosphorus (SRP), ammonium-N (NH$_4$-N) and nitrate/nitrite-N (NO$_3$–N) using a Lachat QC 8000 FIA autoanalyzer (Lachat Instruments, Loveland, CO, U.S.A.). A subset of filtrate samples was digested and analysed for total dissolved phosphorus (TDP) and total dissolved nitrogen (TDN). SRP was analysed using a molybdenum blue technique, NH$_4$–N with a phenolphthalein technique and NO$_3$–N with a cadmium reduction/sulphanilamide technique. TDP and TDN were digested with potassium persulphate and analysed as SRP and NO$_3$–N, respectively.

Filters (47 mm diameter) were analysed for SS by measuring the difference in dry mass before and after filtering, using a Mettler UMT ultra-microbalance (Mettler-Toledo, Columbus, OH, U.S.A.). The subset of these filters used for PP analysis were placed in glass vials and digested with 1 N hydrochloric acid to convert PP to SRP (Stainton et al. 1977), which was then quantified as described earlier. PC and PN filters (25 mm) were analysed using a Perkin Elmer Series 2400 CHN analyzer (Perkin-Elmer, Waltham, MA, U.S.A.).

For those nutrient fractions determined on only a subset of samples (PP, PN, PC, TDN, TDP), concentrations were estimated from stream-specific regressions against fractions that were routinely quantified. Thus, PP, PN and PC concentrations were regressed against SS, TDN was regressed against NO$_3$–N, and TDP was regressed against SRP (Vanni et al., 2001). All regressions had an $r^2 > 0.6$ and most were >0.9. TP concentration was obtained as TDP plus PP, and TN was obtained as TDN plus PN.

Hourly nutrient concentrations were interpolated between samples by a flow-proportionate method that models the effects of flow variations on concentration (Vanni et al., 2001). Hourly concentrations were then multiplied by mean hourly discharge to obtain hourly loading rates, which were then summed to obtain daily and annual loads. To obtain whole catchment loading rates, we divided rates from gaged areas by the proportion of the catchment represented by the gaged area. This assumes that the gaged area is representative of the entire catchment, which seems reasonable given that gaged areas constituted ≥70% of catchment area. N : P loading ratios were calculated by dividing N loading rate by P loading rate for the appropriate interval (daily or annual). All ratios are given in molar units.

To obtain sediment dry mass and bulk density, core samples were weighed pre- and post-drying at 60 °C until a constant mass was achieved. Dry bulk density was determined using the dry mass and original volume of each core sample. For nutrient analyses, dried core samples were ground and homogenised into a fine powder using a mortar and pestle. A subsample was analysed for PC and PN as described earlier. Another subsample was ashed at 550 °C for 4 h and then analysed for inorganic C. Organic C was then determined by the difference between C in
non-ashed and ashed samples. Another subsample was taken and analysed for PP as described earlier. For lake TP and TN analyses, unfiltered water was preserved and processed in the same manner as stream TDN and TDP samples.

**Acton Lake annual N and P budgets**

To gain a better understanding of the factors mediating N and P retention in Acton Lake, we generated budgets for these elements. Because we quantified only PC inputs (and not other forms of C), we did not construct a C budget for Acton. We did not have sufficient data on the other lakes to construct budgets. For Acton, we constructed a budget for calendar year 2001. We were not able to construct a budget for earlier periods because of a lack of data on lake level, which was necessary to estimate outflow from the lake. Thus, the period for which we present the nutrient budget (January–December 2001) differs somewhat from the 2001 water year (October 2000–September 2001).

Inputs of N and P from streams were estimated as described earlier. We estimated total atmospheric N deposition (wet and dry) to the lake surface using data from a meteorological site at the Miami University Ecology Research Center (ERC), which is located only ~5 km from Acton Lake and is part of the US EPA’s Clean Air Status and Trends Network program (CASTNET, http://www.epa.gov/castnet).

Output of N and P from the lake’s outflow (over the dam spillway) was estimated using data on lake level, lake morphometry and concentrations of N and P measured either at the lake’s outflow site (from April–October, as described earlier) or from a site below the spillway (during other months) sampled at weekly intervals. When stream flow is high (i.e. in winter and spring), concentrations are similar at the outflow site and below the spillway; thus, we feel justified using both of these measurements. Nutrient concentrations were linearly interpolated between sampling dates. At hourly time steps, we estimated the volume of water exiting the lake as the difference between water inflow (sum of stream discharge plus precipitation on the lake surface), evaporation and change in lake volume. Lake volume was estimated using data on lake level (continuously recorded with a lake level gauge) and bathymetry. Evaporation was estimated from air temperature and day length (Hamon, 1961). Hourly data were summed to get daily water export rate, which was multiplied by N or P concentration to get nutrient export rates.

We also examined known fluxes from lake sediments to the water column. Nutrient excretion by sediment-feeding fish (gizzard shad, Dorosoma cepedianum) represents an important nutrient flux from sediments to water during summer (Schaus et al., 1997; Vanni et al., 2006a) and was estimated using data on size-specific abundance of fish and per fish excretion rates, as described in Vanni et al. (2006a). Fluxes from sediments to water via microbially mediated processes were obtained from Nowlin, Evarts & Vanni (2005), who estimated these fluxes in Acton Lake using core incubations in summer 1996. Because N and P release rates are probably low (or possibly even negative) during winter (Holdren & Armstrong, 1980; Hupfer & Lewandowski, 2008), we divided the summer rates from Nowlin et al. (2005) by two to get annual rates. To estimate diffusive flux rates across the thermocline, we used rates reported by Nowlin et al. (2005) for N and P and assumed that they applied only to the stratified period (~5 months). Nowlin et al. (2005) calculated diffusive flux using depth-specific N and P concentrations and a vertical exchange coefficient (0.01 cm$^2$ s$^{-1}$), taken from Auer et al., 1993).

Dentrification represents a potentially major loss of N from reservoirs, but we have no data on this process. Thus, we used rates presented by David et al. (2006) for a productive reservoir in an agricultural catchment in Illinois. We also used data on ‘recent’ (1987–2001) N and P burial rates (from Renwick et al., 2005a, as described earlier), as well as the net change in N and P mass in the lake water column over the budget period, to complete the budget.

**Results**

**Nutrient export rates and ratios from catchments**

Nutrient loading rates (expressed per reservoir surface area) were generally highest to the agricultural lake (Acton) and lowest to the forested lake (Burr Oak), although this was not true for all nutrient fractions (Table 2). In all catchments, NO$_3$ dominated export of dissolved inorganic N (DIN, i.e. NO$_3$ + NH$_4$), constituting >80% of DIN export in Burr Oak and >95% in all others. DIN and SRP were more strongly associated with land use differences than were particulate...
nutrient fractions. In fact, PN and PP loading rates were higher for the mixed catchment lake (Pleasant Hill) than the agricultural lake in water year 2000, when water yield was relatively low in the agricultural lake’s catchment (Table 2). Because dissolved fractions constituted a greater proportion of total load for N than for P, differences among lakes in TN loading were more closely associated with land use than were differences in TP loading (Table 2).

The ratio at which dissolved nutrients were exported from catchments to reservoirs (DIN : SRP ratio) was overall highest in the agricultural catchment and lowest in the forested catchment (Table 2), but this ratio varied temporally over three orders of magnitude (Fig. 1a). Absolute variation in DIN : SRP was greatest in the agricultural catchment (5th and 95th percentiles of daily ratios = 23 and 6958, respectively) and lowest in the forested catchment (18 and 269). However, the coefficient of variation (CV, i.e. SD/mean) of daily DIN : SRP ratios was remarkably similar among catchments (1.1, 1.0 and 1.2 in the forested, mixed and agricultural catchments, respectively). Daily SRP loading rates were generally more variable than daily DIN loading rates in all three catchments; CVs for daily SRP loading were 5.1, 2.9 and 4.0, while CVs for DIN were 4.7, 2.1 and 2.3 in Burr Oak, Pleasant Hill and Acton catchments, respectively. Thus, relative variation in DIN : SRP was apparently caused more by variation in SRP than in DIN. Compared to DIN : SRP ratios, particulate nutrient export ratios (PN : PP) were much lower, much more similar among catchments and much less temporally variable (Fig. 1b). Cumulatively, PN : PP export was highest in the agricultural catchment, but the mixed and forested catchments were very similar to each other (Table 2). Total nutrient export ratios (TN : TP) showed among-catchment patterns similar to those for DIN : SRP, except that the relative differences among catchments were less pronounced than those for dissolved export, because of the moderating influence of PN : PP export (Fig. 1c). Even though we observed considerable variability between years in the relative ranking of catchments in terms of nutrient loading and water yield, differences in nutrient export ratios among catchments were similar in the 2 years (Table 2).

Table 2 Water yield and export ratios from the study watersheds during water years 2000, 2001 and cumulative over the 2 years. Water yield and nutrient loading rates represent those from entire watersheds, including ungaged areas

<table>
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<tr>
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<tbody>
<tr>
<td>Water yield [m³ (ha watershed area)⁻¹ year⁻¹]</td>
<td>4109</td>
<td>2996</td>
<td>1936</td>
<td>2230</td>
<td>2254</td>
<td>3006</td>
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<tr>
<td>Nutrient loading rates [kg (ha lake area)⁻¹ year⁻¹]</td>
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<tr>
<td>DIN</td>
<td>32</td>
<td>845</td>
<td>2234</td>
<td>23</td>
<td>616</td>
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<td>Particulate N</td>
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<td>133</td>
<td>69</td>
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<tr>
<td>Total N</td>
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<td>1194</td>
<td>2499</td>
<td>63</td>
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<td>SRP</td>
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<td>10.1</td>
<td>0.9</td>
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<td>108</td>
<td>120</td>
<td>100</td>
<td>106</td>
<td>148</td>
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</tbody>
</table>

DIN, dissolved inorganic N.

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Reservoir nutrients and phytoplankton

Water column nutrient concentrations in reservoirs generally reflected catchment land use and nutrient export rates. TN concentration was consistently highest in Acton (agricultural) and lowest in Burr Oak (forested) (Fig. 2a,b). TN decreased in Acton and Pleasant Hill (mixed catchment) in late summer, at which time concentrations in Pleasant Hill and Burr Oak were very similar to each other. TP concentration was always higher in Acton than in Burr Oak, with Pleasant Hill TP intermediate (Fig. 2c,d). The water column TN : TP ratio was highest in Acton except in late summer when TN : TP converged among lakes (Fig. 2e,f). TN : TP was higher in Pleasant Hill than in Burr Oak in spring and early summer, but this pattern reversed later in the season. In Pleasant Hill and Acton, TN : TP declined seasonally, corresponding to a temporal decline in the ratio at which nutrients were delivered to the reservoirs from streams (Fig. 1). Overall, temporal variability in TN : TP was greater in Pleasant Hill (CV among dates = 0.74) than either Acton (0.60) or Burr Oak (0.29). In Pleasant Hill and Acton, where seasonal TN declines were pronounced, TN was more variable than TP (Pleasant Hill: CV\textsubscript{TN} = 0.42, CV\textsubscript{TP} = 0.38; Acton: CV\textsubscript{TN} = 0.44, CV\textsubscript{TP} = 0.31). However, in Burr Oak the CV for TP (0.27) exceeded that for TN (0.18).

In accordance with nutrient inputs and lake nutrient concentrations, phytoplankton biomass (chlorophyll a) was almost always highest in Acton Lake and lowest in Burr Oak (Fig. 2g,h).

The identity of the limiting nutrient for phytoplankton and the severity of nutrient limitation reflected catchment export ratios and in-lake TN : TP (Fig. 3). Phytoplankton in Burr Oak, which had the lowest TN : TP, were significantly N limited on 8 of 13 dates, while P was limiting on only three dates (Fig. 3a,b). Burr Oak phytoplankton showed a colimitation effect on all but two dates (15 May and 7 August 2001), and usually this effect was quite strong (Fig. 3a,b). In contrast, phytoplankton in Acton, which had the highest TN : TP, were significantly P limited in 9 of 11 experiments and were never significantly N limited. Colimitation occurred twice, but the colimitation effect was generally small (Fig. 3e,f). Nutrient limitation status varied seasonally in Pleasant Hill (Fig. 3c,d), corresponding to declining TN : TP ratios (Fig. 2). P limitation was observed on 6 of 11 dates, but only through mid-August. The severity of N limitation increased seasonally, but N was limiting by itself on only two dates. Pleasant Hill phytoplankton also showed significant colimitation on five dates. Overall, nutrient limitation status and severity were significantly related to lake TN : TP. Pooling all experiments in all lakes (n = 35), the severity of P limitation was positively related to TN : TP (\Delta\text{TP} = -0.463 + 0.327 log(TN/TP); \textit{r}\textsuperscript{2} = 0.448, \textit{P} < 0.0001), while the severity of N limitation was negatively related to TN : TP (\Delta\text{TN} = 0.459 - 0.197 log(TN : TP); \textit{r}\textsuperscript{2} = 0.291, \textit{P} = 0.0008).

\[10000 \text{ (a)} \]
\[100 \text{ Particulate N:P} \]
\[1000 \text{ Total N:P} \]
\[8 \text{ M. J. Vanni et al.} \]
\[10 \text{ (b)} \]
\[100 \text{ (c)} \]
\[10 \text{ (d)} \]
\[10 \text{ (e)} \]
\[10 \text{ (f)} \]
\[10 \text{ (g)} \]
\[10 \text{ (h)} \]

\textbf{Fig. 1} N : P ratios in streams flowing into the three reservoirs. N : P ratios were also calculated on a daily basis. The dashed horizontal line in (c) indicates Total N : P= 50, the approximate boundary between lake phytoplankton N versus P limitation as shown by Guildford & Hecky (2000).
The density of cyanobacteria filaments (including both heterocystous and non-heterocystous forms) was always highest in Acton, the most eutrophic lake (Table 3). All three reservoirs had non-heterocystous genera such as Pseudanabaena and Planktothrix, while Pleasant Hill and Burr Oak had greater representations by potentially heterocystous genera Anabaena, Aphanizomenon and Cylindrospermopsis. Both the density of heterocysts (heterocysts mL\(^{-1}\)) and the number of heterocysts per cyanobacterial filament were highest in Burr Oak, the most N limited reservoir. In contrast Acton, the least N limited reservoir, had the fewest heterocysts per filament. Averaged over all seven dates, Burr Oak had approximately five times more heterocysts mL\(^{-1}\) than the other two reservoirs, and the mean number of heterocysts per cyanobacteria filament showed an order-of-magnitude difference between each reservoir (Table 3). In both years, the number of heterocysts per filament increased over both summers within Pleasant Hill as the severity of N limitation increased (data not shown).

**Carbon, nitrogen and phosphorus burial in reservoir sediments**

Sediment C, N and P concentrations varied among reservoirs and sites (Fig. 4). Within a reservoir, concentrations of all elements were generally highest at outflow sites and lowest at inflow sites. At outflow and middle sites, concentrations of C, N and P were generally higher in Pleasant Hill than in other reservoirs, and concentrations in Acton tended to be higher than those in Burr Oak except for P. At the inflow sites, there were no consistent among-lake differences.
in concentrations. Concentrations of all elements varied with depth over the top 20 cm, especially at the outflow site, but there was little variation below ~20 cm, among reservoirs and sites (Fig. 4).

Sedimentation rate (cm year\(^{-1}\)) over reservoir life-spans was related to land use and was highest in Acton (2.09), intermediate in Pleasant Hill (1.02) and lowest in Burr Oak (0.70). However, when expressed as sediment accumulation (burial) per unit catchment area, the relationship with land use was less clear; these rates were highest in Acton (1326 kg sediment km\(^{-2}\) of catchment area per year) but were much higher in
Fig. 4 Depth profiles of organic C, N and P, as per cent dry mass of sediments, at three sites in the three reservoirs. Points represent means of three sediment cores taken at each sampling site (i.e. nine cores per lake).

Burr Oak (932) than in Pleasant Hill (286). Similarly, C, N and P burial rates per unit reservoir surface area (Fig. 5a–c) were related to land use (Acton > Pleasant Hill > Burr Oak); however, when expressed per unit catchment area, burial rates were higher in Burr Oak than in Pleasant Hill, although Acton rates were the highest among all reservoirs (Fig. 5d–f).

In all lakes, the N : P and C : P of sediments declined with depth over the top ~10 cm (more so in Burr Oak than the other lakes) then was relatively constant with depth (Fig. 6). Sediment C : N increased with depth over the top ~10 cm and was relatively constant in deeper sediments (Fig. 6). In all reservoirs, the declines in C : P and N : P over the top 10 cm resulted from decreases in C and N concentrations as well as increases in P concentration over this depth stratum (Fig. 4).

**Variation in N : P along the flow path from catchments to reservoir sediments**

As nutrients travel from catchments to reservoir water columns and finally to reservoir sediments, two patterns emerge. First, despite great variation in the ratio at which N and P were exported from the three catchments to the reservoirs, the ratio at which these elements were buried in sediments was remarkably similar among reservoirs (Fig. 6). Second, the burial N : P ratio was much lower than the ratio at which N

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*Fig. 5* Catchment export rates (loading rates to lakes) and burial rates in sediments for C, N and P in the three study reservoirs. Loading and burial rates are expressed per m$^2$ lake area in the left-hand panels, and per m$^2$ catchment area in the right-hand panels. N and P export rates are shown for total and particulate fractions (but total C loading was not measured). For all lakes, the black bar represents burial rate over the entire lifespan of the lake. For Acton, the recent burial rate (1987–2001) is also given; recent rates were not available from the other lakes. Numbers above export rates represent relative burial, i.e. burial rate divided by loading rate. The numbers above the particulate bar indicate relative burial based on particulate loading rates, while the numbers above the hatched bar indicate those based on total loading rates. For Acton, the two numbers above the bars are for the entire lifespan of the lake (top number) and for recent burial (bottom number).
and P entered the lakes from catchments (Fig. 7). Lake water column N : P was intermediate between catchment export ratios and burial ratios, in regard to both actual N : P and the relative variation among lakes (Fig. 7). Thus, while the TN : TP export ratio from the agricultural (Acton) catchment was 8.5× that of the TN : TP export ratio from the forested (Burr Oak) catchment (with Pleasant Hill intermediate), the TN : TP ratio in the water column differed by 3.8× among lakes, and that of sediment burial differed only by 1.3× among lakes (Fig. 7).

**Annual N and P budgets for Acton Lake**

As expected for a reservoir with a relatively large agricultural catchment, stream N inputs greatly exceeded N deposition directly onto the lake surface (Fig. 8). Annual exports of N and P via the lake outflow...
were substantial, equal to 74 and 69% of annual stream N and P inputs, respectively. At the annual scale, internal fluxes from sediments to the water column were relatively small compared to stream inputs and the lake outflow. Among the sediment–water fluxes, nutrient excretion by sediment-feeding fish was the greatest, followed by microbially mediated fluxes from oxic sediments (Fig. 8).

The N : P ratio of nutrients exiting the lake’s outflow was only slightly higher than the ratio of stream inputs (114 versus 106; Fig. 8). (Note that TN : TP of stream inputs for ‘2001’ differ somewhat in Table 2 versus Fig. 8 because the former is for water year 2001 while the latter is for calendar year 2001). All of the major measured fluxes from sediments to the water column (microbially mediated release from oxic and anoxic sediments and excretion by sediment-feeding fish) are characterised by a higher N : P than the sediments themselves (Fig. 8).

Discussion

Stoichiometric ratios from inflow streams to reservoir sediments

At the catchment scale, two important stoichiometric trends emerged from this study. The first is that the N : P ratios of buried sediments are very similar among the three reservoirs, even though the reservoirs vary greatly in catchment land use and the N : P ratios at which nutrients are delivered from catchments. The second is that the sediment N : P ratios are quite low compared to ratios at which nutrients are delivered and to ratios in the water columns of the lakes. Together, these two trends suggest that sediment stoichiometry is constrained by a common process or set of processes operating across gradients of catchment land use, nutrient loading ratios and lake productivity.

The relatively low N : P of sediments suggests an important role for denitrification. This hypothesis is supported by the N budget for Acton Lake, specifically the estimates of N and P retention. Based on the balance of catchment stream inputs (I), atmospheric deposition (A), change in mass storage in the water column (ΔS) and export via the lake outflow (O), we can calculate predicted N and P retention (R) in sediments, using only these measured fluxes: R = I + A + ΔS – O (note that ΔS is negative if water column mass declines). According to these calculations, predicted P retention (RP) is 2.0 g P m⁻² year⁻¹ for 2001, while predicted N retention (RN) is 114 g N m⁻² year⁻¹. Observed sediment P burial for the recent period in Acton (1987–2001) is
served denitrification rates of 58.6–117 g N m\(^{-2}\) year\(^{-1}\), fairly close to predicted P retention. However, observed sediment N burial for 1987–2001 is only 4.5 g N m\(^{-2}\) year\(^{-1}\), about 25\(\times\) lower than that predicted based on the balance of measured inputs and outputs. It is likely that denitrification can account for much of this ‘discrepancy’ (i.e. predicted minus observed retention), which is equal to 109.5 g N m\(^{-2}\) year\(^{-1}\) (114 – 4.5). In a reservoir in an agricultural catchment in Illinois, David et al. (2006) observed denitrification rates of 58.6–117 g N m\(^{-2}\) year\(^{-1}\) (depending on assumptions regarding the depth of sediments within which denitrification occurs). Thus, denitrification can plausibly account for this ‘discrepancy’. Given that denitrification probably removes large amounts of N from sediments, it probably contributes significantly to the low N : P ratio of sediment burial. The David et al. (2006) rates seem reasonable for Acton, given that they were measured under similar conditions, i.e. in an agriculturally impacted reservoir with very high nitrate inputs.

The N : P ratio was low even in the surface (top 1–2 cm) sediments of our reservoirs (Fig. 6), suggesting that denitrification rapidly removes N. This view is also supported by studies on the seasonal dynamics of NO\(_3\) in Acton Lake. During thermal stratification, NO\(_3\) concentrations decline at all depths, but the decline is more rapid in the hypolimnion (which becomes anoxic shortly after stratification) and is most pronounced near the sediment–water interface (Nowlin et al., 2005). The decline in the epilimnion is likely because of the uptake by phytoplankton (Horgan, 2005), but the more rapid decline in the hypolimnion is likely because of denitrification. We do not have data on Burr Oak or Pleasant Hill to construct nutrient budgets, but it seems likely that denitrification is also important in these systems. The three reservoirs are similar in terms of the percentage of lake area overlain by anoxic hypolimnetic water, and it is likely that sediments are anoxic a few millimetres below the surface even where the entire water column is oxic.

Several studies suggest that N removal efficiency in reservoirs is mediated by hydraulic properties (Seitzinger et al., 2002; David et al., 2006; Harrison et al., 2009). We compared our measured N removal efficiency with that predicted by Harrison et al. (2009). We used this study (and not the others cited earlier) because it produced a model specific to reservoirs and incorporated more ecosystems and a much wider range of reservoir sizes (\(\geq 0.001\) km\(^2\)) than the others. As in Harrison et al. (2009), we calculated N removal efficiency (%N\(_{\text{rem}}\)) as 100\*[N\(_{\text{in}}\) - N\(_{\text{out}}\)/N\(_{\text{in}}\], where N\(_{\text{in}}\) and N\(_{\text{out}}\) are total N inputs and outputs via streams.

For calendar year 2001, the Harrison et al. (2009) model predicted %N\(_{\text{rem}}\) = 28.8 based on the observed Acton hydraulic residence time (\(T_w\)) of 0.097 year for 2001, compared to our measured estimate of 25.6 (from our nutrient budget; Fig. 8). Thus, the modelled and observed removal efficiencies differed by only \(~12.5\%\). Next, we applied the model to the 2 years (water years 2000–01) for the three lakes, using discharge and lake volumes to estimate hydraulic residence times. The model predicted %N\(_{\text{rem}}\) to be 40.0, 29.5 and 74.1 for Acton, Pleasant Hill and Burr Oak, respectively (%N\(_{\text{rem}}\) was higher in Acton for the 2-year period than in 2001 because \(T_w\) was longer over the 2-year period). All lakes have similar mean depths (Table 1); thus, differences in %N\(_{\text{rem}}\) are due mostly to differences in hydraulic residence time during the study (\(T_w\) = 0.147, 0.123 and 0.425 years in Acton, Pleasant Hill and Burr Oak). The higher retention efficiency in Burr Oak agrees with our observations suggesting that net burial rate (N burial rate/TN loading) is much higher in Burr Oak (Fig. 5). N retention efficiencies reported for other reservoirs vary greatly, but overall our estimated %N\(_{\text{rem}}\) values are within the range reported for others. Harrison et al. (2009) present data for several reservoirs; among these systems, %N\(_{\text{rem}}\) ranges from 0 to 99\% (Table 1 in Harrison et al., 2009). However, the simple mean of these values (using the midpoint for individual references if %N\(_{\text{rem}}\) is reported as a range) is 37\%, very similar to the value we estimate for Acton over the 2-year period and within the range found for Burr Oak and Pleasant Hill.

The longer residence time in Burr Oak should increase denitrification efficiency, all else being equal, compared to the other lakes. However, denitrification rates can be limited by supplies of organic C or NO\(_3\) (Boyer et al., 2006; David et al., 2006). Surface sediment organic C concentrations are similar among these reservoirs (Fig. 4) as are concentrations of dissolved organic C (L. B. Knoll et al., in prep.). However, nitrate supply differs greatly among lakes (Table 2) and NO\(_3\) concentrations vary accordingly, with Burr Oak water column concentration often <10 mg N L\(^{-1}\). Although little is known about the extent to which NO\(_3\) concentration limits
denitrification in the field (Boyer et al., 2006), concentrations this low can limit denitrification rates (Garcia-Ruiz, Pattinson & Whitton, 1998). This suggests that denitrification rates are lower in Burr Oak, although it is not clear if the percentage of N removed via denitrification also would be lower. The higher N burial rates in Burr Oak may also be the result of a greater contribution of allochthonous particles. A greater fraction of TN loading occurs in particulate form in Burr Oak compared to the other lakes, and allochthonous particles have a relatively low N : P (Table 2). Thus, burial of allochthonous material could contribute more to the sediment N : P in Burr Oak than in the other lakes, and in terms of controlling TN retention, may outweigh the importance of potentially lower denitrification rates in this lake.

Thus, the low N : P of these reservoir sediments may result from a variety of processes, including high denitrification rates (which probably increase with nitrate supply); the retention efficiency of allochthonous particles (which increases with hydraulic residence time); and the relatively low N : P of allochthonous particles. Future studies need to construct budgets and follow the fates of all three elements (C, N and P) in sediments, including processes such as denitrification, respiration and the mobility of P in sediments (Carignan & Flett, 1981). Furthermore, this needs to be carried out in several lakes along a land use gradient, to determine whether the convergent sediment N : P that we observed is a general phenomenon, and to ascertain the mechanisms accounting for this pattern.

Regardless of the actual mechanisms accounting for the similar sediment N : P among reservoirs, our results are reminiscent of the scale-dependent stoichiometry of seston C : N : P ratios in the oceans (Sterner et al., 2008). These ratios vary over small spatial scales but when averaged over larger scales, tend to be constrained to a ratio similar to the classic Redfield ratio. Sterner et al. (2008) proposed the ‘biogeochemical mosaic hypothesis’, i.e. that various biogeochemical processes occur under different conditions and are segregated spatially, but at larger scales these processes together yield relatively constrained stoichiometric ratios. In our study, processes such as inputs from catchments, phytoplankton uptake and denitrification are variable over spatial scales (among catchments and reservoirs) and temporal scales. However, in total these processes yield consistent ratios when integrated over a large spatio-temporal scale, i.e. sediment burial in multiple reservoirs over several decades.

The landscape-level importance of reservoirs in carbon and nutrient removal

Reservoirs can be hotspots of carbon and nutrient retention and removal, thereby decreasing downstream transport of these elements (Garnier et al., 1999; Seitzinger et al., 2002; Bukaveckas et al., 2005; Cole et al., 2007; Harrison et al., 2009). At regional scales, the ability of reservoirs to trap and remove nutrients is particularly important if this reduces nutrient transport to coastal areas that display hypoxic ‘dead zones’ (Turner & Rabalais, 2003; Diaz & Rosenberg, 2008). Agricultural areas in the Ohio River drainage, within which our study catchments lie, are major exporters of nutrients contributing to hypoxia in the Gulf of Mexico (Turner & Rabalais, 2004; Royer, David & Gentry, 2006; Alexander et al., 2009). At the scale of the entire Ohio River drainage, reservoirs are apparently important in reducing loading to the Gulf of Mexico, especially for N (Bukaveckas et al., 2005). Recent studies suggest that relatively small reservoirs, such as those we studied, may be particularly important in retaining C (Smith et al., 2002 Downing et al., 2008) and N (Harrison et al., 2009) and that the effectiveness of reservoirs in retaining N may be sensitive to changes in land use (Harrison et al., 2009). Our sediment core data suggest that the fractions of C, N and P inputs buried in sediments decrease with increasing agriculture, if we use ‘recent’ (1987–2001) Acton burial rates (Fig. 5). Whether the trends with land use hold generally across many lakes and regions is unclear and warrants further study.

If the relatively constant C : N : P ratios of sediments we observed in our study reservoirs prove to be a general trend, this has many implications for estimating C, N and P retention in reservoirs, and consequently, for estimating export to coastal areas. In particular, the relatively constant C : N : P of deeper sediments suggests that the long-term retention of any one of these elements can be predicted from retention rates of the others. This would greatly facilitate estimation of large-scale retention rates, e.g. at the scale of the Ohio River or Mississippi drainages. As mentioned earlier, we know of no other studies that have examined the long-term burial of C, N and P, so we cannot evaluate the generality of these trends.
Brahney et al. (2006) found that surface sediment C : N ratio varied threefold (from 9–27 molar) among 11 lakes in British Columbia, suggesting that sediment stoichiometry can be quite variable. The Brahney et al. (2006) study differs from ours in several ways, however. They studied only surface sediments, which may have more variable stoichiometric ratios than sediments integrated over longer periods (Fig. 6). In addition, sediment C : N ratios in the Brahney et al. (2006) lakes were strongly influenced by marine-derived N delivered to the lakes via spawning salmon, and terrestrial influences on C and N supply were viewed as minor. Clearly, more studies are needed to evaluate the variability of sediment C : N : P ratios across many lakes.

_Catchment land use and N- versus P limitation of phytoplankton_

At present, aquatic scientists are engaged in an intense debate as to whether aquatic ecosystems are primarily limited by P or if N limitation also can be persistent (Lewis & Wurtsbaugh, 2008; Schindler et al., 2008; Sterner et al., 2008; Conley et al., 2009). One view is that aquatic systems ultimately should be limited by P, because N fixation by cyanobacteria can compensate for short-term N deficits (Schindler, 1977; Schindler et al., 2008). However, an opposing view is that N limitation (or colimitation by N and P) can persist for ecologically relevant time scales, because N compensation via fixation is a relatively slow process, and because denitrification can remove large amounts of N, increasing the likelihood that N limitation is maintained (Lewis & Wurtsbaugh, 2008; Sterner, 2008; Conley et al., 2009). In our study, N limitation persisted in Burr Oak throughout the entire growing season in both years, a pattern that also is evident in other years (Vanni et al., 2006b). N limitation persisted even though heterocystous (and presumably N-fixing; Findlay et al., 1994) cyanobacteria were common; these taxa can constitute >80% of Burr Oak phytoplankton biovolume (Dickman, Vanni & Horgan, 2006). Note, however, that Burr Oak phytoplankton were secondarily limited by P on all dates (Fig. 3), indicating that they are not far from being P limited. Thus, N fixation apparently comes close to alleviating N limitation, but does fully compensate for the N deficit.

Our results thus support the view that lake phytoplankton can be N limited over ecologically mean-
seems likely that the relationship between N versus P limitation and lake productivity may be mediated in large part by land use.

Thus, our data provide empirical support for a relationship between catchment land use, nutrient export rates and ratios, in-lake N : P ratios, the relative severity of N- and P limitation and the relative abundance of potentially N-fixing cyanobacteria. From the perspective of reversing eutrophication, implications of the relationship between catchment land use and the nature of nutrient limitation are profound. Strategies to reduce agricultural nutrient supply to aquatic ecosystems differ for N versus P and will vary according to the type of agriculture (e.g. reduction of high N : P row crop fertilizers versus low N : P manure wastes). All nutrient reduction strategies involve considerable socio-economic costs (Bryhn & Hakanson, 2009; Conley et al., 2009; Schindler & Hecky, 2009). Thus, it is essential that we further our understanding of the relationships between catchment land use, the N : P ratio of catchment exports to reservoirs, the severity of N and P limitation and the relative retention of N and P by reservoirs.

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