Dust Deposition Changes Production, Chlorophyll-a and Community Composition in Mountain Lakes

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DUST DEPOSITION CHANGES PRODUCTION, CHLOROPHYLL-A,
AND COMMUNITY COMPOSITION IN MOUNTAIN LAKES

by

Jiahao Wen

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Ecology

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UTAH STATE UNIVERSITY
Logan, Utah

2022
ABSTRACT

Dust deposition changes production, chlorophyll-a, and community composition in mountain lakes

by

Jiahao Wen, Master of Science

Utah State University, 2022

Major Professor: Dr. Janice Brahney
Department: Watershed Sciences

Drought and human land use have increased dust emissions and deposition in the western United States. Remote mountain lakes are sensitive to atmospheric deposition, which has the potential to change their chemical and biological condition. However, to date this assertion has not yet been experimentally tested. Using in situ bioassays, we investigated the effects of dust enrichment on the production, chlorophyll-a, and taxonomic composition of phytoplankton communities in three mountain lakes in the American West. We found that dust additions increased chlorophyll-a concentration at all three lakes, but the magnitude differed, ranging from 32% to 226%. This variation may be associated with the pre-existing status of the lake (such as trophic status and attendant nutrient limitation). Surprisingly, bioassays with increased dust additions showed decreased $^{14}$C primary productivity, which we interpret as dust inputs enhancing dark $^{14}$C uptake by stimulating chemoautotrophic bacterial production and phytoplankton allocating energy (ATP) from photosynthesis to assimilate nutrients instead of using the same energy source to fix carbon. The relative abundance of algal species in dust treatments was different from the controls such that mixotrophs to total phytoplankton
ratios increased in dust treatments at Castle Lake possible due to increased DOC. Our study presented experimental evidence on how dust deposition influences remote mountain lakes in the short term which may contrast with longer-term observations. We proposed a hypothesis that the pre-existing status of the lake may affect the responses of dust inputs which needs to be tested in the future.
PUBLIC ABSTRACT

Dust deposition changes production, chlorophyll-a, and community composition in mountain lakes

Jiahao Wen

Increasing quantities of dust emitted from semi-arid soils, agricultural soils, and urban regions are blown to remote mountain lakes in the American West. Remote mountain lakes lacking local nutrient inputs and presenting simple food webs that are easily affected by climate changes. Dust can carry nutrients (e.g., nitrogen and phosphorus) to mountain lakes and potentially enhance algae growth and change algal communities. However, experimental tests of this hypothesis are lacking. Using in situ experiments, we investigated the effects of dust enrichment on the production, biomass, and primary algal species in three mountain lakes in the American West. We found that dust additions increased algal biomass at all three lakes, but the magnitude differed, ranging from 32% to 226%. This variation may be associated with the initial algal biomass before the experiments and nutrient limitation of lakes (limited by nitrogen, phosphorus, or both). Surprisingly, bioassays with increased dust additions showed decreased $^{14}$C primary productivity (the ability to fix carbon). The first reason could be that dust inputs stimulated bacteria that could fix carbon under dark conditions while suppressing algal production that can fix carbon during the day. The second reason could be that nutrient-limited algae allocated energy to uptake dust nutrients instead of fixing carbon after the dust was added. The relative abundance of algal species changed in dust treatments compared to the controls. Mixotrophs (algal species that both photosynthesize
and take up organic matter or eat other plankton) increased at Castle Lake but decreased at the other lakes compared to phototrophs (species that only photosynthesize). Our study presented experimental evidence on how dust deposition influences remote mountain lakes. We proposed the hypothesis that the pre-existing status of the lake may affect the responses of dust inputs. This hypothesis needs to be tested in the future.
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INTRODUCTION

Drought and human land use have dramatically disturbed the global nitrogen (N) and phosphorus (P) cycles leading to cross-ecosystem nutrient fertilization through atmospheric transport (Baron et al. 2000; Wolfe et al. 2001; Brahney et al. 2015b). Nutrient fertilization in lakes typically occurs from lakes being directly connected to human activity within their catchments; however, dust can transfer nutrients to remote ecosystems that would otherwise be free of direct human impact (Morales-Baquero et al. 2006; Brahney et al. 2014, 2015b). Recent studies indicate that dust deposition in the American West has risen by 500% above the late Holocene average following the increased settlement during the nineteenth century (Neff et al. 2008). More recently (>1995), dust emissions have increased by an additional 168% on average, peaking in 2009 in the American West (Brahney et al. 2013; Tong et al. 2017). Dust emissions have also been increasing in other areas such as Tibetan Plateau in China (Wan et al. 2016) and the southern hemisphere (Hooper and Marx 2018; Brahney et al. 2019).

Dust can carry significant amounts of N and P to ecosystems, although dust composition can be expected to vary with biogeography and land use (Neff et al. 2002; Zhang et al. 2018b; Reche et al. 2022; Scholz and Brahney 2022). The bioavailability of dust nutrients in aquatic systems has been of recent interest due to the eutrophication of mountain lake systems (Morales-Baquero et al. 2006; Brahney et al. 2015b; Stoddard et al. 2016), but has been difficult to quantify. Previous research has not considered dust nutrients (particularly, P) as bioavailable due to limited bioavailable P extractions from Saharan dust (Guerzoni et al. 1999; Eijsink et al. 2000). However, dust chemical composition is variable globally and can contain up to 67% organic matter, increasing the
potential for P release (Brahney et al. 2022). Using an atmospheric model combined with
direct measurements, an estimated 40% increase in P deposition has occurred from pre-
industrial times to the present (Brahney et al. 2015b). In addition to elevated dust
emissions, dust-P concentrations may have increased as dust is now increasingly
generated from P-rich substrates such as dry lakebeds and agricultural and semi-arid
soils.

Dust N can contribute a significant fraction to total N deposition flux but
historically has been poorly constrained and underappreciated. Studies have found that
water-soluble organic N in dust can contribute up to 66% of total atmospheric N
deposition in North America (Neff et al. 2002). Dust N mass may vary in space and time
due to biomass burning, agricultural activities, and natural soils with varying amounts of
organic N (Mace et al. 2003; Lawrence and Neff 2009; Brahney et al. 2022). With
increasing dust deposition regionally and decreasing nitrate emissions, dust N might play
an increasingly important role compared to N deposition in rain and gaseous aerosols.

Increasing dust emissions in the American West can affect mountain lake ecology
by changing their chemical and biological conditions with nutrient additions. Several
studies have identified dust-derived P as a driver of mountain lake eutrophication through
observation and correlation (Brahney et al. 2014, 2015b, a). For instance, on a longer
time scale, studies using historical data or paleolimnological approaches reported the
association between dust deposition and increased nutrient concentrations in lakes
(Brahney et al. 2015b), spatial variances of lake responses to dust deposition (Brahney et
al. 2014), altered biogeochemical cycles (Ballantyne et al. 2011), increased primary
production (Brahney et al. 2015a) and chlorophyll-a (chl-a) concentration (Morales-
Baquero et al. 2006), and altered phytoplankton functional assemblages (Jacquemin et al. 2019). However, only one study (Reche et al. 2009) to date has experimentally tested the effect of Saharan dust on lake nutrient status and bacterial community composition.

Dust deposition effects on western US lakes could differ than those using Saharan or synthetic dust (Eglinton et al. 2002; Malm et al. 2004). Sahara dust is dominated by sedimentary minerals with low bioavailable P. In addition, in Saharan dust, much of the P is adsorbed onto iron-rich particles (Krom et al. 1991). Studies have shown that only 10% - 25% of phosphorus in Saharan dust is water leachable (Herut et al. 1999; Baker et al. 2006). US dust generated from agricultural soils or semi-arid soils tend to have organic matter up to 67% (Malm et al. 2004; Brahney et al. 2022), and thus should have higher P bioavailability than Saharan dust (Kattra et al. 2016) because organic P is readily available in lake systems by phosphatase hydrolysis (Correll 1998). Biomass burning and wildfires also contribute to high soluble P concentration in the dust depending on the degree of pyrolyzation (Boy et al. 2008; Bigio and Angert 2019), and thus have the potential to influence aquatic ecosystems (Goldman et al. 1990; Mackey et al. 2013).

The sensitivity of mountain lakes to shifts in deposition within its airshed makes mountain lakes ideal spots to study dust deposition effects. Mountain lakes have been sentinels of dust deposition due to their natural oligotrophic status, small, steep, and bare watershed conditions, as well as low acid neutralizing capacity (ANC) (Catalan et al. 2017; Moser et al. 2019). Mountain lakes are typically formed in exposed rock substrates and located in remote areas with a low extent of human disturbance and thus present oligotrophic status. Their bare watershed landscapes result in low nutrient weathering and leaching into lakes but steep slopes can increase nutrients (e.g., dust deposition) washed
from watersheds into lakes (Brahney et al. 2014). The absence of base cations from chemical weathering and watershed inputs also indicates that dust can lead to an increase of ANC in mountain lakes since dust particles in the American West are rich in calcium-bearing minerals (Skjelkvåle and Wright 1998; Ballantyne et al. 2011; Brahney et al. 2013). Additionally, mountain lakes may be subject to higher rates of dust deposition due to the higher elevation of lakes. High mountains can intercept the dust that are usually mixed and transported above the boundary layer between 1000-1500 m (Catalan et al. 2017). Furthermore, the sensitivity of mountain lakes to dust deposition also depends on dust P species and their relative bioavailability to phytoplankton. Inorganic P in dust is easily leached and available for algae after deposition into the water column, which is important considering P is a key limiting nutrient in the aquatic ecosystem (Schindler 1977). Organic P compounds can be utilized after enzymatic hydrolysis; the hydrolysis efficiency is dependent upon organic P concentration, species and specific phytoplanktonic composition (Boström et al. 1988). Considering that US dust typically is dominated by alkaline particles rich in P and organic matter, mountain lakes in the American West should see a large response to dust deposition (Malm et al. 2004; Brahney et al. 2013). Here we experimentally evaluated the effects of dust deposition using in-situ bioassays in three mountain lake systems of the American West.

METHODS

To evaluate the effect of dust on phytoplankton community composition, we conducted in situ bioassay experiments in three mountain lake systems in the American West. We elected to use a mixed dust sample collected from several sites around the US
on all three lakes to separate the biological response to dust composition from the
differences between attendant lake conditions. The three lakes are Castle Lake, CA,
Flathead Lake, MT, and The Loch, CO. An on-site lab at Castle Lake allowed us to
perform time-dependent bioassay trials as well as quantify production using the $^{14}$C
technique.

**Study Sites**

Castle Lake (41°13′ N, 122°22′ W) is located in the Trinity Mountains (Northern
California) at 1660 meters above sea level (m asl) and is ice-free for 135 days on average.
It is a slightly alkaline meso-oligotrophic lake typically expressing co-limitation of
nitrogen and phosphorus of the phytoplankton community most of the time (Park et al.
2003). The Loch (40°17′ N, 105°39′ W) is located in Rocky Mountain National Park
(Colorado) at 3109 m asl and is ice-covered from October to April. It is a slightly acidic
oligotrophic lake with P limitation due to high N deposition historically (Baron et al.
1991). Flathead Lake (47°53′ N, 114°04′ W) is located between Mission Mountains and
Salish Mountains in northwestern Montana at 881 m asl. It is a large and deep, slightly
alkaline, ultra-oligotrophic lake (Elser et al. 2022). Since nutrient limitations can
frequently shift between seasons and locations, nutrient limitation assays were also
performed coincident with the dust addition assays. The detailed lake characteristics are
shown in Table 1.

**Dust Collection and Determination of Bioavailable Nutrients**

Dust samples were collected using Dry Sampling Units (DSU) in conjunction
with the National Atmospheric Deposition Program (Brahney et al. 2020). The sampler
Table 1 Characteristic of two studied mountain lakes. SA: Surface area. MD: Mean depth. Chl-a: Initial Chl-a concentration.

<table>
<thead>
<tr>
<th>Lake</th>
<th>SA (km²)</th>
<th>MD (m)</th>
<th>CA:LA</th>
<th>Elevation (m)</th>
<th>pH</th>
<th>Chl-a (ug/L)</th>
</tr>
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<tr>
<td>Castle Lake</td>
<td>0.2</td>
<td>11.4</td>
<td>4.2</td>
<td>1660</td>
<td>8.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Flathead Lake</td>
<td>510</td>
<td>39</td>
<td>43.6</td>
<td>881</td>
<td>8</td>
<td>0.9</td>
</tr>
<tr>
<td>The Loch</td>
<td>0.05</td>
<td>1.5</td>
<td>132</td>
<td>3109</td>
<td>6.5</td>
<td>3.5</td>
</tr>
</tbody>
</table>

captures the dry gravitational fallout of dust while eliminating contamination through a series of screens. The screens also act as a wind buffer preventing the excavation of dust from the sampler (Brahney et al. 2020). We collected dust monthly from 11 sites across the western US between 2019-2020 using methods described in (Brahney et al. 2020). Dust samples were frozen until further analyses.

To identify the amount of bioavailable P in dust materials, we used a sequential extraction procedure modeled after the method for P speciation in soils (Moir and Tiessen 2007) and sediments (Ruttenberg 1992). Both methods have been used in previous dust studies (Zhang et al. 2018b; Scholz and Brahney 2022). We combined sediment- and soil-based P fractionation procedures in this study to assess what nutrients are immediately bioavailable (minutes to hours) versus nutrients that would become available through microbial degradation in the medium term (days to weeks) (Fig. 1).

The sequential P extraction procedure consisted of five steps (Fig. 1): 1) The first step extracts readily soluble and freely exchangeable inorganic P (P$_i$), using 0.1 M of KCl; this extraction represents the most bioavailable P. 2) In the second step, 0.5 M of sodium bicarbonate (NaHCO$_3$) is used to release labile P$_i$ and organic P (P$_o$) that is absorbed on soil minerals and a small amount of microbial P with the bicarbonate ion. Bicarbonate extracted P$_i$ is available in the short term and organic P is easily
mineralizable (Moir and Tiessen 2007; Hou et al. 2018). 3) In the third step, we use 0.1 M of sodium hydroxide (NaOH) to extract amorphous and some crystalline Al and Fe phosphates and more labile forms of P\textsubscript{o}. Extracted inorganic P in this step is not readily available due to Al and Fe binding but P\textsubscript{o} correlates well with algal-available P forms in bioassay studies (Sonzogni et al. 1982; Dorich et al. 1985). 4) The fourth step uses acetic acid-sodium acetate solution at pH 4 to extract authigenic and biogenic apatite and Ca-associated P\textsubscript{i} and P\textsubscript{o}, which are also not readily bioavailable as this fraction is stable at the pH of most lake waters and sediments. 5) The residue left after step 4 is unlikely to contain anything but highly recalcitrant Pi. To determine the total P in the last step, we used dry oxidation by combusting samples at 550 °C, followed by digestion with 1 M HCl (Andersen 1976).

For all extractions, we used a ratio of 2:1 (dust: solution). Between each step, dust was rinsed and dried overnight. P\textsubscript{i} was measured using the standard molybdate blue method (Murphy and Riley 1962) and total phosphorus (TP) was measured similarly after the extraction was digested with potassium persulfate. P\textsubscript{o} was obtained by subtracting P\textsubscript{i} from TP. The residual in the last step was analyzed for total TP only. P measurements were conducted on SpectraMax® M2 spectrometer (Fig. 1).

**Lake Bioassay Experiments**

We used sequential leaching to estimate the amount of P released in each experimental trial in the absence of biological activity. Here we define bioavailable P as the combination of KCl extracted P, NaHCO\textsubscript{3} extracted P, and NaOH extracted P\textsubscript{o}. As such, every 1 mg of the mixed dust materials releases 0.1 μg KCl extracted P (the most bioavailable P) and 0.75 μg bioavailable P (including KCl extracted P) (Fig. 1). Average
Fig. 1 A flow chart of dust P fractionation and bioavailability and experimental design. Bio- P includes KCl extracted P, NaHCO$_3$ extracted P, and NaOH extracted P$_o$ (green backgrounds). OP includes extracted P$_o$ from NaHCO$_3$, NaOH, and acetic buffer (dotted lines).

dust deposition rates to three lake sites (estimated from nearby NADP sites) were 1.8 to 25 g m$^{-2}$ year$^{-1}$ and TP concentrations in dust ranged from 1.1 to 5.4 mg g$^{-1}$. Based on dust-P loadings to the lake and the final phosphate concentration, we set up three gradients of dust addition. The low dust addition (12 mg/L) mimicked a dust event to the lake. The medium dust addition (24 mg/L) mimicked dust accumulated all summer. The high dust addition (40 mg/L) mimicked an input level in which all winter snowpack dust is delivered to the lake during snowmelt. Because Flathead Lake has a much larger size and thus a larger epilimnion volume to dilute dust inputs than the other two lakes, at Flathead Lake dust was added at 5 mg/L, 10 mg/L, and 20 mg/L (Fig. 1).
In-situ bioassay experiments were conducted using a buoy-frame-anchor system in July and August 2021. At each site, slightly different methods were used based on available facilities and distinct lake morphologies. We collected lake water using an integrated water sampler (one 2.5-m clear PVC tube with two rubber stoppers) and removed large zooplankton with a 180-um mesh. We dispensed the mixed lake water into 250-ml clear incubation bottles (in the Flathead Lake experiment, we used 4.5 L of clear incubation bottles). Each treatment had three replicates. Pre-mixed and weighed dust materials were added into filled bottles and shaken. All bottles were attached to polyvinyl chloride (PVC) frame with zip ties. The frame was attached to a buoy and an anchor using ropes. All the bottles were suspended and incubated for 96 h in the epilimnion (about 1-2 m under the surface, receiving about 70% of coming light). The bottles were rotated and shaken at least once every day during the bioassay. To determine nutrient limitation during the bioassay, three replicate incubations were conducted for N addition treatment, P addition treatment, and N+P addition treatment. Nutrients were added, N as KNO\textsubscript{3} and P as KH\textsubscript{2}PO\textsubscript{4}, to reach a final concentrations of 448 ug/L N and 62 ug/L P (Fig. 1).

**Determination of Chlorophyll-a and Primary Production**

At Castle Lake, we conducted primary productivity (PPR) experiments in parallel with the bioassay. Three light samples and one dark bottle sample from each dust treatment and the control were labeled with inorganic $^{14}\text{C}$ in the form of NaHCO\textsubscript{3} and then incubated in 150-ml glass bottles at the same depth as our primary bioassay experiment for 4 hours. The PPR samples were incubated at 48 and 96 hours of the primary bioassay to determine temporal trends of primary production (Goldman 1988).
At the end of the bioassay, we separated water into subsamples for the following analyses. We filtered water samples with MF-Millipore 0.45µm MCE membrane filters (HAWP02500) and dried filters for 24 hours. Filters were counted on a pico-counter (IPC-650, Protean Instrument Corporation). PPR was calculated by subtracting dark bottle PPR from light bottle PPR.

For the determination of chl-a concentration, the sample was passed through pre-ignited (450 °C, 4 h) acid-rinsed ADVANTEC 0.3-µm glass fiber filters. The filters were wrapped in tinfoil and kept frozen until analyses. Chl-a analysis followed the fluorometric method (Welschmeyer 1994). To determine the daily chl-a concentration variation, we examined relative fluorescence units (RFU) variation during the incubation at Castle Lake. We measured RFU at 44 hours, 68 hours, 78 hours, and 96 hours using a Turner Designs 10-AU fluorometer.

**Determination of Nutrients and Phytoplankton Community**

Filtrates from the chl-a analyses were used for C, N, and P analyses. Filtrates were acidified with trace metal grade HCl to pH 2. Total dissolved P (TDP) and soluble reactive phosphorus (SRP) concentrations were determined following the ascorbic acid method and persulfate digestion method on SpectraMax® M2 spectrometer (Baird et al. 2017). Organic P concentrations were calculated from TDP and SRP. Total dissolved nitrogen (TDN), total dissolved carbon (TDC), and inorganic carbon (IC) were determined by the Skalar C/N analyzer directly while dissolved organic carbon (DOC) was estimated from TDC and IC. We also measured the fluorescence index (FI) to determine the source of DOC in the dust treatments (Fellman et al. 2010). Unfiltered water subsamples were preserved with 50% glutaraldehyde to a final concentration of 2%
for phytoplankton community analysis. Samples were stored in dark bottles and cooled, and immediately sent to the BSA Environmental Services for community composition and biovolume quantification.

**Statistical Analyses**

Chl-a concentrations were examined for differences across the control, dust, and nutrient treatments using analysis of variance (ANOVA) for each lake. We used natural log response ratios (LRR) to calculate the effects of dust and nutrient treatments on chl-a concentrations in three lakes. LRR has been frequently used as an effect size metric in the ecological research (Cabreroz et al. 2020). The equation was as follows:

\[
LRR_{\text{treatment}} = \ln \frac{\text{Chl-a}_{\text{treatment}}}{\text{Chl-a}_{\text{control}}}
\]  

(1)

To determine the daily chl-a increase rates across dust addition gradients, we conducted a linear regression analysis of daily RFU in dust treatments and the control and compared slopes of regression lines using ANOVA. The final chl-a concentrations from the nutrient addition bioassays in each lake were used to determine the lake nutrient limitation proposed by (Elser et al. 2009) which is defined in 5 classifications: single N or P limitation, synergistic N-P co-limitation, strict N-P co-limitation, sequential co-limitation by N or P, and dual limitation. Net primary production rates (PPR) were examined for differences across the control and dust treatments using analysis of variance (ANOVA).

To characterize the effects of dust treatments on phytoplankton community composition, we first estimated the relative abundance of mixotrophs to the total
And then we visualized differences in community composition in each lake using Principal Coordinates Analysis (PCoA) based on a Bray–Curtis distance matrix with the ‘vegan’ package in R. Bray–Curtis distance was developed by (Bray and Curtis 1957) to calculate (dis)similarity in community composition. PCoA is able to present visual group information based on distance (dissimilarity) matrix. Bray–Curtis distance based PCoA has been commonly used in microbial and phytoplanktonic studies (e.g. Paliy and Shankar 2016; Aanderud et al. 2019; Xu et al. 2022). To quantify the effects of dust treatments on phytoplankton size variations, we first classified size structure from biovolume based on equivalent spherical diameter (ESD) (Finkel et al. 2010). We calculated biomass for diatoms and other cells using regression equations (2), and (3) according to (Strickland 1970) based on laboratory data. This method has been used in recent publications (Soria-Piriz et al. 2017). To better visualize how dust additions influence the size structures in three lakes, we normalized biomass to describe the trend only. All analyses and figures were conducted in R version 4.1.3 (R Development Core Team 2021).

\[
\log C = 0.76 \log V - 0.29 \tag{2}
\]

\[
\log C = 0.94 \log V - 0.60 \tag{3}
\]

Where \( C \) is carbon mass (pg cell \(^{-1}\)) and \( V \) is cell volume (\( \mu \text{m}^3 \) cell \(^{-1}\)).

To estimate the contribution of bacterial productivity between treatments, we assume that \(^{14}\text{C} \) dark uptake rate represents the chemoautotrophic bacteria productivity because chemoautotrophic microorganisms contribute to large fractions of \(^{14}\text{C} \) uptakes in the dark (Swan et al. 2011; Callieri et al. 2014). We also calculated the percentages of \(^{14}\text{C} \)
dark uptake rate (one replicate) to light uptake (three replicates) after 48 and 96 hours at Castle Lake to determine the contributions from bacterial activities (Gieskes et al. 1979).

RESULTS

Nutrient Limitations and Chlorophyll-a Concentrations

Our studies revealed that three mountain lakes had different nutrient limitation conditions during the bioassay (Fig. 2A). At Castle Lake, only N+P addition significantly increased chl-a concentration by 151%, indicating strict co-limitation. At Flathead Lake, P addition significantly increased chl-a concentration by 243% while N+P additions had a super-additive effect (341%), suggesting sequential co-limitation with P. At The Loch, only P significantly increased chl-a concentration by 72%, which indicated that The Loch was limited by P alone (Fig. 2A). When comparing the effect sizes of nutrient additions on chl-a, we found that N addition had negative influences on chl-a concentration at The Loch and weak influences at other two lakes (LRR$_N < 0.3$). P addition had the largest effect at Flathead Lake (LRR$_P = 1.23$) and the smallest at Castle Lake (LRR$_P = 0.07$). NP addition influenced Flathead Lake substantially (LRR$_{NP} = 1.48$) while affecting The Loch by the lowest amount (LRR$_{NP} = 0.46$) (Fig. 2B).

At the end of the bioassay, dust stimulated chl-a concentrations at three lakes by 1.32 to 3.26-fold (Fig. 2A). Dust addition gradients had a clear positive relationship with chl-a increase. Low dust treatments increased chl-a concentration at Castle Lake and The Loch by 1.48-fold and 1.32-fold, respectively but had no clear relationship at Flathead Lake (Fig. 2). Medium and high dust treatments stimulated chl-a by 2.33 and 3.26-fold (Castle Lake), 1.63 and 1.8-fold (The Loch), respectively (Fig. 2). At Flathead Lake, we
only saw a response for the high dust treatment (1.86-fold, Fig. 2). Log response ratios revealed that Castle Lake had the largest responses for all dust treatments with the highest LRR values and The Loch had the lowest increase of chl-a from low to high dust treatments (Fig. 2B). The high dust treatment at Flathead Lake substantially elevated LRR values from LRR\textsubscript{low} and LRR\textsubscript{med} (Fig. 2B).

**Fig. 2** A) Chlorophyll-a concentrations at the end of bioassay in controls, dust, and nutrient treatments in three mountain lakes. Significant differences between treatments for each lake are indicated by capital letters. B) Log response ratios (LRRs, effect sizes, left y-axis) and fold changes (right y-axis) for chl-a concentration in dust and nutrient treatments over the control in three lakes.

During the bioassay at Castle Lake, relative fluorescence units (RFU) consistently increased along the incubation time in dust treatments (Fig. 3). From 44 hours to 96 hours, RFU increased 1.69, 2.08, and 2.12-fold at low, medium, and high dust treatments, respectively. In controls, RFU decreased at 68 and 96 hours and reached a peak at 78 hours. Compared to the control, RFU increased 1.27, 1.38, 1.67-fold (44 hours), 1.67, 2.11, 2.36-fold (68 hours), 1.53, 1.99, 2.35-fold (78 hours), and 1.85, 2.47, 3.01-fold (96 hours) in low, medium, and high dust treatments, respectively (Table 2).
Fig. 3 RFU variations during the incubation at Castle Lake. Statistical differences in slopes between treatments were indicated by letters.

Table 2 Fold changes in RFU in dust treatments compared to controls at Castle Lake.

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
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<td>44 h</td>
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<td>96 h</td>
<td>1.85</td>
<td>2.47</td>
<td>3.01</td>
</tr>
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</table>
**14C Primary Production**

Our 14C experiment showed that PPR decreased with increasing dust concentration. PPR in the low dust treatment had the highest PPR (29 mg C m⁻³ h⁻¹) and was significantly different from the PPR in the high dust treatment (18 mg C m⁻³ h⁻¹) and the control (22 mg C m⁻³ h⁻¹). PPR in medium dust treatment (26 mg C m⁻³ h⁻¹) was significantly higher than PPR in high dust treatment (Fig. 4).

![Graph showing net primary production rates (PPR) (mean ± se) at the end of bioassay experiment at Castle Lake. Statistical differences in PPR between treatments are indicated by letters.](image)

**Fig. 4** Net primary production rates (PPR) (mean ± se) at the end of bioassay experiment at Castle Lake. Statistical differences in PPR between treatments are indicated by letters.

During the bioassay experiment (after 48 hours), percentages of dark 14C uptake to light 14C uptake (%) increased from the control (3.4 %), low (6.2 %), medium (10.48 %) to high (17.14 %) dust treatments. At the end of the bioassay (after 96 hours), percentages of dark 14C uptake to light 14C uptake had a 0.68, 0.75, 0.53 and 0.63 -fold change in the control, low, medium, and high dust treatments, respectively, compared to 48 hours (Fig. 5). Similar to the pattern after 48 hours, percentages of dark 14C uptake to light 14C uptake
Fig. 5 A) Estimated bacterial productivities from raw dark $^{14}$C uptake values. B) The percentage of dark $^{14}$C uptake to light $^{14}$C uptake in $^{14}$C experiment.

(%) increased from the control (2.2 %), low (4.7 %), medium (5.7 %) to high (10.9 %) dust treatments after 96 hours (Fig. 5).

Estimated bacterial productivities from dark uptake rates in our $^{14}$C experiment showed that bacterial productivities increased along dust addition treatments, after both 48 and 96 hours. The bacterial productivities increased 1.91, 2.82, and 5.2-fold (48 hours), and 2.72, 2.93, and 4.07-fold (96 hours), in low, medium, and high dust treatment, respectively, compared to the control (Fig. 5).

**Phytoplankton Community Composition**

The community composition (indicated by the relative abundance of the main phytoplankton genus) was altered by dust treatments in three lakes, particularly in Flathead Lake. The community composition in the control was separated from low dust treatment by axis 1 at Castle Lake, which explained 71% of the variation (Fig. 6). Low dust treatments were separated from other treatments at The Loch by axis 2 which
explained 16% of the variation (Fig. 6). All treatments at Flathead Lake were separated by axis 1 and 2 which explained 68% of the variation together (Fig. 6).

Weighted scores for species explained changes on dominant species and occurrence of new species, which drove separations of treatments at each lake (Fig. 6). At Castle Lake, abundance of dominant species such as *Ochromonas* *spp.* and *Chlamydomonas* *spp.* were the highest in low dust treatments than other treatments (Fig. 6 & Table A1). *Eucapsis* *spp.* were found at treatments except for low dust treatments. At Flathead Lake, abundance of *Ochromonas* *spp.* and *Chlamydomonas* *spp.* were the highest in controls while abundance of *Chlorella* *spp.* and *Chroococcus* *spp.* were the highest in low dust treatments (Fig. 6 & Table A1). Abundance of *Ochromonas* *spp.* increased with increasing dust additions while not found in controls. *Synedra* *spp.* were found in medium and high dust treatments while *Eucapsis* *spp.* were found only in high dust treatments (Fig. 6 & Table A1). At The Loch, the separation of low dust treatments and other controls were mainly derived by new species including diatoms (e.g., *Nitzschia* *spp.*, *Cocconeis* *spp.* etc.) and cyanobacteria and changes on dominant species (decreased abundance of *Chrysochromulina* *spp.* compared to other treatments) (Fig. 6). *Dinobryon* *spp.* were mainly found in medium dust treatments. Abundance of *Chlorella* *spp.* and *Chlorococcum* *spp.* were higher in controls than dust treatments (Fig. 6 & Table A1).

The abundance of mixotrophs relative photoautotrophs increased at Castle Lake but decreased at the other two lakes (Fig. 7). Abundance in photoautotrophs such as *Eucapsis* *spp.* decreased 21% to 100% at Castle Lake but increased up to 2.2-fold at The Loch in high dust treatments relative to controls (Table A1). Mixotrophs such as
Ochromonas spp., increased in abundance in dust treatments by up to 2.45-fold at Castle Lake but decreased up to 79% at Flathead Lake relative to controls (Table A1).

**Fig. 6** Principal coordinates analysis (PCoA) with Bray–Curtis distance showing the effects of dust addition on the relative abundance of the main phytoplankton genera in the three study lakes. Weighted scores were calculated and visualized for species contributing to PCoA results.
**Fig. 7** Mixotroph : photoautotrophs abundance ratio (mean ± se) at the end of the experiments in controls, dust, and nutrient treatments in three mountain lakes.

**DISCUSSION**

The objectives of this study were to use *in-situ* bioassay experiments to investigate the impacts of dust on chl-a, primary production, and community composition in mountain lakes. The three mountain lakes (Castle Lake, Flathead Lake, and The Loch) provided a wide range of initial lake conditions to explore both general and site-dependent relationships between dust deposition and lake responses. In general, we found that dust additions led to ecosystem shifts in all three lakes, though the manifestation differed in each system. Specifically, we observed that 1) Dust addition increased chl-a concentration at three lakes but to different extents; 2) Increasing dust inputs reduced $^{14}$C primary productivity at Castle Lake; 3) Dust additions increased microbial activity and changed phytoplankton community composition.
While all lakes showed a positive chl-a response to dust additions, the degree of oligotrophy in each lake appears to have moderated the effect of dust deposition. We suggest that the more moderate response observed at Flathead Lake is a direct result of its ultraoligotrophic status and lower phytoplankton concentrations at the start of the experiment (Table 1). Because algae growth rate (cell division) is associated with initial biomass (e.g., chl-a concentration), the duration of the experiment and starting algal concentration may dictate the degree of chl-a response. Our finding is consistent with previous findings that dust increased chl-a concentration by 10% to 280% in marine ecosystems (Liu et al. 2013; Chien et al. 2016; Zhang et al. 2018a) and across field observations in lake ecosystems (Brahney et al. 2014).

The attendant nutrient deficiencies appear to be another factor moderating the effect of dust deposition. Because dust additions include both labile N and P, dust additions have the potential to produce a larger effect than P additions alone. This was observed at N&P co-limited Castle Lake. At Castle Lake, chl-a concentrations in dust treatments were higher than in the P-alone addition treatment (Fig. 2). Another supporting observation is that total N concentrations in water after 96 hours at Castle Lake were lower in the medium and high dust treatments than in the controls and low dust treatments, indicating N utilization by algae, while total N concentrations remained higher than controls in dust treatments at The Loch (Table A2).

The finding that chl-a increased while PPR decreased in Castle Lake experiments is unexpected and there are several possible explanations. One, because biomass-specific primary productivity (PPR/chl) is size-dependent, and changes of total productivity are usually enhance by large-sized phytoplankton (Malone 1980), we hypothesized that the
low dust treatment stimulates larger algae, while the high dust treatment selects for smaller-sized cells. We tested this hypothesis by examining the size distribution of the plankton in each treatment. However, we found only weak evidence supporting this explanation (Fig. A1). For example, we see a trend that low and medium treatments stimulated larger sized species (e.g., 50-100 μm) while high dust treatments stimulated smaller-sized cells (e.g., 0-10 μm), but not at Castle Lake where we observed the decrease in production. Second, dust may release toxic materials such as As, Pb, Sb, Cr, Cu, and Cd (Brahney et al. 2014) and thus suppress algal growth and production (Paytan et al. 2009). This may be more likely when dust is anthropogenic in origin, which often includes high metal concentrations from industry and transportation (Marín et al. 2017). In this study, we mixed dust materials emitted from areas with different land uses, including mining, and distances to urban areas, which may indicate dust metal toxicity from some sites. We think that dust toxicity is feasible in our experiments. Future tests with macro and micronutrients (such as K, Ca, Mg, Fe, Mo, Ni, Mn, Cu, Zn) and toxic elements (such as As, Pb, Sb, Cr, Cu, and Cd) in dust would provide stronger evidence.

Another possible explanation is that dust inputs could stimulate dark $^{14}$C uptake (carbon dioxide fixation that occurs in the dark) by stimulating chemoautotrophic bacterial production. First, the supporting evidence for this explanation is that dark $^{14}$C uptake and bacterial productivity (estimated from dark $^{14}$C uptakes) increased relative controls, low, medium to high dust treatment, either after 48 or 96 hours (Fig. 5). Usually, light and dark bottles are incubated together to correct for the influences of carbon uptake into phytoplankton and other microbes that is independent of light. Studies have reported that in most conditions, the dark carbon fixation rate was less than 5% of
the light carbon fixation (Howarth and Michaels 2000). However, high dark $^{14}$C uptake (e.g., >10% of light bottles) may occur via non-photosynthetic carboxylation processes in the dark by chemoautotrophs and heterotrophs (Gieskes et al. 1979; Markager 1998). Our estimation of bacterial productivities suggests that higher dust inputs appear to stimulate a higher chemoautotrophic bacterial production (Harris et al. 1989). The stimulation of chemoautotrophic production with dust additions is consistent with marine experiments (Marañén et al. 2010; Rahav et al. 2018). For instance, an observational study revealed that bacterial production increased by 25% in coastal waters influenced by dust storm events (Rahav et al. 2018). In a bioassay experiment in the ocean, Marañén et al. (2010) found that Saharan dust addition stimulated bacterial production while suppressing primary production. However, these results contrast a ten-year observational study which found Saharan dust inputs stimulated primary production in the marine environment but not the bacterial production (González-Olalla et al. 2018). The inconsistency could result from differences in observation times. Dust may stimulate bacterial activities at the outset; however, increasing nutrient availability from dust over the long term may lead to greater algal production. In our study, increasing bacterial activities with increasing dust additions likely resulted from nutrients stimulating chemoautotrophs. Nevertheless, labile DOC additions from dust may stimulate heterotrophs as well (Fig. 8). This may be important because high nutrient additions from higher dust loads may lead to faster algal turnover rates and more extracellular release of DOC. To test this idea, we determined the fluorescence index (FI) which can differentiate DOC sources (Fellman et al. 2010). The results showed that dust treatments had lower FI than controls, suggesting that inputs of DOC in dust treatments (Table A3). Taken together, our results suggest that the elevated
addition of both nutrients and DOC stimulated chemoautotrophic and heterotrophic bacteria, which may explain our contrasting observations of increasing chl-a and decreasing PPR (Fig. 8).

**Fig. 8** The influences of dust inputs on A) carbon paths and biological activities and B) $^{14}$C primary productivity. Dotted lines indicate negative effects.

An additional possibility is that phytoplankton allocate energy (ATP) from photosynthesis to assimilate nutrients instead of using that ATP to fix carbon (C) in nutrient-limited conditions in the short-term (Fig. 8). The supporting evidence for this hypothesis is an increasing chl-a quota with decreasing PPR (Fig. 2 & Table A4). Theoretically, nutrient-limited phytoplankton have low photosynthesis and growth rates and are thus low in RNA and protein. Nutrient deficiency usually increases the potential
to take up nutrients by increasing the active transport of nutrients into the cell (Cembella et al. 1982). Active transport needs the energy to drive nutrient uptake; this energy comes from ATP hydrolysis. ATP synthesis has at least four distinct metabolic pathways, two of which are cyclic and non-cyclic photophosphorylation which need light (Beardall et al. 2001). Therefore, energy derived from the light reactions of photosynthesis can be utilized preferentially for nutrient uptake while reducing the allocation of energy to C fixation (Healey 1979; Beardall et al. 2001). Additionally, nutrient-limited phytoplankton are generally rich in carbohydrates and lipids and thus it is not necessary to increase C capture. Presumably, the most direct finding under this theory is that nutrient spikes may suppress C fixation in the short term. Dust nutrients will leach at a slow rate and thus may suppress C fixation in the short term; however, the overall increase in available nutrients from dust additions through time may stimulate primary production in the longer term, as has been observed elsewhere (Brahney et al. 2014; González-Olalla et al. 2018). PPR results in our dust addition experiments were consistent with early studies that found that enrichments of nitrate, ammonium, or phosphate reduced C fixation by 10% - 50% over controls from 6 hours to 1.5 days after nutrients were added (Falkowski and Stone 1975; Lean and Pick 1981; Diaz et al. 2001). Thus, although PPR decreased, chl-a concentration per cell increased with increasing dust inputs (Table A4). These contrasting PPR and chl-a results have been found in previous studies examining nutrient uptake of cultured algae in the short-term (Falkowski and Stone 1975; Healey 1979). In other studies, ATP content was found as a reliable index to cell growth after nutrient additions (Falkowski and Stone 1975; Healey 1979; Beardall et al. 2001). These energy gains
(ATP) may help algae assimilate external nutrients for growth, instead of fixing C in the short term (Falkowski and Stone 1975; Healey 1979).

*In situ* observations have suggested that dust deposition can modify phytoplankton community composition. Here, we found supporting evidence in the PCoA results where the relative abundance of the main phytoplankton genus differed in dust treatments relative to the controls (Fig. 6). Occurrence of new species or disappearance of old species in dust treatments contributed largely to the separation between treatments indicates that dust additions have potentials to shift community composition. Dust additions stimulated diatoms is consistent with other observational studies in mountain lakes, which suggests that dust Ca may play a role (Jiménez et al. 2018). Changes of dominant species such as *Ochromonas spp.* and *Chlamydomonas spp.* in dust treatments were opposite indicates that dust derived functional differences in phytoplankton composition may depend on lake conditions. As we found that the relative mixotroph abundance increased at Castle Lake but decreased at the other two lakes (Fig. 7). Our results revealed that dust mass have different influences on community composition, which is similar to another experimental study in ocean (Zhang et al. 2019). Accumulated N and P with increasing dust additions meet the growth requirement of certain species may explain consistent increases in dust treatments. Some species have preferences for certain nutrient ranges may explain that species were found only in one dust treatment. However, the evidence of community composition changes at present is weak considering the limitations of the study. First, there were very low phytoplankton counts at all sites (20 – 139 x 10^8 μm^3 L^-1) with exceptionally low counts at Flathead Lake (20 x 10^8 μm^3 L^-1). Low algal counts may limit the ability of dust fertilization to alter the
resident community because it may take a longer time to see the significant results.

Large varieties in chl-a quota at Flathead Lake also help to explain little change in chl-a concentrations but the biggest shift in community composition at Flathead Lake (Table A4 & Fig. 6). Second, dust composition is variable and thus includes different nutrient species and mass. Various N:P ratios in dust may have different effects in the phytoplankton community in lakes with different nutrient limitations. Here we used mixed dust materials from areas with different land uses which homogenized their organic matter / bioavailable nutrient concentrations. Nevertheless, our findings suggest at least two implications. First, the increase in mixotroph abundance may be associated with elevated DOC inputs from dust (Fig. 7&8). As we discussed above, the elevated DOC concentrations in higher dust treatments likely fueled bacteria at Castle Lake. This could also lead to a competitive advantage of bacteria over phytoplankton for the inorganic nutrients (Carney et al. 2016). Thus, mixotrophs may increase in response to dust inputs because their main competitors for inorganic nutrients decreased (Thingstad et al. 1996). Previous studies have also indicated that global change-induced DOC inputs could increase heterotrophic and mixotrophic processes in high-altitude mountain lakes (Dory et al. 2022). Second, changes in community composition after dust inputs may depend on initial lake conditions such as nutrient limitation status. For example, studies have found that some species have specific physiological characteristics for N assimilation or have high N demands (Fernandez and Galvan 2007; Olivier 2017). These N-sensitive species (e.g., *Chlamydomonas spp.*, *Chlorococcum spp.*) increased in relative abundance in response to dust addition in NP co-limited Castle Lake but decreased at other lakes (Table A1).
CONCLUSION

Our experimental data support previous assertions that dust-derived nutrient and DOC additions may influence community composition and production in mountain lake ecosystems. However, the manifestation of the effects appears to rely on both the attendant nutrient limitation and likely on the dust composition. In summary, our data suggest that 1) The degree of oligotrophy and nutrient limitation types of lakes may modify dust fertilization efficiency. 2) Dust inputs stimulated dark $^{14}$C uptake by stimulating chemoautotrophic bacterial production. 3) Dust inputs of labile DOC could stimulate microbial respiration which in turn decreases primary production. 4) Dust-induced nutrient and DOC inputs may influence the relative abundance of mixotrophs to autotrophs. Our study provides experimental evidence about how dust deposition influences remote mountain lakes in the short term but such effects may differ from those seen over the long term. Our data also imply possible eutrophication and stimulation of microbial food webs in mountain lakes under enhanced dust emission scenarios.

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Table A1 Relative abundance (mean ± se) of primary genus presented in dust treatments and controls in three lakes.

<table>
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<tr>
<th>Lakes</th>
<th>Treatments</th>
<th><em>Ochromonas. spp</em></th>
<th><em>Chlamydomonas. spp</em></th>
<th><em>Chlorococcum. spp</em></th>
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</thead>
<tbody>
<tr>
<td>Castle Lake</td>
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<td>0.055 ± 0.02</td>
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<tr>
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<td>Medium</td>
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<tr>
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<td>0.068 ± 0.006</td>
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<tr>
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<tr>
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</tbody>
</table>
Table A2  Concentrations of total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), and organic phosphorus (OP) in the water after 96 hours at Castle Lake and The Loch.

<table>
<thead>
<tr>
<th>Lakes</th>
<th>Treatments</th>
<th>TDN (mg L⁻¹)</th>
<th>TDP (μg L⁻¹)</th>
<th>SRP (μg L⁻¹)</th>
<th>OP (μg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castle Lake</td>
<td>Low</td>
<td>0.26</td>
<td>52.80</td>
<td>7.83</td>
<td>44.97</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.18</td>
<td>30.80</td>
<td>2.83</td>
<td>27.97</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0.17</td>
<td>18.80</td>
<td>1.17</td>
<td>17.63</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.23</td>
<td>4.80</td>
<td>2.83</td>
<td>1.97</td>
</tr>
<tr>
<td>The Loch</td>
<td>Low</td>
<td>0.56</td>
<td>44.80</td>
<td>26.17</td>
<td>18.63</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.44</td>
<td>10.80</td>
<td>6.17</td>
<td>4.63</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0.46</td>
<td>8.80</td>
<td>4.50</td>
<td>4.30</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.39</td>
<td>4.80</td>
<td>2.83</td>
<td>1.97</td>
</tr>
</tbody>
</table>

Fig. A1 Variations of normalized biomass based on phytoplankton size (equivalent spherical diameter, ESD) in dust treatments and the control at A) Castle Lake, B) Flathead Lake, and C) The Loch. Biomass were normalized to describe the trend.
**Table A3** DOC concentration indicated by fluorescence index (FI) in water after 96 hours at Castle Lake and The Loch. Higher FI indicates DOC derived from extracellular release and leachate from bacteria and algae while lower FI suggests DOC derived from terrestrial plants and soils.

<table>
<thead>
<tr>
<th>Lakes</th>
<th>Treatments</th>
<th>FI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castle Lake</td>
<td>Low</td>
<td>1.58</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>1.81</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>2.09</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>1.64</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1.89</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>1.77</td>
</tr>
<tr>
<td>The Loch</td>
<td>Control</td>
<td>1.52</td>
</tr>
</tbody>
</table>

**Table A4** Chlorophyll-a concentration per cell (mean ± se) at the end of bioassay in controls, dust, and nutrient treatments in three mountain lakes. Significant differences were indicated by capital letters between treatments for each lake.

<table>
<thead>
<tr>
<th>Lakes</th>
<th>Treatments</th>
<th>Chlorophyll-a concentration per cell (μg 10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castle Lake</td>
<td>Low</td>
<td>0.056 ± 0.0001 B</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.068 ± 0.003 B</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0.098 ± 0.005 A</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.029 ± 0.006 C</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>0.312 ± 0.132 A</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.883 ± 0.434 A</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0.460 ± 0.212 A</td>
</tr>
<tr>
<td>The Loch</td>
<td>Control</td>
<td>1.577 ± 1.076 A</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>0.102 ± 0.075 A</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.031 ± 0.006 A</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0.124 ± 0.036 A</td>
</tr>
<tr>
<td>Flathead Lake</td>
<td>Control</td>
<td>0.021 ± 0.003 A</td>
</tr>
</tbody>
</table>