Hydrobiologia
DOI 10.1007/s10750-016-2758-y

PRIMARY RESEARCH PAPER

Phytoplankton responses to nitrogen enrichment in Pacific Northwest, USA Mountain Lakes

Jason J. Williams · Marc Beutel · Andrea Nurse · Barry Moore · Stephanie E. Hampton · Jasmine E. Saros

Received: 6 January 2016 / Revised: 31 March 2016 / Accepted: 1 April 2016

Abstract Limited information is available about threshold lake nitrogen concentrations necessary to stimulate phytoplankton species and biomass responses in remote nitrogen-limited mountain lakes. We conducted in situ enrichment bioassays in mountain lakes within Mount Rainier, North Cascades, and Olympic National Parks in Washington State, USA to characterize phytoplankton species and biomass responses to nitrogen enrichment, and associated dissolved inorganic nitrogen (DIN) concentration thresholds. Based on biomass and growth measurements, phytoplankton were nitrogen-limited or co-limited by nitrogen and phosphorus in the nine bioassay lakes. We identified 20 taxa that responded to nitrogen enrichment, and estimated response thresholds using nitrogen Monod half-saturation constants (K_s) for 18 of these taxa. DIN thresholds in nitrogen-limited lakes were 13 μg N l^{-1} for any increase in chlorophyll *a*, and 25 μg N l^{-1}, for an increase beyond typical inter-annual chlorophyll *a* variation. K_s values ranged from 0.02 to 77 μg N l^{-1} across most N-responsive taxa, and diatom K_s values were higher than those previously quantified in U.S. Rocky Mountain lakes. Approximately, 75% of sampled mountain lakes in the parks have summer dissolved inorganic nitrogen concentrations below biomass response thresholds. This finding suggests that phytoplankton in park mountain lakes are likely

Handling editor: Luigi Naselli-Flores

Data accessibility Associated raw chlorophyll *a*, phytoplankton cell density, and water chemistry data are available at http://datadryad.org; doi:10.5061/dryad.4fp90.

Electronic supplementary material The online version of this article (doi:10.1007/s10750-016-2758-y) contains supplementary material, which is available to authorized users.

J. J. Williams (✉) · M. Beutel
Department of Civil & Environmental Engineering, Washington State University, Pullman, WA, USA
e-mail: jason.williams2@wsu.edu

A. Nurse · J. E. Saros
Climate Change Institute, University of Maine, Orono, USA

B. Moore
School of the Environment, Washington State University, Pullman, USA

S. E. Hampton
Center for Environmental Research, Education, and Outreach, Washington State University, Pullman, USA

Present Address:
M. Beutel
School of Engineering, University of California, Merced, USA

Published online: 21 April 2016

sensitive to future deposition-induced lake nitrogen enrichment.

Keywords Nitrogen deposition · National parks · Nutrient limitation · Mountain lakes · Phytoplankton · Pacific Northwest

Introduction

Phytoplankton in remote mountain lakes are among the most sensitive ecological indicators of atmospheric nitrogen (N) deposition (Pardo et al., 2011). Many mountain lakes have naturally low N concentrations, watershed characteristics that facilitate efficient flux of deposited N to lakes, and N-limited phytoplankton growth (Baron et al., 2011). Anthropogenic N deposition has increased lake nitrate, and thereby altered phytoplankton species composition, increased phytoplankton biomass, and shifted nutrient limitation from N-limitation to P-limitation in many remote lakes throughout the northern hemisphere (Bergstrom & Jansson, 2006; Elser et al., 2009a; Baron et al., 2011). Threshold or 'critical load' deposition rates for diatom community changes in Western U.S. mountain lakes are 1.0–1.5 kg wet inorganic N ha^{-1} $year^{-1}$ (Baron, 2006; Saros et al., 2010; Sheibley et al., 2014), which is lower than those for other ecological indicators (Pardo et al., 2011). Deposition-induced phytoplankton changes in mountain lakes therefore have been used as an indicator of early stage eutrophication in aquatic ecosystems, and to develop target deposition rates in regional air quality agreements (Porter & Johnson, 2007).

To assess or monitor deposition-induced phytoplankton changes, it is important to characterize phytoplankton responses to lake N increases, and nutrient concentration thresholds associated with those responses in lakes with N-limited phytoplankton growth. Once quantified, thresholds can be used to inform monitoring, assess the number of lakes where thresholds are exceeded, calculate and map critical load deposition rates (Nanus et al., 2012), or help explain phytoplankton community changes observed in sediment cores (Saros et al., 2005; Michel et al., 2006; Arnett et al., 2012). At the species-level, N deposition effects on mountain lake phytoplankton in the Western U.S. have been assessed using indicator diatom species *Asterionella formosa* Hassall and *Fragilaria crotonensis* Kitton. These species have N and P resource requirements that allow rapid growth in response to relatively modest lake N increases (Saros et al., 2005; Michel et al., 2006; Arnett et al., 2012; Nanus et al., 2012), and their relative abundance increased in sediment cores along with increasing N deposition (Saros et al., 2003, 2005; Wolfe et al., 2003; Baron, 2006). N concentration thresholds for species responses in N-limited lakes have been defined as Monod half-saturation growth constants (K_s) quantified in nutrient-enrichment bioassays. K_s values have been quantified for *F. crotonensis* (0.1–0.4 μg N l^{-1}) (Michel et al., 2006), *A. formosa* (2.5 μg N l^{-1}), and a handful of other diatom species (Michel et al., 2006) in the Rocky Mountains, and used to calculate and map critical loads (Nanus et al., 2012). These K_s values indicate that N concentrations near or below detection can induce growth of these species. However, variation in phytoplankton K_s values within and across Western U.S. regions is not clear because K_s has only been quantified for a few diatoms within a few mountain lakes in the Rockies (Michel et al., 2006; Arnett et al., 2012; Nanus et al., 2012). N deposition can also stimulate the growth of soft-bodied taxa that cannot be studied retrospectively in sediment cores (Nydick et al., 2003; Gardner et al., 2008), but may be useful indicators of N enrichment for lake monitoring programs.

At the biomass-level, the mass ratio of dissolved inorganic nitrogen to total phosphorus (DIN:TP) has been used to define thresholds for changes in phytoplankton biomass nutrient limitation in mountain lakes (Morris & Lewis, 1988; Bergström, 2010). Nutrient-enrichment bioassays indicate lakes with DIN:TP mass ratio below 1.5 are typically N-limited, lakes with DIN:TP 1.5–3.4 are co-limited by N and P, and lakes with DIN:TP >3.4 are P-limited (Bergström, 2010). The DIN:TP criterion has helped assess lake sensitivity to N deposition and identify regional patterns of N deposition effects (Murphy et al., 2010; Baron et al., 2011), but it only predicts nutrient limitation shifts for biomass growth. DIN:TP ratios do not indicate the magnitude of biomass response expected in response to lake N increases, which may be relevant for some management applications. They also do not provide information about species or community responses. It is possible that N increases

induce species-level responses in lakes with co-limited or P-limited biomass growth.

The objectives of this study were to characterize phytoplankton species and biomass responses to N enrichment, and to define associated N response thresholds in mountain lakes within Mount Rainier (MORA), North Cascades (NOCA), and Olympic (OLYM) national parks. In the Western U.S., many mountain lakes sensitive to N deposition are located within federal lands, where laws require their protection from air pollution (Fenn et al., 2003). Characterizing phytoplankton responses to N and associated N response thresholds in sensitive lakes is a first step toward calculating and mapping mountain lake critical load N deposition rates (Nanus et al., 2012), which are needed to inform air quality management (Porter & Johnson, 2007; Burns et al., 2008). When combined with lake monitoring data, N response thresholds can be used to estimate the number of mountain lakes above or below thresholds. We used in situ nutrient-enrichment experiments in nine mountain lakes to characterize phytoplankton species and biomass responses to N enrichment, and associated response thresholds. We then compared experimentally defined thresholds to N concentrations observed in park mountain lakes, and estimated the number of sampled mountain lakes above and below thresholds and inferred sensitivity of park mountain lakes to N deposition.

Methods

Study area

Study lakes are located in three national parks within the Cascade and Olympic mountain ranges (Fig. 1). Annual precipitation rates range from 100 to 500 cm across the parks and are strongly influenced by terrain, elevation gradients, and weather systems moving east from the Pacific Ocean. Most park mountain lakes range from 1,000 to 2,000 m elevation, are cirque, tarn, or moraine-dammed lakes formed by past glacial activity, and are typically ice-free from mid-July through October. Park mountain lakes are oligotrophic, and frequently have N and P concentrations below or near detection limits. Nitrogen deposition rates measured at park National Trends Network (NTN) monitoring sites range from 0.38 to 3.24 kg wet inorganic N ha^{-1} $year^{-1}$ (http://nadp.sws.uiuc.edu/data/ntn/). There is no significant trend in annual N deposition rates at the parks from the 1980's to the present (Du et al., 2014). However, N deposition rates at park NTN sites are frequently near or above the 1–1.5 kg wet inorganic N ha^{-1} $year^{-1}$ critical load associated with diatom community changes in the intermountain West. Sheibley et al. (2014) documented increases in the relative abundance of *A. formosa* and *Fragilaria tenera* Smith in a sediment core from Hoh Lake in OLYM as wet inorganic N deposition rates increased above 1–1.2 kg N ha^{-1} $year^{-1}$ in the 1969–1975 timeframe. However, multiple sediment cores in the three parks and elsewhere in the Pacific Northwest with similar or higher deposition rates indicate few occurrences of diatom indicator species, diverse phytoplankton communities that differ substantially among lakes, and no evidence for historic deposition-induced diatom changes (Saros, 2009; Sheibley et al., 2014). These patterns could indicate that critical loads are not yet widely exceeded, phytoplankton growth is not N-limited, or that diatom taxa responsive to N deposition in the Pacific Northwest is different from those in other regions.

Nutrient-enrichment bioassays

We conducted bioassays in three mountain lakes above 1,200 m within each park where available data suggested that N-limitation was likely or N-sensitive diatom species were present (Table 1). Lakes above 1,200 m were selected to exclude lakes that may have nitrogen inputs from nitrogen fixing red alder plants (Clow & Campbell, 2008). Selected lakes were remote and accessible only by foot. Bioassays were conducted during late summer (August or September) in 2013 (NOCA) or 2014 (MORA and OLYM). Our general approach was modeled after other studies (Nanus et al., 2012; Slemmons & Saros, 2012). We collected lake water, divided it among 1 l containers to create experimental treatments, incubated containers in the lake for 7–11 days, and quantified changes in phytoplankton biomass and species densities resulting from treatment nutrient additions. Bioassay treatments were designed to (i) classify species and biomass nutrient limitation, (ii) quantify Monod growth curves and K_s values for N-sensitive species, and (iii) quantify biomass responses to an increasing N concentration

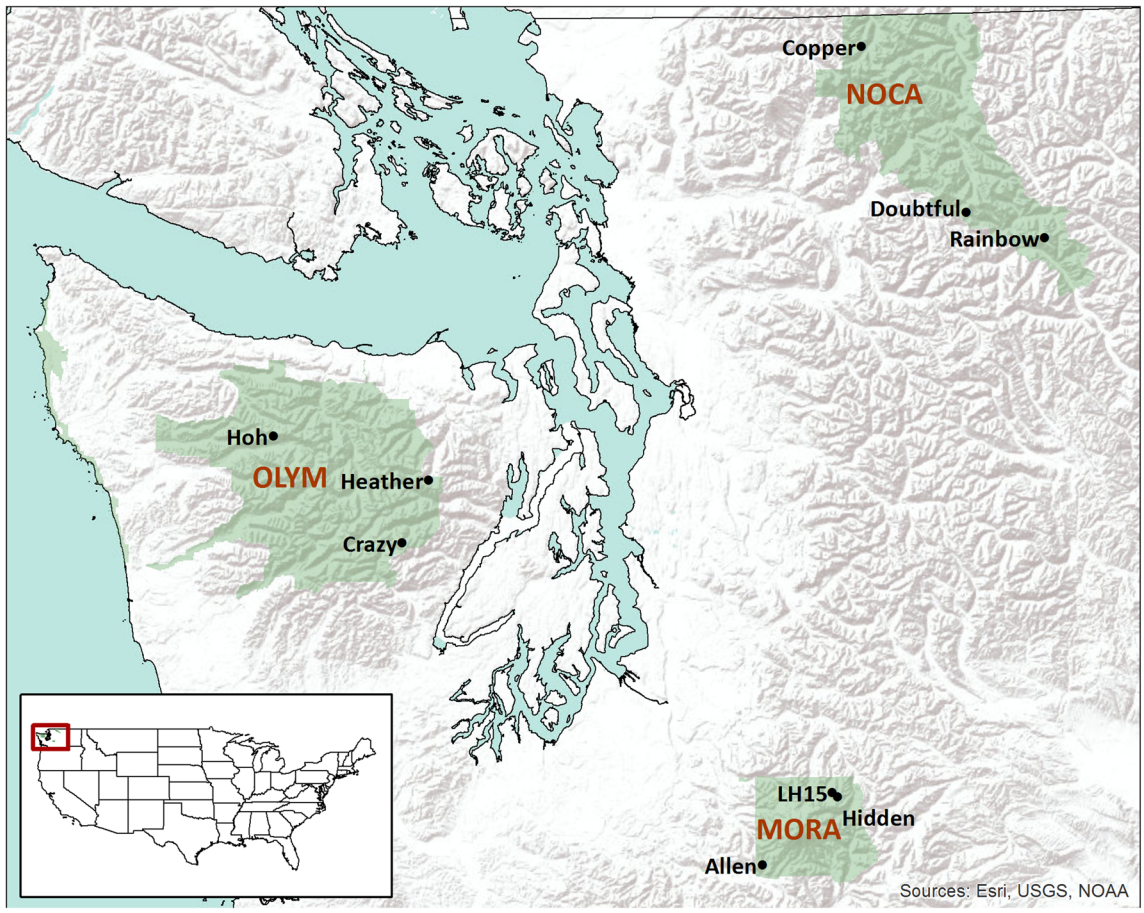

Fig. 1 Locations of Mount Rainier National Park (MORA), North Cascades National Park (NOCA), and Olympic National Park (OLYM), with bioassay lakes indicated in each park

Table 1 Physical characteristics, incubation depth, and experiment duration for study lakes

Park	Lake	Elevation (m)	Area (km^2)	Max depth (m)	Secchi depth (m)	Incubation depth (m)	Incubation length (d)	Incubation temperature (°C)
North Cascades (NOCA)	Rainbow	1,717	0.06	10.4	10.4	5.5	7	17.3
	Doubtful	1,642	0.12	17.7	9.5	4.75	7	13.8
	Copper	1,601	0.05	22.6	19*	9	7	7.6
Mount Rainier (MORA)	LH15	1,659	0.03	10	10	5	7	16.8
	Hidden	1,806	0.02	7	7	3.5	7	16.5
	Allen	1,397	0.02	7.2	7	3.5	7	16.3
Olympic (OLYM)	Crazy	1,457	0.01	7.7	7.7	3.5	11	14
	Heather	1,589	0.004	6.6	6.6	3	8	7.9
	Hoh	1,383	0.07	14.9	11	5.5	8	14.5

Secchi disk depth recorded by NPS in 2011. A Secchi disk depth of 13 m was recorded on Aug 29, 2013 during cloudy skies and heavy rain

gradient. Each of these aspects is discussed in greater detail below.

At each lake, water for experiments was collected from approximately one half of the Secchi depth, or at half the maximum lake depth ("mid depth") if the Secchi disk was visible on the lake bottom. The collection depth was also used as the bioassay incubation depth. One half the Secchi depth typically has sufficient light intensity for phytoplankton growth, but does not cause algal photo inhibition (Morris & Lewis, 1988). A 30–40 l volume of water was collected into 4 l LDPE cubitainers using a battery-powered submersible sampling pump and Tygon tubing. The pump and Tygon tubing were flushed with lake water for 2–3 min prior to collecting experimental water. Large-bodied zooplankton were screened out using 100 µm Nitex mesh on the pump intake. Collected water was subsampled to establish the initial chlorophyll *a* (Chl *a*), nutrient chemistry, and phytoplankton community composition. Triplicate Chl *a* samples were collected by filtering a known volume of water through 0.7-µm Whatman GF/F filters. Filters were placed in petri dishes and wrapped in an aluminum foil. Three 60 ml subsamples were preserved with Lugol's iodine solution to determine initial phytoplankton community composition and species densities. A 60 ml subsample was filtered through a pre-rinsed 0.4-µm polycarbonate filter into an HCl-washed bottle for analysis of dissolved nitrate, ammonium, and ortho-phosphate. An additional 60 ml subsample was filtered for analysis of dissolved silica (Si). A120 ml unfiltered subsample was collected into an HCl-washed bottle for analysis of total phosphorus (TP). All bottles were rinsed three times with sample water before filling. Filters and water samples were stored in a cooler bag packed with snow in the field. Upon return, filters and N and P samples were frozen, and Si and phytoplankton samples were refrigerated until analysis.

After subsampling, collected water was divided among clean 1 l LDPE cubitainers that were pre-rinsed three times with lake water. To classify species and biomass nutrient limitation, four treatments were established (control, N, P, N + P) using concentrations similar to those in previous bioassays (Elser et al., 2009b; Slemmons & Saros, 2012; Bergström et al., 2013). Treatments receiving N had 112 µg N l^{-1} added in the form of $NaNO_3$; treatments receiving P had 31 µg P l^{-1} added as NaH_2PO_4. These treatments were run in triplicate. To quantify growth kinetics of key species as well as biomass responses to an increasing N concentration gradient, six additional treatments were established in triplicate with 0.14, 0.7, 1.4, 7, 14, and 56 µg N l^{-1}. Phosphorus (31 µg P l^{-1} added as NaH_2PO_4) and silica (2.8 mg Si l^{-1} added as Na_2SiO_3) were also added to these N gradient treatments to isolate responses to N additions. N gradient concentrations were selected to mimic those in similar experiments in the Rocky Mountains (Nanus et al., 2012). The 56 µg N l^{-1} treatment was not used at NOCA; it was added in 2014 to bioassays conducted at MORA after relatively small biomass responses were observed in the 14 µg N l^{-1} treatment at NOCA in 2013. Cubitainers were suspended in each lake for 7–11 days, depending on the access constraints (Table 1). Three replicate buoys, each with one treatment replicate, were anchored to the lake bottom. After incubation, each cubitainer was subsampled for Chl *a* and phytoplankton taxonomy as described above. Water temperatures were recorded at the incubation depth using a submersible digital thermometer at the beginning or end of the experiment, or continuously during the experiment using a temperature logger.

Laboratory analyses

Chl *a* filters were extracted in 10 ml of 90% acetone within 3 weeks of collection (USEPA method 445.0). The extract was then clarified by centrifugation and analyzed for Chl *a* using a Shimadzu RF-501 spectrofluorometer (emission at 670 nm, excitation at 430 nm light). Chl *a* samples were not corrected for pheophytin interference. Detection limits, defined as three times the mean of filter blanks, were 0.04 µg l^{-1} or less in each analytical run, and all results were above detection limits. Water samples were analyzed for nutrients using standard methods (APHA, 2005). N and P analyses were performed using a Seal Analytical AA3 Auto-Analyzer at the WSU School of the Environment Water Quality Laboratory according to standard methods (APHA, 2005). Detection limits were 1–2 µg N l^{-1} for nitrate plus nitrite, 4 µg N l^{-1} for ammonium, 2 µg P l^{-1} for ortho-phosphate and TP, and 2 µg N l^{-1} for TN. Si analyses were performed at the Oregon State University Cooperative Chemical Analytical Laboratory using a Technicon Auto-Analyzer II using standard method 4500-SiO_2 E. Phytoplankton samples were settled in an Utermöhl -style chamber and identified to the lowest practical level (typically species or genus, based on

Wehr & Sheath (2003) under ×400 or 600 magnification on a Nikon TS100 inverted microscope. A minimum of 300 individuals were counted per sample.

Nutrient limitation classification

Nutrient limitation of biomass growth was classified using an approach similar to Slemmons & Saros (2012). A one-way ANOVA and Tukey HSD means separation test were applied to Chl *a* data from the control, N, P, and N + P limitation treatments. Data were log-transformed, ANOVA homogeneity of variance and normality assumptions were tested with Levene's and Shapiro-Wilks tests, and significant differences between means were assessed at $\alpha = 0.05$. Statistical analyses were performed using R (version 3.1.1) (www.r-project.org). If only the N or P treatment was significantly greater than the control, growth was classified as 'N-limited' or 'P-limited.' If a single-element treatment was significantly greater than the control, and N + P > single element > control, growth was classified as 'serial N limitation' or 'serial P limitation.' Growth was classified as 'simultaneous co-limitation' if only N + P > control, or 'independent co-limitation' if both N > control and P > control (Harpole et al., 2011). If only significant decreases relative to the control were observed, growth was classified as 'constrained.' Growth was classified as 'not limited' if there were no significant differences between treatments and the control. Nutrient limitation of individual algal taxa was classified by applying the same procedure to cell densities. Taxon-specific growth responses were also described by calculating cell density relative responses (RR_{taxa} = (1 + treatment mean cell density)/(1 + control mean cell density) to quantify the magnitude of responses relative to the control. Changes in phytoplankton community composition were also characterized using nonmetric multidimensional scaling and permutation ANOVA (PERMANOVA); community analyses and results are described in the Supplemental Information.

Species N response thresholds

At the taxa level, our approach was to identify N-responsive taxa based on nutrient limitation classifications, and calculate N K_s values for those taxa. We defined N-responsive taxa as those that were abundant (>1% total lake cell density) and N-limited, serial N-limited, or were co-limited and showed a strong Monod response in at least one lake. We assumed co-limited taxa that showed a strong Monod response could potentially be responsive to lake N increases. Cell densities were highly variable within limitation treatments for some taxa, which may have confounded some nutrient limitation classifications. Results from the control, N gradient treatments (0.14, 0.7, 1.4, 7, 14, and 56 μg N l^{-1}, plus 31 μg P l^{-1}, and 2.8 mg l^{-1} Si), and 112 μg N + 31 μg P treatments were used to construct Monod growth curves. The 112 μg N + 31 μg P treatment does not include Si, but was used because initial lake Si concentrations and observed growth responses indicated that Si did not limit growth (see results). Growth rates (μ, day^{-1}) were calculated as follows:

$$\mu = \frac{\ln(F) - \ln(I)}{T},$$

where F is the cell concentration at the end of the incubation (cells ml^{-1}), I is the mean cell concentration (cells ml^{-1}) from triplicate initial lake water samples, and T is the incubation time (d) (Table 1). Growth curves were created by fitting the Monod equation to experimental data using nonlinear least squares regression with the 'nls' function in R:

$$\mu = \mu_m\left(\frac{S}{K_s + S}\right)$$

where μ_m is the maximum specific growth rate (d^{-1}), S is the dissolved inorganic nitrogen (DIN = NO_3–N + NH_3–N) concentration (μg N l^{-1}) calculated as the sum of initial and added DIN, and K_s is the half-saturation coefficient for growth (S when $\mu = \mu_m/2$). Growth affinities (μ_m:k_s) were calculated to characterize the relative competitive ability of each taxon in each lake; higher values indicate a greater initial growth curve slope and thus theoretically a greater competitive ability.

Biomass response thresholds

N concentration thresholds were quantified for any increase in biomass (Chl *a*), and for an increase in biomass beyond typical biomass inter-annual variation. Biomass relative response ($RR_{biomass}$) was calculated for each treatment replicate as the ratio of treatment Chl *a* to control mean Chl *a*. Simple linear regression was applied to the same treatments used for

Monod growth curves to estimate two N biomass criteria: the DIN concentration for any increase in biomass relative to the control ($RR_{biomass} > 1$), and the DIN concentration required to increase biomass above typical lake inter-annual variability. We defined typical inter-annual variability as the average of Chl *a* coefficients of variation (CV) observed across 21 mountain lakes sampled annually by the National Park Service (NPS) in late summer 2009–2013. A CV value was calculated for each lake and then used to calculate an average Chl *a* CV ($CV_{mean} = 0.73$, range = 0.26–1.6). Data for calculating CV_{mean} were provided by NPS (NPS, unpublished data). DIN (initial DIN + added N) was used as the predictor variable and lake mean RR as the response variable in regressions, using data from the N gradient and 112 µg N + 31 µg P treatments. Regressions were performed for (1) individual lakes, (2) using data from all lakes, and (3) using data from only N-limited and serial N-limited lakes (Table 3) for comparison. Best-fit regression equations were solved for DIN where $RR_{biomass} = 1$ or where $RR_{biomass} = (1 + CV_{mean})$ to estimate criteria within and across lakes.

We used biomass thresholds calculated from bioassays to estimate the number of mountain lakes at each park where DIN (NO_3–N + NH_3–N) exceeds thresholds. DIN concentrations in park lakes were estimated using a database with 675 DIN observations encompassing 125 mountain lakes above 1,200 m sampled between 1988 and 2014 at MORA, 82 lakes sampled between 1989 and 2013 at NOCA, and nine lakes sampled between 2005 and 2013 at OLYM. Some lakes in the database were sampled only once, and some lakes were sampled multiple times. Detection limits were 1 µg NO_3–N l^{-1} and 5–10 µg NH_3–N l^{-1} across observations in the database. Because detection limits were variable and in some cases near threshold concentrations for phytoplankton responses, we calculated a lower bound and upper bound mean DIN concentration for each lake. Lower bound values were calculated by substituting 0 µg N l^{-1} for NO_3–N and NH_3–N values below detection. Upper bound values were calculated by substituting the detection limit for NO_3–N and NH_3–N values below detection. We then compared lower and upper bound lake mean DIN to thresholds, to generate lower and upper bound estimates for the number of lakes where DIN exceeds thresholds.

Results

Bioassay initial conditions and enclosure effects

Initial nutrient and Chl *a* concentrations are presented in Table 2. The DIN:TP ratio was less than 1.5 and therefore predicted biomass N-limitation in four lakes (Rainbow, Hidden, Allen, and Heather). Initial Si concentrations ranged from 0.7 to 3.3 mg l^{-1}, and thus were above concentrations typically associated with Si-limited diatom growth (<0.5 mg Si l^{-1}) (Reynolds, 2006). Initial lake mean Chl *a* concentrations were 0.05–0.65 µg l^{-1} in lake water prior to nutrient additions. Mean Chl *a* concentrations in control treatments were generally similar to, but slightly lower than, initial Chl *a* (Table 3). Differences in initial and control treatment Chl *a* within each lake were 3–40 times smaller than treatment responses observed for significant treatments. An exception was Hoh Lake, where initial mean Chl *a* (0.59 µg l^{-1}) was substantially higher than control mean Chl *a* (0.11 µg l^{-1}). The difference between the initial and control at Hoh was larger than that between the N-only treatment and the control, but less than that between the N + P treatment and the control (Table 3). At the level of individual algal taxa, we assessed enclosure effects by calculating RR_{encl} (control mean density/initial mean density). In the few cases with substantial negative enclosure effects (control mean/initial mean <0.5), treatment effects on cell density were many orders of magnitude larger than enclosure effects. No Chl *a* or taxa results were excluded from analyses due to enclosure effects. RR_{encl} values for all abundant taxa are provided in Supplemental Information Table S1.

Nutrient limitation classification

Biomass increased in response to N or N + P, but not to P alone (Table 3). Phytoplankton growth was classified as N-limited or serial N-limitation in four lakes, and as simultaneous co-limited by N and P in five lakes. In N-limited lakes, N addition increased biomass 2–3 times relative to the control (Table 3). N + P addition significantly increased biomass relative to the control in all 9 lakes ($P < 0.05$). N + P addition increased Chl *a* concentrations 3–10 times relative to the control, with the largest increases in co-limited lakes (Table 3).

Table 2 Initial nutrient and Chl *a* concentrations. Values are averages where $N = 3$

Park	Lake	*N*	Total P (μg P l^{-1})	Ammonium (μg N l^{-1})	Ortho-phosphate (μg P l^{-1})	Nitrate plus Nitrite (μg N l^{-1})	Si (mg l^{-1})	DIN:TP[a]	Chl *a*[b] (μg l^{-1})
NOCA	Rainbow	3	7	5	2	<1	0.90	0.9	0.65
	Doubtful	3	5	9	2	7	0.65	3.2	0.33
	Copper	3	4	6	2	5	0.63	2.6	0.28
MORA	Hidden	1	6	<4	<2	<2	2.66	1.00	0.63
	LH15	1	6	5	3	15	1.98	3.33	0.25
	Allen	1	8	<4	<2	<2	2.34	0.75	0.12
OLYM	Heather	1	7	<4	<2	3	1.23	1.00	0.05
	Crazy	1	4	<4	<2	3	0.71	1.75	0.2
	Hoh	1	6	<4	<2	6	1.22	1.67	0.59

[a] DIN:TP calculated as [(nitrate + nitrite) + (ammonium)]/TP; detection limits were used for calculations when results were below detection

[b] Chl *a* values are means of triplicate samples

Table 3 Mean ± standard error chlorophyll *a* (μg/l) concentrations from control, N, P, and N + P treatments and inferred limitation type

Park	Lake	Control	P	N	N + P	Limitation type
NOCA	Copper	0.18 ± 0.02	0.21 ± 0.03	0.23 ± 0.04	0.53 ± 0.01*	Simultaneous co-limitation
	Doubtful	0.30 ± 0.03	0.27 ± 0.04	0.97 ± 0.05*	3.21 ± 0.36*^	Serial N-limitation
	Rainbow	0.53 ± 0.14	0.47 ± 0.07	1.07 ± 0.16	5.31 ± 0.49*	Simultaneous co-limitation
MORA	Hidden	0.27 ± 0.12	0.12 ± 0.02	0.47 ± 0.06	2.2 ± 0.74*	Simultaneous co-limitation
	Allen	0.08 ± 0.01	0.1 ± 0.01	0.20 ± 0.03*	0.64 ± 0.12*^	Serial N-limitation
	LH15	0.13 ± 0.01	0.05 ± 0.01	0.12 ± 0.03	1.13 ± 0.43*	Simultaneous co-limitation
OLYM	Crazy	0.12 ± 0.05	0.09 ± 0.01*	0.27 ± 0.04	1.3 ± 0.05*	Simultaneous co-limitation
	Heather	0.04 ± 0.01	0.04 ± 0.00	0.1 ± 0.02*	0.12 ± 0.04*	N-limitation
	Hoh	0.11 ± 0.01	0.12 ± 0.02	0.22 ± 0.03*	0.86 ± 0.04*^	Serial N-limitation

*Denotes treatments significantly different from the control ($P < 0.05$, Tukey HSD), and ^ denotes cases where N + P > N > control ($P < 0.05$, Tukey HSD)

Nutrient limitation classifications for abundant N-responsive taxa are presented in Table 4. Nutrient limitation classifications for all abundant taxa in each lake are presented in Supplemental Information (Table S1). Most of the abundant algal taxa responded to N and/or N + P addition, but not to addition of P alone (Table 4). Among abundant taxa, there was only one instance of P-limitation; N-limitation, co-limitation, and no response to enrichment were most common (Table 4, Table S1). Within any given lake, 27–50 algal taxa were observed. Diatom, chrysophyte, chlorophyte, and cyanophyte taxa were numerically most abundant (Supplemental Information Fig. S2, Table S1).

Species response thresholds

Monod parameters for abundant N-responsive taxa are presented in Table 4 and in Table S1. Monod curves for abundant N-responsive taxa are presented in Supplemental Information (Figs. S3–S7). We identified 20 distinct N-responsive taxa, meaning they were N-limited, serial N-limited, or were co-limited and showed a strong Monod response in at least one lake. Eight of these taxa were diatoms, seven were chlorophytes, two were cyanophytes, and three were chrysophytes. Monod curves were constructed for 18 of 20 N-responsive taxa (Table 4, Figs. S3–S7). Monod curves could not be fit to responses observed for some

Table 4 Nutrient limitation classification and Monod parameters for abundant N-responsive taxa

Division	Taxa	Lake	Limitation	μ_{max} (d^{-1}) ± SE	K_s (μg N l^{-1}) ± SE	μ_m: K_s
BAC	*Aulacoseira alpigena*	LH15	N	0.49 ± 0.05*	27.17 ± 6.64*	0.02
BAC	*Aulacoseira pusilla* 6 μm	Allen	S. co-lim.	0.12 ± 0.02*	3.90 ± 3.31	0.03
BAC	*Aulacoseira pusilla* 6 μm	Crazy	Serial N	–	–	–
BAC	*Aulacoseira pusilla* 6 μm	LH15	Serial N	–	–	–
BAC	*Cyclotella* <7 um distinguenda	LH15	Serial N	–	–	–
BAC	*Cyclotella* <7 um distinguenda	Crazy	Serial N	–	–	–
BAC	*Discostella glomerata*	Heather	N	–	–	–
BAC	*Discostella glomerata* 7-8 μm	Crazy	N	0.06 ± 0.03*	9.46 ± 5.76	0.01
BAC	*Discostella glomerata* 7-8 μm	LH15	I. co-lim.	3.73 ± 3.22	782 ± 761	0.00
BAC	*Discostella stelligera*	Doubtful	Serial N	–	–	
BAC	*Fragilaria capucina*	Crazy	S. co-lim.	0.51 ± 0.01*	1.50 ± 0.21*	0.15
BAC	*Fragilaria crotonensis*	Allen	S. co-lim.	0.63 ± 0.10*	32.12 ± 13.52*	0.02
BAC	*Fragilaria crotonensis*	Crazy	Serial N	0.30 ± 0.01*	6.25 ± 0.83*	2.14
BAC	*Fragilaria tenera*	Crazy	Serial N	0.34 ± 0.02*	24.67 ± 4.76*	0.01
BAC	*Fragilaria tenera*	Hidden	Serial N	–	–	–
BAC	*Fragilaria tenera*	Hoh	S. co-lim.	0.42 ± 0.04*	14.52 ± 3.61*	0.02
CHL	*Ankistrodesmus* sp.	Allen	S. co-lim.	0.64 ± 0.54	77.60 ± 128	0.01
CHL	Coccoid green alga sp.	Allen	N	0.51 ± 0.04*	0.05 ± 0.05	10.65
CHL	Coccoid green alga <5 μm sp.	LH15	N	0.28 ± 0.04*	9.95 ± 5.87	0.03
CHL	*Gonium* sp.	Hidden	Serial N	0.45 ± 0.15*	10.20 ± 12.43	0.04
CHL	Nonmotile, unicellular green algae sp.	Heather	S. co-lim.	0.21 ± 0.04*	13.49 ± 6.62	0.01
CHL	*Schroederia* sp.	Allen	S. co-lim.	0.17 ± 0.06*	16.38 ± 18.84	0.01
CHL	*Schroederia* sp.	LH15	N	0.40 ± 0.06*	57.0 ± 17.4*	0.01
CHL	*Tetraspora* sp.	LH15	S. co-lim.	0.02 ± 0.07*	0.57 ± 16.51	0.04
CHR	*Chrysolepidomonas* sp.	Allen	I. co-lim.	0.25 ± 0.02*	0.02 ± 0.04	11.14
CHR	*Chrysolepidomonas* sp.	LH15	I. co-lim.	0.82 ± 0.12*	27.3 ± 9.95*	0.03
CHR	Unknown unicellular Chrysophyta 5–7 μm	Heather	N	–	–	
CHR	Unknown unicellular Chrysophyta 5–7 μm	LH15	N	0.15 ± 0.04*	36.70 ± 24.0	0.00
CHR	Unknown unicellular *Chrysophyta* 10 μm	Allen	Serial N	0.40 ± 0.03*	0.06 ± 0.07	6.50
CYA	Coccoid cyanobacteria sp.	Allen	Co-limitation	0.26 ± 0.10	18.26 ± 22.68	0.01
CYA	*Synechocystis* sp.	Hoh	Serial N	0.38 ± 0.03*	14.13 ± 2.51	0.02
CYA	Microcystis sp. colony	Crazy	N	0.41 ± 0.31	129 ± 162	0.00
CYA	*Synechocystis* sp.	Heather	Serial N	–	–	–

Corresponding Monod curve plots are provided in the Supplemental Information (Figures S3–S7)

Notes: abundant N-responsive taxa are taxa with >1% lake total cell density that were N-limited, serial N-limited, or were co-limited and showed a strong Monod growth response. μ_{max} is the maximum growth rate, K_s is the Monod half-saturation constant, and μ_{max}:K_s is the taxon growth affinity

* Indicates statistically significant Monod parameters. − Indicates a Monod curve could not be constructed

BAC Bacillariophyta, *CHL* Chlorophyta, *CHR* Chrysophyta, *CYA* Cyanophyta, *UND* undetermined

taxa. K_s ranged from 0.02 to 77 μg DIN l^{-1} across most N-responsive taxa. K_s exceeded 100 μg DIN l^{-1} in two cases where responses appeared more linear than Monod-like (*Discostella glomerata* Bachmann in LH15, *Microcystis* sp. colony in Crazy). Maximum specific growth (μ_m) ranged from 0.1 to 0.6 for most

taxa, but was higher for the taxa mentioned above which showed a linear response (Table 4). Growth affinities (μ_m:K_s) ranged from 0.01 to 11, with all but four values between 0.01 and 0.05 (Table 4). Multiple Monod curves were constructed for *F. crotonensis*, *F. tenera*, coccoid green alga sp., *Tetraspora sp.*, and coccoid cyanobacteria, and parameters for these taxa varied substantially among lakes (Table 4). *Cyclotella distinguenda* Hustedt showed serial N-limited growth in two lakes, but a Monod curve could not be fit to data in the gradient treatments (Table 4). Monod curves were not constructed for taxa in NOCA lakes. Taxon-level responses were most prominent in N + P treatments in NOCA lakes, with limited responses in gradient treatments. The 56 μg N l^{-1} treatment was not used at NOCA, which also likely affected our ability to construct Monod curves. *Discostella stelligera* (Cleve & Brunow) Houk & Klee in Doubtful Lake was the only abundant taxon at NOCA that responded to N alone. Other abundant taxa responded only to N + P or did not respond at all (Supplemental Information Table S1).

Biomass response thresholds

There was a positive relationship between DIN and $RR_{biomass}$ in each lake (Fig. 2; Table 5). The relationship was linear in all lakes except Hoh Lake (OLYM), where it was logarithmic (Table 5). Regression models explained >65% of variation in $RR_{biomass}$ in all lakes except Heather Lake (OLYM) ($r^2 = 0.48$) (Table 5). DIN criteria for any increase in biomass ($RR_{biomass} > 1$) and for an increase beyond typical inter-annual variation ($RR_{biomass} > 1.73$) ranged from 7 to 33 μg DIN l^{-1} and 13–67 μg DIN l^{-1} across lakes, respectively (Table 5). The type of lakes included in the regression (N-limited versus co-limited biomass growth) did not strongly affect threshold values (Table 5). When upper and lower bound lake mean DIN in sampled lakes was compared to the 13 μg N l^{-1} threshold for any increase in biomass, 33–45 lakes (26–36%) at MORA, 22–28 lakes at NOCA (27–34%), and 0 lakes at OLYM exceeded the threshold. Using the 25 μg N l^{-1} threshold, 17–19 lakes at MORA (13–15%), 6–10 lakes at NOCA (7–12%), and 0 lakes at OLYM exceeded the threshold.

Discussion

This study characterized phytoplankton responses to N enrichment in mountain lakes, and estimated DIN thresholds associated with those changes. At the level of individual algal taxa, we identified 20 taxa that responded to N additions, and quantified N K_s values for 18 of those taxa (Table 4). At the biomass-level, we estimated DIN concentrations necessary for any increase in Chl *a* (13 μg N l^{-1}) and for a Chl *a* increase beyond typical lake inter-annual variation (25 μg N l^{-1}). Species and biomass-level results indicate that phytoplankton in park mountain lakes are sensitive to future increases in lake N, and a wide variety of taxa may respond to N increases. Growth of phytoplankton biomass and of individual algal taxa was primarily limited by N or co-limited by N and P in our study lakes (Supplemental Information Table 1). Only two instances of P-limitation for individual algal taxa were observed. The DIN concentration thresholds we quantified for biomass and taxon-level responses suggest relatively low DIN concentrations could stimulate growth of total biomass or growth of specific taxa. Comparing experimentally defined response thresholds to DIN concentrations in sampled lakes indicates many lakes currently have summer DIN concentrations below thresholds. Future lake N increases therefore will likely stimulate phytoplankton changes. We estimate 26% of sampled lakes at MORA and NOCA, and no sampled lakes at OLYM, have DIN concentrations that exceed the biomass response threshold (13 μg N l^{-1}), which is similar to or below species-level thresholds quantified for many taxa.

N-responsive phytoplankton taxa

Our results suggest that *F. tenera and F. crotonensis* could be used as indicator species of N enrichment in the Pacific Northwest; both species responded to N enrichment in multiple lakes here, and have increased along with N deposition in Western U.S. sediment cores (Saros et al., 2003, 2010; Sheibley et al., 2014). *F. tenera* responded to N addition in three of our study lakes, including Hoh Lake, where increased in relative abundance in a sediment core as N deposition rates began to exceed 1–1.2 kg N ha^{-1} $year^{-1}$ in the 1969–1975 timeframe (Sheibley et al., 2014). Several other diatom species also responded to N addition in one or more lakes, including *Aulacoseira alpigena*

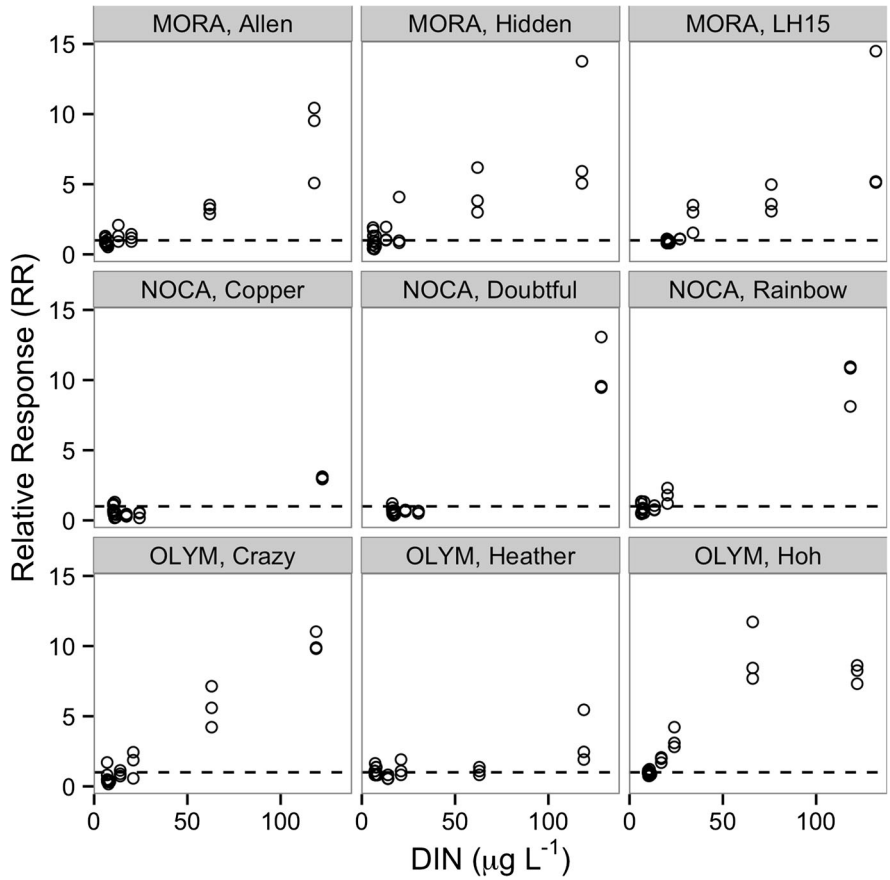

Fig. 2 Relationship between biomass relative response ($RR_{biomass}$) and DIN across gradient treatments. $RR_{biomass}$ = treatment replicate Chl *a* divided by control mean Chl *a*. The dashed horizontal line indicates $RR_{biomass} = 1$

Table 5 Threshold DIN concentrations required for $RR_{biomass} = 1$ and $RR_{biomass} = 1 + CV_{chla}$

Model	Regression equation	r^2	*P*	RR = 1 (µg DIN/l)	RR = 1 + CV_{chla} (µg DIN/l)
All lakes	RR = 0.159 + 0.063*DIN	0.69	<0.001	13	25
N-limited lakes only	RR = 0.226 + 0.062*DIN	0.66	<0.001	13	24
Allen (MORA)	RR = 0.306 + 0.064*DIN	0.86	<0.001	11	22
Hidden (MORA)	RR = 0.525 + 0.065*DIN	0.70	<0.001	7	19
LH15 (MORA)	RR = −0.333 + 0.063*DIN	0.67	<0.001	21	33
Copper (NOCA)	RR = 0.268 + 0.022*DIN	0.85	<0.001	33	67
Doubtful (NOCA)	RR = −1.18 + 0.092*DIN	0.95	<0.001	24	32
Rainbow (NOCA)	RR = 0.137 + 0.083*DIN	0.97	<0.001	10	19
Crazy (OLYM)	RR = −0.176 + 0.088*DIN	0.97	<0.001	13	22
Heather (OLYM)	RR = 0.739 + 0.018*DIN	0.48	<0.001	15	55
Hoh (OLYM)	RR = −7.25 + 3.46*ln(DIN)	0.88	<0.001	11	13

$1 + CV_{chla} = 1.73$; CV_{chla} is the average of coefficients of variation (CV) observed in 21 mountain lakes NPS staff have sampled at least once annually in late summer 2009–2013

(Grunow) Krammer, *Aulacoseira pusilla* (Meister) Tuji & Houki, *C. distinguenda*, *D. glomerata*, *D. stelligera* and *Fragilaria capucina* Desmazieres (Table 4). These species may be useful indicators of N enrichment, but there is less supporting evidence from sediment cores. *Aulacoseira* species were

replaced by *A. formosa* and *F. tenera* in some Rocky Mountain lakes as N deposition increased (Wolfe et al., 2003; Hobbs et al., 2010), and did not show substantial changes over time in lakes examined by Sheibley et al. (2014). In some sediment cores, *Aulacoseira* decreases were attributed to warming (Catalan et al., 2013). The abundance of small centric diatoms such as *Cyclotella* and *Discostella* is strongly affected by complicated interactions among warming, light, and nutrients, which makes assigning causal factors to changes in sediment cores difficult (Saros & Anderson, 2015; Saros et al., 2014). In addition, although *D. stelligera* responded to N here (Table 4) and in other bioassays (Arnett et al., 2012), it often competes with other N-sensitive species. In Rocky Mountain lakes, *A. formosa* and *D. stelligera* frequently co-occur, but the abundance of these two species is often inversely related (Arnett et al., 2012).

In addition to diatoms, we identified seven chlorophyte, three cyanophyte, two chrysophyte taxa that responded to N enrichment (Table 4). Several of these taxa responded to N in multiple lakes, and had K_s values similar to or lower than those observed for diatoms (Table 4). The lowest K_s values were observed for nondiatom taxa, including *Chrysolepidomonas sp.* (0.02 μg N l^{-1}), 'coccoid green alga sp.' (0.05 μg N l^{-1}), and 'unknown unicellular *Chrysophyta* 10 μm' (0.06 μg N l^{-1}) in Lake Allen. Chlorophyte, chrysophyte, and cyanophyte increases were also observed in mountain lakes in response to N additions in other regions. Increases in chlorophyte and cyanophyte taxa have also been observed in bioassays conducted in Snowy Range (Nydick et al., 2004) and Colorado (Gardner et al., 2008) mountain lakes.

Phytoplankton response thresholds

Thresholds developed in this study were based on the treatments that may have alleviated or prevented P-limitation or co-limitation for some taxa because 31 μg P were added along with N in the gradient treatments. We added P in the gradient treatments to use methods consistent with previous studies (Michel et al., 2006; Arnett et al., 2012; Nanus et al., 2012), and to quantify thresholds under N-limiting conditions. In cases of initial co-limitation or N-limitation, P additions may have alleviated or prevented P-limitation, respectively. In the case of serial N-limitation, P additions likely caused response magnitudes to be larger than if only N were added. Our bioassays therefore may overestimate growth responses and underestimate N response thresholds for cases of initial co-limitation or serial N-limitation. For example, in lakes with co-limited and serial N-limited biomass growth, addition of N alone resulted in smaller relative response of biomass than predicted by regressions (Tables 3, 5). The National Park Service faces legal mandates to protect park resources from air pollution that motivate adopting a precautionary approach to monitoring and assessing N deposition effects. Our N thresholds are protective of all park mountain lakes because they assume N limits phytoplankton growth.

Diatom μ_m values quantified in this study were similar to those quantified for diatoms in the Rocky Mountains, but our K_s values were consistently higher. Our *F. crotonensis* K_s values were 6.2 μg N l^{-1} in Crazy Lake and 32.1 μg N l^{-1} in Lake Allen, whereas Michel et al. (2006) quantified K_s values less than 1 μg N l^{-1} in Beartooth Mountains lakes. K_s for *D. stelligera* and *A. formosa* in the Rocky Mountains range from 0.04 to 2.5 μg N l^{-1} (Michel et al., 2006; Arnett et al., 2012; Nanus et al., 2012). In contrast, diatom K_s values in this study ranged from 3.5 to 50 μg N l^{-1}. K_s values frequently have standard errors that exceed 100% of K_s (Grover et al., 1999; Michel et al., 2006). However, our diatom K_s values are higher than those in the Rockies, even after accounting for this uncertainty. The higher K_s values in this study indicate greater N requirements for these taxa in our study lakes. These greater requirements could result from sub-optimal light, temperature, or other conditions during our in situ incubations, all of which can raise nutrient requirements. Many of our study lakes had high transparency, often reaching the lake bottom. Photoinhibition is thus one potential explanation for higher N requirements compared to previous studies. Our K_s values for *F. tenera* (14.5 μg N l^{-1} in Hoh Lake, and 24.7 μg N l^{-1} in Crazy Lake) were also higher than we expected. Because Sheibley et al. (2014) documented an increase in *F. tenera* in Hoh Lake, we expected *F. tenera* K_s to be near or below current Hoh Lake DIN concentrations, but DIN concentrations measured in this study and by NPS in Hoh Lake are consistently below 10 μg N l^{-1}. The reason for this discrepancy is not clear.

At the biomass-level, DIN:TP (Bergström, 2010) successfully predicted biomass nutrient limitation patterns in 5 of 9 lakes. DIN:TP correctly predicted co-limitation in Copper, LH15, and Crazy lakes, and correctly predicted N-limitation (N or serial N-limitation) in Heather and Allen lakes. Discrepancies between predicted and observed biomass nutrient limitation are not entirely surprising given that thresholds developed by Bergström (2010) were designed to accurately discriminate between N- and P-limitation in 50% of cases. In addition, DIN:TP can be affected by how results below detection are handled when calculating DIN:TP. DIN:TP may also be prone to misclassification if concentrations are near detection limits or analytical precision is low relative to concentrations. Our results indicate one cannot assume biomass nutrient limitation patterns based on DIN:TP, or observed in nutrient-enrichment experiments, are representative of taxon-level patterns. We observed taxon-level responses to N addition among diatom indicator species and other taxa in lakes where biomass growth was classified as co-limited. For example, Crazy Lake had co-limited biomass growth, but we observed serial N-limitation and strong Monod responses for indicator diatoms *F. crotonensis* and *F. tenera.* In addition, nonmetric multidimensional scaling plots indicated distinct effects of N and N + P additions on community structure in Hidden, LH15, and Crazy lakes, all of which had co-limited biomass growth (Supplemental Information, Fig. S1).

We estimated that DIN concentrations of 13 μg N l^{-1} are required for an increase in biomass, and a concentration of 25 μg N l^{-1} is necessary to increase biomass beyond typical lake inter-annual variation. These thresholds are similar to those estimated by Heard (2013) based on experiments in Sierra Nevada mountain lakes. Heard (2013) enriched lake water with P and a range of N concentrations and used Michaelis–Menten and Monod growth models to estimate the 10, 50, and 90% 'effective doses.' or N concentrations that resulted in 10, 50, and 90% of saturated biomass growth rates (μg day^{-1}). In contrast to those experiments, this study used a smaller N concentration gradient (0–112 μg N l^{-1} vs. 0–700 μg N l^{-1}), did not observe biomass growth saturation except perhaps in Hoh Lake (Fig. 5), and modeled biomass responses using linear regression and RR values. Despite these methodological differences, our DIN thresholds (Table 5) overlap with Heard's estimated 10% (4.6–12.5 μg N l^{-1}) and 50% (14–56 μg N l^{-1}) effective doses (Heard, 2013). Daggett et al. (2015) conducted similar bioassays using additions of nitrogen only in boreal lakes in the upper midwest U.S. In a N-limited lake at Isle Royale National Park, Daggett et al. (2015) observed a linear Chl *a* response to a nitrate gradient, an increase in Chl *a* relative to the control at 2.2–4.5 μg NO_3–N l^{-1}, and a 2-fold increase in Chl *a* (RR $\sim$ 2) at 9 μg NO_3–N l^{-1} (Daggett et al., 2015, Fig. 3b). In N-limited lakes across these three studies, DIN concentrations above 5–15 μg DIN l^{-1} initiated a Chl *a* response, and the response magnitude continued to increase with lake DIN. This suggests a DIN threshold of 5–15 μg N l^{-1} may be a relatively robust indicator of the onset of Chl *a* increases in remote oligotrophic N-limited lakes. However, the biomass response magnitude that occurs as DIN concentrations exceed this threshold will depend on a variety of factors. Biomass growth will shift from N-limitation to P-limitation (Bergstrom & Jansson, 2006), and the response magnitude will increase with TP concentration (Filstrup et al., 2014; Dolman & Wiedner, 2015). Increasing lake temperature (Bergström et al., 2013) and dissolved organic matter concentrations (Daggett et al., 2015) will also increase response magnitude. Other factors, such as light availability, ultraviolet radiation (Williamson et al., 2010), zooplankton grazing (Vinebrooke et al., 2014), or phytoplankton species composition may suppress or mediate response magnitude.

We compared our Chl *a* threshold (13 μg N l^{-1}) to DIN concentrations in sampled mountain lakes at each of the three parks. Applying N criteria developed in N-limited lakes to all mountain lakes in a park is a conservative (protective) screening-level exercise because it assumes all lakes have N-limited phytoplankton. Considering that 70% of nitrate and 70% of ammonia observations in the lake survey database were below detection limits, and NPS faces legal mandates that warrant a precautionary approach, this is likely a reasonable assumption for management and monitoring purposes. We estimated approximately 25% of sampled lakes at MORA and NOCA, and 0 out of 9 sampled lakes at OLYM have DIN concentrations that exceed the 13 μg N l^{-1} threshold. We therefore concluded that phytoplankton in the majority of park mountain lakes are sensitive to and are relatively unimpacted by anthropogenic N deposition. In contrast to lakes in the eastern Rocky Mountains where N deposition effects are well documented (Elser et al., 2009b), we observed N-limitation and co-limitation of

phytoplankton growth rather than P-limitation, and park lakes have lower nitrate and Chl *a*. Several phytoplankton taxa had K_s values below 13 μg N l^{-1}, and thus more than 25% of lakes may have DIN concentrations that exceed some taxon response thresholds for individual taxa. However, it is more difficult to infer lake sensitivity using K_s values. In cases where K_s was estimated for a taxon in multiple lakes, K_s varied substantially across lakes (Table 4). In addition, some K_s values were below detection limits, and limited information is available about the distribution of phytoplankton taxa in park lakes. Biomass thresholds were more consistent across lakes, and were consistent with those quantified in other regions.

Conclusions

At least three conditions must be met for N deposition to induce phytoplankton changes in mountain lakes. N deposition inputs must increase lake N concentrations, N-limited phytoplankton must be present, and lake N increases must be sufficient to stimulate growth of N-limited phytoplankton. Our results indicate phytoplankton in park mountain lakes of the Pacific Northwest of the US are sensitive to future increases in lake N. Based on our results, we expect future lake N increases to increase phytoplankton biomass, and stimulate growth of diatom, chlorophyte, chrysophyte, and cyanophyte taxa. Diatom species that have increased in relative abundance throughout the Western U.S. along with increasing N deposition rates will also likely respond to lake N increases in the northwest. Growth stimulation of taxa can be expected in both lakes with N-limited and co-limited biomass growth. However, it is still not clear whether N deposition is a primary driver of mountain lake N concentrations in these three parks. In the Western U.S., lake nitrate is positively correlated with watershed characteristics that decrease watershed biological N uptake and increase hydrologic flow rates, but is not always positively correlated with N deposition rates (Clow & Sueker, 2000; Clow et al., 2010; Nanus et al., 2012). In some regions, and at small spatial scales, N deposition is not among the variables that best explains regional patterns of mountain lake nitrate (Clow et al., 2010; Nanus et al., 2012). Future research should use multivariate analyses to identify atmospheric and watershed factors that influence regional lake N and P concentrations, and phytoplankton threshold exceedances.

Acknowledgements This research was funded by the National Park Service Air Resources Division and National Park Service North Coast and Cascades Learning Network (Task Agreement No. P13AC00955) through the Pacific Northwest Ecosystem Studies Unit. Charlie Elverson, Jordan Youngmann, Mikell Bova, and Camden Nix assisted with field work. We thank National Park Service staff members that assisted this project, including: Tonnie Cummings, Tamara Blett, Reed Glesne, Carmen Welch, Ashley Rawhouser, Regina Rochefort, Barbara Samora, Rebecca Lofgren, and Steve Fradkin. The Oregon State University Cooperative Chemical Analytical Lab (CCAL) analyzed lake Si samples. Comments from two anonymous reviewers improved the manuscript.

References

American Public Health Association, 2005. Standard Methods for the Examination of Water and Wastewater, 21st ed. American Public Health Association, Washington, DC.

Arnett, H. A., J. E. Saros & M. A. Mast, 2012. A caveat regarding diatom-inferred nitrogen concentrations in oligotrophic lakes. Journal of Paleolimnology 47: 277–291.

Baron, J. S., 2006. Hindcasting nitrogen deposition to determine an ecological critical load. Ecological Applications 16: 433–439.

Baron, J. S., C. T. Driscoll, J. L. Stoddard & E. E. Richer, 2011. Empirical critical loads of atmospheric nitrogen deposition for nutrient enrichment and acidification of sensitive US Lakes. BioScience 61: 602–613.

Bergström, A.-K., 2010. The use of TN:TP and DIN:TP ratios as indicators for phytoplankton nutrient limitation in oligotrophic lakes affected by N deposition. Aquatic Sciences 72: 277–281.

Bergstrom, A.-K. & M. Jansson, 2006. Atmospheric nitrogen deposition has caused nitrogen enrichment and eutrophication of lakes in the northern hemisphere. Global Change Biology 12: 635–643.

Bergström, A.-K., C. Faithfull, D. Karlsson & J. Karlsson, 2013. Nitrogen deposition and warming—effects on phytoplankton nutrient limitation in subarctic lakes. Global Change Biology 19: 2557–2568.

Burns, D. A., T. Blett, R. Haeuber & L. H. Pardo, 2008. Critical loads as a policy tool for protecting ecosystems from the effects of air pollutants. Frontiers in Ecology and the Environment 6: 156–159.

Catalan, J., S. Pla-Rabés, A. P. Wolfe, J. P. Smol, K. M. Rühland, N. J. Anderson, J. Kopáček, E. Stuchlík, R. Schmidt, K. A. Koinig, L. Camarero, R. J. Flower, O. Heiri, C. Kamenik, A. Korhola, P. R. Leavitt, R. Psenner & I. Renberg, 2013. Global change revealed by palaeolimnological records from remote lakes: a review. Journal of Paleolimnology 49: 513–535.

Clow, D. W., & D. H. Campbell, 2008. Atmospheric deposition and surface-water chemistry in Mount Rainier and North

Cascades National Parks, U.S.A., water years 2000 and 2005–2006. U.S. Geological Survey Scientific Investigations Report 2008-5152, 37 p.
Clow, D. W. & J. K. Sueker, 2000. Relations between basin characteristics and stream water chemistry in alpine/subalpine basins in Rocky Mountain National Park, Colorado. Water Resources Research 36: 49–61.
Clow, D. W., L. Nanus & B. Huggett, 2010. Use of regression-based models to map sensitivity of aquatic resources to atmospheric deposition in Yosemite National Park, USA. Water Resources Research 46: W09529.
Daggett, C. T., J. E. Saros, B. M. Lafrancois, K. S. Simon & A. Amirbahman, 2015. Effects of increased concentrations of inorganic nitrogen and dissolved organic matter on phytoplankton in boreal lakes with differing nutrient limitation patterns. Aquatic Sciences 77: 511–521.
Dolman, A. M. & C. Wiedner, 2015. Predicting phytoplankton biomass and estimating critical N: P ratios with piecewise models that conform to Liebig's law of the minimum. Freshwater Biology 60: 686–697.
Du, E., W. de Vries, J. N. Galloway, X. Hu & J. Fang, 2014. Changes in wet nitrogen deposition in the United States between 1985 and 2012. Environmental Research Letters 9: 095004.
Elser, J. J., T. Andersen, J. S. Baron, A.-K. Bergstrom, M. Jansson, M. Kyle, K. R. Nydick, L. Steger & D. O. Hessen, 2009a. Shifts in Lake N: P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. Science 326: 835–837.
Elser, J. J., M. Kyle, L. Steger, K. R. Nydick & J. S. Baron, 2009b. Nutrient availability and phytoplankton nutrient limitation across a gradient of atmospheric nitrogen deposition. Ecology 90: 3062–3073.
Fenn, M. E., J. S. Baron, E. B. Allen, H. M. Rueth, K. R. Nydick, L. Geiser, W. D. Bowman, J. O. Sickman, T. Meixner & D. W. Johnson, 2003. Ecological effects of nitrogen deposition in the western United States. BioScience 53: 404–420.
Filstrup, C. T., T. Wagner, P. A. Soranno, E. H. Stanley, C. A. Stow, K. E. Webster & J. A. Downing, 2014. Regional variability among nonlinear chlorophyll—phosphorus relationships in lakes. Limnology and Oceanography 59: 1691–1703.
Gardner, E. M., D. M. McKnight, W. M. Lewis & M. P. Miller, 2008. Effects of nutrient enrichment on phytoplankton in an Alpine Lake, Colorado, U.S.A. Arctic, Antarctic, and Alpine Research 40: 55–64.
Grover, J. P., R. W. Sterner & J. L. Robinson, 1999. Algal growth in warm temperate reservoirs : nutrient-dependent kinetics of individual taxa and seasonal patterns of dominance. Archiv für Hydrobiologie 145: 1–23.
Harpole, W. S., J. T. Ngai, E. E. Cleland, E. W. Seabloom, E. T. Borer, M. E. S. Bracken, J. J. Elser, D. S. Gruner, H. Hillebrand, J. B. Shurin & J. E. Smith, 2011. Nutrient co-limitation of primary producer communities. Ecology Letters 14: 852–862.
Heard, A. M., 2013. Global Change and Mountain Lakes: Establishing Nutrient Criteria and Critical Loads for Sierra Nevada Lakes. PhD Dissertation, University of California, Riverside.
Hobbs, W. O., R. J. Telford, H. J. B. Birks, J. E. Saros, R. R. O. Hazewinkel, B. B. Perren, É. Saulnier-Talbot & A. P. Wolfe, 2010. Quantifying recent ecological changes in remote lakes of North America and Greenland using sediment diatom assemblages. PloS One 5: e10026.
Michel, T. J., J. E. Saros, S. J. Interlandi & A. P. Wolfe, 2006. Resource requirements of four freshwater diatom taxa determined by in situ growth bioassays using natural populations from alpine lakes. Hydrobiologia 568: 235–243.
Morris, D. & W. M. Lewis, 1988. Phytoplankton nutrient limitation in Colorado Mountain Lakes. Freshwater Biology 20: 315–327.
Murphy, C. A., P. L. Thompson & R. D. Vinebrooke, 2010. Assessing the sensitivity of alpine lakes and ponds to nitrogen deposition in the Canadian Rocky Mountains. Hydrobiologia 648: 83–90.
Nanus, L., D. W. Clow, J. E. Saros, V. C. Stephens & D. H. Campbell, 2012. Mapping critical loads of nitrogen deposition for aquatic ecosystems in the Rocky Mountains, USA. Environmental Pollution 166: 125–135.
Nydick, K. R., B. M. Lafrancois, J. S. Baron & B. M. Johnson, 2003. Lake-specific responses to elevated atmospheric nitrogen deposition in the Colorado Rocky Mountains, U.S.A. Hydrobiologia 510: 103–114.
Nydick, K. R., B. M. LaFrancois, J. S. Baron & B. M. Johnson, 2004. Nitrogen regulation of algal biomass, productivity, and composition in shallow mountain lakes, Snowy Range, Wyoming, USA. Canadian Journal of Fisheries and Aquatic Sciences 61: 13.
Pardo, L. H., M. E. Fenn, C. L. Goodale, L. H. Geiser, C. T. Driscoll, E. B. Allen, J. S. Baron, R. Bobbink, W. D. Bowman & C. M. Clark, 2011. Effects of nitrogen deposition and empirical nitrogen critical loads for ecoregions of the United States. Ecological Applications 21: 3049–3082.
Porter, E. & S. Johnson, 2007. Translating science into policy: Using ecosystem thresholds to protect resources in Rocky Mountain National Park. Environmental Pollution 149: 268–280.
Reynolds, C., 2006. Ecology of Phytoplankton. Cambridge University Press, Cambridge.
Saros, J. E., 2009. Determining critical loads to subalpine lakes in the Pacific Northwest. U.S. Forest Service:, http://www.fs.fed.us/air/documents/PNWCL-FinalReport.pdf.
Saros, J. E. & N. J. Anderson, 2015. The ecology of the planktonic diatom Cyclotella and its implications for global environmental change studies. Biological Reviews of the Cambridge Philosophical Society 90: 522–541. doi:10.1111/brv.12120.
Saros, J. E., S. J. Interlandi, A. P. Wolfe & D. R. Engstrom, 2003. Recent changes in the diatom community structure of lakes in the Beartooth Mountain Range, USA. Arctic, Anarctic, and Alpine Research 135: 18–23.
Saros, J. E., T. J. Michel, S. J. Interlandi & A. P. Wolfe, 2005. Resource requirements of *Asterionella formosa* and *Fragilaria crotonensis* in oligotrophic alpine lakes: implications for recent phytoplankton community reorganizations. Canadian Journal of Fisheries and Aquatic Sciences 62: 1681–1689.
Saros, J. E., D. W. Clow, T. Blett & A. P. Wolfe, 2010. Critical nitrogen deposition loads in high-elevation lakes of the western US inferred from paleolimnological records. Water, Air, & Soil Pollution 216: 193–202.

Saros, J. E., K. E. Strock, J. Mccue, E. Hogan & N. J. Anderson, 2014. Response of Cyclotella species to nutrients and incubation depth in Arctic lakes. Journal of Plankton Research 36: 450–460.

Sheibley, R. W., M. Enache, P. W. Swarzenski, P. W. Moran & J. R. Foreman, 2014. Nitrogen deposition effects on diatom communities in lakes from three National Parks in Washington State. Water, Air, & Soil Pollution 225: 1–23.

Slemmons, K. E. H. & J. E. Saros, 2012. Implications of nitrogen-rich glacial meltwater for phytoplankton diversity and productivity in alpine lakes. Limnology and Oceanography 57: 1651–1663.

Vinebrooke, R. D., M. M. Maclennan, M. Bartrons & J. P. Zettel, 2014. Missing effects of anthropogenic nutrient deposition on sentinel alpine ecosystems. Global Change Biology 20: 2173–2182.

Wehr, J. D., & R. G. Sheath, 2003. Freshwater algae of North America: Ecology and classification. Academic Press.

Williamson, C. E., C. Salm, S. L. Cooke & J. E. Saros, 2010. How do UV radiation, temperature, and zooplankton influence the dynamics of alpine phytoplankton communities? Hydrobiologia 648: 73–81.

Wolfe, A. P., A. C. Van Gorp & J. S. Baron, 2003. Recent ecological and biogeochemical changes in alpine lakes of Rocky Mountain National Park (Colorado, USA): a response to anthropogenic nitrogen deposition. Geobiology 1: 153–168.