

# Nutrient indicators of agricultural impacts in the tributaries of a large lake

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## Abstract

Lake Simcoe in Ontario, Canada, is a large lake surrounded by a mix of urban, agricultural, and less developed areas and is showing adverse effects from excess nutrient inputs, including low hypolimnetic oxygen concentrations. Knowledge of both the quantity and quality of nutrients and seston entering the lake is important because large reductions in phosphorus (P) loads have been proposed to help restore the lake and its coldwater fishery. We examined land use effects on P quality (i.e., bioavailability) and its relationship to seston in the tributaries of Lake Simcoe. Indicators of agricultural impacts were examined in 13 tributaries of Lake Simcoe, which were selected to represent a range of land use types. Bioavailability of P was assessed through analysis of different forms of P and stoichiometric indicators of nutrient status in seston. Nutrient sources were examined using the  $\delta^{15}\text{N}$  of seston. The percentage of cropland in the subwatershed had a strong relationship with P as reflected in higher soluble reactive P concentrations and lower indicators of P deficiency. Cropland land use effects were complicated; they contributed highly bioavailable P to a P deficient lake, and at the same time, contributed high seston loads causing turbidity, resulting in light deficiency. In the Lake Simcoe watershed, animal manure application on cropland could be a source of nutrients related to the  $\delta^{15}\text{N}$  variability and, correspondingly, bioavailable P. Management efforts should therefore include best management practices to reduce manure application to croplands and to prevent runoff from areas where manure is stored.

**Key words:** agriculture, Lake Simcoe, land use, manure, nonpoint sources of phosphorus, phosphorus limitation, streams, seston

## Introduction

Increased urbanization, agricultural activities, and changes to wetlands have large impacts on the amount and chemical nature of nutrients produced in the watersheds of many large lakes and how they are transported through aquatic systems. Streams draining these altered catchments show higher nutrient concentrations and changes in their stoichiometric ratios compared with their less developed counterparts (Bennett et al. 2001). Likewise, light regimes within streams are substantially altered through removal of riparian vegetation for urban or agricultural development

as well as increased turbidity as a result of these changes in land use.

Agricultural and urban activities have been well documented as potential sources of nitrogen (N) and phosphorus (P), which can ultimately lead to the eutrophication of aquatic ecosystems (e.g., Carpenter et al. 1998). Agriculture increases inorganic (nitrate;  $\text{NO}_3^-$ ) and organic N and dissolved P concentrations as a result of livestock waste and fertilizer application to cropland (e.g., Howarth et al. 1996). Further, urbanization tends to increase ammonium ( $\text{NH}_4^+$ ),  $\text{NO}_3^-$ , and dissolved P concentrations in runoff waters because of discharge of sewage and septic

effluents, as well as lawn fertilizer application (Hatt et al. 2004, O'Brien and Wehr 2010).

These changes are generally correlated with population density and impervious surface area (Walsh et al. 2005); however, relationships between land use and the forms of nutrients (an indicator of bioavailability) in streams are not always straightforward. For example, in 2 Canadian tributaries of Lake Ontario, the amount of total P (TP: determined from whole water samples and includes both the dissolved and particulate fractions) exported has declined over the last ~50 years (Malkin et al. 2010). Interestingly, in both of these urbanizing watersheds, the proportion of soluble reactive phosphorus (SRP: an operationally defined P fraction, thought to represent the most bioavailable form of P) has also declined (Malkin et al. 2010). In contrast, in primarily agricultural tributaries to Lake Erie, there is evidence that loading of SRP has increased, despite declines in TP loading. These trends have been attributed to changes in agriculture practices affecting the delivery of SRP to the lake (Joosse and Baker 2011).

Stream nutrient chemistry is controlled by the balance of exogenous and terrestrial inputs to a stream and by internal processing within a stream. The nutrient spiralling concept (Webster 1975) emphasizes the use, transformation, and retention of nutrients within stream ecosystems. Nutrient spiralling controls nutrient transport to downstream aquatic systems; thus, improved understanding of P cycling within the stream will facilitate efforts to mitigate P loading effects on downstream ecosystems.

Receiving waters can be profoundly affected by these inputs, especially if the inputs contain nutrients that limit the growth of primary producers in the receiving water, such as P for most temperate freshwaters. In both the Thames and Sandusky rivers, for example, P fluxes were strongly influenced by processes that retain and cycle P, and patterns in P retention were linked to differences in P sources and speciation, hydrology, and land use (Jarvie et al. 2011). In addition to nutrient loading from tributaries to large lake systems, algal loading to lakes may be important. This concept has been suggested by Conroy et al. (2008), who hypothesized that phytoplankton discharged by tributaries formed critical inoculums that resulted in an increase in offshore phytoplankton growth rates in Lake Erie.

The objective of this study was to examine variations in P bioavailability and nutrient sources across a range of land uses in tributaries to Lake Simcoe, Canada. We predicted that agricultural and urban land uses would have higher P bioavailability than other land uses. We also used the natural abundance of  $\delta^{15}\text{N}$  stable isotopes to assess nutrient sources and to differentiate among land use effects. We focused on the seston in the streams because

the sources of particulate organic matter (POM) and its ability to transport nutrients to coastal areas are of increasing global concern due to increasing urban and agricultural pollution of many streams (Howarth et al. 1996, Vitousek et al. 1997).

## Study site

Lake Simcoe, this study's focal watershed, is the largest lake in southern Ontario, Canada, with the exclusion of the Laurentian Great Lakes. The Lake Simcoe watershed (LSW) has a total area of 2899 km<sup>2</sup> and is fed by 35 tributaries, the majority of which originate in the southern portion of the watershed and flow northward before discharging into the lake (Palmer et al. 2011). These catchments vary in terms of stream order, land use, slope, P flux, and rainfall. Land use in the watershed is predominately agricultural (47%) with approximately 2000 crop and livestock operations, including the province's largest cultivated marsh for market gardening (the Holland Marsh), collectively generating over C\$500 million annually (Winter et al. 2007). The watershed is also home to some of the fastest growing urban centres in Canada with a projected population of 642 000 by 2031 (Palmer et al. 2011). Tributary P inputs accounted for 62% of the TP loading to the lake (OMOE 2009), and excessive P loading has been the most significant cause of water quality impairment in Lake Simcoe and its tributaries (Winter et al. 2007).

## Methods

### Field sampling

Tributaries in 13 different subwatersheds of Lake Simcoe (Fig. 1), representing the largest tributaries to the lake, were sampled in August and October 2008 and on a monthly basis from June to November in 2009. The sample sites were located at the mouths of the tributaries and are long-term monitoring sites for the Ontario Ministry of the Environment (OMOE) and the Lake Simcoe Region Conservation Authority (LSRCA; Table 1). Average daily flow rates (discharge; m<sup>3</sup> s<sup>-1</sup>) for each of the 13 tributaries corresponding with our sampling dates and sites were obtained from the LSRCA and represent a combination of gauged (O'Connor et al. 2011), and ungauged (modelled) sites (Table 1). Dissolved oxygen concentrations, pH, and temperature were obtained with a YSI multi-parameter probe (Model 85W; YSI Inc., Yellow Springs, Ohio, USA) from the middle of the water column at each site. Vertical profiles (10 cm increments) of photosynthetically active radiation (PAR) were measured with a Li-Cor cosine underwater quantum

sensor (Model LI-192SA; Li-Cor, Lincoln, Nebraska, USA). The vertical attenuation coefficient for PAR:  $k_p$ , was determined from the linear regression of the natural logarithm of irradiance versus depth.

Water sampling was conducted in the middle of the water column, and sample water was collected into 20 L darkened, low-density polyethylene containers, which were immediately placed in coolers until processed and filtered within 12 h at Trent University (Peterborough, Ontario, Canada). All of the water was prescreened through a 200  $\mu\text{m}$  mesh nylon (Nitex) filter to remove large debris and vascular plant fragments, as well as large invertebrates.

Land use classifications for the LSW were prepared by the LSRCA in 2008 using Ecological Land Classification (ELC) and Land Use Classifications (LSEMS 2006). The mapping was undertaken on a subwatershed basis using orthophotography; a grid was created for each subwatershed, and hard copies of air photos were overlain with 5 m contour and watercourse layers. The 13 tributaries sampled differed in trophic status and represented a variety of land uses including agriculture (crops and pasture), wooded areas, urban (pervious and impervious), wetlands, sod farms/golf courses, roads, quarries, and transition (open water, cultural meadow, open alvar) sites (Table 1; LSRCA, April 2011, unpubl. data).

### Biological variables

Because the periphyton communities differed greatly among our study systems depending on the substrate type and depth, we chose to use seston (a proxy for phytoplankton) as comparable representatives of the primary producers in these diverse systems. To characterize the phytoplankton populations in the 13 tributaries, representative samples from July 2009 were preserved with Lugol's iodine, followed by formaldehyde, and algae were subsequently identified to species resolution and counted using an inverted microscope. Phytoplankton counting followed the Utermohl technique (Utermohl 1958), wherein half the chamber was counted at magnifications of 225 $\times$  for large or less numerous cells, and a 200  $\mu\text{m}$  wide strip was counted at magnifications of 900 $\times$  for small numerous cells. If cells were so numerous as to exceed 10 cells per field, random fields were counted. Results were reported as biomass. Sample water for chlorophyll *a* (Chl-*a*) analysis was filtered onto glass fiber filters (GFF: nominal pore size 0.7  $\mu\text{m}$ , 47 mm) and then frozen ( $-20\text{ }^\circ\text{C}$ ) and stored in the dark before passive extraction with 90% acetone. The extracts were quantified by fluorometry with corrections for phaeopigments (via acidification); the fluorometer was calibrated annually with pure Chl-*a* (Furgal et al. 1998).

### Water chemistry

Phosphorus bioavailability was examined using the different forms of P (TP, total dissolved P [TDP], particulate P, and SRP), as well as stoichiometric indicators of nutrient status of the seston (particulate N:particulate P ratios and particulate C:particulate P ratios; Healey and Hendzel 1979).

Whole water TP samples (including both the dissolved and particulate fractions), filtered (0.2  $\mu\text{m}$ ) TDP (OMOE 2007a), and nitrate+nitrite (herein referred to as  $\text{NO}_3^-$ ) samples (OMOE 2007b) were analyzed using standard colourimetric methods. Samples were filtered (0.45  $\mu\text{m}$ ) and acidified for analysis of dissolved organic carbon (DOC) using a Shimadzu TOC-V<sub>CPH</sub> Analyzer (Shimadzu Scientific Instruments, Columbia, Maryland, USA).

To avoid cell breakage during forced vacuum pump (<5 psi) filtration, passive filtration with dialysis membrane was employed (Taylor 2010) to determine SRP concentrations. Sample water was incubated in 5 L covered containers on the bench top at room temperature for 12 h. Dialysis tubing (Spectra/Por Biotech cellulose ester, 100 000 m.w. cut-off, 30 mm diameter) was filled with Milli-Q water, sealed at both ends, and placed in the containers to equilibrate for 12 h. The dialyzate was then analysed according to Stainton et al. (1977) with a detection limit of 12 nM P.

Sample water for particulate organic carbon (POC) and N analyses was filtered onto precombusted (450  $^\circ\text{C}$  for 4 h) GFF (nominal pore-size 0.7  $\mu\text{m}$ , 47 mm), which were immediately dried and stored in a desiccator until analysis. Carbonates were removed from the POC filters by fumigation using concentrated hydrochloric acid (37%) in a desiccator for 4 h. The particulate N samples were non-acidified and thus analyzed independently to POC (Brodie et al. 2011). The dried filters were analyzed on a MACRO CNS analyzer (Elementar, Hanau, Germany). Particulate P was measured using the persulphate digestion method in an autoclave (Parsons et al. 1984).

### Stoichiometric indicators of nutrient deficiency

POC:Chl-*a* ratios were calculated to represent both a general indicator of nutrient status, as well as an indicator of phytoplankton adaptation and acclimation to changing light conditions (Healey and Hendzel 1979). We applied molar ratios of particulate C: particulate P and particulate N: particulate P as stoichiometric indicators of P deficiency in phytoplankton (Healey and Hendzel 1979). According to the P limitation criteria for particulate C:P ratios, values >258 are indicative of severe P deficiency, 129–258 are indicative of moderate P deficiency, and values <129 are not considered to be P deficient. The P limitation criteria

**Table 1.** Summary of Lake Simcoe subwatershed characteristics including the annual average TP load from 2004–2007 (OMOE and LSRCA 2009) and area of the 13 study tributaries. Trophic status was determined from TP concentrations measured during this study and classified according to Wetzel (2001). The discharge (Q) and attenuation coefficient ( $k_d$ ) arithmetic means and standard error of the mean in parentheses are shown for the sampling dates for this study ( $n = 8$ ). Land use distribution is shown by the percentage of each subwatershed covered by the 10 land uses identified (based on data from the LSRCA 2008).

Tributary	Trophic Status	TP loading $\text{kg yr}^{-1}$	Area $\text{km}^2$	Q $\text{m}^3 \text{s}^{-1}$	$k_d$ $\text{m}^{-1}$	Ag Crops %	Ag Pasture %	Wooded Area %	Urban P %	Urban IP %	Wetland %	Sod/Gf %	Road %	Quarry %	Trans %
East Holland River	E	8937	247	0.96 <sup>a</sup> (0.20)	3.20 (0.61)	17	11	16	7	23	10	5	4	0	7
West Holland River	E	7659	105	1.33 <sup>b</sup> (0.21)	2.02 (0.29)	43	18	16	6	5	4	0	2	0	6
Hotohkiss Creek	M	4514	38	0.23 <sup>b</sup> (0.03)	1.17 (0.70)	1	3	10	7	57	3	0	14	2	4
Black River	M	3705	375	1.45 <sup>a</sup> (0.24)	1.76 (0.15)	21	16	21	6	3	23	3	1	1	6
Pefflerlaw Brook	M	3203	446	2.86 <sup>a</sup> (0.37)	2.60 (0.68)	22	22	20	5	3	17	2	1	2	6
Beaver River	M	3179	327	1.59 <sup>a</sup> (0.23)	1.73 (0.22)	28	35	8	2	1	19	1	1	1	4
Leonards Creek	M	2675	107	0.65 <sup>b</sup> (0.10)	1.10 (0.10)	27	18	17	4	14	11	1	2	1	5
Bluffs Creek	O	2483	75	0.46 <sup>b</sup> (0.06)	0.80 (0.14)	10	25	28	5	12	11	0	2	2	5
Talbot River	M	2008	71	2.20 <sup>b</sup> (0.28)	1.04 (0.13)	8	44	20	1	2	12	3	1	2	6
Maskinonge River	E	1118	63	0.19 <sup>a</sup> (0.02)	2.57 (0.28)	43	15	9	3	4	8	13	2	0	4
Whites Creek	M	1053	105	0.63 <sup>b</sup> (0.08)	2.70 (1.02)	20	40	13	1	1	19	0	1	0	5
Lovers Creek	M	804	60	0.44 <sup>a</sup> (0.10)	1.50 (0.15)	27	7	17	5	18	13	2	6	0	4
Hawkestone Creek	O	318	48	0.27 <sup>a</sup> (0.05)	1.73 (0.16)	11	23	29	3	2	21	1	1	2	7

Ag = agricultural; P = pervious; IP = impervious (excluding roads); Sod/GF = sod farm/golf course; Trans = transition; O = oligotrophic; M = mesotrophic; E = eutrophic <sup>a</sup>gauged discharge; <sup>b</sup>modeled discharge

for molar particulate N:P ratios (Healey and Hendzel 1979) states that values >22 are indicative of P deficiency, while values <22 are not considered to be P deficient.

### $\delta^{15}\text{N}$ stable isotope analyses

Sample water for POM isotopic analysis (defined as particles between 0.7 and 33  $\mu\text{m}$ ) was obtained by filtering lake water that was previously screened with a 33  $\mu\text{m}$  mesh onto precombusted (450 °C for 4 h) GFF (nominal pore-size 0.7  $\mu\text{m}$ , 47 mm), which were immediately dried and stored in a dessicator until analysis. Nitrogen isotope ratios of POM samples were determined using a Euro Elemental Analyzer and a Micromass IsoPrime Continuous Flow Isotope Ratio Mass Spectrometer in the Water Quality Centre at Trent University.  $^{15}\text{N}$  stable isotope values were expressed as  $\delta$  (units of parts per thousand; i.e., per mil [‰]) and calculated as follows:

$$\delta\text{‰} = \left( \frac{R_{\text{sample}}}{R_{\text{reference}}} - 1 \right) * 1000, \quad (1)$$

where  $R_{\text{sample}}$  and  $R_{\text{reference}}$  are the sample and reference (pure gas) isotope ratios ( $^{15}\text{N}/^{14}\text{N}$ ). Several international and internal standards were used for calibration of the isotopic data. The calibration standards were USGS 40 ( $\delta^{15}\text{N} = -4.5\text{‰}$ , National Institute of Standards and Technology) and USGS 41 ( $\delta^{15}\text{N} = 47.6\text{‰}$ , National Institute of Standards and Technology). Analytical reproducibility was  $\pm 0.2\text{‰}$ .

### Statistics and data analysis

Prior to analysis, variables were transformed to meet the conditions of normality as assessed by a Shapiro-Wilk test ( $p > 0.05$ ) with the exception of the  $\delta^{15}\text{N}$  values, which did not require transformation. All land cover measures were arcsine transformed; flow, Chl-*a*, SRP, TP, particulate P,  $\text{NO}_3^-$ , and DOC concentrations were  $\log_{10}$  transformed, and particulate C:P, particulate N:P, TDP, and POC:Chl-*a* were  $\log_{10}(X+1)$  transformed (Zar 2010).

Tributary water quality can be influenced by stream flow and time of year (e.g., seasonality). Pearson correlation tests determined that there was no relationship between flow (Table 1) and any of our water chemistry measurements; therefore, we concluded that in our dataset, tributary flow rates did not significantly influence nutrient concentrations, and thus we did not flow-adjust our water chemistry.

To address the fundamental limitation presented by multicollinearity of land-cover class percentages (King et al. 2005) we used hierarchical partitioning analysis (HP; Chevan and Sutherland 1991) to evaluate the independent influence of the 10 land-use conditions on each measured

variable. HP is a protocol in which all possible models in a multiple regression setting are jointly considered to attempt to identify the most likely causal factors; it is a proven method for identifying environmental variables that are most likely to be causal drivers of pollutant concentrations in streams (Mac Nally 2000, Hatt et al. 2004). HP allowed the identification of variables whose independent correlation with the dependent variable was strong, in contrast to variables that have little independent effect but have a high correlation with the dependent variable resulting from joint correlation with other independent variables.

Our HP analyses were carried out with the hier.part package (Walsh and McNally 2008) in the statistical software R version 2.14.0 (R Development Core Team 2011) using multiple linear regression models and  $R^2$  as a measure of model fit. Due to the potential bias associated with the use of 10 explanatory variables (Olea et al. 2010), we ran the model 100 times using different ordering of the 10 variables; the results of these tests were similar. Variables that independently explained a larger proportion of variance than could be explained by chance were then identified by comparison of the observed value of independent contribution to explained variance (I) to a population of Is from 100 randomizations of the data matrix. Significance was accepted at the upper 95% confidence limit (Z-score  $\geq 1.65$ : Mac Nally 2002).

Because HP analyses does not report on the nature (directionality) of the relationships for the predictors it identifies, we subsequently employed Pearson correlations and reported the correlation coefficients ( $r$ ; Table 2). For the correlations,  $p$ -values were corrected for potential false discovery rates based on the significance level of 0.05 (Benjamini and Hochberg 1995).

The most important land use variables identified by HP and correlation analyses were the percentage of cropland, wooded area, and sod farms/golf courses (each had 3 significant relationships with nutrient indicators; Fig. 2; Table 2). The ranges in the percentage of each of the 13 subwatersheds covered by these 3 land uses were 1–43% (cropland), 8–29% (wooded area), and 0–13% (sod farms/golf courses; Table 1). Although the relationships with sod farms/golf courses could be important, due to the small percentage of the watershed this land use represented in the dataset it was deemed not suitable for further detailed analyses. Additionally, cognizant of the water quality concerns associated with Lake Simcoe, we chose to focus on land uses that had a negative impact on water quality (i.e., a positive relationship with nutrient concentrations); therefore, we did not further examine the relationships associated with wooded area in the watershed.

Because the percentage of cropland in the subwatershed had a strong relationship with the  $\delta^{15}\text{N}$  (POM; indicator of

**Table 2.** Correlation matrix for the predictors identified as significant by hierarchical partitioning (Fig. 2) of land use effects on nutrient indicators in the tributaries to Lake Simcoe. Values represent correlation coefficients ( $r$ ).

Parameter	Ag (crops)	Wooded Area	Urban (IP)	Sod/Gf	Quarries
POC:Chl- <i>a</i>	<b>-0.227*</b>		0.175		
Nitrate			<b>0.509*</b>		
TP		<b>-0.438*</b>		<b>0.493*</b>	
TDP				<b>0.515*</b>	<b>-0.606*</b>
Particulate P		<b>-0.451*</b>			
SRP	<b>0.316*</b>				
C:P		<b>0.389*</b>			
N:P				<b>-0.239*</b>	
$\delta^{15}\text{N}$ (POM)	<b>0.585*</b>				

Correlations that were significant at  $p < 0.05$  and after correcting for false discovery rate are printed in bold and with an asterisk (\*), respectively.

nutrient sources), combined with its positive relationship with P bioavailability, we felt that detailed examination of the relationships associated with cropland was warranted. Thus, the 13 tributary stations were categorized by percentage in 10% increments of cropland into 4 groups ranging from 0 to 50% (there were no sites in the 30–40% range). Two-way analysis of variance (ANOVA) was used to subsequently test for differences by month and cropland category, and a Tukey-Kramer post hoc test was employed with a significance value of  $p < 0.05$  (Table 3).

## Results

### Predictors of land use effects on water quality parameters

The relationships between the 10 different land uses in the LSW and 11 representative water quality parameters were examined. The HP analyses revealed that 5 of the 10 land uses had significantly greater independent explanatory power than the other land uses (Fig. 2). The independent influences of the 5 different land uses (cropland, wooded area, urban impervious, sod farms/golf courses, and quarries) on water quality were not consistent across the variables measured. Detailed analyses of the significant relationships (as identified by HP) were conducted, revealing that the most important land uses were the percentage of cropland, wooded area, and sod farms/golf courses (Fig. 2; Table 2). Increases in the percentage of cropland and sod farms/golf courses were further identified through correlation analyses as the 2 land uses having a detrimental effect on water quality (Table 2). Due to the low percentage of sod farms/golf courses in the LSW (Table 1), and because the tributary with 13% of its area dedicated to this land use (Maskinonge; Fig. 1) also had

43% cropland in its subwatershed, it was impossible to separate the effects of sod farm/golf courses and cropland land uses, which further limited our analyses of these potential impacts. As a result, detailed analyses were only undertaken to examine the effect of the percentage of cropland on indicators of water quality over the growing season.

There was a significant, positive relationship between phytoplankton biomass and Chl-*a* concentrations ( $r = 0.885$ ,  $p < 0.0005$ , data not shown), indicating that Chl-*a* concentrations were representative of phytoplankton at the mouths of the 13 tributaries to Lake Simcoe. The average tributary Chl-*a* concentration was  $1.92 \pm 0.29 \mu\text{g L}^{-1}$ , with values ranging from  $0.04 \mu\text{g L}^{-1}$  (Leonards) to  $18.6 \mu\text{g L}^{-1}$  (West Holland). Agriculturally dominated tributaries with 40–50% cropland, such as the West Holland River, had significantly higher (3.4 times) Chl-*a* concentrations than all other tributaries, with no significant differences among months, as indicated by ANOVA (Table 3). Despite the range in trophic status (Table 1), Chl-*a* concentrations were not significantly related to any of the 10 land use categories (Fig. 2); however, the percentages of cropland and urban (impervious) areas were significant predictors of POC:Chl-*a* ratios and explained 20 and 26%, respectively, of the variance (Fig. 2; Table 2). These 2 land uses had opposite effects on the POC:Chl-*a* ratio, as evidenced by the significantly negative relationship with the percentage of cropland, and the significantly positive relationship with the percentage of urban (impervious) land area (Figs. 2 and 3; Table 2). Neither the monthly differences between POC:Chl-*a* ratios nor the cropland area as indicated by ANOVA was significant (Fig. 3; Table 3).

DOC concentrations were not significantly related to any of the 10 land use categories (Fig. 2); however, there were significantly higher DOC concentrations in

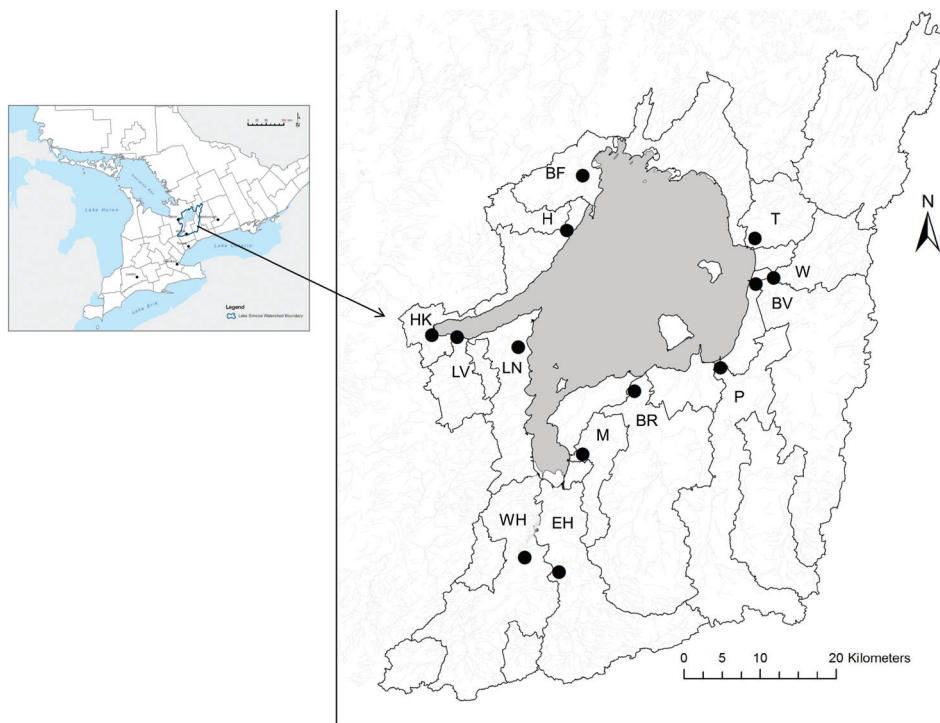
tributaries dominated (40–50%) by cropland relative to tributaries with 0–10% cropland (Table 3). Urban (impervious) area was a significant predictor of  $\text{NO}_3^-$  concentrations and explained 21% of the variance, resulting in a significantly positive relationship (Fig. 2; Table 2). There was also an association between  $\text{NO}_3^-$  concentrations and the percentage of cropland, with significantly lower  $\text{NO}_3^-$  concentrations in tributaries with 40–50% more cropland than the other 3 categories (Table 3). The higher percentage of cropland was associated with higher DOC concentrations but lower  $\text{NO}_3^-$  concentrations, while tributaries with a high percentage of urban (impervious) area had higher  $\text{NO}_3^-$  concentrations.

The percentage of wooded area and sod farms/golf courses were significant predictors of TP concentrations and explained 20 and 18%, respectively, of the variance. These 2 land uses had opposite effects on TP concentrations, as shown by the significantly negative relationship with the percentage of wooded area and the significantly positive relationship with the percentage of sod farms/golf courses (Fig. 2; Table 2). Throughout the growing season, TP concentrations decreased from June to November; however, these differences were not significant (Fig. 4; Table 3). Tributaries with 40–50% cropland had significantly higher (2.4 times) TP concentrations than all other tributaries, as indicated by ANOVA (Table 3). The relationship between TP load (Table 1) and land use was also

examined, and the only significant positive correlations were between TP load and the percentage of urban pervious ( $r = 0.631$ ,  $p < 0.0001$ ) and impervious ( $r = 0.315$ ,  $p = 0.004$ ) land areas.

The percentage of sod farms/golf courses and quarries were significant predictors and explained 18 and 25%, respectively, of the variance in TDP concentrations (Fig. 2; Table 2). Once again, these relationships trended in opposite directions, with TDP concentrations having a significant positive relationship with the percentage of sod farms/golf courses and a significant negative relationship with the percentage of quarries (Fig. 2; Table 2). As with TP concentrations, tributaries with 40–50% cropland had significantly higher (2.5 times) TDP concentrations than all other tributaries, and there were no significant differences among months, as indicated by ANOVA (Fig. 4; Table 3).

The relationship between TDP and SRP for this dataset has been previously analyzed (Whitehead et al. 2011) and showed a significant positive linear relationship. The percentage of wooded area was a significant predictor of particulate P concentrations (25% of the variance), with a significantly negative relationship (Fig. 2; Table 2), and displayed the same relationships with percentage of cropland and among months as both TP and TDP concentrations (Fig. 4; Table 3). The percentage of cropland was a significant predictor of SRP concentrations (explained 15% of the variance), and they had a positive relationship



**Fig. 1.** The Lake Simcoe study area, Ontario, Canada; watershed is delineated in black, showing the 13 sampled tributaries to Lake Simcoe. BR = Black River; BV = Beaver River; W = Whites Creek; T = Talbot River; P = Pefferlaw Brook; M = Maskinonge; WH = West Holland River; EH = East Holland River; LV = Lovers Creek; BF = Bluff's Creek; HK = Hotchkiss Creek; LN = Leonards Creek; H = Hawkestone Creek.

**Table 3.** Two-way ANOVA and Tukey-Kramer post hoc comparisons between percentage of cropland and between month effects on indicators of agricultural land use. Post hoc tests were conducted if ANOVA factors were identified as significant ( $p < 0.05$ ). The letters for the post hoc comparison indicate statistical significance ( $p < 0.05$ ); the relationship between identical letters is not statistically significant, whereas the relationship between different letters is significant.

Parameter	% of cropland	Post hoc test				Seasonal	Post hoc test					
		0–10	10–20	20–30	40–50		Jn	Jl	A	S	O	N
Chl- <i>a</i>	$F_{3,99}=7.639, p<0.0005$	a	a	a	b	$F_{5,97}=1.415, p=0.226$						
POC:Chl- <i>a</i>	$F_{3,98}=1.767, p=0.159$					$F_{5,96}=1.435, p=0.219$						
DOC	$F_{3,79}=17.905, p<0.0005$	a	b	bc	c	$F_{5,79}=0.760, p=0.581$						
Nitrate	$F_{3,79}=7.141, p<0.0005$	a	a	a	b	$F_{5,79}=1.718, p=0.140$						
TP	$F_{3,99}=13.032, p<0.0005$	a	a	a	b	$F_{5,97}=1.619, p=0.162$						
TDP	$F_{3,74}=11.055, p<0.0005$	a	b	ab	c	$F_{5,72}=0.745, p=0.592$						
Particulate P	$F_{3,99}=10.696, p<0.0005$	a	a	a	b	$F_{5,97}=0.586, p=0.711$						
SRP	$F_{3,84}=5.228, p=0.002$	a	ab	a	b	$F_{5,81}=0.759, p=0.582$						
C:P	$F_{3,98}=6.332, p=0.001$	a	ab	bc	c	$F_{5,96}=0.935, p=0.462$						
N:P	$F_{3,98}=1.314, p=0.274$					$F_{5,96}=4.513, p=0.001$	ab	ab	a	b	b	ab
$\delta^{15}\text{N}$ (POM)	$F_{3,52}=10.937, p<0.0005$	a	b	b	b	$F_{4,51}=0.324, p=0.861$						

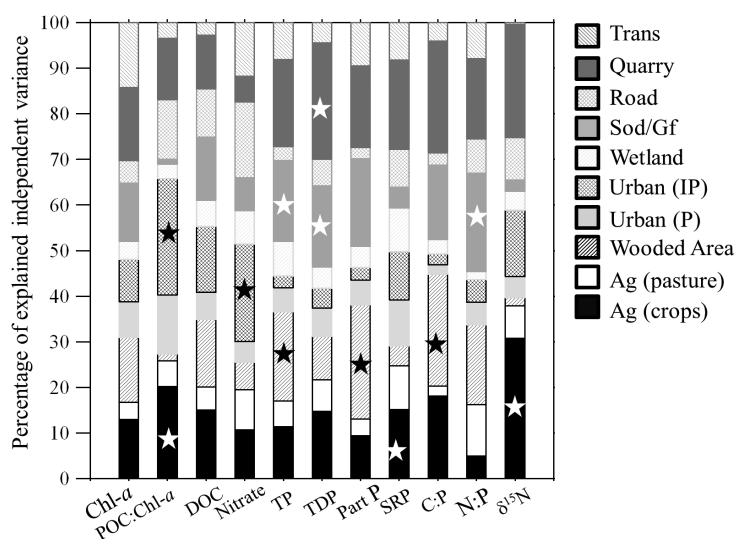
Jn = June; Jl = July; A = August; S = September; O = October; N = November

(Fig. 2; Table 2). Tributaries with 40–50% of cropland area had significantly higher SRP concentrations than the 0–10% (4.5 times lower) and the 20–30% (3.1 times lower) categories (Fig. 4; Table 3). There were also no significant differences among months, as indicated by ANOVA (Figs. 3 and 4; Table 3). Tributaries with a higher percentage of wooded area had lower P concentrations,

while tributaries with a higher percentage of sod farms/golf courses and cropland had higher P concentrations.

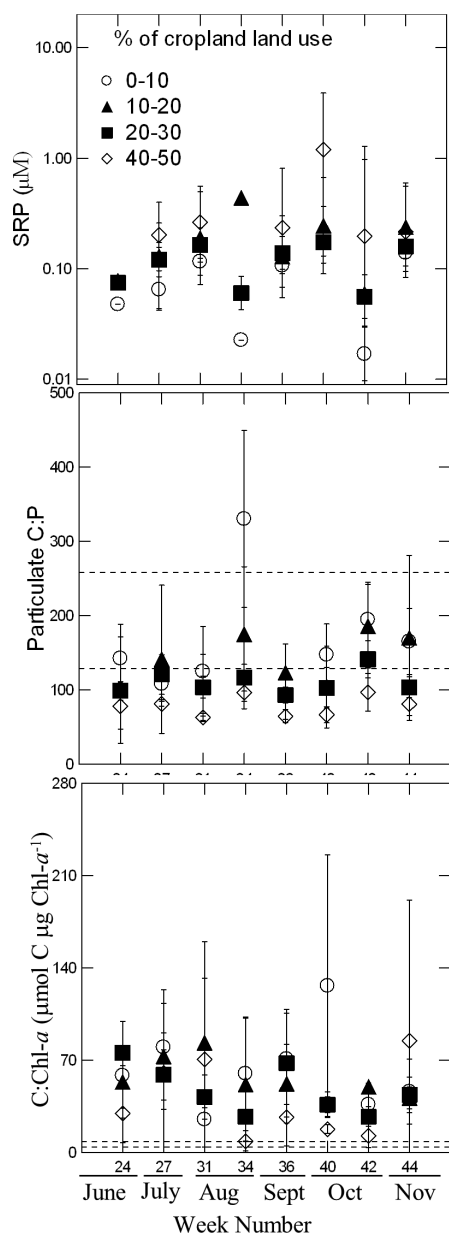
Molar ratios of particulate C:P and N:P were used as stoichiometric indicators of P deficiency. The average particulate C:P ratio for all of the study sites was not considered to be P deficient ( $123 \pm 7, n = 102$ ). The HP analysis showed that the percentage of wooded area was a significant predictor of particulate C:P ratios (25% of the variance) and displayed a positive relationship (Fig. 2; Table 2). Particulate C:P ratios indicating moderate P deficiency were significantly higher in the 0–10% cropland category compared to the 40–50% cropland category, where they were not considered to be P deficient (Fig. 3; Table 3). There were no significant differences in C:P ratios among months (Fig. 3; Table 3), although we did observe an increase in P deficiency in the early fall with values in the moderately P deficient range. There was also an increase to extreme P deficiency in particulate C:P ratios at the end of August in the 0–10% cropland area category (Fig. 3).

The average particulate N:P ratio for all of the study sites was considered to be P deficient ( $44 \pm 9, n = 102$ ). The percentage of sod farms/golf courses were significant predictors of particulate N:P ratios (22% of the variance) and had a negative relationship (Fig. 2; Table 2). There were significant differences among months, with August having significantly higher ratios than both



**Fig. 2.** Hierarchical partitioning results for variables on 10 land uses. Variables marked with a star were identified as significant independent correlates ( $Z \geq 1.65$ ) over the hierarchy of models. Agcrops = agriculture (crops); Agpast = agriculture (pasture); Urbanperv = urban (pervious); Urbanimperv = urban (impervious); Sodgolf = sod farm/golf course; trans = transition.





**Fig. 3.** Monthly cropland land use (%) patterns in SRP concentrations (log scale,  $n = 88$ ), particulate C:P ratios ( $n = 103$ ), and POC:Chl-*a* ratios ( $n = 102$ ). Error bars represent one standard error of the mean of monthly measurements over the study duration. The 13 tributary stations were grouped by percentage of cropland land use into 4 groups shown in legend (there were no sites in the 30–40% cropland land use range). In the particulate C:P molar ratios figure, the dashed lines represent criteria for P deficiency as defined by Healey and Hendzel (1979). Values above the upper dashed lines are indicative of severe P deficiency, while values between the upper and lower dashed lines are indicative of moderate P deficiency, and values below the dashed lines are not considered to be P deficient. In the POC:Chl-*a* figure, the dashed lines represent criteria for nutrient deficiency as defined by Healey and Hendzel (1979). Values above the upper dashed lines are indicative of severe nutrient deficiency.

September and October, as indicated by ANOVA (Table 3). Land use with a higher percentage of cropland had significantly lower indicators of P deficiency than nonagricultural tributaries, and the maximum indicator of P deficiency occurred in August.

### Nutrient sources

The average  $\delta^{15}\text{N}$ -POM for our dataset was  $5.08 \pm 0.27\%$  ( $n = 56$ ). Results from our HP analysis revealed that the percentage of cropland area was a significant predictor of  $\delta^{15}\text{N}$ -POM (31% of the variance) and had a positive relationship (Fig. 2; Table 2). Tributaries that had a low percentage (0–10%) of cropland in their subwatershed had significantly lower (1.9 times)  $\delta^{15}\text{N}$  values than all other categories, with no significant differences among months, as indicated by ANOVA (Fig. 5; Table 3). The average  $\delta^{15}\text{N}$  of POM of the 2 tributaries (Maskinonge and West Holland rivers) with the highest percent of cropland area (40–50%) was  $6.7 \pm 0.8\%$  ( $n = 9$ ).

### Discussion

Our data suggest that land use strongly influenced P bioavailability in streams within the LSW. Of the 10 land uses assessed, the most important were the percentage of cropland, wooded area, and sod farms/golf courses. Increases in the percentage of cropland and sod farms/golf courses were further identified as the 2 land uses having a detrimental effect on water quality in the Lake Simcoe tributaries. Our study design did not allow us to directly test the effect of sod farms and golf courses; however, we believe they may be important and should be the focus of future studies. Although the tributaries of the LSW covered a large range in both size and discharge, the ones with the highest percentage of cropland were large rivers. These rivers were identified as having a strong relationship with P bioavailability as reflected in the higher P concentrations (for all forms measured) and lower indicators of P deficiency.

Tributaries with a high percentage of cropland also displayed indications of poor light environments, evidenced by high light attenuation coefficients and possibly resulting in augmented Chl-*a* concentrations due to low light conditions. Our seston-based nutrient status measurements indicate that assimilation of P by phytoplankton may have been occurring within the tributaries, suggesting that nutrients measured at the watershed outlets are affected by processes that retain and cycle P. One major concern about seston stoichiometry and interpretation in freshwater is the possible contribution of terrestrial detritus to the suspended particles found in streams. We acknowledge that the seston represents a combination of

live and dead phytoplankton, bacteria, and detritus and are concerned that allochthonous detritus can make a substantial contribution to the POM, distorting the stoichiometric ratios away from the actual ratios of the *in vivo* phytoplankton. We assumed, however, that a major fraction of detritus is derived from autotrophs; thus, the seston carries the stoichiometric and biochemical footprints of the phytoplankton.

### Nutrient deficiency in streams

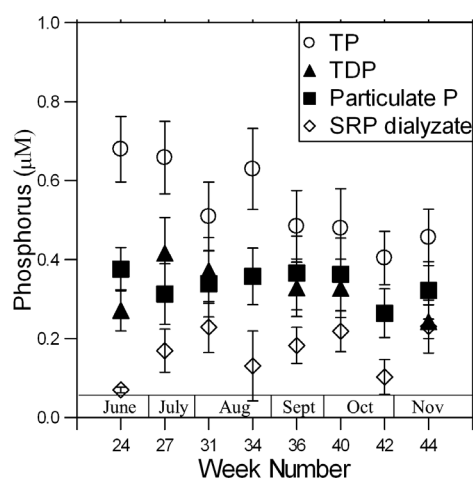
Over the growing season, our study streams were considered moderately or not P deficient, depending on the indicator used; however, the agricultural tributaries with a high percentage of cropland always indicated P sufficiency with low particulate N:P and particulate C:P ratios. Row-crop agriculture is associated with higher nutrient enrichment of stream ecosystems due to extensive channel modifications than low-intensity agricultural livestock production in pastures, which can have a much less pronounced effect (Strayer et al. 2003). A study in north-central Pennsylvania, USA, in no-till agricultural soils found that SRP concentrations increased 3- to 28-fold above background levels whenever manure was broadcast to nearby field soils (Kleinman et al. 2009).

High TP loading for streams associated with golf courses (Winter and Dillon 2006) and sod farm agriculture (Chebud et al. 2011) have been previously reported. Unfortunately, the low percentage of these land uses in the LSW, combined with the overlapping effect of cropland dominance in the same subwatershed, prevented us from fully examining these relationships with nutrient concentrations. Urbanization has also been associated with high SRP concentrations (Wiley et al. 1990) and increases in algal abundance and decreases in nutrient limitation (Bowman et al. 2005). For example, in the majority of the 36 streams studied, urban land use was found to relieve P limitation of periphyton (lower N:P ratios), while increasing the N limitation (greater C:N ratios) relative to reference streams (O'Brien and Wehr 2010). We saw no evidence of this in the LSW, but the previous studies measured periphyton while we report on suspended seston responses to urban land use. Although we did find that the percentage of urban area was correlated with TP load, the lack of a relationship with nutrient deficiency and algal abundance may also be due to an under-representation of urban streams in our study because only one tributary (Hotchkiss Creek) was predominately urban (57%). In addition, Hotchkiss Creek runs directly through the City of Barrie with a population density of 1668 km<sup>-2</sup>, while the O'Brien and Wehr (2010) study was located in south-eastern New York State, USA, with population densities close to 8000 km<sup>-2</sup>. Due to the mixed land use nature of our study streams, we may not

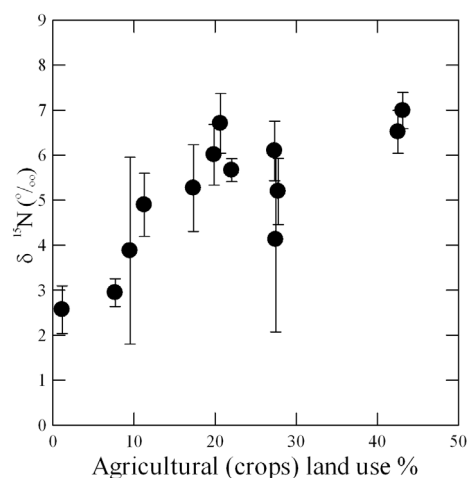
have had sufficient sod farm/golf courses or urban areas to truly assess these relationships.

### Seston stoichiometry

There was good agreement between our particulate C:P and particulate N:P ratios as indicators of P deficiency in our study streams, as reflected by their inverse relationships with SRP concentrations; however, our use of particulate C:P and particulate N:P ratios to indicate P deficiency in streams should be interpreted with caution. Typically, a combination of physiological assays and seston nutrient ratios are preferred for assessing the nutrient status of phytoplankton (Healey and Hendzel 1980). We found that physiological assays to assess



**Fig. 4.** Monthly distribution of the forms of phosphorus (P). Weekly means of P concentrations including total P (TP;  $n = 103$ ), total dissolved P (TDP;  $n = 78$ ), particulate P (PP;  $n = 103$ ), and soluble reactive P (SRP;  $n = 88$ ). Error bars represent one standard error of the mean.



**Fig. 5.** Seasonal means of  $\delta^{15}\text{N}$  (‰) of particulate organic matter (POM;  $n = 56$ ) with error bars representing one standard error of the mean.

nutrient deficiency of phytoplankton in streams are not reliable due to high microbial activity (RL North, Trent University, 2012, unpubl. data) and benthic processes; thus, we relied on seston stoichiometry as one of the simplest and most comparable ways of characterizing nutrient status of phytoplankton. Due to the predominance of heterotrophic activity in streams (Dodds et al. 2004), it is imperative to gauge the effect of land use on C availability as well as nutrient availability (i.e., a stoichiometric approach) to understand fully how heterotrophic nutrient status may be mitigated by other controlling factors (Johnson et al. 2009). The use of particulate ratios is also appealing because they are an integrative metric that has a “memory” of nutrient conditions (Davies et al. 2010) in the upstream environment.

Correction for detrital contributions to particulate nutrient concentrations is complicated and operates under the assumption that the POC:Chl-*a* ratio is constant. Multiple influences, such as light and nutrient limitation may interact in opposing ways to produce a realized POC:Chl-*a* ratio differing significantly from that which would be produced by a single process alone. Nutrient limitation can lead to marked increases in POC:Chl-*a* ratios (Healey and Hendzel 1979), making them unreliable indicators of terrestrial influence.

### Light environment as related to land use

The discordance between POC:Chl-*a* ratios and the nutrient status indicators (particulate C:P and N:P), implies that the POC:Chl-*a* ratios may be more indicative of light conditions than nutrients in this study. Although the average POC:Chl-*a* ratio for all of the study sites could also be considered nutrient deficient ( $52 \pm 4$ ,  $n = 102$ ; Healey and Hendzel 1979), we assumed that the differences in the POC:Chl-*a* ratios between land uses was indicative of light deficiency (low POC:Chl-*a* ratios). We acknowledge that differences in detritus load from upstream locations could also contribute to differences in the ratio; however, the decreasing POC:Chl-*a* ratios with increasing percentage of cropland we observed were a reflection of increasing Chl-*a* concentrations and not decreases in the particulate C concentrations.

Phytoplankton populations at our agriculturally impacted sites seem to be adapted and/or acclimated to low light availability, possibly as a result of high turbidity within the water column as indicated by the high light extinction coefficients. Agricultural cropland provides bioavailable P and seston to downstream systems while also changing the light environment, which the phytoplankton are responding to by increasing Chl-*a* content per cell; thus, light can become the limiting factor in agriculturally impacted streams. Our data indicate that light availability

is a strong driver of algal biomass in our study streams, potentially obscuring the effects of land use on nutrient deficiency, an observation consistent with the results of a broad-scale study in the USA (Johnson et al. 2009).

### Stable nitrogen isotopes as indicators of anthropogenic nutrient sources

The  $\delta^{15}\text{N}$  value of POM can be used as an indicator of anthropogenic nutrient inputs to aquatic systems by tracking the source of particulate matter (Kendall et al. 2001). The extent and magnitude of N fractionation is difficult to estimate, however; therefore, our use of  $\delta^{15}\text{N}$ -POM to trace origins of N is recognized as a qualitative technique. Although N isotope geochemistry is complex and affected by a suite of processes including denitrification, N assimilation, and  $\text{N}_2$  fixation, increasingly enriched  $\delta^{15}\text{N}$  values are often associated with an increasing input from animal wastes (Kendall et al. 2001, Wayland and Hobson 2001). Our  $\delta^{15}\text{N}$ -POM values can thus be interpreted as indicators of agricultural impacts because we assume the N signal becomes incorporated in the aquatic life that eventually becomes POM, serving as an integrator of underlying isotopic variability in dissolved inorganic N. A survey of N isotopic composition of POM from 4 large river systems across the United States concluded that elevated  $\delta^{15}\text{N}$  values at some sites reflected inputs from animal waste. They report an average  $\delta^{15}\text{N}$ -POM of  $4.4 \pm 3.4\%$  (Kendall et al. 2001), similar to our dataset average. Nitrogen derived from organic manure usually has  $\delta^{15}\text{N}$  values around 5‰ and is often further elevated through ammonia volatilization during storage and application resulting in values between 5 and 20‰ (Macko and Ostrom 1994, Kendall et al. 2001).

Using primary consumer stable N isotopes, Vander Zanden et al. (2005) were able to quantitatively link urban and agricultural land use in the watershed with elevated biotic  $\delta^{15}\text{N}$ . Other studies have also found strong correlations between agricultural land use and  $\delta^{15}\text{N}$  in streams (Harrington et al. 1998, Anderson and Cabana 2005). Godwin et al. (2009) found that the  $\delta^{15}\text{N}$  content of periphyton increased from the headwaters to downstream and attributed it to anthropogenic inputs of N to the stream. Their values were characteristic of agricultural runoff enriched with fertilizers and domestic animal waste. The use of the  $\delta^{15}\text{N}$ -POM signature in our dataset as an integrator of upstream land cover suggests that manure could be an important source of nutrients to the tributaries. The observed increasing  $\delta^{15}\text{N}$  values with the percentage of cropland area seem to be related to potential animal manure sources that are frequently applied to agricultural lands in both the West Holland (LSRCA 2010) and Maskinonge River (LSRCA 1998) subwatersheds.

## Management implications for Lake Simcoe

On average, the TP concentrations for the Lake Simcoe tributaries are below the Provincial Water Quality Objective (PWQO) for TP ( $0.97 \mu\text{M}$ ); however, the seasonal average for 3 of the 13 subwatersheds, exceed this threshold. The two predominately agricultural (cropland) tributaries, the West Holland and Maskinonge, had seasonal mean TP concentrations of 1.89 and 2.11  $\mu\text{M}$ , respectively, indicating that agricultural land use plays a substantial role in contributing to TP concentrations; however, the Maskinonge subwatershed also had the highest percentage of sod farm/golf courses. The tributary draining a large percentage (34%) of urban area and road, the East Holland, also exceeded the PWQO with a seasonal mean TP concentration of 2.60  $\mu\text{M}$ .

Extensive efforts to reduce TP loading from the LSW have been somewhat successful, resulting in a TP load reduction from more than 100 tonnes  $\text{yr}^{-1}$  during the 1990s to 72 tonnes  $\text{yr}^{-1}$  in 2002–2007 (OMOE and LSRCA 2009); however, a better understanding of the net effects of P retention and release in watersheds is important for targeting remediation and restoration measures most effectively (Jarvie et al. 2011). In scenario modelling of the Black River, Whitehead et al. (2011) found that even if the best P reduction management scenario for that subwatershed was employed, TP concentration and load reductions of only 6.6 and 5.7%, respectively, were predicted. If the quantity of TP supplied by certain subwatersheds cannot be addressed, perhaps implementing management strategies targeting the quality of P would be successful.

Budgets of phosphates in runoff from over-fertilized soils show that a proportionately small loss of bioavailable P can still be highly significant in promoting aquatic production (Reynolds and Davies 2001, Joosse and Baker 2011). As such, the bioavailable P load resulting in eutrophication was calculated to be only 1–2% of a typical fertilizer application to agricultural lands (Reynolds and Davies 2001). Promisingly, Jarvie et al. (2011) found that in-stream processes in the Thames (UK) and Sandusky (USA) rivers under low flows may regulate the delivery of nutrients and modify the timing of delivery in a way that may reduce ecological impacts to downstream at times of greatest eutrophication risk. Thus, in-stream processes in the LSW tributaries could provide an important ecosystem service in reducing ambient P concentrations. In this study, however, this retention seems to be insufficient, given the high concentrations still detected at the mouths of the tributaries.

In a mixed agricultural landscape such as the LSW, a key management question is to determine whether row crop or animal agriculture is causing a greater proportion

of nutrient inputs. This work indicates that row crop agriculture is an important factor contributing to eutrophication, but manure is also an important source of nutrients, reflected by the farming practice of using manure from livestock on croplands as a source of fertilizer. Limited storage capacity for manure in the LSW may lead to applications during periods that are not ideal for crop fertilization, resulting in excess nutrient runoff, such as occurred in the Conesus Lake catchment of New York, USA (Makarewicz et al. 2009). Runoff from manure storage facilities in the subwatersheds is also a possibility. Reducing nutrient loads requires an understanding of nutrient sources and how they are transported from catchments to aquatic ecosystems.

Best Management Practices (BMPs) have been successfully implemented in other watersheds, including the Conesus Lake catchment where BMPs had profound effects on nutrient and soil losses (Makarewicz et al. 2009). The only significant decreases of TP and SRP concentrations occurred in watersheds where considerable effort went into managing manure and where dairy cows were removed completely (Makarewicz et al. 2009). Reduction of anthropogenic nutrient loading through BMPs in agriculture is an important management goal for improving water quality and reflects a shift toward watershed-based approaches for addressing water quality issues. Through the Lake Simcoe Farm Stewardship Program there are currently 105 BMPs implemented in the LSW, some of which address livestock management; however, the ones related to manure application to crops including improved manure storage and handling, manure treatment, and manure land application represent <1% of the BMPs implemented (LSFSP 2011). The effects of these management efforts are not yet clear. Our results suggest that nutrient management approaches that include a focus on manure as well as inorganic fertilizers will be particularly effective.

## Conclusions

In the LSW, agricultural land use was identified as an important source of nutrients to the tributaries. While the area of urban land was correlated with TP load, the percentage of cropland area exhibited a strong relationship with bioavailable P. Cropland also seems to be the dominant nutrient source supporting seston, and by extension, is a contributor of both nutrients and seston to Lake Simcoe. Although P deficiency of phytoplankton was detected in the Simcoe tributaries, specifically the ones not heavily influenced by agriculture, light also seems to play an important role. Agricultural land use effects are complicated; they contribute highly bioavailable P in addition to high seston loads, causing turbidity

with subsequent light deficiency. The results of this study provide new insight into the relative influence of nutrient sources and transformations on  $\delta^{15}\text{N}$  of seston in streams. In the LSW, we suggest that manure application on agricultural cropland could be a potential driver of  $\delta^{15}\text{N}$  variability and correspondingly, bioavailable P.

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