Nutrient Budgets Calculated in Floodwaters Using a Whole-Ecosystem Reservoir Creation and Flooding Experiment

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Abstract Flooding in forested areas can release nutrients via organic matter flushing and organic matter decomposition and contribute to nutrient loading in aquatic ecosystems. Organic matter content may set an upper limit for nutrient release during flooding, but variation in timing, depth, duration of inundation, and site organic matter substrate inhibit comparison across sites and events. We used data collected during a whole-ecosystem flooding experiment conducted at the IISD-Experimental Lakes Area (ELA) in northwestern Ontario to examine the relationship between site organic matter content and nutrient release on both a short-term (first 3 weeks) and annual scale (open-water season). Three upland forest sites with differing amounts of organic matter stored in soil and vegetation were flooded to create experimental reservoirs. We hypothesized that the magnitude of nutrient release would be related to the organic matter content in each site. We found that nutrient concentrations increased relative to low-nutrient water pumped into each reservoir at both scales, but there was no relationship between site organic matter content and short-term total nitrogen (TN) and total phosphorus (TP) concentrations or annual TN and TP fluxes. Additionally, N and P release rates differed, decreasing TN:TP ratios in reservoirs and outflows relative to inflows. Nutrient concentrations increased immediately after inundation each year and continued for 5 years of repeated flooding. Predicted increases in flooding due to changing precipitation patterns and reservoir creation may cause forested areas to be a long-term nutrient source to aquatic ecosystems. Over time, nutrient accumulation may affect water quality, shift biological communities, and influence ecosystem functioning.

1. Introduction

When terrestrial areas are inundated stored organic matter in soils and vegetation may be exported laterally to aquatic ecosystems during event-based floods and/or decompose and become a source of nutrients (Grimard & Jones, 1982; Hall & St. Louis, 2004; Matthews et al., 2005; Paterson et al., 2019). Soil nutrient stores in areas that experience high frequency flooding may often become depleted and the magnitude of nutrient release may decrease with each flooding event (Paradis & Saint-Laurent, 2017). Alternatively, upland forest areas that have not previously flooded may have larger stores of nutrients that could be released upon inundation. Potential increases in precipitation due to climate change may exacerbate flooding (Burn & Whitfield, 2015) and increase nutrient release to boreal aquatic ecosystems. Thus, estimating the potential for forested areas to release nutrients under flooded conditions is important for establishing a baseline nutrient release response to flooding.

An important cause of flooding in boreal areas is the creation of reservoirs and there is increasing evidence that reservoirs affect the delivery of nutrients from continents to the ocean (Maavara et al., 2015). Increased nutrient fluxes associated with flooding organic matter-rich forested areas can stimulate algal blooms, a "trophic upsurge" of greater secondary productivity, and changes in the composition of invertebrate and fish communities (Lucotte et al., 1999; Ostrofsky & Duthie, 1980; Paterson et al., 2019). Releases of nutrients from flooded soils may be especially important in systems that have low initial nutrient concentrations, such as in boreal regions. In some cases, changes in water quality and fish populations have had detrimental social and economic effects on communities living on or downstream of new reservoirs (e.g., Lucotte...
et al., 1999; Quinn, 1991; Rosenberg et al., 1997). A better understanding of the controls on nutrient releases to floodwaters is essential for predicting how future inundation will influence nutrient loading to aquatic ecosystems and how to mitigate associated societal consequences.

During flooding, saturated soils leach nutrients into overlying water, which can transfer into aquatic ecosystems and inundation further stimulates microbial metabolism and decomposition of organic matter (Kelly et al., 1997; Ostrofsky & Duthie, 1980; Paterson et al., 1997, 2019). Nutrient leaching is the dominant process responsible for nutrient release into floodwaters at the onset of flooding, but as the period of inundation progresses, decomposition takes over and maintains nutrient fluxes until the system becomes limited by oxygen or decomposable organic matter substrate (France et al., 1997; Manzoni et al., 2010). Factors such as substrate C:N ratio, litter type, and oxygen availability control decomposer activity. Decomposers tend to respond to high substrate C:N by decreasing both C and N use efficiency, which can allow N mineralization in the presence of N-poor substrates (Manzoni et al., 2008). In addition, inundation may initially stimulate decomposer activity, but microbial oxygen use may result in anoxia and eventually decrease decomposition, slowing nutrient release (Moore et al., 2005). While decomposer responses are variable, the store of nutrients in flooded organic matter may set an upper limit on the total amount of nutrients available for release and, consequently, may determine the size and duration of ecosystem-scale nutrient fluxes following inundation. If this is the case, designing reservoirs to minimize flooding of areas with high nutrient storage may help to decrease negative impacts on water quality associated with impoundment. Alternatively, removing organically rich top-soils and vegetation prior to impoundment may help to mitigate impacts on water quality (Calder et al., 2018; Campbell et al., 1975).

Flooding may result in higher nutrient loads due to leaching and decomposition and may also contribute water with highly variable N:P ratios to receiving waterbodies. Shifting N:P ratios in floodwaters relative to source waters may result from differential physical settling of particulate nutrients or differing N and P release rates during leaching and decomposition. Floodplains adjacent to aquatic systems may increase hydrologic residence time and promote particulate nutrient settling (Nee & Hupp, 2007), which tends to retain P and alter N:P ratios in nutrient loads to downstream systems. A similar effect occurs in older reservoirs, where longer hydrologic residence times favor particulate P settling and higher P retention, relative to N (Cook et al., 2010; Grantz et al., 2014). During decomposition, foliar litter tends to lose P faster than N (Moore et al., 2005), decreasing N:P ratios, especially at the onset of flooding when leaching is high (Manzoni et al., 2010). Changing N:P ratios may affect cyanobacterial abundance or the predominance of *Daphnia* in zooplankton communities (Hessen et al., 2013; Smith, 1990). Therefore, understanding how decomposition, leaching, and particle settling interact to shift N:P ratios in floodwaters can improve our understanding of the impacts of flooding on downstream systems.

In this study, we use data collected during a whole-ecosystem flooding experiment to quantify N and P in floodwaters and investigate the role of site organic matter content in nutrient release. The unique design of this experiment allowed us to partition the importance of organic matter quantity for nutrient release in floodwaters while maintaining consistency with respect to other site characteristics such as nutrient inputs, temperature, and inundation depth, duration, and frequency. The FLooded Upland Dynamics EXperiment (FLUDEX) at the IISD-Experimental Lakes Area (ELA) in northwestern Ontario examined whether the amount of stored organic matter in 3 newly created reservoirs affected fluxes of greenhouse gases, methyl mercury, and nutrients (Bodaly et al., 2004). Although there was not a relationship between site organic matter content and GHG budgets (Matthews et al., 2005; Venkiteswaran et al., 2013) or mercury budgets (Hall et al., 2005), the potential relationship between nutrient release and organic matter content has not been previously explored. Here, we calculate TN (total nitrogen) and TP (total phosphorus) budgets for the three experimentally flooded FLUDEX reservoirs to quantify the contribution of N (nitrogen) and P (phosphorus) released into floodwaters. We also assess the impacts of repeated flooding by considering nutrient concentrations within the first three weeks of inundation in the spring of each flooding year to assess nutrient depletion in flooded sites.

We hypothesized that the magnitude and duration of nutrient fluxes in the experimental reservoirs would be related to the amount of terrestrial carbon and associated nutrients stored in each reservoir as organic matter. We expected that annual N and P fluxes would be greatest in the reservoir with the most carbon (i.e., highest organic matter content) and lowest in the reservoir with the least carbon (lowest organic matter...
content). We used measurements of parameters such as temperature and dissolved oxygen (DO) taken within the experimental reservoirs to identify potential mechanisms that explain patterns of N and P release into floodwaters.

2. Methods

2.1. Site Description

FLUDEX was initiated in 1999 at the IISD-ELA in northwestern Ontario. Three upland forest sites with naturally varying amounts of stored organic carbon were selected and flooded from May or June through September from 1999 to 2003 (Figures S1, S2, and S3). Sites were characterized as either low (30,900 kg C ha\(^{-1}\)), medium (34,900 kg C ha\(^{-1}\)), or high (45,860 kg C ha\(^{-1}\)) carbon sites based on carbon content measured during vegetation surveys and soil cores collected prior to flooding (see Hall et al., 2005 for full inventories of C in litter, soil, and vegetation in each reservoir and Bodaly et al., 2004 for photographs of the reservoirs). We use site carbon content as a proxy for site organic matter content because organic matter is commonly measured and expressed as units of carbon, although it also contains other elements in abundance (e.g., oxygen, hydrogen, nitrogen, sulphur, and phosphorus). The high carbon FLUDEX site had the most potentially labile or non-woody vegetation. 60% of the total carbon was stored in above ground vegetation and 40% was stored in soil and litter. This site was relatively flat, with moist soil covering about half of the site and a drier treed community covering the other half. This site was dominated by jack pine forest (\textit{Pinus banksiana}) with an understory composed primarily of \textit{Sphagnum} spp. and Labrador tea (\textit{Rhododendron} spp.). Bedrock occurred at a mean depth of 35 cm below the soil. The medium carbon site was dominated by dry jack pine forest with bedrock a mean depth of 47 cm below the soil surface. In the medium carbon site, 79% of carbon was stored in above ground vegetation and 20% was stored in soils and litter. This site contained the least amount of C stored in soils and litter. The low carbon site was located on a ridge-top that was approximately three quarters covered by a dry jack pine forest. Above ground vegetation and soil including litter comprised 64% and 36% of the stored carbon, respectively. This site had thin soils with bedrock mean depth of 15 cm below the soil surface and some exposed bedrock (Bodaly et al., 2004; Matthews et al., 2005). Overall, C and N bulk masses measured in pre-flooded soils were like those previously reported for boreal regions of Canada (Oelbermann & Schiff, 2008). Following flooding, the low, medium, and high carbon reservoirs had mean water depths of 1.2 m ± 0.05, 0.9 m ± 0.15, and 1.0 m ± 0.11 and surface areas of 0.63, 0.50, and 0.74 ha, respectively.

2.2. Reservoir Set Up, Hydrology, and Experimental Design

Reservoir walls were constructed of gravel, plastic, and plywood (where dike heights were less than 1 m) and wood, cement, and plastic (dike heights >1 m) built at low-lying site contours (see photos in Bodaly et al., 2004). A layer of polyethylene sheeting was sandwiched between plywood sheets to prevent seepage through the walls. Water was continuously pumped into each reservoir through aluminum irrigation pipes from nearby oligotrophic Roddy Lake and flows were managed using manually controlled valves (Hall et al., 2005). In-line flow meters were installed in the pipes leading to each reservoir to measure input volumes. Water continuously exited reservoirs over v-notch weirs installed in the dike walls and there was always measurable outflow. Continuous water level recorders in each site were used to calculate reservoir volumes based on bathymetric maps and water volumes leaving over the v-notch weirs (Venkiteswaran et al., 2013). Reservoirs were drained at the end of each flooding season, usually the end of September, from valves at the bottom of reservoir walls (Hall et al., 2005). As a result, the flooding regime in the FLUDEX reservoirs was analogous to the shoreline regions of boreal hydroelectric reservoirs that are typically flooded in the spring and summer and exposed in winter following drawdown to meet increased power demands. Precipitation data were collected at the ELA meteorological site, which is located less than 1 km from the experimental reservoirs (Matthews et al., 2005). Evaporation was measured using Class A evaporation pans in the reservoirs and at the ELA meteorological site (Venkiteswaran et al., 2013). Evaporation occurring on rain-free days was used to estimate losses from the reservoirs and was approximately 2.2 mm day\(^{-1}\). Each reservoir had a small catchment that provided direct runoff (Figures S1, S2, and S3) and the areas varied among the three reservoirs (low carbon: 0.09 ha, medium carbon: 0.73 ha, high carbon: 4.78 ha).
runoff volume was estimated using a gauged sub-catchment nearby (northwest inflow to Rawson Lake), which was scaled to direct runoff area for each reservoir (Hall et al., 2005).

Temporal scale is an important factor in understanding flooding dynamics. Our experimental design did not capture event-based flooding and short-term (i.e., first 72 h), so high resolution initial flooding measurements are not available. Rather, we have focused on two longer but different timescales: (a) initial nutrient fluxes occurring during the first 3 weeks of inundation and (b) annual fluxes occurring over the full flooding season. Further, continuing the experiment for five consecutive years allowed us to consider the effect of repeated flooding across multiple years.

2.3. Chemistry

Water chemistry was sampled weekly during the first month of each flooding season and then biweekly thereafter at the inflow, weir outflow, and within the reservoirs. Samples were collected at more than ten sites distributed throughout each reservoir at depths from 0.5 to 2 m. Analyses for particulate phosphorus (PP), total dissolved phosphorus (TDP), particulate nitrogen (PN), total dissolved N (TDN), NH₄, NO₂, and NO₃ used methods described in Stainton et al. (1977). Dissolved N and P were operationally defined as passing through a GF/C filter, which has a nominal pore size of 1.2 μm. Minimum detection limits were 1.8 μg L⁻¹ for TDN and 0.58 μg L⁻¹ for TDP. Minimum detection limits for PN and PP were 10 μg L⁻¹ and 0.2 μg L⁻¹, respectively. We calculated TN and TP by summing dissolved and particulate fractions. TN and TP were measured at different depths and we separated them into surface (≤0.5 m), middle (0.6–1 m), and bottom (1.1–2 m) depths. A YSI temperature/oxygen probe was used to measure dissolved oxygen and temperature at three depths: 0.5 m (surface), 1.0 m (middle), and 1.5 m (bottom) at various sampling sites within reservoirs (Hall et al., 2005). Precipitation chemistry was determined from sampling at the ELA meteorological site and occurred on an event-based schedule, whenever precipitation volumes in the collectors exceeded 850 mL.

2.4. Nutrient Budgets

We calculated nutrient budgets for the duration of each open-water season for each year to assess the overall release of nutrients following reservoir creation. We also compared nutrient concentrations at the onset of flooding in each year to better understand how repeated flooding of dry soil and organic matter affects nutrient release. We considered the first three weeks to be the most important period of nutrient release because reservoir N and P were consistently highest during the first three weeks of flooding in each year (see Supplemental Information). We constructed annual water and nutrient budgets based on previous calculations for carbon (Matthews et al., 2005) and mercury (Hall et al., 2005) where:

\[
Net_{\text{Water, TN, TP}} = \sum O_{\text{Water, TN, TP}} - \sum I_{\text{Water, TN, TP}}
\]

and \(\sum I_{\text{Water, TN, TP}}\) was the sum of inputs for water, TN, or TP and \(\sum O_{\text{Water, TN, TP}}\) was the sum of the outflows in each year. Inputs included pumped inflow, precipitation, and runoff. Outputs included weir outflow, seepage through reservoir walls and bedrock cracks, evaporation (water budget only), and drain outflow. Seepage was not measured directly, therefore, it was calculated as the residual term in the water budget. Mass inputs and outputs of N and P were estimated by multiplying inflow and outflow volumes by the average TN and TP concentrations between chemistry sampling dates. We calculated precipitation N and P inputs by multiplying total precipitation by reservoir surface area and then multiplying by average N and P concentration between precipitation chemistry sample dates. We did not account for nitrogen gas phases in balances. Hendzel et al. (2005) found that the reservoirs were net sinks for N₂O and there were no extended periods of anoxia after 1999 that might promote denitrification. We also considered significant nitrogen fixation unlikely because TN : TP ratios were greater than a mass ratio of 10, which is above levels at which nitrogen fixing bacteria predominate (Flett et al., 1980). Nitrogen fixing cyanobacteria were not observed in any of the reservoirs for the duration of the experiment (D. Findlay, unpublished data).

The main sources of error in TN and TP budgets were estimated water budget components. Errors in individual TN and TP concentrations were approximately 1.4% and 1.7%, respectively. Errors associated with estimates of pump inflow, weir outflow, water level, and precipitation were likely small and have been
estimated to be about 5% (Yanai et al., 2018). Error was estimated at 15% for evaporation pans and 18% for estimated direct runoff using data from the Lake 239 East Inflow (Hall et al., 2005; Winter 1981). The largest sources of error were TP and TN losses via seepage and the bedrock crack in the low carbon reservoir.

2.5. Runoff

The direct runoff area for each reservoir accounts for the land around the reservoir contributing runoff and is equivalent to catchment area. Direct runoff areas for individual reservoirs were delineated using topographical maps based on aerial photographs taken in 1982 and 1991 (Hall et al., 2005). Runoff chemistry was determined using weekly data collected from the nearby Lake 239 East Inflow (EIF). This site has a similar forest composition to the FLUDEX uplands and contains no wetlands. TN and TP runoff was estimated as mass per area at the Lake 239 EIF and then multiplied by each reservoir’s direct runoff area.

2.6. Drain

Reservoirs were drained at the end of each flooding season to protect the dike walls and mimic drawdown in shallow areas of hydroelectric reservoirs during the winter. Once water levels dropped below the base of the v-notched weirs, remaining water was removed using drain pipes at the lowest point of each reservoir. Drain volumes were not measured explicitly but were estimated using the reservoir volume prior to drawdown, calculated using maps and water level loggers (Hall et al., 2005). Chemistry sampling only occurred at the drain in 1999 and 2000, therefore drain TN and TP concentrations were estimated from water volumes and weir concentrations for all years to maintain consistency. TN and TP concentrations at the weir in each reservoir on the last sampling date of each flooding season were used as a proxy for drain concentration. At this point in the flooding season, TN and TP concentrations were similar throughout all depths of each reservoir and the weir concentration was representative of the concentrations found throughout the reservoir at drawdown.

2.7. Seepage

Seepage losses of water under or through the reservoir walls were estimated as the residual term in the water balances. Seepage estimates were verified using annual seepage surveys, where seepage was channeled into small streams and periodically measured. Chemistry in the seepage was not measured explicitly but was estimated using weir concentrations. Daily seepage was estimated by subtracting daily reservoir water inputs from daily outputs via the weir and evaporation. Daily seepage volumes were then totaled between chemistry sampling dates and multiplied by weir nutrient concentrations to find TN and TP masses leaving the reservoirs via seepage. In the low carbon reservoir, water was also lost to a fracture in the bedrock (Hall et al., 2005). Because seepage was estimated daily as the residual term in the water budget, seepage in the low carbon reservoir also contained water losses to the bedrock fracture.

2.8. Periphyton

Dense mats of periphyton developed in the FLUDEX reservoirs, growing on flooded trees and vegetation. Nutrients released during flooding potentially accumulated in periphyton and would not be measured in the outflows discussed above. As a result, we estimated the annual mass of N and P in periphyton in each reservoir for the years 2000–2003. Wooden dowels (1.3 cm diameter) were hung in each reservoir at random locations prior to flooding to serve as a substrate for periphyton collection. Dowels extended from above the surface of the flooded reservoir to the bottom. From 2000 to 2002, five dowels from each reservoir were collected up to four times per flooding season. In 2003, dowels were only collected once at the end of the flooding season in September. Periphyton was washed from the surface of the dowels, homogenized in a blender, and analyzed for N and P composition. Total masses of N and P stored in periphyton in each reservoir were estimated by extrapolating average N and P mass per area on dowels over the entire submerged tree surface area in each reservoir. Submerged tree surface area was estimated by collecting vegetation from areas of sparse, medium, and densely vegetated areas at multiple distances above the ground and mapping the distribution of these vegetation densities over the area of each reservoir. Submerged tree surface
areas calculated per reservoir area were 3.03, 3.25, and 2.92 m² m⁻² in the low, medium, and high carbon reservoirs, respectively. Periphyton estimates represent a broad quantification of nutrient storage within reservoirs but should be interpreted with caution. Although both dowels and submerged vegetation were wood, the nature of the surfaces necessarily differed and the estimates of submerged tree surface area were prone to error. For this reason, we did not include N and P storage in periphyton in our nutrient budget calculations.

### 2.9. Statistical Analyses

We used a repeated measures analysis of variance (ANOVA) to test whether annual TN (kg ha⁻¹ m⁻²), TP (kg ha⁻¹ m⁻²), and molar TN:TP fluxes were significantly different among the three reservoir sites (low, medium, and high carbon). Prior to analysis, we used Shapiro-Wilks tests to check for normality in each of the response variables. TN and TN:TP values were normally distributed but TP values were not. Therefore, we log transformed TP to meet the assumption of normality and used the transformed values for all subsequent statistical analyses. We fit linear mixed models for each nutrient flux, with nutrient flux and site as fixed effects and year as a random effect to account for repeated measures. We created mixed effects models using the “nlme” package (Pinheiro et al., 2018) and completed each ANOVA using the “stats” package in R 3.5.2 (R Core Team, 2018). We calculated the between-measure variance as the variation of the mean of each measure from the grand mean and the between-treatment mean by summing values for all years for each treatment and dividing by the number of years. We calculated the variance between treatments as the variation of the mean of each treatment from the grand mean. We calculated the grand mean by summing all TN, TP, or TN:TP values for all treatments and all years and dividing by the number of values. Total variance was the variation of each value from the grand mean and residual variance was the difference between total variance, between treatment variance, and between-measures variance.

### 3. Results

#### 3.1. Hydrology

In all reservoirs, the greatest proportion of the water input (>90%) was from the pumped inflow (Table 1). In the medium and high carbon reservoirs, the greatest portion of the outputs (>70%) was the weir outflow. Seepage was the largest proportion of output (53%) in the low carbon reservoir due to cracks in the bedrock. Other components such as seepage through dike walls (20%), evaporation (7%), and drained water (7%)

### Table 1

| Year | High carbon | Medium carbon | Low carbon |
|------|-------------|---------------|------------|
|      | 1999        | 2000          | 2001       | 2002       | 2003       | 1999        | 2000          | 2001       | 2002       | 2003       | 1999        | 2000          | 2001       | 2002       | 2003       | 2004       | 2005       | 2006       |
| Inputs (m³) | 85,556 | 108,339 | 91,518 | 89,123 | 91,702 | 74,427 | 74,164 | 77,240 | 54,323 | 58,804 | 99,445 | 112,441 | 109,440 | 93,651 | 117,435 |
| Weir | 60,195 | 80,214 | 63,392 | 63,193 | 60,299 | 60,756 | 57,249 | 63,037 | 44,340 | 44,479 | 33,515 | 42,517 | 50,518 | 35,243 | 43,839 |
| Drain | 6,801 | 6,801 | 6,801 | 6,801 | 6,801 | 6,801 | 6,801 | 6,801 | 6,801 | 6,801 | 6,801 | 6,801 | 6,801 | 6,801 | 6,801 |
| Evaporation | 2,165 | 2,331 | 2,313 | 1,980 | 2,202 | 1,475 | 1,575 | 1,550 | 1,338 | 1,500 | 1,859 | 1,985 | 1,922 | 1,622 | 1,906 |
| Seepage | 16,327 | 18,924 | 18,944 | 17,080 | 22,332 | 12,929 | 11,074 | 8,386 | 4,379 | 4,547 | 5,579 | 5,079 | 4,983 | 4,669 | 6,573 |
| Total | 85,487 | 108,270 | 91,449 | 89,054 | 91,633 | 79,427 | 74,164 | 77,240 | 54,323 | 58,804 | 99,445 | 112,299 | 109,298 | 93,509 | 117,293 |
made up much less of the total outputs and runoff (5%) and precipitation (3%) made up small proportions of the inputs.

### 3.2. Concentrations of Nutrients in Inflows and Outflows

TN and TP concentrations in the reservoir outflows always exceeded inflow concentrations from oligotrophic Roddy Lake, indicating that the reservoirs were a nutrient source during flooding (Figure 1). Considering only the first 3 weeks of flooding in each year, the greatest differences in nutrient concentrations between the inflow and outflow occurred in 1999, the first year of flooding. In the low and medium carbon reservoirs, the highest outflow TN concentrations recorded during the study occurred within the first 3 weeks of flooding in the first year, but concentrations in the high carbon reservoir outflow did not peak at the beginning of the study and were consistent among all flooding seasons (Figure 1). The highest outflow TN concentration was in the low carbon reservoir and occurred within the first week of flooding in 1999 (Figure 1a). Outflow concentrations in the medium carbon reservoir were greatest during the first week of flooding in 1999 and 2003 (Figure 1a). The highest TP concentrations were measured in the first three weeks of flooding in 1999 (Figure 1b). Molar TN:TP varied between 12 and 64 and was generally two times larger in the inflow than the outflow in all years (Figures 1c and S6). In all reservoirs, outflow TN:TP was lowest within the first three weeks of each flooding season and increased toward the end (Figure S6). In the low and medium carbon reservoirs, average annual TN:TP in the outflow was lowest in the first flooding season in 1999 (21 and 19, respectively; Figure S6) and increased with each flooding season. In the high carbon reservoir, annual average TN:TP in the outflow was also lowest in 1999 (19), but slightly increased to about 23 in 2000 and remained steady in 2001 and 2002. In 2003, the high carbon outflow reached an average TN:TP of 34 (Figure S6).

### 3.3. Internal Cycling/Conditions

Within the first three weeks of flooding, average water temperatures in the reservoirs were within 1.5°C of each other and there was no thermal stratification. In most cases, temperatures were lowest within the first and last weeks of flooding (Figure S7). In the first flooding season, DO concentrations were lowest in all reservoirs and especially low DO occurred three weeks after flooding began, reaching close to 0 mg L⁻¹ (Figures 2 and S8). The lowest DO concentration within the first week of flooding in 1999 occurred in the medium carbon reservoir, followed by the high carbon, and then low carbon reservoirs. In general, average DO concentrations increased with each subsequent flooding season. In the low carbon reservoir, average DO concentrations remained consistent and only slightly increased from 1999 to 2003. In the medium and high carbon reservoirs, average DO concentrations increased between 1999 and 2003. We found that DO and TDN were negatively correlated in all reservoirs (low carbon: \( R^2 = 0.18, \ p < 0.05 \); medium carbon: \( R^2 = 0.32, \ p < 0.05 \); high carbon: \( R^2 = 0.061, \ p < 0.05 \); Figure 3a) in the first three weeks of each flooding season. During the same period, we found a negative correlation between DO and TDP concentrations in all reservoirs (low carbon: \( R^2 = 0.51, \ p < 0.05 \); medium carbon: \( R^2 = 0.65, \ p < 0.05 \); high carbon: \( R^2 = 0.24, \ p < 0.05 \); Figure 3b).

Over the five flooding seasons, all reservoirs experienced peak N and P concentrations within the first three weeks of flooding in each year (see supporting information). TP concentrations within each reservoir during the first three weeks of flooding ranged from 11 to 69 μg L⁻¹, 13 to 579 μg L⁻¹, 11 to 142 μg L⁻¹ in the low, medium, and high carbon reservoirs, respectively. TP concentrations were lowest in the low carbon reservoir and highest in the medium carbon reservoir at the onset of flooding in all five flooding seasons (Figure 4). TN concentrations were highest during the first week of flooding in 1999 in the medium (2657 μg L⁻¹) and high carbon reservoirs (949 μg L⁻¹). However, concentrations were highest in the third week of flooding in 1999 in the low carbon reservoir (692 μg L⁻¹).

### 3.4. Annual Fluxes

Total outputs of TN and TP exceeded inputs for all reservoirs in each year, generating consistently positive net fluxes over the entire period of flooding (Figure 5). Total annual TN (\( F = 0.66, \ p = 1.18 \)) and TP (\( F = 0.66, \ p = 0.54 \)) fluxes were not significantly different among reservoirs. The largest TP fluxes occurred
During the first year of flooding in 1999 with values of 2.59, 4.16, and 2.00 kg ha\(^{-1}\) in the low, medium, and high carbon reservoirs, respectively. TP fluxes typically decreased with each subsequent flooding season, but the reservoirs continued to be TP sources for five years. TN fluxes were always positive and did not markedly decrease after the initial flooding season (Figure 5). There was no discernible pattern in N in the low carbon reservoir (Figure 5). The largest TN fluxes in the medium carbon reservoir occurred in the first three years of flooding (27.67 kg ha\(^{-1}\), 16.57 g ha\(^{-1}\), and 17.74 kg ha\(^{-1}\), respectively). In the high

Figure 1. Total nitrogen (TN) concentration (a), total phosphorus (TP) concentration (b), and TN:TP (c) in water pumped into reservoirs and in water leaving the low, medium, and high carbon reservoirs over weirs during the first three weeks of flooding in June 1999 to 2003.
carbon reservoir, TN fluxes were largest in the last two years of flooding in 2002–2003 (14.38, 20.10 kg ha\(^{-1}\), respectively). Neither TP nor TN fluxes were related to site carbon content (Figure 5), as we hypothesized. However, both TN and TP fluxes varied similarly in each reservoir (Figure 5). During five years of repeated flooding, the low, medium, and high carbon reservoirs released a total of 73.30, 81.29, and 58.75 kg ha\(^{-1}\) TN and 6.10, 7.85, and 5.96 kg ha\(^{-1}\) TP, respectively. TP and TN fluxes were not significantly correlated with residence time or average water temperature.

Annual TDP and TDN fluxes were positive in all reservoirs throughout the experiment (Figure 6). TDN comprised the majority of TN in all reservoirs for all years and TDN flux patterns in each reservoir were similar to TN flux patterns. PN fluxes were positive in all reservoirs during the first three years of flooding but was negative in the medium carbon reservoir in 2002 (Figure 5). TDP fluxes were largest in the first year of flooding in 1999 in all reservoirs reaching 1.5, 2.3, and 0.84 kg ha\(^{-1}\) in the low, medium, and high carbon reservoirs, respectively. PP fluxes were generally positive in all reservoirs for all years except for in the low carbon reservoir in 2003 (Figure 5). PP fluxes were largest during the first year of flooding in 1999. PP fluxes generally decreased after each flooding season in the low and medium carbon reservoirs but were more variable in the high carbon reservoir.

**Figure 2.** Dissolved oxygen (DO) concentrations measured at the surface, middle, and bottom of the low, medium, and high carbon reservoirs during the first three weeks of flooding in June 1999 to 2003.

**Figure 3.** Linear regressions between log TDN and log DO (a) and log TDP and log DO (b) internal reservoir concentrations on each chemistry sampling day and for each of three depths during the first three weeks of flooding in the low, medium, and high carbon reservoirs.
On average, we found that molar TN:TP flux ratios were largest in the low carbon reservoir (34), followed by the medium carbon reservoir (27), and smallest in the high carbon reservoir (25, Figure 5). However, TN:TP flux ($F = 0.92, p = 0.44$) was not statistically different among reservoirs. TN:TP flux ratios were generally lowest in the first year of flooding in 1999 with values of 14, 15, and 12 in the low, medium, and high carbon reservoirs, respectively. TN:TP ratios generally increased with each flooding season. Decreases in TP fluxes generally drove changes in TN:TP ratios since TN fluxes were relatively consistent among all years. On average, TDN:TDP fluxes were smallest in the high carbon reservoir (42), followed closely by the medium carbon reservoir (43), and largest in the low carbon reservoir (50). TDN:TDP flux was smallest during the first year of flooding in all reservoirs (low: 20, medium: 18, high: 16). There was no clear pattern of TDN:TDP increase or decrease in any of the reservoirs. The largest TDN:TDP ratios occurred in 2001 in the low and medium carbon reservoirs (80 and 63, respectively) and in 2000 in the high carbon reservoir (60). Average PN:PP was largest in the high carbon reservoir (13), followed by the low carbon reservoir (9), and smallest in the medium carbon reservoir (6). PN:PP fluctuated from year to year in each of the reservoirs and there was no discernible pattern.

Following flooding of the FLUDEX reservoirs, we observed dense mats of periphyton growing on flooded trees and vegetation. These mats potentially represented important stores of N and P that were not measured in our initial mass-balance calculations. Beginning in year 2, we measured periphyton N and P at the end of each flooding season, which represented the net accumulation for the flooding season. Estimates of nutrient storage in periphyton ranged from 14 to 9,519 g ha$^{-1}$ for N and 1–681 g ha$^{-1}$ for P, respectively (Figure 6). Periphyton was a substantial pool of N and P in all reservoirs at the end of the flooding season in

Figure 4. Total nitrogen (TP) (a) and total phosphorus (TN) (b) concentrations measured at the surface, middle, and bottom of the low, medium, and high carbon reservoirs during the first three weeks of flooding in June 1999 to 2003.
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2000, equaling about half of the annual N and P fluxes from reservoirs. N mass in periphyton was greatest in the medium carbon reservoir (9,519 g ha$^{-1}$), followed by the high carbon reservoir (5,141 g ha$^{-1}$), and then the low carbon reservoir (3,646 g ha$^{-1}$). Periphyton P mass followed the same pattern in 2000 with values of 681, 385, and 295 g ha$^{-1}$ in the medium, high, and low carbon reservoirs, respectively. In 2001 and 2002, N and P masses were largest in the high carbon reservoir, smaller in the medium carbon reservoir, and smallest in the low carbon reservoir. In 2003, periphyton N mass was highest in the low carbon reservoir and lowest in the medium carbon reservoir, while periphyton P mass was highest in the high carbon reservoir and lowest in the low carbon reservoir. Periphyton N:P molar ratios were relatively consistent in all reservoirs from 2000 to 2002, with an average ratio of 31 ± 4 (Figure 6c). In 2003, N:P increased to 53 in the high carbon reservoir and decreased to 24 in the low carbon reservoir, while N:P in the medium carbon reservoir slightly increased to 36.

4. Discussion

Our results indicate that flooding of organic matter causes N and P to be released into overlying water and leads to an initial decrease in N:P ratio, but do not support the hypothesis that the magnitude of N and P release is correlated with the amount of stored organic matter. Overall, the highest TN and TP concentrations within the three reservoirs occurred during the first flooding season. Within each flooding season, reservoir TN and TP concentrations were highest during the first three weeks of flooding. Total annual TN and TP fluxes did not significantly vary across sites for each year of the study, potentially due to insufficient
Figure 6
variation in site carbon content. However, the medium carbon reservoir typically released the most TN and TP, while the low carbon reservoir released the least. With repeated flooding, P release declined, but N release continued, thus dramatically increasing N:P ratios and decoupling N and P fluxes across years.

Elevated N and P concentrations immediately following inundation (week one) were likely due to leaching and export from soils, but decomposition in subsequent weeks maintained high nutrient concentrations. DO concentrations gradually decreased reaching 0 mg L⁻¹ during the first three weeks of flooding in 1999 in response to microbially driven decomposition. After the initial nutrient release, TDN and TDP concentrations were generally inversely related to average DO concentrations (Figure 3), which resulted from the decomposition of flooded organic matter. Additionally, previous studies that quantified by-products of decomposition such as CO₂ and CH₄ showed that the three reservoirs were sources of these gases for all five years of flooding (Matthews et al., 2005; Venkiteswaran et al., 2013). Others have observed the role of flooding in stimulating microbial metabolism and organic matter decomposition in flooded boreal forest soils and litters (Hall & St. Louis, 2004; Kim et al., 2014). Low DO concentrations caused by the decomposition of flooded organic matter presumably increased the desorption of phosphorus from flooded sediments through changes in redox conditions (Orihel et al., 2017). TP fluxes substantially declined after the first flooding season but reservoirs continued to be a net TP source over five years of repeated flooding. The occurrence of low DO concentrations also declined substantially after the first year of flooding. Previous studies have also demonstrated that soils and organic matter can continue to release P after repeated flooding (Tian et al., 2017), including soils within frequently re-flooded zones on the outer edges of reservoirs (Ma et al., 2010). TN fluxes remained elevated throughout all flooding seasons.

Although TN and TP fluxes remained positive over the five years of flooding, N and P were released at different rates throughout the duration of each flooding season. There is some evidence from terrestrial studies that P is preferentially mineralized over N from organic matter (Marklein et al., 2015), which may have caused P stores to be depleted more quickly and lead to decreased P fluxes after the first year of flooding. The pattern of high initial P release followed by lower P release with repeated flooding has also been demonstrated in studies considering wetland restoration and reflooding (Ardón et al., 2010; Kinsman-Costello et al., 2014). N release was sustained for at least 5 years, while P release decreased with each flooding season. Therefore, the relative amounts of N and P released from flooding may not be equal and changes in N:P ratios can be expected.

Changes in N:P availability may affect biota living downstream of and in flooded ecosystems. Very low N:P ratios may induce algal N-limitation and cause undesirable increases in nitrogen-fixing cyanobacteria abundance (Smith, 1983). Changes in N:P ratios may also affect the production of algal toxins (Van de Waal et al., 2014). In turn, these changes may affect invertebrates and fish. In our experiment, however, N:P mass ratios in all reservoirs were greater than values suggested to favor cyanobacteria (Smith 1983, 1990), but this may not always be the case.

Overall, internal TN and TP concentrations and fluxes were not related to site carbon content, refuting our hypothesis that the total amount of organic matter substrate was the main factor controlling the magnitude of ecosystem TN and TP release throughout five years of flooding. We found no correlations between nutrient fluxes, residence times, water temperatures, watershed runoff, precipitation, or C:N ratios, which were all similar among reservoirs (Hall & St. Louis, 2004). Additionally, variation in runoff and precipitation inputs among reservoirs likely does not explain differences in nutrient fluxes because these budget components were small compared to pumped inflow, which had identical quality for all reservoirs. The lack of a relationship between site carbon content and nutrient release may be due to differences in dominant litter types within each reservoir. A previous study noted that different litter types present in these reservoirs decomposed at different rates even though internal temperature and oxygen conditions were similar (Hall & St. Louis, 2004). For example, the organic matter in the medium carbon site may be more labile and decompose faster than the organic matter in the high carbon site, explaining why nutrient release from the medium carbon reservoir may be greater in the short time span of this study. However, it is also possible that N and P release may have persisted longer in the sites with more stored organic carbon, had flooding.
continued for additional years. Still, we did not detect significant differences in TN and TP release among reservoirs within the 5 years of study.

Flooding substantially altered N:P ratios in water leaving the reservoirs. TN:TP ratios were low in reservoir outflows in the first year but tended to increase after each flooding season. Outflow TN:TP was generally lower than inflow TN:TP indicating that these reservoirs were a greater relative source of P than N. If N and P sequestered in periphyton were included in annual fluxes from the FLUDEX reservoirs, then the initial decrease in N:P ratio at the onset of flooding in 1999 would have been slightly less pronounced because periphyton stored more N relative to P. Previous studies have often shown that N:P ratios in reservoir outflows tend to be higher than inflows in older reservoirs (Cook et al., 2010; Grantz et al., 2014). As reservoirs age, they frequently shift to become net P sinks due to longer residence times, which elevates sedimentation of particulate P relative to N (Maavara et al., 2015). Changes in the N:P of reservoir outflows have the potential to affect downstream plankton community composition and biogeochemical cycling (Gillespie et al., 2015; Haxton & Findlay, 2008). For example, increased inputs of low N:P water can stimulate undesirable increases in cyanobacterial abundance and promote harmful algal blooms (Paerl et al., 2011). Thus, flooding may result in important, ecologically relevant local impacts, and downstream impacts.

An understanding of factors affecting nutrient cycling in inundated systems requires examination at the ecosystem scale, which was an important advantage of our study. We were also able to utilize an experimental design where we controlled the timing and extent of flooding and important ecosystem characteristics. Opportunities to examine processes affecting nutrient fluxes in naturally flooded systems or newly created reservoirs are rare and often do not allow for the collection of critical baseline data on soil conditions and water inputs. While our project had these advantages, there were also many limitations. Because of the cost and scale of building and maintaining the experimental reservoirs, our replication was low (n = 3) and the range of ecosystem characteristics (carbon content) may have been insufficiently broad to detect effects. The effects of inundation may vary considerably in other areas with different soil types, vegetation, and input waters. Although the length of our study (5 years) was comparatively long, there was still insufficient time to observe potentially important longer term changes that may take decades to occur. Finally, the effects of flooding may vary with different patterns of inundation (for example, daily cycling in hydroelectric reservoirs) or water flows.

Despite these uncertainties, our results indicate that flooding in forested areas can contribute to increased nutrient loading that may persist for several years of repeated inundation. In aquatic ecosystems with low N and P concentrations, nutrients mobilized during flooding can be an important biogeochemical component and support increased primary productivity. Thus, predicted increases in precipitation and flooding in pristine boreal regions of Canada (Burn & Whitfield, 2015) and associated nutrient release will be particularly problematic because boreal aquatic ecosystems tend to be nutrient poor. However, these large pulses of N and P may also accumulate and contribute to algal blooms within and downstream of flooded sites that already have high nutrient concentrations. Nutrient enriched floodwaters can change N:P ratios in receiving ecosystems and affect primary producer communities (Smith, 1983). Therefore, we suspect that increased incidences of flooding landscapes as a result of reservoir creation or increased precipitation will contribute nutrients to nearby waterways, potentially exacerbating current problems associated with excess nutrients such as eutrophication and degraded water quality. Additionally, we suggest that managers account for forested areas and their potential contribution to nutrient release when trying to mitigate flood-induced nutrient loading to aquatic ecosystems.

Data Availability Statement

Data will be available online following journal acceptance at https://doi.org/10.6084/m9.figshare.12962612.

Acknowledgments

At the FLUDEX project. Volunteers for their time and dedication continued for additional years. Still, we did not detect significant differences in TN and TP release among reservoirs within the 5 years of study.

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