The influence of an in-network lake on the timing, form, and magnitude of downstream dissolved organic carbon and nutrient flux

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Abstract
Within fluvial networks, lakes can be sinks or sources of dissolved organic carbon (DOC) and nutrients, yet the controls over sink-source behavior remain unclear. We investigated the influence that an in-network lake exerted on DOC and nutrient export. Our investigation consisted of: (1) injecting a conservative tracer to determine lake travel times and flow paths; (2) sampling lake inflow, outflow, and surrounding groundwater to determine water and nutrient budgets; and, (3) sampling internal lake profiles to ascertain in-lake physico-chemical patterns through time. Conservative tracer data indicated considerable in-lake retention and combined with inflow-outflow discharge measurements revealed a decoupling of kinematic and solute pulses. Nitrate (NO₃) was the dominant form of dissolved inorganic nitrogen (DIN) at lake inflow whereas ammonium (NH₄) became the dominant component at lake outflow. The lake was a sink for NO₃-N and PO₄, but a source for NH₄-N, DON, TDN, and DOC. We observed hydrologic controls on DOC concentrations and export patterns, but redox controls on DIN dynamics. Our results indicate that lakes within fluvial networks can be sources of dissolved organic material and reduced nitrogen (NH₄) while simultaneously being sinks for NO₃-N and PO₄-P. Determining controls on sink-source behavior and the cumulative effect of lakes on DOC and nutrient budgets is a necessary first step toward improved understanding of the role of lakes in network- to regional-scale dynamics.

1. Introduction
Interactions between streams and lakes (stream-lake interactions) can influence water, carbon, and nutrient flux, and lakes are increasingly being studied in the context of a connected fluvial network [Winter, 1999; Jones, 2010; Lottig et al., 2011, 2013; Soranno et al., 2015]. Within the network and relative to the streams they are connected to, lakes have longer residence times, and are often retention zones for water [Krasnostein and Oldham, 2004], and sediment [Arp et al., 2007; Myers et al., 2007]. While extended residence times in lakes could result in lakes functioning as dissolved organic carbon (DOC) and inorganic nutrient sinks, results to date have been inconsistent indicating that lakes can function as both sources and sinks [Kling et al., 2000; Brown et al., 2008], and/or sinks [Kang et al., 2016] of DOC and nutrients. Determining the reasons for these inconsistent results and elucidating the drivers of sink-source behavior of in-network lakes is necessary to determine the cumulative effect of lakes on network DOC and inorganic nutrient budgets, but remains a challenge.

Carbon and nutrient transformation within- and flux from- lakes are ultimately the result of combined physical and biological processes. From a physical perspective, the travel time of inflowing water is a primary driver, yet remains poorly constrained in lake biogeochemical models and associated export estimates [Hanson et al., 2011]. Hydrologic travel times of inflowing stream water through lakes is partially a function of mixing between the stream inflow and receiving lake water. As inflow streamwater enters a lake, the buoyancy of the inflow water relative to the buoyancy of lake water (i.e., temperature and associated density differences), determines the depth at which stream inflow inserts into the lake [Martin and McCutcheon, 1999]. Warm inflow streamwater that is positively buoyant (less dense) will insert into surface waters, and often become spatially distributed due to wind driven mixing [Martin and McCutcheon, 1999]. Conversely, colder, negatively buoyant inflow water generally plunges vertically until it reaches a depth of neutral buoyancy (i.e., density of inflow and lake water are equal), at which point the inflow will intrude into the water.
column, often a stratified metalimnion [Serruya, 1974; Hebbert et al., 1979; Romero and Imberger, 2003]. Subsequent to intrusion, streamwater will mix with lake water in relation to density gradients, stratification, turbulent diffusion, and entrainment in vertical and lateral dimensions over the proceeding days, weeks, and months [Rueda et al., 2006; Owens et al., 2014]. As a function of these mixing dynamics, some of the inflow water mixes little, traveling quickly to the outlet due to thermal and density driven isolation, while other water may mix and reside in the lake for longer periods. Seasonal and diurnal variability in patterns of stream insertion, lake stratification, and stream-lake mixing can generate lateral and vertical heterogeneity. Water may mix and reside in the lake for longer periods. Seasonal and diurnal variability in patterns of stream insertion, lake stratification, and stream-lake mixing can generate lateral and vertical heterogeneity in the distribution of inflow water, DOC, nutrients, and associated biological activity in lakes [Rueda and MacIntyre, 2009; Van de Bogert et al., 2012]. As such, it is necessary to quantify the distribution of hydrologic travel times and flow paths to determine the influence of physical transport processes on DOC and nutrient dynamics in linked stream-lake systems [Hanson et al., 2011].

From a biological perspective, lakes can be locations of high biogeochemical activity within the fluvial network [Cole et al., 2007; Harrison et al., 2009; Tranvik et al., 2009]. While lakes cover only ~3.7% of the land surface [Downing et al., 2006; Verpoorter et al., 2014], they can exert a disproportionate influence on global carbon and nitrogen cycling as a result of high biological processing [Cole et al., 2007; Downing, 2010]. Modeling estimates suggest that nearly half of the organic carbon that enters inland waters annually from the terrestrial environment is buried in lake sediments or vented as carbon dioxide or methane [Tranvik et al., 2009; Bastviken et al., 2011], and that lakes can be important to global nitrogen cycles, removing 19.7 Tg N yr$^{-1}$ globally [Wolheim et al., 2008; Harrison et al., 2009]. While modeling approaches have highlighted the role of small lakes in fluvial network biogeochemical dynamics and budgets [Epstein et al., 2013], we currently lack sufficient process-based data and understanding necessary to properly constrain these estimates. Furthermore, while lakes can influence network biogeochemical dynamics, there is limited information on the physical transport processes that in part drive DOC and nutrient fluxes. Often studies of physical transport in lakes (physical limnology) do not incorporate biogeochemical dynamics, and conversely, studies of mechanistic biology in lakes often lack physical transport characterization.

As a step toward improved understanding of linked physical and biological processes in lakes, we assessed: in-lake travel times and mixing, and quantified the timing, magnitude, and form of down-network DOC and nutrient fluxes, from lake inflow to outflow to address the following questions:

1. What are the dynamics of through lake travel times and flow paths?
2. How does an in-network lake influence the timing, form, and magnitude of down-network nutrient flux?

2. Methods

2.1. Study Site Description

Our research site was the Bull Trout Lake Watershed (44.302961, −115.2564899), located in the Sawtooth Mountains, central ID (Figure 1). Bull Trout is an undeveloped high elevation watershed (2117–2600 m) that drains an area of 11.4 km$^2$ located at the headwaters of the South Fork Payette River (Figure 1b). The Sawtooth Mountains receive low atmospheric deposition of nitrogen (0.6 kg-N ha$^{-1}$ yr$^{-1}$) [National Atmospheric Deposition Program, 2008], are drained by low nutrient streams, and contain oligotrophic lakes [Budy et al., 1995]. Thirty-year average annual precipitation is 108 cm, 64% of which is snow, and 2008 annual precipitation was 108.5 cm (Banner Summit snow pack telemetry, SNOTEL #312, 2140 m elevation located <2 km NE of Bull Trout Lake). Land cover in the watershed is dominated by lodge pole pine (Pinus contorta) in the uplands, with willows (Salix spp.), sedges (Carex spp.), and grasses in the riparian areas. Upstream of Bull Trout Lake (BTL), valley fill is composed of mixed Pleistocene till and Holocene alluvium and colluvium, while downstream fill is predominantly glacial outwash [Kilsgaard et al., 2003]. BTL is a terminal moraine lake, typical of the region, with an alluvial wetland at the lake inflow (see Figure 1). Average depth across the lake is 4.3 m, and the deepest region of the lake is located at the lake inflow where depths approach 15 m.

2.2. Experimental Infrastructure and Design

Our investigation consisted of three main approaches: (1) injecting conservative tracers to determine lake travel times and flow paths; (2) sampling of lake inflow, outflow, and surrounding groundwater (GW) to determine water, DOC, and nutrient budgets; and (3) sampling internal lake profiles to ascertain in-lake patterns through time. Our experimental design and infrastructure included: 3 stream sampling locations...
(upstream, inflow, and outflow), 13 in-lake sampling stations, and 4 lake-GW piezometer nests (Table 1 and Figure 1c). The upstream site was located 1.5 km upstream of the lake inflow where the stream exits the forested headwater region and enters the alluvial valley wetland (Table 1 and Figure 1c). The inflow site was located 100 m upstream of the lake edge in the alluvial wetland, where local vegetation consists of willows (Salix spp.), sedges (Carex spp.), and grasses (Table 1 and Figure 1c). The outflow site was located 20 m downstream of the lake edge, where the stream channel was wide and shallow, with slow moving water (Table 1 and Figure 1c). The 13 in-lake sampling locations were distributed across the lake and piezometer nests were located at the northeast, northwest, southeast, and southwest corners of the lake to assess lake-GW interactions (Figure 1).

2.3. Tracer Injection of Rhodamine-WT and Lithium

We injected Rhodamine-WT (RWT, 7.4 kg) and lithium (Li, 29.5 kg) just upstream of lake inflow for 48 h, from 21 to 23 June 2008 to determine flow paths and travel times through the lake. We injected the tracers at a constant flow rate (250 mL min⁻¹) using an FMI metering pump (Syosset, New York), and injectate was maintained at a constant RWT to Li ratio (RWT/Li = 0.25). RWT and Li both have their advantages and disadvantages, which is why we used both simultaneously. Advantages of RWT include low detection limit (0.01 ppb), detection over multiple orders of magnitude (0.01–1000 ppb), and the ability to measure in-situ (real-time) with sensors. Conversely, a disadvantage associated with use of RWT is the potential for nonconservative behavior associated with sorption and photo decay [Lin et al., 2003]. The advantages of Li include very low background concentration and conservative transport behavior [Thies et al., 2002]. A disadvantage of Li is that it cannot be sensed in-situ, so grab samples must be used. Accordingly, the RWT tracer allowed us to develop tracer breakthrough curves (BTCs) with far more data

Table 1. Physical and Hydrologic Characteristics of Sampling Locations*

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Watershed Area (km²)</th>
<th>Elevation (m)</th>
<th>Average Runoff (mm h⁻¹)</th>
<th>Total Runoff (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upstream</td>
<td>8.8</td>
<td>2122</td>
<td>0.160</td>
<td>540.8</td>
</tr>
<tr>
<td>Inflow</td>
<td>9.5</td>
<td>2115</td>
<td>0.180</td>
<td>608.9</td>
</tr>
<tr>
<td>Outflow</td>
<td>11.4</td>
<td>2115</td>
<td>0.181</td>
<td>609.5</td>
</tr>
</tbody>
</table>

*Average and total runoff are from 5/9 to 9/26.
points than one could generate with grab samples, while the Li provided a verification of transport behavior, albeit with fewer data points than RWT.

To quantify tracer dynamics, we: (1) measured tracer concentrations for 1 week preinjection to establish background concentrations; and (2) measured tracer concentrations during and for 90 days postinjection at inflow, outflow, and within the lake. At each of these sampling locations we collected grab samples for laboratory analysis of Li and RWT, and measured in-situ RWT tracer concentrations with sensors (described in next section).

2.4. Stream, Lake, and Groundwater Measurements and Sampling

We recorded water levels (i.e., stage) real-time at: upstream, inflow, lake site 4 (L4), and outflow at 15 min intervals using TruTrack, Inc. capacitance rods (resolution +/− 1mm). We measured stream discharge at daily to weekly intervals across the range of flow states using either salt dilution gauging [Kilpatrick and Cobb, 1985] or velocity area gauging [Dingman, 2002] to develop stage-discharge rating curves for real-time discharge calculation. The outflow site was located directly at the lake outflow and due to the wide nature of the stream, low velocities, and shallow depths, accurate stream discharge measurements were not possible at this location (Figure 1c). The hydrograph for outflow was developed by area adjusting streamflow yield measured just downstream (Figure 1c).

Our research design included both in-situ measurements and grab sampling approaches. All grab samples (stream, lake, and GW) were analyzed for injected tracers (RWT and Li), DOC, and nutrients (laboratory analysis described next section). We collected streamwater grab samples at inflow and outflow at hourly to weekly intervals, and weekly to bi-weekly intervals at upstream (Figure 1). Our higher resolution sampling bracketed the time of the tracer injection when tracer concentrations were changing rapidly. We also measured in-situ (real-time) RWT concentrations at inflow, outflow, and within the lake (described below) using Turner Designs, Inc. Cyclops 7 fluorometry probes. The Cyclops probes were shielded to ensure sunlight did not interfere with fluorometric readings, and we calibrated the Cyclops probes using standards made with stream or lake water to account for background fluorescence. In-stream measurements at inflow and outflow occurred at 2 min to hourly intervals, which allowed us to develop high-resolution BTCs for analysis of lake travel times and tracer mass recoveries. Real-time RWT data were then verified against grab sample/lab analysis data for RWT and Li. Lastly, we combined high-resolution BTCs developed from in-situ RWT sensors with Li BTCs developed from grab samples to provide multiple lines of evidence regarding lake travel times and transport patterns.

We selected 13 sampling locations distributed across the lake that spanned the range of inflow, littoral, lake-center, and outflow locations (Figure 1c). We measured RWT and temperature-depth profiles, and collected water samples at all 13 lake sampling locations at daily to weekly intervals, again with higher frequency when concentrations were changing rapidly. At each location measurements were made and samples were collected at 0.5, 1.5, 3, 6, 9, and 12 m depths (depending on maximum depth at given location). Lake RWT and temperature-depth profiles were collected using a Cyclops 7 probe bundled with a Druck pressure transducer, thermocouple wire, measuring tape, and global positioning system. Lake water grab samples were collected using 1/4 inch flexible plastic tubing and a peristaltic pump (Geo Pump, Medina, NY). The tubing was outfitted with a weight and attached to a measuring tape in order to collect water from desired lake depths. Prior to sampling, lake water from the desired depth was circulated through the tubing to completely purge and rinse the sampling tube. All lake samples were analyzed for injected tracers (RWT and Li), DOC, and nutrients (laboratory analysis described section 2.5) to assess spatial and temporal dynamics of tracer transport, and DOC and nutrient concentrations.

Piezometer nests were located at the northeast, northwest, southeast, and southwest corners of the lake (Figure 1). Each nest consisted of two piezometers completed to different depths in order to determine vertical GW flow gradients at each location (e.g., from GW toward lake or vice versa). Piezometers were 1.5 inch diameter PVC pipes, open only at the completion depths (which ranged from 55 to 224 cm), and we completely evacuated piezometers and allowed them to recharge prior to sampling. We measured depth to water and collected water samples from the piezometer nests at bi-weekly intervals from mid-June to mid-September to determine GW, DOC, and nutrient concentrations and to determine hydraulic gradients between the lake and GW.
All grab samples (stream, lake, and GW) were collected in acid washed, 60 mL high density polyethylene bottles. Bottles were rinsed three times with filtered water before sampling. Samples were field-filtered with 0.7 μm glass fiber filters (GF/F Whatman International, Ltd., Maidstone, UK) and stored in the dark and frozen until analysis for DOC, nutrient, and injected tracer concentrations.

2.5. Chemical Analysis

All stream, lake, and GW samples were analyzed for RWT, Li, nitrate-nitrogen (NO$_3$-N), ammonium-nitrogen (NH$_4$-N), phosphate-phosphorous (PO$_4$-P), total dissolved nitrogen (TDN), and dissolved organic carbon (DOC). RWT was measured with a Turner 10-AU fluorometer and Li was analyzed using a Metrohm 820 ion chromatograph (Herisau, Switzerland) with a 250 mm by 4 mm column and a detection limit of 2 μg L$^{-1}$. NO$_3$-N, NH$_4$-N, and PO$_4$-P were analyzed with a colorimetric method on Seal QuAAtro (Milwaukee, WI) Segmented Flow Analyzer with a detection limit of 0.5 μg L$^{-1}$ for NO$_3$-N and PO$_4$-P, and 1 μg L$^{-1}$ NH$_4$-N. TDN and DOC were analyzed using oxidative combustion on Shimadzu TOC-V (Kyoto, Japan) with a detection limit of 0.02 mg L$^{-1}$. Dissolved organic nitrogen (DON) was determined by difference from TDN and dissolved inorganic nitrogen (DIN).

2.6. Concentration and Flux Analyses

We evaluated spatial and temporal concentration patterns across the stream sampling sites, the lake, and GW piezometers. We tested differences in mean concentrations between inflow and outflow for statistical significance using a Student’s two tailed t-test with unequal variance assumption and significance level of $\alpha = 0.05$. We examined relationships between discharge and DOC and nutrient concentrations using linear regression. We calculated mass flux for each stream sampling location as the product of concentration (μg L$^{-1}$) and site Q (L s$^{-1}$) at the time of sampling. Lake sink-source behavior was then evaluated as the mass balance of flux at outflow minus inflow. Lake storage was determined from in-lake concentration and hypsometry patterns.

3. Results

3.1. Lake Transport Dynamics

Water temperatures in the surface layers of the lake (0 – 10 m) warmed over the course of the summer but remained relatively constant −7.5°C at depths greater than 10 m (supporting information Figure S1). Epilimnetic waters reached a maximum temperature of −18°C on 8/13 and then began to cool (supporting information Figure S1). We measured RWT concentrations continuously, and Li concentrations semicontinuously at outflow (supporting information Figure S2). Analysis of both Li and RWT tracers produced similar estimates of tracer mass recoveries, in-lake tracer retention, and travel times from start of injection at inflow to arrival at outflow (supporting information Figure S2 and Table 2). We used in-lake tracer sampling to evaluate the transport and mixing of tracer within the lake through both space and time. Inflow tracer water initially inserted into the lake metalimnion and areas of high (and low) tracer concentration were evident (Figure 2). A spatial snap-shot of RWT concentrations taken on a lake cross-section (sites L5 – L8) perpendicular to flow from the lake inflow to outflow on 6/22 (1 day after injection began), indicates areas of high (red) and low (blue) tracer concentrations immediately after injection (Figure 2). These data suggest a plunging inflow, with insertion of tracer inflow water into the lake metalimnion and limited interaction with the epi- and hypo-limnions on this cross section (Figure 2). However, tracer mixing through the lake was evident as time since injection increased (Figure 2). While tracer concentrations were highest in the metalimnion immediately postinjection, they were greatest in the deeper lake strata (hypolimnion) at longer times since injection (Figure 2). These data indicate that while metalimnetic waters were rapidly loaded with inflow tracer water, they also promptly flushed (Figure 2). Conversely, while hypolimnetic waters took longer to be loaded with inflow tracer water, they did not flush as readily (relative to the metalimnion) and retained tracer for longer periods (Figure 2). Consequently, while some tracer (and water) was rapidly transported through the lake, other tracer (and water) was stored for considerable (>90 days) lengths of time (Figure 2 and Table 2).

3.2. Lake, Stream, and Groundwater Dissolved Organic Carbon and Nutrient Concentrations

We developed depth-profiles of nutrients for all lake sites through time, but present representative data from lake site 7 (L7, Figure 1). Both NO$_3$-N and NH$_4$-N concentrations in the lake were typically low and
were always less than 20 μg L\(^{-1}\) (Figures 3a and 3b). There was a NO\(_3\)-N peak of 19 μg L\(^{-1}\) on 7/16, but concentrations were otherwise below 5 μg L\(^{-1}\) (Figure 3a). NH\(_4\)-N concentrations varied between 1 and 11 μg L\(^{-1}\), and did not demonstrate strong patterns with depth or over time (Figure 3b). There was a peak in PO\(_4\)-P concentrations at all depths on 6/19, with a maximum concentration of 111 μg L\(^{-1}\) at 3 m (Figure 3c). Outside of this one event, PO\(_4\)-P concentrations were low and were always less than 7 μg L\(^{-1}\) for all depths (Figure 3c). There were 12.7 mm of rain between 6/16 and 6/17, which may have contributed to this PO\(_4\)-P pulse. Average summer nutrient concentrations in the lake were 1 μg L\(^{-1}\) for NO\(_3\)-N, 6 μg L\(^{-1}\) for NH\(_4\)-N, and 13 μg L\(^{-1}\) PO\(_4\)-P (Table 3). However, the mean of the PO\(_4\)-P concentrations was heavily skewed by the pulse event and the median of PO\(_4\)-P concentrations was 0.5 μg L\(^{-1}\) PO\(_4\)-P, which is equal to the detection limit. DON concentrations varied with time and across depths between 10 and 83 μg L\(^{-1}\) (Figure 3d). DON concentrations generally decreased with time for all depths except 9 m, where concentration increased from June to August (Figure 3d). TDN trends were similar to DON and DON comprised 50–98% of TDN.
DOC concentrations varied between 624 and 1859 mg L\(^{-1}\) and generally decreased at all depths through time (Figure 3f).

Maximum stream runoff at all gauging locations occurred during the end of May with two additional (though smaller) runoff peaks during early and late June (Figure 4). NO\(_3\)-N concentrations were highest at all stream sampling sites during early May, declined to low levels (<10 mg L\(^{-1}\)) by the beginning of June, and remained low for the duration of the season (Figure 4b). Average seasonal (May–October) NO\(_3\)-N concentrations decreased in the downstream direction, while NH\(_4\)-N concentrations increased moving downstream (Table 3). There was considerable temporal variability in NH\(_4\)-N concentrations, particularly at inflow and outflow (Figure 4). There was a peak in PO\(_4\)-P of 48 μg L\(^{-1}\) on 5/24 at upstream, but otherwise PO\(_4\)-P concentrations were low.
concentrations were less than 13 μg L⁻¹ at this site (Figure 4b). There was a simultaneous peak of in PO₄-P of 179 μg L⁻¹ on 5/24 at inflow, but concentrations had fallen to 3 μg L⁻¹ by 5/30 at this site (Figure 4c). From 5/30 to 10/3 PO₄-P concentrations at inflow were generally less than 5 μg L⁻¹, with one peak of 37 μg L⁻¹ observed on 6/19 (Figure 4c). This PO₄-P peak was simultaneously observed on 6/19 within the lake (111 μg L⁻¹ at L7, Figure 3c) and at outflow (76 μg L⁻¹, Figure 4d). The PO₄-P peak that occurred at inflow on 5/24 was not simultaneously observed at outflow (concentration of 3 μg L⁻¹ PO₄-P on 5/24 at outflow). Instead, down-network transport induced a lag and a peak of 34 μg L⁻¹ (compared to 37 μg L⁻¹ at inflow) was observed on 5/26 at outflow, 2 days later than the PO₄-P peak at inflow (Figure 4). Unfortunately, in-lake sampling had not begun on 5/24 – 5/26 because the lake was still ice covered, so we are unable to compare the timing of lake nutrient dynamics to inflow-outflow patterns for this particular PO₄-P pulse.

DON, TDN, and DOC concentrations were low in early May, rose with the hydrograph to maximum concentrations during peak runoff, and subsequently declined with the stream hydrograph at all sites (Figure 5). DON comprised the majority of TDN and consequently DON and TDN patterns were similar (Figure 5 and Table 3). DON comprised 68% of TDN at upstream, 76% at inflow, and 74% at outflow over the May to October time period (Figure 5). Average seasonal (May – October) DON concentrations increased in the downstream direction and were 27 μg L⁻¹ at upstream, 29 μg L⁻¹ at inflow, and 46 μg L⁻¹ at outflow (Table 3). Similarly, average seasonal DOC concentrations increased in the downstream direction from 1020 μg L⁻¹ at upstream, to 1092 μg L⁻¹ at inflow, and 1403 μg L⁻¹ at outflow (Table 3). Ratios of DOC:DON were 37.8 at upstream, 38.3 at inflow, and 30.5 at outflow, and DOC:TDN ratios followed similar patterns (Table 3). Groundwater concentration of DON (160 μg L⁻¹) and TDN (521 μg L⁻¹) were particularly high, while DOC (1800 μg L⁻¹) concentrations were only slightly higher than surface water values (Table 3). In addition to having high DON and TDN, GW samples also had high NH₄-N (358 μg L⁻¹) concentrations and C:N ratios of GW samples were 11.3 (DOC:DON) and 3.5 (DOC:TDN, Table 3).

Table 3. Average Concentrations for the Period of Study (May–October) for Sampling Locations Along With Dissolved Organic Carbon (DOC) to Dissolved Organic Nitrogen (DON) and DOC to Total Dissolved Nitrogen (TDN) Ratios

<table>
<thead>
<tr>
<th>Location</th>
<th>NO₃-N (μg L⁻¹)</th>
<th>NH₄-N (μg L⁻¹)</th>
<th>PO₄-P (μg L⁻¹)</th>
<th>DON (μg L⁻¹)</th>
<th>TDN (μg L⁻¹)</th>
<th>DOC (μg L⁻¹)</th>
<th>DOC:DON</th>
<th>DOC:TDN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upstream</td>
<td>7</td>
<td>5</td>
<td>6</td>
<td>27</td>
<td>40</td>
<td>1020</td>
<td>37.8</td>
<td>25.5</td>
</tr>
<tr>
<td>Inflow</td>
<td>5</td>
<td>4</td>
<td>12</td>
<td>29</td>
<td>38</td>
<td>1111</td>
<td>38.3</td>
<td>29.2</td>
</tr>
<tr>
<td>Lake</td>
<td>1</td>
<td>6</td>
<td>13</td>
<td>37</td>
<td>44</td>
<td>1400</td>
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</tr>
<tr>
<td>Outflow</td>
<td>3</td>
<td>8</td>
<td>8</td>
<td>46</td>
<td>57</td>
<td>1403</td>
<td>30.5</td>
<td>24.6</td>
</tr>
<tr>
<td>Groundwater</td>
<td>3</td>
<td>358</td>
<td>6</td>
<td>160</td>
<td>521</td>
<td>1800</td>
<td>11.3</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Figure 4. (a) Snow water equivalent (SWE) and rain inputs from the Banner Summit SNOTEL site located <2 km NE of Bull Trout Lake; (b) runoff hydrograph, nitrate (NO₃-N), ammonium (NH₄-N), and phosphate (PO₄-P) concentration time series for upstream; (c) runoff hydrograph, NO₃-N, NH₄-N, and PO₄-P concentration time series for inflow; and (d) runoff hydrograph, NO₃-N, NH₄-N, and PO₄-P concentration time series for outflow.
We evaluated relationships between Q and DOC and nutrient concentrations using linear regression, and found statistically significant relationships between Q and PO₄-P, DON, TDN, and DOC concentrations (Figure 6). Relationships between Q and NO₃-N and NH₄-N concentrations were not statistically significant (Figure 6). NO₃-N, PO₄-P, DON, TDN and DOC were positively correlated with Q, while Q and NH₄-N were negatively related. Discharge explained a greater amount of variation in DOC concentrations than in other constituents (Figure 6).

Average concentrations of NH₄-N, DON, TDN, and DOC were statistically greater at outflow relative to inflow (two tailed t-test, $a=0.05$, Table 4). Concentrations were 103% (NH₄-N), 59% (DON), 52% (TDN), and 26% greater at outflow relative to inflow. Average concentrations of NO₃-N and PO₄-P were 28% (NO₃-N) and 37% (PO₄-P) lower at outflow relative to inflow, although these differences were not statistically significant (Table 4). We also assessed relationships between NO₃-N and NH₄-N, and DOC and NO₃-N and NH₄-N to evaluate the potential for stoichiometric [Taylor and Townsend, 2010] and thermodynamic [Helton et al., 2015] tradeoffs and controls on DOC and nutrient flux. We observed a tradeoff between NO₃-N and NH₄-N, where one or the other could be found in high concentration but never were both simultaneously high (Figure 7). High concentrations of NH₄-N and DOC occurred in GW samples, and NH₄-N and DOC concentrations were comparatively low in streamwater (Figure 7b).

### 3.3. Lake Storage and Stream Fluxes of Dissolved Organic Carbon and Nutrients

We combined lake hypsometry with nutrient concentrations sampled at all water column depths at all lake sampling sites to estimate the mass of DOC and dissolved nutrients contained within the lake water column over the June to September lake sampling period. In-lake storage of DIN was greatest during early June and declined as the summer progressed (Figure 8). DIN storage in the lake ranged from 2 to 23 kg and was dominated by NH₄-N, which comprised 62–95% of total in lake DIN (Figure 8b). Accordingly, NO₃-N consistently comprised a relatively small fraction of lake DIN (Figure 8b). In-lake PO₄-P content was generally less than 3 kg, with one increase in PO₄-P concentration on 6/19 that resulted in 85 kg of in-lake PO₄-P (Figure 8c). The mean of in-lake PO₄-P storage was 10 kg, but this was heavily skewed by the 6/19 PO₄-P increase as indicated by the median in-lake PO₄-P storage of 1 kg (Figure 8c). In-lake TDN content ranged from 36 to 70 kg (Figure 8d). On 6/9 DON accounted for 49% of the in-lake TDN but otherwise accounted for 77 – 95% (Figure 8d). Dissolved organic carbon (DOC) storage in the lake was considerably higher than DON. Lake storage of DOC ranged from 1299 to 2024 kg and was greatest during peak runoff and declined slightly as the season progressed (Figure 8). In-lake content of organic constituents (DON and DOC) was more stable relative to storage of inorganic nutrients (NH₄-N, NO₃-N, and PO₄-P) over the June–September period.
Specifically, inorganic nutrient mass (NH$_4$-N, NO$_3$-N, and PO$_4$-P) in the lake was initially high during early June and subsequently decreased with the snowmelt hydrograph, whereas DON and DOC mass remained more stable with time (Figure 8).

Total seasonal NO$_3$-N flux decreased in the downstream direction (Figure 9a). This pattern shifted for NH$_4$-N where greatest flux occurred at outflow (41 g ha$^{-1}$), followed by upstream (24 g ha$^{-1}$), then inflow (19 g ha$^{-1}$), Figure 9b). The majority of cumulative NO$_3$-N flux occurred by mid-June at each of the three stream sampling sites (Figure 9a). Conversely, NH$_4$-N cumulative mass flux increased steadily throughout the season (Figure 9b). In fact, 82% of the cumulative NO$_3$-N flux occurred by the first week of June while only 35% of the total NH$_4$-N flux had occurred by this time (Figures 9a & 9b). Increases in cumulative PO$_4$-P flux were punctuated by pulse events on 5/24, 5/26, and 6/19 that transported large amounts of PO$_4$-P and accounted for large proportions of the total cumulative flux (Figure 9c). Early season dynamics dominated PO$_4$-P flux at upstream and inflow with 95% and 85% of the total flux occurring by 6/1 at upstream and inflow respectively (Figure 9c). Conversely, at outflow only 24% of the total PO$_4$-P flux had occurred by 6/1, and cumulative flux was dominated by a pulse event during the 6/19 – 6/22 time period (Figure 9c).

DON fluxes increased in the downstream direction (Figure 9d). Patterns of TDN flux were similar to DON, and total fluxes amounted to 210 g ha$^{-1}$ at upstream, 254 g ha$^{-1}$ at inflow, and 460 g ha$^{-1}$ at outflow (Figure 9e). At inflow, DON comprised 79%, NH$_4$-N contributed 7%, and NO$_3$-N accounted for 14% of the total TDN flux. Conversely, at outflow DON comprised 85%, NH$_4$-N contributed 9%, and NO$_3$-N accounted for 6%

![Regression analysis of streamwater dissolved nutrient and carbon concentrations versus stream discharge for: (a) nitrate (NO$_3$-N); (b) ammonium (NH$_4$-N); (c) phosphate (PO$_4$-P); (d) dissolved organic nitrogen (DON); (e) total dissolved nitrogen (TDN); and (f) dissolved organic carbon (DOC). Data are shown for each of the three stream sampling sites: upstream, inflow, and outflow.](image)

![Table 4. Summary of Average Differences Between Inflow and Outflow Concentrations](table)

<table>
<thead>
<tr>
<th></th>
<th>NO$_3$-N</th>
<th>NH$_4$-N</th>
<th>PO$_4$-P</th>
<th>DON</th>
<th>TDN</th>
<th>DOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflow-outflow difference (µg L$^{-1}$)</td>
<td>-1.4</td>
<td>4.1</td>
<td>-4.4</td>
<td>17.1</td>
<td>19.8</td>
<td>291.8</td>
</tr>
<tr>
<td>Inflow-outflow difference (%)</td>
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<td>103</td>
<td>-37</td>
<td>59</td>
<td>52</td>
<td>26</td>
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<tr>
<td>Confidence interval</td>
<td>±2.5</td>
<td>±2.2</td>
<td>±14.7</td>
<td>±11.7</td>
<td>±11.5</td>
<td>±239.7</td>
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<tr>
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<td>-0.598</td>
<td>2.928</td>
<td>3.432</td>
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<tr>
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<td>0.533</td>
<td>0.005</td>
<td>0.001</td>
<td>0.018</td>
</tr>
<tr>
<td>Observations</td>
<td>65</td>
<td>65</td>
<td>60</td>
<td>65</td>
<td>65</td>
<td>65</td>
</tr>
</tbody>
</table>

*A negative difference indicates a decrease in concentration from inflow to outflow; a positive difference indicates an increase. Concentrations of NH$_4$-N, DON, TDN and DOC were significantly greater at outflow relative to inflow at a significance level of $\alpha = 0.05$. Both NO$_3$-N and PO$_4$-P concentrations were lower at outflow relative to inflow, however these differences were not statistically significant.
of the TDN flux. Similarly to DON, DOC fluxes increased in the downstream direction (Figure 9f). The cumulative fluxes of all constituents (NO$_3$-N, NH$_4$-N, PO$_4$-P, DON, TDN, and DOC) were dominated by early season exports, specifically: 92% of the NO$_3$-N, 73% of the NH$_4$-N, 91% of the PO$_4$-P, 86% of the DON, 85% of the TDN, and 85% of the total DOC fluxes at the watershed outlet (outflow) had occurred by 7/1 indicating large percentages of these fluxes took place during peak runoff periods (Figure 9).

Stream DIN fluxes were comprised primarily of NO$_3$-N (62% of total DIN flux) at inflow and NH$_4$-N (58% of total DIN flux) at outflow (supporting information Figure S3). Daily DIN fluxes were greatest during peak runoff and declined with the stream hydrograph at both inflow and outflow (supporting information Figure S3). There was consistently greater flux out of the lake than into the lake for all constituents except for NO$_3$-N and PO$_4$-P, indicating the lake was a source of NH$_4$-N, DON, TDN, and DOC; and a sink for NO$_3$-N and PO$_4$-P (Figure 10). During peak runoff, there was greater NO$_3$-N flux out of the lake but this trend reversed after 6/1 (Figure 10a). The NH$_4$-N mass balance from inflow to outflow of the lake increased fairly linearly with time, indicating consistently more NH$_4$-N leaving than entering the lake as streamflow (Figure 10b). The lake was a source of DON during the early part of the season (prior to 6/15), but input-output mass balances were more balanced and leveled off after 6/15 (Figure 10d). During the 5/1 – 9/1 time period there was 179 g ha$^{-1}$ greater TDN flux out of than into the lake, 91% of which was comprised by DON (Figure 10e). DOC dynamics were similar to DON although the magnitude of DOC flux was considerably larger; during the 5/1 – 9/1 time period there was 2,222 g ha$^{-1}$ greater DOC flux out of than into the lake (Figure 10f). For all dissolved constituents early season dynamics (before 7/1) were important in setting the inflow-outflow mass balances (Figures 9 and 10).

4. Discussion

4.1. What Are the Dynamics of Through Lake Travel Times and Flow Paths?

Travel times and flow paths between lake inflows and outflows are a function of initial insertion, transport, and mixing [Andradottir et al., 2012; Ayala et al., 2014; Owens et al., 2014]. In turn, these transport (travel
times) and mixing (flow paths) processes can exert strong influence on the spatial and temporal dynamics of within lake biogeochemical processing and productivity [Van de Bogert et al., 2012]. We observed fast and slow flow systems through Bull Trout Lake (BTL), associated with inflow and mixing dynamics. During the tracer injection (6/21 – 6/23), inflow water plunged and inserted into intermediate depths [Wetzel, 1983]. Some of this inflow water and tracer moved rapidly to the lake outlet, potentially due to low mixing as a result of density driven isolation. However, mixing and entrainment of a portion of inflow water was evident in both lateral and vertical dimensions initially and through time postinjection. Water and tracer that entered deeper strata tended to remain there for considerable lengths of time, while surface and intermediate layers flushed more quickly. This variability in flow paths and mixing led to considerable variability in through-lake travel times. For instance, while there was a rapid transport system associated with modal travel times, the tracer BTC was heavily skewed toward longer travel times and demonstrated power law tailing. Although power law tailing is often associated with subsurface exchange in small streams [Haggerty et al., 2000, 2002; Cardenas et al., 2008], these data suggest that the combination of fast flow pathways and less mobile regions of surface water storage in the lake can also produce power law tailing behavior. One result of this spatial and temporal heterogeneity of stream-lake mixing is that lake outlet grab samples integrate a distribution of travel times and flow paths. Accordingly, interpreting outlet signatures should be improved if done in conjunction with information about physical transport and mixing processes. Additionally, spatial and temporal heterogeneity in mixing and travel times influence DOC and nutrient residence times and the strength of interaction between solutes and biology. If information were available, incorporating physical mixing and transport heterogeneity into lake biogeochemical models should help constrain processing and associated flux estimates relative to models that assume a fully mixed lake with a singular residence time.

Tracer mass recovery at lake outflow indicates that a substantial proportion of the RWT (59%) and Li (63%) injected at inflow was retained in the lake over the 90 day sampling period (Table 2). Because sampling...
ended near the onset of winter, it is likely that much of this tracer entered flow paths that did not exit the lake until the following snowmelt season or even later. Although considerable tracer was retained in the lake, the outflow total runoff was slightly greater than inflow total runoff during this same timeframe. This demonstrates the ability of lakes to decouple kinematic and solute pulses [Lottig et al., 2013]. Additionally, although lakes may have limited hydrologic buffering capacities during high flows [Arp et al., 2006], they may retain and transform considerable dissolved nutrient loads, and thus maintain high biogeochemical buffering capacities during these periods. Theoretically, DOC and nutrients that are retained within the lake for longer periods of time should experience greater biological processing. Because in-lake transport (e.g., travel times and flowpaths) of surface- and ground-water inputs to lakes is a result of initial insertion and

Figure 9. Cumulative mass fluxes for upstream, inflow, and outflow sites for (a) nitrate (NO$_3$-N); (b) ammonium (NH$_4$-N); (c) phosphate (PO$_4$-P); (d) dissolved organic nitrogen (DON); (e) total dissolved nitrogen (TDN); and, (f) dissolved organic carbon (DOC). Watershed outlet runoff is shown in each panel for context.

Figure 10. Nutrient mass balances from inflow to outflow. Values above the dashed lines indicate the lake was a source for that constituent, while values below the dashed lines indicate the lake was a sink for that constituent. The pie chart in panel E indicates that dissolved organic nitrogen (DON) accounted for 91% (163 g ha$^{-1}$) and dissolved inorganic nitrogen (DIN) accounted for 9% (16 g ha$^{-1}$) of the net positive total dissolved nitrogen (TDN) export from inflow to outflow.
subsequent mixing (turbulent diffusion and entrainment), these physical transport mechanisms can have a direct impact on in-lake biological processing. It is however important to note that the travel times important for and relevant to biological nutrient processing are solute (e.g., dissolved conservative tracer), not hydrologic or kinematic travel times. Accordingly, it is important to consider in-lake solute travel times and flow paths when interpreting in-lake biological processing and outlet nutrient flux; when possible these dynamics should be incorporated into lake processing models and export estimates.

4.2. How Does an In-Network Lake Influence the Timing, Form, and Magnitude of Down-Network Dissolved Organic Carbon and Nutrient Flux?

We observed shifting streamwater composition along the network from upstream to inflow to lake to outflow. We found that the lake was a source for NH₄-N, DON, TDN, and DOC but a sink for NO₃-N and PO₄-P over the 5/1 – 9/1 timeframe. During the early season, the lake was originally a source for NO₃-N but became a sink as the season progressed. This could be a combined result of limited storage [Arp et al., 2007], kinetic (temperature), and biomass constraints during early snowmelt periods. During snowmelt runoff, high flow and limited storage could potentially lead to shortened travel times and less opportunity for biological NO₃-N processing (e.g., assimilation and denitrification). However, other studies in the region have demonstrated the ability of lakes to attenuate snowmelt NO₃-N pulses [e.g., Brown et al., 2008; Epstein et al., 2012], and the early snowmelt NO₃-N inflow-outflow source behavior we observed could be related to other hillslope and watershed processes [Creed et al., 1996; Creed and Band, 1998; Perrot et al., 2014] beyond lake control.

DIN composition shifted from inflow to outflow and the lake was a sink for NO₃-N and a source for NH₄-N. Specifically, DIN flux at inflow was comprised primarily by NO₃-N (64%), but was 58% NH₄-N at lake outflow. This shifting composition of DIN observed from inflow to outflow is likely due to a few mechanisms. First, uptake of NO₃-N has been shown to be fairly high in both stream [Covino et al., 2010] and lake [Epstein et al., 2012] compartments of the Bull Trout network. Second, contributions of NH₄-N from near-lake GW and the lake sediments (i.e., mineralization of organic material) likely contributed increased NH₄-N to lake outflow. Rapid water column uptake of inflow NO₃-N has been shown to induce greater in-lake residence times for NO₃-N relative to hydrologic residence times [Epstein et al., 2012]. As NO₃-N is taken up and incorporated into biomass, upon death it settles to lake sediments, which can be mineralized and released as NH₄-N. As such, rapid NO₃-N uptake and decomposition of organic mater in lake sediments and near lake wetland soils are likely responsible for the observed shifts in inflow-outflow DIN composition. Our results also suggest thermodynamic controls on DIN dynamics, and support the notion that redox condition is an important driver of the relationships between DOC, NO₃-N and NH₄-N concentration patterns [Helton et al., 2015]. While it has been suggested that stoichiometry can control DIN dynamics along fluvial networks, our results indicate redox controls on these observed patterns.

In addition to redox influences, we also observed hydrologic controls on DOC and nutrient fluxes. The relationship between stream discharge (Q) and concentration was strongest for DOC. There did not appear to be a dilution effect or source depletion; in fact DOC concentration increased with Q across the range of flows. Relationships between Q and concentration also increased for NO₃-N, DON and TDN but the patterns were not as strong as those for DOC. On the other hand, there was no relationship between Q and NH₄-N or PO₄-P. NH₄-N concentrations were particularly nonresponsive to flow. This is potentially related to redox controls on NH₄-N patterns (described above), in-lake mineralization, and contributions from GW sources. Hydrologic processes did have influence on PO₄-P, with peaks in PO₄-P concentrations at higher flows; but PO₄-P was typically in very low concentrations presumably due to high biological demand. Under such high demand, biological controls would dominate concentration patterns and hydrology would have less influence.

While the lake was a slight sink for NO₃-N, it was a strong sink for PO₄-P. These PO₄-P dynamics could be the result of several retention mechanisms. First, because PO₄-P has high binding capacity, it often settles and is buried in lake sediments [Wodaka et al., 1985]. Second, due to the low availability of P in these study systems [Marcarelli and Wurtsbaugh, 2007, Goodman et al., 2010], it may be a limiting resource and consequently in high biological demand. Similar studies in other regions have documented greater P relative to N-retention, and these results have been interpreted as indicative of P limitation [Verburg et al., 2013]. Bull Trout Lake attenuated PO₄-P pulses that occurred on 5/24 and 6/19, and this attenuation was particularly
areas, and residence times of lakes with relatively standard uptake rates, can result in substantial PO4 uptake. The strong reduction in mass was initially surprising, the uptake rates required to produce this reduction in mass were actually equivalent (if not on the low side) to published values for both lakes [Lean and White, 1983] and streams in the region [Davis and Minshall, 1999]. As such, combining the large volumes, benthic areas, and residence times of lakes with relatively standard uptake rates, can result in substantial PO4 uptake within lakes. In addition to biological uptake, PO4-P can also be retained in lakes through physical sedimentation and sorption processes. Combined physical and biological retention mechanisms can result in substantial PO4 retention within lakes along fluvial networks.

The source behavior of the lake in terms of NH4-N, DON, TDN, and DOC is likely related to both in-lake productivity and contributions from near-lake GW sources. Increased residence time, higher water temperature, and large surface area within the lake and in lake sediments can contribute to high levels of productivity. This increased productivity is potentially responsible for the observed DOC, DON, and TDN source dynamics and changing C:N ratios (Table 3). The low C:N ratios at outflow relative to upstream ratios, are suggestive of in-lake productivity (autochthonous carbon) and support the notion that the source behavior is related to production. Along with in-lake production, contributions from GW could also be partly responsible for the NH4-N, DOC, DON, and TDN source behavior of the lake and changing C:N ratios. Because near-lake GW had high NH4-N, DON, TDN, and DOC concentrations along with low C:N ratios, it is challenging to decipher between influences from GW contributions and in-lake production. Studies of lakes in similar settings have found that GW was a substantial contributor, accounting for 30 – 74% of annual lake outflow [Hood et al., 2006]. Conversely, other studies have suggested in-lake productivity was responsible for the DOC source behavior of lakes, but largely disregarded GW sources [Goodman et al., 2011]. Given that the near-lake wetland GW had NH4-N concentrations of 358 µg L⁻¹ (Table 3), it seems likely that contributions from this source influenced inflow-outflow NH4-N balances. Ammonification and mineralization processes in lake sediments and near-lake wetland soils combined with low hydraulic conductivities and oxygen concentrations could lead to the build up of NH4-N in these source areas [Mitsch and Gosselink, 1993].

Large portions of the lake margins of BTL are occupied by wetlands, and hydrologic connections between the wetlands and lake could provide considerable NH4-N, DON, and DOC to the lake. In fact, wetlands have been noted to exert strong influence over total nitrogen, phosphorous, DON, and DOC concentrations in the streams and lakes they hydrologically connect to [Devito et al., 1989; Schiff et al., 1998; Martin and Soranno, 2006]. Although we observed source behavior for NH4-N, DON, TDN, and DOC, this is by no means universal and results from other studies indicate considerable variability in sink-source behavior of lakes in relation to these constituents [Tranvik et al., 2009; Lottig et al., 2013; Kang et al., 2016]. It does appear that in low nutrient fluvial networks, such as our study site, lakes can be sources of organic material and reduced nitrogen (NH4-N) to downstream locations while simultaneously being a sink for NO3-N and PO4-P. Current estimates suggest that small lakes are considerably more abundant than previously recognized; as such it is important to quantify the role small in-network lakes can have on hydrologic and biogeochemical fluxes. Determining the relative importance of lakes in network and regional DOC and nutrient budgets requires data driven analyses such as those presented here.

5. Summary

We observed both fast and slow flow systems through the lake, along with heterogeneity in mixing of inflow tracer. Tracer injected to the lake inflow had a modal travel time of 3.5 days from start of injection to arrival at lake outflow (800 m straight line distance). In addition to this fast flow system, a slow flow system retained injected tracer and led to long tailing behavior observed in the outflow tracer breakthrough curve. This heterogeneity in tracer travel times was related to mixing dynamics, as tracer that mixed with epilimnetic waters was retained for considerable periods while tracer from hypo- and meta-limnetic regions...
flushed more quickly. Additionally, we observed considerable in-lake retention of injected tracers and 59% (Li) and 63% (RWT) of the total mass injected at inflow was not recovered at outflow over the 90 day sampling period. This indicates that some tracer and associated water entering the lake during the 2 day injection period (6/21 – 6/23) had in-lake travel times of longer than 90 days. Our tracer data combined with inflow-outflow discharge measurements indicated a decoupling of hydrologic (kinematic) and solute pulses through the lake. This decoupling of kinematic and solute pulses increased in-lake dissolved nutrient travel times and opportunity for biological processing. We found that DIN shifted from being composed of 64% NO3-N at inflow to 58% NH4-N at outflow. This shifting DIN composition was likely a function of in-lake NO3-N uptake, along with NH4-N contributions from lake sediments and near-lake GW sources. Together, these processes led to differential timing of total N component fluxes, and 82% of the cumulative NO3-N flux had occurred by the first week of June while only 35% of the total NH4-N flux had occurred by this time. Our data and analyses indicated redox controls on DIN dynamics but hydrologic controls on DOC patterns. The lake was a sink for NO3-N and PO4-P, but a source for NH4-N, DON, TDN, and DOC. Our results suggest that lakes within low nutrient fluvial networks can be sources of dissolved organic material and reduced nitrogen (NH4-N) to downstream communities while simultaneously being sinks for NO3-N and PO4-P. Given the abundance of lakes in fluvial networks of mountainous watersheds, determining the cumulative effect of lakes on DOC and inorganic nutrient budgets is a necessary first step toward improved understanding of network- to regional-scale dynamics in these systems.


NADP (National Atmospheric Deposition Program) (2008), Atmospheric integrated research monitoring network, NADP, Champaign, Ill.


