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Periphytic algal biomass as a bioindicator of phosphorus concentrations in agricultural headwater streams of southern Ontario

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ABSTRACT

Algal blooms in Lake Erie have worsened in recent decades and are driven by diffuse export of phosphorus (P) from a large stream network that drains predominately agricultural land. Given the diffuse nature of nonpoint source pollution, best management practices (BMPs) must target areas where P levels are high. This requires long-term watershed-wide monitoring programs that do not currently exist in many jurisdictions. Instead of conventional nutrient analyses that can be costly and time-consuming, we propose the use of periphyton biomass as a bioindicator of trophic status in low-order streams, where agricultural runoff first enters watercourses. We carried out 2-week in-stream bioassays to measure periphytic algal biomass (CHL_{peri}) in 19 low-order streams in southern Ontario across an agricultural gradient (8 % to 89 %). CHL_{peri} was significantly related to total P (TP) concentration (r^2 = 0.46; p = 0.0015) but was not significantly related to soluble reactive P (SRP). A relationship between TP and turbidity (r^2 = 0.52; p = 0.0007) is consistent with previous observations of increasing SRP uptake in streams draining agriculturally-dominated landscapes. Stream temperature (°C) was correlated with the proportion of agricultural land (R = 0.55; p = 0.019) and may reflect the warming effects of the sun in unshaded agricultural streams. This method involving substrate rods (Peristix) is cost-effective, requires very little training, and yielded data that were significantly related to TP concentrations in agricultural streams. We recommend that environmental agencies and landowners use this bioassay to identify areas for implementing BMPs to reduce P export from the Lake Erie watershed.

Introduction

Eutrophication is a growing problem threatening freshwater and marine ecosystems on a global scale. Within the Great Lakes Basin, Lake Erie is enduring cyanobacterial blooms in the western basin and macroalgal (*Cladophora*) overgrowth in the eastern basin. Eutrophication at this scale impacts not only ecosystem health, but human health and economic activities (Le Moal et al., 2019; Paerl and Huisman, 2008). It is widely recognized that phosphorus (P) is the key driver contributing to the over-productivity in Lake Erie and is the most appropriate parameter for nutrient management (Dove and Chapra, 2015; Maccoux et al., 2016; Scavia et al., 2014). As early as 1972, the Great Lakes Water Quality Agreement had identified total P (TP; both dissolved and particulate P fractions) as the driver of eutrophication in Lake Erie and focused remediation measures on decreasing TP loading to the lake (De Pinto et al., 1986; Scavia et al., 2014; Wilson et al., 2019). Through this focus, there was widespread adoption of no-till agriculture in an effort to reduce concentrations of particulate-bound P and sediment runoff from soil tillage (Smith et al., 2015). This change in agricultural practice contributed to measurable declines in particulate loading until the 1990s, when compacted soil conditions under no-till management exacerbated P transport and the development of preferential pathways of P runoff (Smith et al., 2015).

The recent occurrence of algal blooms and overgrowth in Lake Erie has been attributed to nonpoint source pollution from agriculture throughout the drainage basin (Daloğlu et al., 2012; Forster et al., 2000; Wilson et al., 2019). While point sources were largely responsible for P loading to the lake prior to 1972, most (88 to 93 %) of P loading is now from nonpoint sources originating from agricultural land (Wilson et al., 2019). To address the cyanobacterial blooms, the International Joint Commission updated binational P targets for the western basin, recommending a reduction in spring TP and dissolved inorganic P (which we will refer to as soluble reactive P; SRP) to 40 % of the 2008 loading (USEPA and Environment Climate Change Canada, 2015). By comparison, there has been no proposed reduction in P loading to the eastern

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basin to address *Cladophora* overgrowth because there has been insufficient research on the source of nearshore eutrophication.

Understanding pathways of diffuse P export is necessary to mitigate nonpoint nutrient pollution to Lake Erie, and this requires knowledge on both the spatial and temporal patterns of P loading from the network of low-order streams into larger tributaries. Unfortunately, lotic eutrophication is not as widely studied as lentic eutrophication and there is a recognized research gap in how rivers contribute to P loading, even though research from U.S. tributaries on TP and SRP is growing (Bridgeman et al., 2012; Daloğlu et al., 2012; Dolan and Chapra, 2012; Joosse and Baker, 2011; Kane et al., 2014). The literature has shown that nutrients in the Maumee and Sandusky Rivers were delivered from their agricultural watersheds (>70 % agricultural land) to Lake Erie at variable rates (Wilson et al., 2019), and were strongly influenced by distinct periods of high flows; from 2002 to 2013 in the Maumee River, Baker et al. (2014) reported that 70 to 90 % of P and nitrogen (N) loads were delivered during the highest 20 % of river flows. As pointed out by Wilson et al. (2019), the two primary strategies to address high P loading in the western basin are identifying the most effective on-farm best management practices (BMPs) and targeting BMP deployment to farms at greatest risk of P loss. Though peak storm flows account for most of the nutrient loading to Lake Erie, focusing on nonpoint agricultural P loss through identifying high-risk areas remains central to reducing overall P loading to Lake Erie.

Small, well-defined drainage areas in large spatially heterogeneous watersheds export a significant amount of nonpoint source water, P, N, and sediment at watershed outflows (Pionke et al., 2000). Delineating these hotspot drainage areas is a major challenge for watershed planners as it often requires resource-intensive sampling methods. Typically, watershed P monitoring is conducted on the larger rivers within the stream network. Monitoring lower-order streams draining small watersheds requires trained technicians and dedicated resources that may exceed the capacity of conservation authorities and government agencies. However, monitoring at the scale of low-order, or even headwaters, is beneficial because the extreme spatial heterogeneity exhibited by low-order streams can be delineated (Bothwell, 1985). At this scale, researchers have the ability to delineate hotspots that act as point sources in agricultural landscapes. In terms of the primary strategies for reducing P loads to Lake Erie (Wilson et al., 2019), monitoring P concentration in small streams draining agricultural land can assist in targeting areas with the greatest risk for P runoff. Delineating priority areas helps watershed managers target areas for assistance and BMP implementation, but this can only be done through long-term, watershed-wide monitoring. To achieve monitoring at this scale, alternative strategies are needed.

Traditional water-quality monitoring methods require trained personnel and laboratory equipment to analyze P fractions (i.e., SRP and TP). These costs can be a deterrent when planning watershed-management strategies. Bioindicators of water quality are a form of biomonitoring and an effective alternative to current monitoring methods that eliminate the costs and time associated with traditional methods. Biomonitoring provides a tool for assessing multiple environmental stressors at different temporal and spatial scales (Nichols et al., 2016). Previous studies have used biomonitoring methods to estimate water-quality conditions in aquatic ecosystems, including the use of benthic macroinvertebrates (Hilsenhoff, 1988), zooplankton (Lougheed and Chow-Fraser, 2002), wetland macrophytes (Croft and Chow-Fraser, 2007), and periphyton growth (McCormick and Stevenson, 1998; McNair and Chow-Fraser, 2003). A biomonitoring approach to long-term water-quality testing not only lowers costs, but also amplifies monitoring capacity as bioindicators are accessible for volunteer use in citizen-science initiatives.

The purpose of this study was to evaluate the response of periphyton to P concentration in low-order streams across southern Ontario. We propose the use of periphytic algal biomass as a bioindicator of trophic status in low-order streams, where agricultural runoff first enters watercourses. Periphyton is comprised of a community of attached algae and dominates primary production in lotic systems (Lamberti, 1996). Periphytic algae are suitable for quantifying the nutrient status in streams because they are immobile and respond to stress quickly (Lowe and Pan, 1996; Welch et al., 1988). To increase applicability of our findings, we chose 19 sites that drain a variety of land uses in southern Ontario from primarily urban subwatersheds to those that are primarily agricultural. By demonstrating the utility of this bioassay for assessing nutrient status in low-order streams, we hope to provide a simple tool for farmers and water quality managers to collaborate on delineating areas of high P concentration, and to focus implementation of BMPs in these areas to reduce P export to Lake Erie.

Methods

Study sites

The 19 low-order streams we sampled between 2016 and 2019 are located throughout southern Ontario (Table 1; Fig. 1). Streams were chosen based on order, accessibility and land-use drainage type. For the purpose of this study, we defined "low-order" streams as first, second and third-order streams. We delineated stream order using the Strahler method of stream ordering (Strahler, 1957) and determined site accessibility based on distance from a road crossing and proximity to private land. Streams were deliberately chosen to represent a variety of land-use types within their respective subwatersheds, including those that drain farms, forest plots, wetlands and urban land (Table 1).

Watershed delineation

We delineated subwatersheds of all streams within the Lake Ontario watershed using a geographic information system (GIS; ArcMap 10.7; ESRI Inc., Redlands, California). Sampling stations in the Lake Ontario watershed were situated at a drainage point within their respective subwatersheds. We reclassified land-use types from the Southern Ontario Land Resource Information System (SOLRIS) v.2 (digital data acquired between 2009 and 2011; Ontario Ministry of Natural Resources and Forestry, 2015) into six main categories for this study including forest, wetland, open water, agriculture, urban and barren land. We calculated land-use percentages by dividing the number of pixels of each land-use type by the total number of pixels in the subwatershed to account for size differences among them.

Sampling stations in the Lake Erie watershed were not situated at a drainage point. In ArcMap 10.4.1, we used the provincial digital elevation model (DEM; Ontario Open Government, 2021) to delineate the Lake Erie subwatersheds. First, we used the Fill tool to eliminate sinks in the DEM. We then created the Flow Direction and Flow Accumulation raster layers consecutively. Next, we used the sample locations shapefile to create pour points using the Snap Pour Point tool. We then delineated the subwatersheds using the Watershed tool. We used the same method we applied for the Lake Ontario subwatersheds to reclassify land use and calculate land-use percentages for the Lake Erie subwatersheds.

Periphytic algae

We adapted McNair and Chow-Fraser's (2003) method, which had been developed for protected coastal wetland systems in the

Table 1
Location of 19 low-order streams in the tertiary watersheds of Lake Erie (E) and Lake Ontario (O) and associated land uses in their subwatersheds. W=% wetland; A=% agricultural; U=% urban; F=% forested.

Tertiary Watershed	Site Name	Site Code	Year	Land use			
				W	A	U	F
Grand R. (E)	Boston Creek	BC	2019	4.41	70.57	21.49	3.53
Grindstone Cr. (O)	Clappison-Bridgeview	CL	2016	0.61	38.56	16.27	44.56
Grand R. (E)	Cockshutt Road	CS	2019	1.15	89.83	2.97	5.28
Redhill Cr. (O)	Eramosa Karst	ER	2017	1.23	55.18	38.11	4.69
Grand R. (E)	Erbs Road	ERB	2018	12.55	59.31	7.64	20.48
Redhill Cr. (O)	Felker Creek	FC	2017	1.19	26.71	63.42	8.30
Grand R. (E)	Home Farm	HM	2018	12.82	58.54	14.49	13.87
Grand R. (E)	Kincardine Street	KS	2019	11.25	58.65	17.55	12.35
Grindstone Cr. (O)	Medad Tributary	ME	2016	22.92	46.97	13.18	16.05
Grindstone Cr. (O)	Millgrove Tributary	MI	2016	16.92	66.87	13.89	2.27
Grindstone Cr. (O)	Mount Nemo	MT	2016	9.31	63.46	16.00	11.23
Grindstone Cr. (O)	Pleasantview Tributary	PL	2016	0.09	58.99	21.48	19.29
Grindstone Cr. (O)	Sassafras Tributary	SA	2016	1.87	46.63	12.28	39.10
Spencer Cr. (O)	Sherman Falls	SF	2017	3.97	10.81	48.47	36.74
Burlington (O)	Shoreacres Creek	SH	2017	1.33	9.47	60.14	28.83
Spencer Cr. (O)	Sulphur Springs	SS	2017	2.58	9.81	8.48	78.73
Spencer Cr. (O)	Tiffany Falls	TF	2017	4.94	8.02	39.28	47.69
Burlington (O)	Tuck Creek	TU	2017	1.20	13.69	37.14	47.64
Spencer Cr. (O)	Valens Conservation	VA	2017	17.83	70.06	2.44	9.67

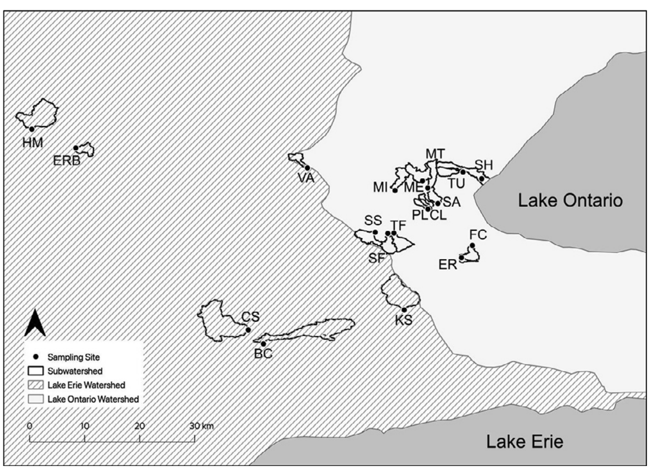

Fig 1. Map of southern Ontario, showing the locations and subwatersheds of 19 headwater stream sites sampled from 2016 to 2019 within the Laurentian Great Lakes Basin.

Great Lakes Basin. The artificial substrate is a 6 ft. long commercially available curtain rod (0.6-cm diameter; purchased from Johnson's Plastics, Etobicoke, ON). These rods (henceforth referred to as Peristic for one and Peristix for multiple) were pre-scored with a file at 5-cm intervals so that they can be easily broken off and stored as 5-cm segments after the incubation period. Just prior to deployment, the surface of the entire Peristic was cleaned with isopropyl alcohol (this removes any oils and dirt that might have prevented colonization by algae). Depending on available space, we spaced out ten to fifteen rods at least 1-m apart and inserted them at least 5 cm into the substrate to prevent them from falling over during high-discharge events. We shortened them so that they only protruded 20 cm from the water surface. To avoid shading from riparian vegetation, we also ensured that the Peristix were at least 3 m from the stream bank.

At the end of the incubation period, we extricated the Peristix from the substrate by holding the tip of the rod and pulling it straight out of the substrate. We used pliers to snap out 5-cm segments in triplicate or quadruplicate from the middle of each rod (~15 cm above where the rod had been inserted into the substrate). Each segment was placed in a glass vial, wrapped in foil to prevent further photosynthesis, stored in a cooler with ice packs, and then kept in a freezer until they could be processed for determination of chlorophyll-α (CHL-α).

Timing and duration of incubation period

Because the Peristix can be deployed throughout the summer months (June, July or August) and the incubation period can vary from two to four weeks (McNair and Chow-Fraser, 2003), we wanted to directly test the effect of timing and duration of incubation periods on the periphytic measurements. We conducted the experiments in two streams (Home Farm and Erbs Road) located in the upper reaches of the Grand River watershed, with 58 % and 59 % agricultural land in watersheds, respectively (Table 1). We conducted periphyton assays during June, July and August using a 2-week incubation period (actual number of days were from 12 to 15 days); in addition, we also conducted 4-week incubation bioassays during July and August (actual number of days either 27 or 28 days). We were forced to vary the number of days for these 2- or 4-week incubation periods because we noticed that the rods were very easily dislodged by high-discharge events and therefore ended experiments early to avoid encountering storms.

Physico-chemical conditions

At the end of the incubation period, physical measurements including stream temperature (°C), specific conductivity (μS/cm), dissolved oxygen ($mg \cdot L^{-1}$) and pH were taken at each site with an in Situ Aqua Troll 600 sonde. A HACH® 2100Q turbidimeter was used to measure water turbidity (NTU) in triplicate water samples collected from the stream. Aliquots of the stream water were also collected at mid-stream depth and placed in acid-washed Corning™ snap-seal containers for analysis of TP; 60 mL of this raw water was also filtered through 0.45-μm syringe filters (25-mm diameter, Maple Lab Systems, Mississauga, Ont.) and stored in snap-seal containers for SRP analysis. All water samples for P measurements were kept in the cooler and immediately frozen upon return to McMaster University (usually within 8–12 h).

Laboratory methods

We followed the procedure used by McNair and Chow-Fraser (2003) to analyze periphytic CHL-α samples (CHL_{peri}) by filling the vials containing the Peristix with 10 mL of 90 % acetone and placing them in the freezer for 96 h. Following the extraction period, the acetone was placed into centrifuge tubes and centrifuged for 5 min at 3500 RPM. We then used a Milton Roy 301 spectrophotometer (Fisher Scientific, Toronto, Ont.) to measure the absorbance of the acetone solution to estimate CHL_{peri} (McNair and Chow-Fraser, 2003; Chow-Fraser, 2006). Equation (1) was used to calculate CHL_{peri} (μg cm^{-2} day^{-1}). All CHL_{peri} samples were processed within 2 months of collection.

$$\left[\left(\frac{28.4 \cdot Abs\,665 \cdot \mathrm{Vol\,extracted}}{\mathrm{surface\,area} \cdot \mathrm{path\,length}}\right)\right] \cdot \mathrm{incubation\,period}^{-1} \qquad (1)$$

where *Abs* 665 is the absorbance of extracted chlorophyll at 665 nm (using spectrophotometer); Vol extracted is the volume of extractant (10 mL), surface area is the surface area of the 5-cm rod segment (i.e. 2πrL or 2π0.3cm5cm), the path length is that of the cuvette (1 cm) and incubation period is in days. Note that since no algae grows on the surface of the two ends of the segment, this is not included in the surface area calculation.

Water samples for P determination were processed at McMaster University within 2 months of sampling. After potassium persulfate digestion in an autoclave for 50 min (120 °C, 15 psi), TP concentrations were determined with the molybdenum blue method (Murphy and Riley, 1962). SRP concentrations were also determined with the molybdenum blue analysis.

Statistical methods

Statistical analyses were performed with SAS JMP software version 14 for MacIntosh (SAS Institute Inc.). For regression analyses, data were either $\log_{10}$-tranformed or proportions were arcsine-transformed. All means reported are arithmetic.

Results

Relating phosphorus to land use

The low-order streams in this study flow through subwatersheds that range from 8 % to 89 % agricultural land use. The lowest in-stream SRP concentration was 7.9 μg L^{-1}, measured at a forested site within a conservation area (site SS), while the highest concentration was 46.1 μg L^{-1}, measured in a stream that drains a subwatershed with 70 % agricultural land (site BC; Table 2). The overall mean SRP concentration for the 19 sites was 26.4 ± 2.54 (±SE) μg L^{-1}. We found a significant relationship between SRP concentration and proportion of agricultural land use that was a quadratic function, with the axis of symmetry at ~50 % agricultural land (Fig. 2a; r^2 = 0.38, p = 0.021). Except for one point (BC), all other concentrations were lower than those at 50 % agricultural land.

The lowest in-stream TP concentration was 16.4 μg L^{-1}and was measured in the same forested stream containing the lowest SRP concentration (site SS) while the highest TP concentration was 211.0 μg L^{-1}, measured in a stream draining the highest proportion (90 %; site CS) of agricultural land of all subwatersheds sampled in this study (Table 2). Overall, the mean in-stream TP concentration was 85.94 ± 14.06 μg L^{-1}. Unlike the curvilinear relationship with SRP, the relationship between TP and proportion of agricultural land use was linear (Fig. 2b; r^2 = 0.35, p = 0.008). We also found a highly significant linear relationship between TP and turbidity (Fig. 3; r^2 = 0.52, p = 0.0007).

Table 2
Mean periphytic chlorophyll-*a* biomass accumulation (CHL_{peri}) on acrylic rods, SRP, and TP concentration at each stream site, presented in descending order of CHL_{peri} biomass. Refer to Table 1 for explanation of Site Code.

Site Code	CHL_{peri}, μg cm^{-2} day^{-1}	SRP, μg L^{-1}	TP, μg L^{-1}
KS	0.856	35.66	183.27
FC	0.722	21.67	59.76
CS	0.489	12.07	211.00
HM	0.451	36.13	186.87
SA	0.427	39.44	78.90
BC	0.388	46.06	116.50
ME	0.240	42.98	170.35
MT	0.239	16.70	124.09
TU	0.183	29.76	43.57
MI	0.113	20.24	63.29
VA	0.103	21.67	35.95
PL	0.084	18.22	38.01
SS	0.066	7.86	16.43
ER	0.053	30.24	88.81
SH	0.047	9.29	22.62
SF	0.044	23.09	55.00
ERB	0.037	30.30	31.89
TF	0.032	26.43	45.95
CL	0.027	33.88	60.60

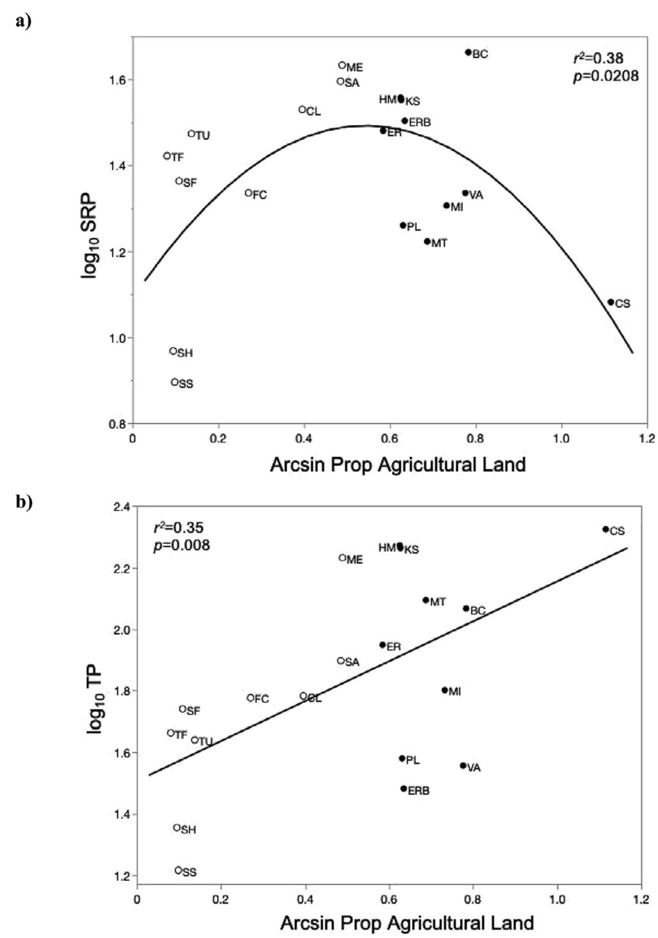

Fig 2. Water-column concentration of a) $\log_{10}$ soluble reactive phosphorus (SRP) and b) $\log_{10}$ total phosphorus (TP) in headwater streams vs arcsine proportion of agricultural land in watersheds. Letter codes correspond to codes for streams in Table 1. Sites with <50 % agricultural land-use are plotted with open symbols; those with >50 % are plotted with closed symbols.

Timing and duration of incubation period

We first measured the total periphytic algal biomass on our Peristix for both incubation treatments (2 weeks and 4 weeks)

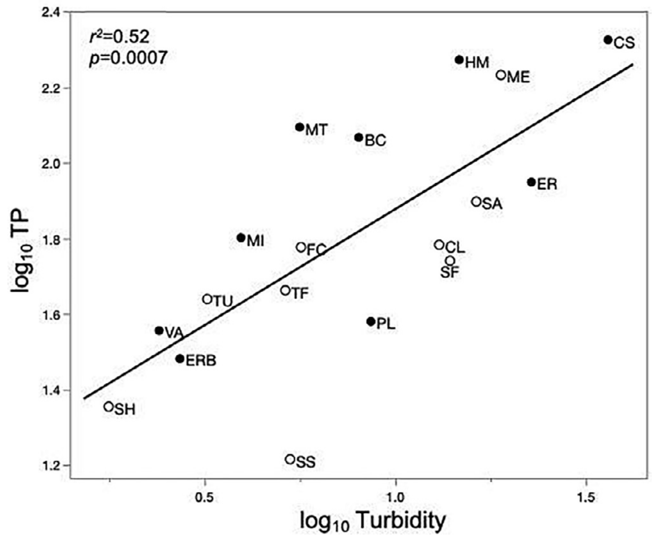

Fig 3. Relationship between total phosphorus concentration (μg L^{-1}) and turbidity (NTU). Sites with <50 % agricultural land-use are plotted with open symbols; those with >50 % are plotted with closed symbols.

(Fig. 4a). Regardless of length of incubation periods, CHL$_{peri}$ concentrations measured at the Home Farm site were significantly higher than those measured at the Erbs Road site (3-factor ANOVA; $p < 0.0001$). As expected, total periphytic algae for the 4-week incubation were also significantly higher than that for the 2-week incubation period, when standardized by site and month (July or August), except for the Home Farm site in August. Interpretation of results associated with timing of incubation was less straight forward. For rods grown at the Home Farm site, we found no significant effect of timing for the 2-week incubations; however, for rods grown at the Erbs Road site, we found significant differences between June and August data, although we did not find a significant difference between June and July data nor between July and August data. For the 4-week incubations, we found no significant differences between July and August data for the Erbs Road site, but we did find a significant difference between July and August data for the Home Farm site. We also noticed that some of the Peristix incubated for 4 weeks had fallen over during August, presumably because of a heavy storm; heavy rains may have caused some of the periphytic algae to become washed off the rods, and this may have resulted in unexpectedly low CHL$_{peri}$, and fallen rods were not included in analyses.

We also analyzed the data by calculating a daily rate of increase (Fig. 4b). As was the case for total periphytic biomass, the daily increase for the Home Farm site was significantly higher than that for the Erbs Road site (3-factor ANOVA; $p < 0.0001$); however, we did not find any significant effect of incubation month or incubation duration for the Home Farm site ($p = 0.6463$). By contrast, there were significant differences between 2- and 4-week incubation periods for the Erbs Road data (2-way ANOVA; $p < 0.0001$), although effect of timing of incubation was only significant when comparing June and August data (Tukey-Kramer post-hoc test; $p < 0.05$). Based on these results, we decided to calculate daily growth rates and to standardize the incubation period to 2-weeks to reduce chance of encountering high-discharge events.

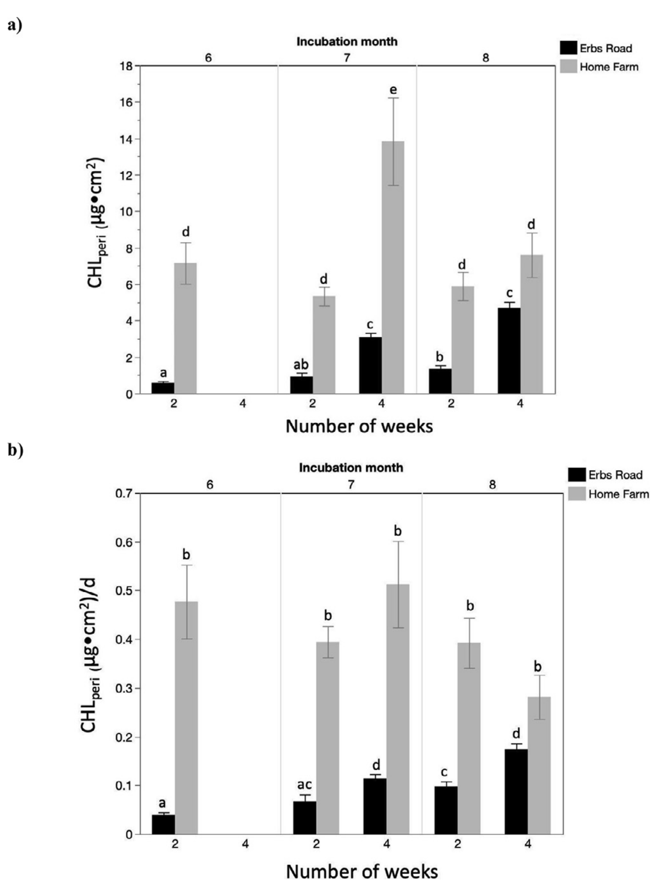

Fig 4. a) Total periphytic algae (CHL$_{peri}$) and b) daily periphytic algal growth measured at Erbs Road (ERB) and Home Farm (HM) during June (6), July (7) and August (8). Rods were incubated for either two or four weeks. Bars with the same letters in each figure indicate that mean values were statistically similar.

In-situ periphytic algal bioassays

The standardized CHL$_{peri}$ daily growth rates varied among the 19 low-order streams. The lowest CHL$_{peri}$ rate was 0.027 μg cm^{-2} d^{-1}, measured at a site draining forested and urban land that was situated on the border of a conservation area (site CL). By comparison, the highest CHL$_{peri}$ concentration was 0.856 μg cm^{-2} d^{-1}, measured in a stream draining a sub-watershed containing approximately 58 % agricultural land, along with a mixture of urban, wetland and forested land (site KS; Table 2). Overall, mean daily CHL$_{peri}$ was 0.24 ± 0.057 μg cm^{-2} d^{-1}. We found a strong positive relationship between CHL$_{peri}$ and TP concentration (Fig. 5; $r^2 = 0.46$, $p = 0.0015$). When we removed data for sites draining land with < 50 % agriculture (open symbols), the regression analysis yielded a significantly ($p = 0.0014$) higher r^2-value (0.74 vs 0.46). CHL$_{peri}$ concentration was not linearly related to either SRP or proportion of agricultural land in subwatersheds.

We regressed proportion of SRP (i.e., SRP:TP) against TP concentration and found a highly significant linear relationship (Fig. 6; $r^2 = 0.44$, $p = 0.0020$); streams with higher TP concentrations were associated with lower proportion of soluble P. We also conducted a Spearman's correlation analysis between CHL$_{peri}$ concentration and environmental variables and land cover (Table 3). CHL$_{peri}$ was significantly and positively correlated with in-stream temperature and TP, and negatively correlated with forested land cover. We also found that in-stream temperature was positively correlated with the proportion of agricultural land in subwatersheds ($R = 0.55$, $p = 0.0187$).

Comparison of low-order streams and coastal wetlands

Data from this study were combined with those from McNair and Chow-Fraser (2003) to compare relationships between coastal wetlands and low-order streams. We obtained a highly significant

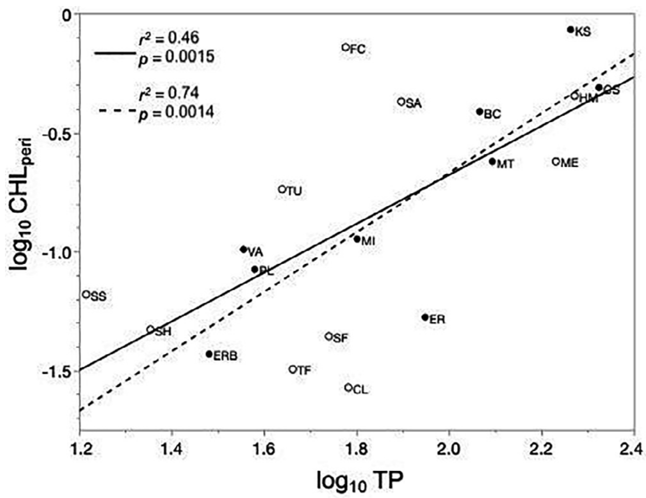

Fig 5. Relationship between $\log_{10}$ CHL_{peri} (μg cm^{-2} day^{-1}) and $\log_{10}$ total phosphorus (μg L^{-1}). Open symbols correspond to sites <50 % agricultural land use in watersheds; closed symbols correspond to sites >50 % agricultural land use in watersheds. Regression through all sites is the solid line; regression through sites with >50 % agricultural land use only is the dotted line.

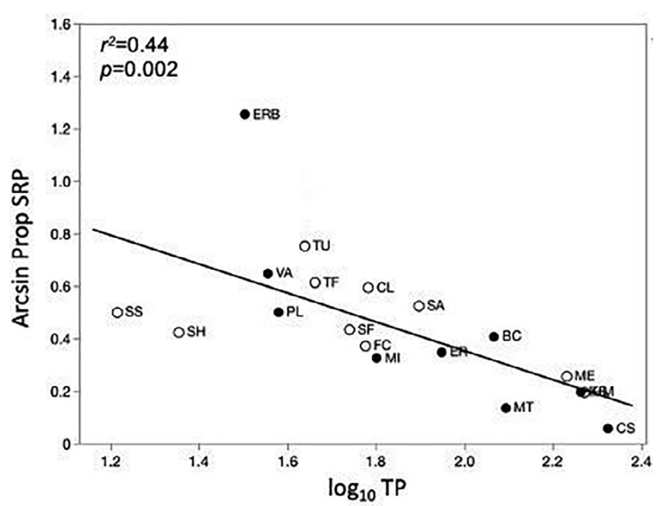

Fig 6. Inverse relationship between the arcsine proportion of soluble to total phosphorus concentration and total phosphorus concentration. ERB was excluded from the linear regression analysis. Sites with <50 % agricultural land-use are plotted with open symbols; those with >50 % are plotted with closed symbols.

Table 3
Spearman's Correlation between CHL_{peri} (μg cm^{-2} day^{-1}) and environmental and land-cover variables. All bolded *p*-values indicate significant correlation with CHL_{peri}.

Variable	*R*	*P*
% Urban	−0.1544	0.5280
% Agriculture	0.4105	0.0808
% Forested	−0.4912	**0.0327**
% Wetland	0.1088	0.6576
pH	0.0424	0.8675
Temperature (°C)	0.6939	**0.0014**
Specific Conductance (μS/cm)	0.0671	0.7914
Turbidity (NTU)	0.3664	0.1348
Dissolved Oxygen (mg/L)	0.0103	0.9698
Soluble Reactive Phosphorus (μg·L^{-1})	0.2045	0.4011
Total Phosphorus (μg·L^{-1})	0.6526	**0.0025**

linear relationship between CHL_{peri} concentration and TP concentration for all data (Fig. 7; r^2 = 0.37, p < 0.0001). We also found CHL_{peri} concentration to decrease significantly with increasing proportion of SRP for both streams and wetlands (r^2 = 0.18, p = 0.0037), and separately for stream data (Fig. 8; r^2 = 0.31, p = 0.0130).

Discussion

Low-order streams are unique ecosystems with spatial heterogeneity and close connections to surrounding landscapes. Factors including nutrient cycling, flow variability, grazing and light variability distinguish periphytic growth characteristics in low-order streams from lentic water bodies. Specifically, the retention time of water in low-order streams is very short and dissolved nutrients are exported at a higher rate than are particulate-bound nutrients due to the flashier flow compared to lentic systems (Essington and Carpenter, 2000). Elevated stream discharge (during and following storms) can scour benthic algae from substrate surfaces, reduce the accumulation of periphyton, and lead to underestimation of $CHL_{peri.}$ A subset of our streams ranged from 0.4 to 32.6 L·s^{-1} in discharge and are similar to reported baseflow discharges of headwater streams in published periphyton accrual studies (Biggs et al., 1999; Kiffney and Bull, 2000), therefore we assume the effects of scouring from elevated flows were minimal in this study. Bioassays involving measurement of benthic algal biomass in low-order streams should not include high discharge events during the incubation period. In our study, we reduced the risk of encountering storm events by shortening the incubation period from 4 to 2 weeks.

We found a positive correlation between periphyton biomass and temperature (Table 3), which is likely the indirect effect of benthic algae having increased light availability in streams that have no or minimal riparian vegetation. To illustrate this, both ER and ERB had moderately high concentrations of SRP (30.24 and 30.30 μg·cm^{-1}, respectively), but both streams were well shaded by overhanging vegetation; this is likely the reason why water temperature at both sites were relatively low (10.2 and 11.2 °C, respectively) as was their CHL_{peri} (0.05 and 0.04 μg cm ^{2} d^{-1}, respectively).

The quadratic function relating SRP to proportion of agricultural land use in subwatersheds may be the result of the soluble form of P being adsorbed to suspended particles in streams that drain heavily agricultural watersheds (Fig. 2a). This is supported by the significant increase in TP with proportion of agricultural land in subwatersheds (Fig. 2b) and the significant linear relationship between TP and turbidity (Fig. 3). Elevated levels of suspended sed-

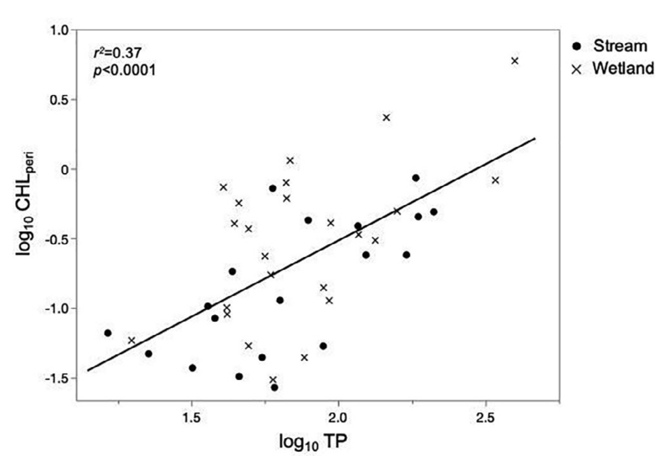

Fig 7. Relationship between periphytic chlorophyll α biomass (CHL_{peri}; μg m^{-2} day^{-1}) and total phosphorus (TP; μg L^{-1}) from 19 headwater streams and 23 wetlands within the Great Lakes Basin. Data for wetlands were taken from McNair and Chow-Fraser (2003).

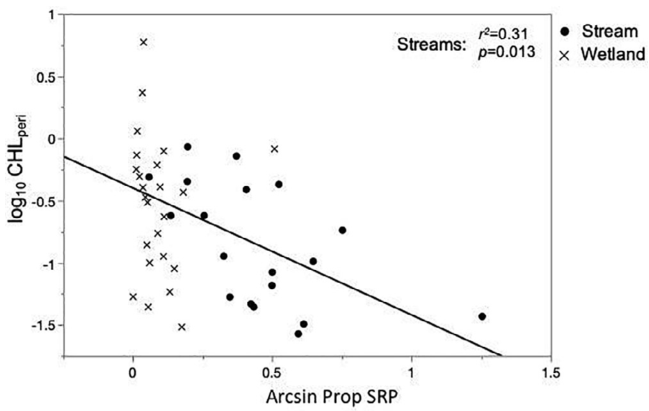

Fig 8. Relationship between periphytic chlorophyll α biomass (CHL$_{peri}$; µg cm^{-2} day^{-1}) and proportion of SRP from 19 headwater streams (solid circle) and 23 wetlands (x) within the Great Lakes Basin. Data for wetlands were taken from McNair and Chow-Fraser (2003). Regression through stream data only is the solid line.

iment in streams act as a sink for SRP, causing removal of soluble nutrients from water column to sediment (Essington and Carpenter, 2000; Owens and Walling, 2002). Hall (2003) also showed that streams respond to ecological disturbance by retention mechanisms that limit nutrient export at the watershed outlet. There is a possibility that the observed decrease in ambient SRP concentration with agricultural land in subwatersheds is also due to uptake by periphyton biomass; however, we did not explicitly test for this through a partitioning experiment. A partitioning experiment should be done in the future to apportion the uptake of SRP by biomass from the binding of SRP to suspended solids in agricultural streams. Due to the rapid uptake and sedimentation of SRP in productive streams draining agricultural landscapes, TP is a more reliable predictor of benthic algal biomass, which can track eutrophication in low-order streams across the full disturbance gradient (Fig. 5).

CHL$_{peri}$ on the Peristix was significantly related to TP in headwater streams in southern Ontario and wetlands across the Great Lakes Basin and there was no observed difference in periphytic algal response to TP in streams and in Great Lakes wetlands (Fig. 7). This finding confirms the effectiveness of periphytic algae as a bioindicator for TP across ecosystems in the Great Lakes Basin where periphytic algal communities may differ. Lack of significance between CHL$_{peri}$ and SRP in wetlands is consistent with our findings across streams in southern Ontario and complements previous studies confirming that SRP is not a suitable predictor for periphyton accumulation.

In this study, we did not account for variations in other biotic or abiotic factors that may have influenced the rate of periphytic biomass accumulation. For instance, we have assumed that grazing effects of the benthic macroinvertebrate community (Taylor et al., 2002) are uniform across site without first testing this assumption. The type and amount of riparian vegetation (emergent vegetation vs forest canopy) are known to influence light availability and thus limit algal growth in streams, and this is related to the wetted width of streams (Hill and Fanta, 2008). Headwater streams draining agricultural plots often course through fields and are void of riparian shading, as supported by the significant correlation between stream temperature and proportion of agricultural land, but this is not always the case (e.g., ER and ERB). To standardize for light availability, we recommend removing all overhanging branches and riparian vegetation in the area where Peristix are to be installed.

The use of Peristix as a bioindicator of trophic status in low-order agricultural streams can be operationalized into a citizen-science monitoring protocol and can be effectively used at both watershed and land-parcel scales. To monitor at the watershed scale and target areas with high P concentration, we recommend coordinating Peristix deployment and subsequent collection with a network of participants with access to low-order streams draining agricultural plots. To effectively organize a coordinated monitoring effort, we recommend conservation agencies or other like-mandated organizations embed this protocol into existing long-term monitoring practices. Agencies can procure the materials (i.e., Peristix, glass vials, acetone, etc.; see *Methods*) and garner participation from local landowners, farmers, and students from secondary or post-secondary institutions. Data collection should be synchronous so that the Peristix will yield comparable data within and across watersheds. We recommend agencies schedule a standardized two-week incubation period in July or August so that all participants deploy and collect the Peristix on, or as close as possible to, the same day (see *Methods* for data collection methodology), and then preserve 5-cm segments of Peristix in a −20 °C freezer until agencies can arrange to pick them up or for the volunteers to drop them off at a convenient location within the community. If agencies are equipped with a spectrophotometer, they can process the Peristix samples at the drop-off location, or they can contract processing to another facility (such as a university) with a spectrophotometer. Results should be communicated back to the participants in a timely manner and should be publicly accessible to the greater community through the agency's website or through a formal report. Results can be interpreted at the watershed scale, by comparing CHL$_{peri}$ biomass levels and determining P hotspots in need of nutrient-management assistance; they can also be interpreted at the land-parcel scale, where landowners or farmers can determine the degree of P loss from their land based on the level of CHL$_{peri}$ biomass collected and implement appropriate remedial actions.

Conclusions

The results of this study confirm the utility of daily periphytic growth rate as an indicator of TP in low-order streams, especially those draining small agricultural watersheds. Our results suggest that TP is the more appropriate form of P to track lotic eutrophication, while the periphyton-SRP relationship remains obscure, lending credence to previous observations on periphyton dynamics that suggest that growth of benthic algae is co-limited by physical and biological factors in streams that are confounding (Welch et al., 1988). Additionally, the materials used in this bioassay are inexpensive and widely available, and the effort required to install and remove rods are suitable for citizen-science programs. A coordinated program involving students in secondary and post-secondary institutions, in partnership with educators and farmers would be a cost-effective way to amplify long-term monitoring and provide valuable information for basin-wide nutrient management of Lake Erie.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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