Received: 30 April 2021 | Revised: 8 May 2022 | Accepted: 19 May 2022

DOI: 10.1111/fwb.13956

ORIGINAL ARTICLE

Freshwater Biology WILEY

Biomass, community composition and N:P recycling ratios of zooplankton in northern high-latitude lakes with contrasting levels of N deposition and dissolved organic carbon

Ann-Kristin Bergström[1] | **Danny C. P. Lau**[1,2] | **Peter D. F. Isles**[3] | **Anders Jonsson**[1] | **Irena F. Creed**[4]

[1]Department of Ecology and Environmental Science, Umeå University, Umeå, Sweden

[2]Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden

[3]Watershed Management Division, Vermont Department of Environmental Conservation, Montpelier, Vermont, U.S.A.

[4]Department of Physical and Environmental Sciences, University of Toronto – Scarborough Campus, Toronto, Ontario, Canada

Correspondence
Ann-Kristin Bergström, Department of Ecology and Environmental Science, Umeå University, Umeå, Sweden.
Email: ann-kristin.bergstrom@umu.se

Funding information
Knut och Alice Wallenbergs Stiftelse, Grant/Award Number: 2016.0083; Oscar and Lili Lamms Minne; Formas, Grant/Award Number: 215-2010-922; Swedish Research council, Grant/Award Number: 621-2010-4675

Abstract

1. Global changes are causing decreases in inorganic nitrogen (N) concentrations, increases in coloured dissolved organic carbon (DOC) concentrations, and decreases in dissolved inorganic N to total phosphorus ratios (DIN:TP) in northern lakes. The effects of these changes on phytoplankton and zooplankton biomass and the N:P recycling ratio of zooplankton remain unresolved.
2. In 33 Swedish headwater lakes across subarctic-to-boreal gradients with different levels of N deposition (low N in the north [Västerbotten, boreal; Abisko, subarctic] vs. high N in the south [Värmland, boreal; Jämtland, subarctic]), we measured water chemistry, phytoplankton biomass (chlorophyll-*a* [Chl-*a*], Chl-*a*:TP), seston mineral quality (C:P, N:P), as well as zooplankton biomass, community composition, and C:N:P stoichiometry. We estimated nutrient imbalances and the N:P recycling ratios of zooplankton using ecological stoichiometry models.
3. There was a large-scale gradient from low lake DIN and DIN:TP in the north to high DIN and DIN:TP in the south, with lower DIN:TP in lakes coinciding with higher DOC within each region. Lower lake DIN was associated with lower phytoplankton biomass (lower Chl-*a*:TP). Lower lake DIN:TP was associated with richer seston mineral quality (lower seston C:P and N:P) and higher zooplankton biomass.
4. Zooplankton community composition differed in the north vs. south, with a dominance of N-requiring calanoid copepods with high N:P in the north and P-requiring cladocerans with low N:P in the south. Also, greater differences in zooplankton community composition were found between subarctic regions (with lower DOC) than between boreal regions (with higher DOC), suggesting that increases in lake DOC and associated declines in lake DIN:TP reduce differences in zooplankton community composition.
5. The combination of lower lake DIN, higher lake DOC, and lower lake DIN:TP led to reduced zooplankton N:P recycling ratios, possibly by reducing seston

N:P and/or by enhancing calanoid copepod dominance in the zooplankton community.

6. Our findings suggest that the combination of declining N deposition and increasing lake browning in northern high-latitude lakes will reduce phytoplankton biomass, but will concurrently enhance seston mineral quality and probably also zooplankton biomass and their recycling efficiency of P relative to N.

KEYWORDS

biomass, C:N:P stoichiometry, community composition, plankton, subarctic-to-boreal

1 | INTRODUCTION

Global environmental changes, driven by changing climate, recovery from acidification, and changes in land-cover and land-use activities, have resulted in the depletion of essential nutrients such as nitrogen (N) and phosphorus (P) (Canham et al., 2012; Eimers et al., 2009; Huser et al., 2018; Isles et al., 2018) and the enrichment of terrestrial coloured dissolved organic carbon (DOC; also called lake browning) in northern lakes (de Wit et al., 2016; Finstad et al., 2016; Monteith et al., 2007). These environmental changes are influencing both phytoplankton and zooplankton biomass (Creed et al., 2018; Solomon et al., 2015). Recent studies have shown that declining N deposition together with lake browning will reduce lake water dissolved inorganic N to total P (DIN:TP) ratios (Bergström et al., 2020; Isles et al., 2018) and promote N limitation for phytoplankton (Isles et al., 2020). Concurrently, these changes are associated with lower phytoplankton biomass (Bergström & Jansson, 2006) but higher phytoplankton mineral quality (Bergström et al., 2020). However, it is still not clear to what extent changes in lake water DIN:TP and DOC and associated changes in phytoplankton biomass and mineral quality might influence trophic transfer efficiency (i.e., the efficiency of energy and carbon transfer across trophic levels; see Sterner & Hessen, 1994) impacting zooplankton productivity in northern lakes.

To grow and reproduce efficiently, zooplankton require sufficient phytoplankton biomass (Brett et al., 2009; Taipale et al., 2013; Wenzel et al., 2021) and phytoplankton mineral quality (Persson et al., 2007; Sterner & Hessen, 1994). Several empirical and model studies have illustrated that the relationship between phytoplankton biomass and lake DOC appears to be unimodal. This is because of the influence of DOC on both nutrient availability (positive effect) and light availability (negative effect) (Bergström & Karlsson, 2019; Isles et al., 2021; Kelly et al., 2018; Vasconcelos et al., 2019). Decreasing lake DIN:TP (Isles et al., 2018) is therefore expected to interact with increasing DOC (Isles et al., 2020) to alter phytoplankton biomass available for zooplankton (Bergström & Karlsson, 2019; Deininger et al., 2017a; Isles et al., 2021).

In addition to phytoplankton biomass, changes in lake DOC and DIN:TP, which influence light relative to nutrient availability (Sterner et al., 1997), also affect phytoplankton mineral quality measured in terms of seston C:N:P stoichiometry. A higher phytoplankton mineral quality is associated with lower seston C:P and N:P. Thus, in clear-water subarctic lakes, seston C:P and N:P are lower in lakes with lower lake DIN:TP and lower atmospheric N deposition than in those with higher lake DIN:TP and higher atmospheric N deposition (Bergström et al., 2020). Further, in these subarctic lakes, when lake DOC increases, seston C:P and N:P decline (Bergström et al., 2020; Sterner et al., 1997). Whether comparable patterns in seston C:N:P stoichiometry exist in brown-water boreal lakes remains unknown. Compared to their subarctic counterparts, boreal catchments are more efficient in retaining DIN from N deposition (Bergström, 2010; Elser et al., 2009; Hessen, 2013), and in releasing TP bound to terrestrial dissolved organic matter (Bergström et al., 2018; Hessen, 2013; Isles et al., 2020). In combination, these processes lower DIN:TP and light availability in boreal lakes (Isles et al., 2020). Light availability will limit the extent to which phytoplankton can change their C:N:P stoichiometry following changes in lake DIN:TP (Bergström et al., 2021; Deininger et al., 2017a; Sterner et al., 1997). Responses in phytoplankton mineral quality to changes in lake DIN:TP and DOC are therefore expected to be different from the responses in phytoplankton biomass (Deininger et al., 2017a; Bergström et al., 2020; Isles et al., 2021). Consequently, changes in phytoplankton biomass might not necessarily translate into changes in zooplankton biomass (McCauley & Kalff, 1981), due to concurrent changes in phytoplankton mineral quality (Bergström et al., 2021; Deininger et al., 2017b).

Another unknown is the extent to which differences in lake DIN:TP ratio and DOC concentration across subarctic and boreal lakes affect the N:P recycling ratio of zooplankton. In contrast to phytoplankton, zooplankton have been considered relatively homeostatic in terms of their stoichiometry, with high N:P stoichiometry among copepods and low N:P stoichiometry among cladocerans especially for *Daphnia* (Andersen & Hessen, 1991). However, the degree of homeostasis and variation in N:P stoichiometry can vary between species and taxonomic groups of zooplankton (Bergström et al., 2018; Hood & Sterner, 2010), and can be influenced by temperature (Bullejos et al., 2014), size distributions (Elser et al., 1988), growth rates (Elser et al., 2000; Vrede et al., 2002), reproduction modes (Ventura & Catalan, 2005), and ontogeny (Villar-Argaiz et al., 2002). Although these different factors influence the N:P stoichiometry of zooplankton, phytoplankton should have a greater capacity in changing their N:P stoichiometry than do zooplankton (Diehl, 2007; Sterner & Hessen, 1994), and the N:P recycling ratio of zooplankton is likely to be lower in lakes with low N deposition, low DIN:TP, and low seston N:P (Bergström et al., 2015). However, the extent to which the N:P recycling ratios of zooplankton are impacted by changes in levels of N deposition is likely to be moderated by lake DOC and its impact on lake DIN:TP (see above; Isles et al., 2020), as well as by its impact on the zooplankton community composition where increasing DOC and nutrient concentrations seem to favour cladocerans (low N:P) over calanoid copepods (high N:P) (Bergström et al., 2018; Pace, 1986).

In this study, we measured water chemistry, phytoplankton biomass (chlorophyll-*a* [Chl-*a*] and Chl-*a* per unit of lake water TP [Chl-*a*:TP]), seston mineral quality (C:P, N:P), as well as zooplankton biomass, community composition, and C:N:P stoichiometry in 33 Swedish headwater lakes across subarctic-to-boreal gradients with different levels of N deposition (low N in the north [Västerbotten, boreal; Abisko, subarctic] vs. high N in the south [Värmland, boreal; Jämtland, subarctic]). We estimated the long-term trends in N deposition in the study sites in 1997–2017 using national monitoring data. We also estimated nutrient imbalances and the N:P recycling ratios of zooplankton using ecological stoichiometry models. We used all these data to explore whether differences in lake water DIN:TP and DOC induced differences in phytoplankton biomass (in terms of Chl-*a* and Chl-*a*:TP) and phytoplankton mineral quality (in terms of seston C:P and N:P), and their subsequent effects on zooplankton biomass, C:N:P stoichiometry, and zooplankton N:P recycling ratio. Our predictions were that:

1. Lake DIN:TP is high in lakes with low DOC concentrations and declines in lakes with higher DOC concentrations.
2. Phytoplankton biomass is higher (Chl-*a* and Chl-*a*:TP is higher) but phytoplankton mineral quality is lower (seston C:P and N:P is higher) in southern lakes with higher lake DIN and DIN:TP (Jämtland [subarctic] and Värmland [boreal]) compared to northern lakes with lower lake DIN and DIN:TP (Abisko [subarctic] and Västerbotten [boreal]).
3. Phytoplankton mineral quality is higher (seston C:P and N:P is lower) in lakes with higher DOC concentrations.
4. Zooplankton biomass increases with increasing phytoplankton biomass and mineral quality.
5. Zooplankton community composition shifts from dominance of calanoid copepods (high N:P) to dominance of cladocerans (low N:P) with increasing lake DIN:TP and DOC concentration.
6. The N:P recycling ratio of zooplankton is lower in northern lakes with lower lake DIN and DIN:TP than in southern lakes and declines with higher lake DOC concentration.

Our findings provide insights into the effects of ongoing changes in lake water chemistry. Decreases in DIN:TP reflect decreases in atmospheric N deposition caused by reduced N emissions and hence a reverse of a previous anthropogenic perturbation, with lakes presumably gradually reverting to something closer to their *natural* state with regards to N. In contrast, increases in DOC reflect increases in lake browning caused, at least in part, by climate change and hence an anthropogenic perturbation that is moving lakes away from their *natural* state, both in terms of its effect on DIN:TP (as DOC increases, TP increases), and on light availability. We explored the cumulative effects of these environmental stressors in subarctic and boreal lakes.

2 | METHODS

2.1 | Site description

The lakes were selected to represent environmental gradients in DIN:TP ratios (north with relatively low DIN:TP ratios compared to the south because of increasing atmospheric N deposition toward the south; Isles et al., 2018) and DOC concentrations (subarctic with lower terrestrial DOC loadings to lakes compared to boreal lakes; Bergström et al., 2018). A total of 33 headwater lakes were sampled (Table S1, Figure 1) at four different study sites: two in the north (Abisko: subarctic-north, eight lakes;, and Västerbotten: boreal-north, nine lakes) and two in the south (Jämtland: subarctic-south, seven lakes; and Värmland: boreal-south, nine lakes) (Bergström et al., 2020; Isles et al., 2020). Lakes were situated at 227–622 m above sea level, and ranged in size between 0.6 and 69.3 ha, with no or little development in their catchments (Bergström et al., 2020; Isles et al., 2020). Lakes were sampled three times between mid-June and mid-September in 2016 (Abisko, Värmland, and Västerbotten) or 2017 (Jämtland), soon after ice-out and before the onset of stratification (i.e., mid to late May for boreal lakes in Värmland and Västerbotten, respectively; and early to mid-June for subarctic lakes in Jämtland and Abisko, respectively), once in the middle of summer when lakes were stratified, and once in early autumn before circulation. Sampling dates differed among the study sites because of differences in ice-out, stratification, and circulation periods of the lakes. During our sampling period, the study lakes varied in nutrient (total dissolved N [TDN]): 62–439 μg/L; DIN: 1–26.5 μg/L; TP: 3–29 μg/L) and DOC (1.9–20 mg/L) concentrations, and in molar $\log_{10}$DIN:TP ratios (0.2–26.5; Table S1; Lau et al., 2021).

2.2 | Climate, N deposition, and long-term lake monitoring data

Climate data from the years that the lakes were sampled were obtained from the Swedish Meteorological and Hydrological Institute (SMHI; https://www.smhi.se). Mean annual air temperature and mean annual precipitation were: −2°C and 600 mm (Abisko, subarctic-north, 2016), 1°C and 700–1,000 mm (Jämtland, subarctic-south, 2017), 3°C and 500–600 mm (Västerbotten, boreal-north, 2016), and 5°C and 500–700 mm (Värmland, boreal-south, 2016). Annual N deposition data (total wet + dry deposition) for NO_3^- and NH_4^+ from 1997 to 2017 were obtained from the SMHI (https://www.smhi.se/klimatdata/miljo/ atmosfarskemi) for each of the study lakes and then averaged across sites to get annual N deposition estimates for each region. We standardised the data by using Z scores to compare relative trends in N deposition (see Isles et al., 2018).

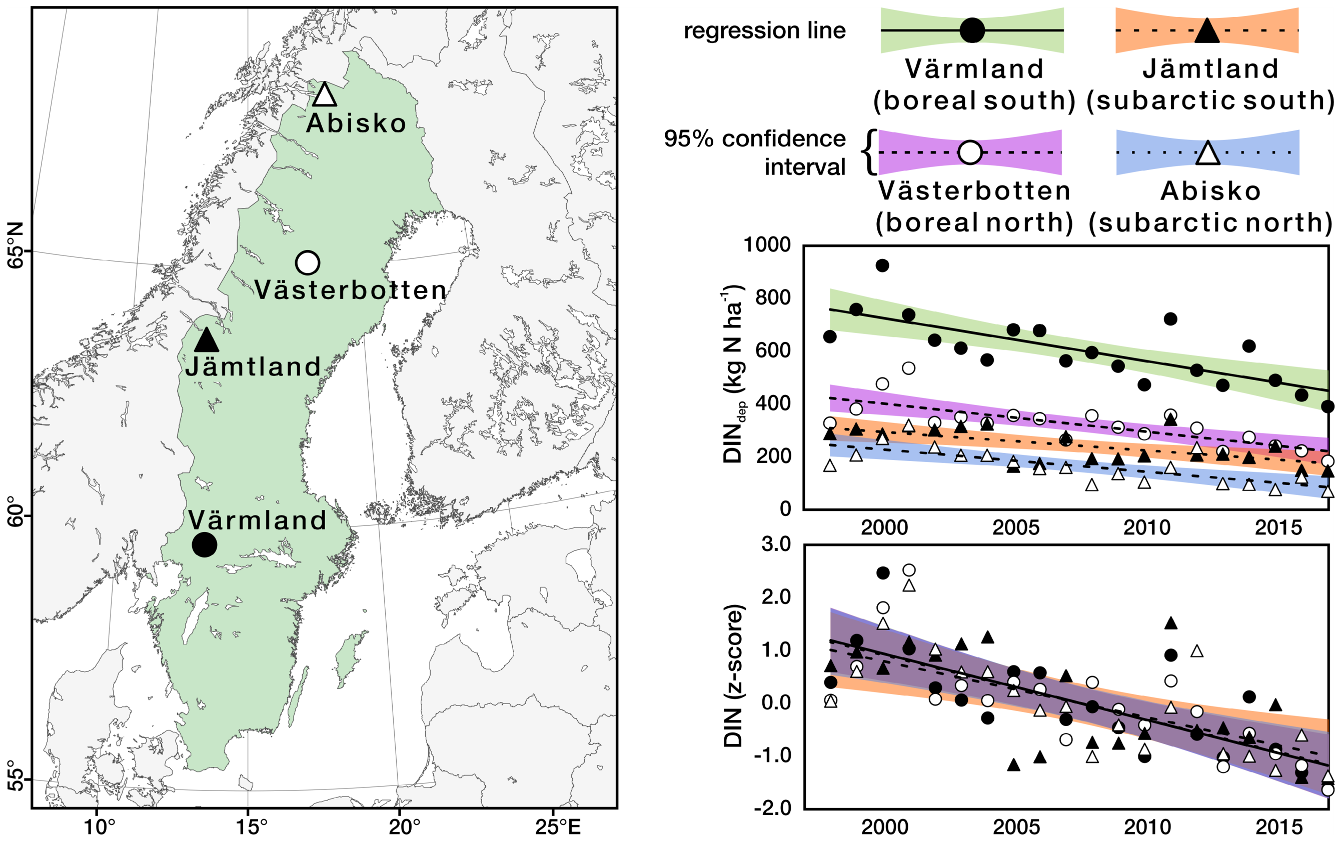


FIGURE 1 The location of the study sites (Värmland [boreal], Västerbotten [boreal], Jämtland [subarctic], and Abisko [subarctic]), and long-term trends in atmospheric DIN deposition (kg ha^{-1} year^{-1}; in absolute rates and expressed as *Z*-scores)

2.3 | Lake sampling and analysis for physicochemical and biological parameters

Water temperature and photosynthetically active radiation (PAR) were measured at 0.5 m increments in the water column, using an YSI Pro-DO (YSI Incorporator) sensor and a spherical quantum sensor (LiCOR Li-250; Licor Biosciences). The light extinction coefficient (k_d) was estimated as the slope of the linear regression between *ln*(PAR) and lake depth and was used as a proxy for light availability.

Lake physicochemical and biological parameters were measured using methods used in Bergström et al. (2018, 2020). Composite water samples from 0.5–1.0 m depth were taken, and subsamples were used for analyses of nutrients, Chl-*a*, and seston C:N:P stoichiometry. Unfiltered subsamples were frozen until analysis for TP. Unfiltered subsamples were also used for Chl-*a* analyses, which were kept in dark for 2–3 h, after which 100 mL was filtered through Whatman glass-fibre filters (0.7 µm; Whatman Arbor Technologies, Ann Arbor, MI, U.S.A.) and then kept frozen (−20°C) until analysis. Filtered (0.45 µm; Sartorius AG) subsamples were kept frozen until analysis for concentrations of DIN (ammonium plus nitrate and nitrite), TDN, and DOC. Filtered water for DOC analyses was further acidified before freezing (100 µl 1.2 M HCl per 10 ml of sample).

Total dissolved N and DOC were measured using a TOC/TN analyser (IL-550, Lange; EVISA), and DIN and TP were measured using a flow-injection analyser (FIA-star 5000; FOSS Inc.; see detection limits in Bergström et al., 2013). Chl-*a* was extracted in 95% ethanol for 24 hr in the dark, then measured using a luminescence spectrophotometer (LS-55; Perkin Elmer) at 433 nm excitation and 673 nm emission wavelengths. Seston C:N:P stoichiometry was determined by filtering known volumes of prescreened composite water samples (filtered through a 50-µm net) onto pre-combusted (550°C, 4 hr) and acid washed (1.2 M HCl) Whatman GF/F filters (Whatman Arbor Technologies). Seston C and N contents were measured using a Costech ECS 4010 elemental analyser (Costech International S. P. A.). Analyses for seston P were made according to Swedish standard (ISO 15681-1, rev 4) using the molybdate blue method after hydrolysis with persulphate using a FIAstar 5000 (FOSS Inc.) following the procedure in Bergström et al. (2015).

Samples for zooplankton biomass were taken by vertical net hauling (100-µm mesh net) from the deepest point of the lake (starting 1 m above the lake bottom). Zooplankton biomass samples were preserved with Lugol's iodine and taxa were identified and counted using inverted microscopy. Zooplankton biomass in dry weight (DW) was estimated using established length–weight regressions for individual taxa (Bottrell et al., 1976; Deininger et al., 2017b). Zooplankton samples for C:N:P analyses followed the same sampling procedure as for biomass, with the exception that several hauls (100-µm mesh net) were performed in order to collect sufficient amounts of zooplankton for C:N:P analyses. After collection, zooplankton bulk samples were transferred to plastic bottles and then left at 4°C and dark overnight (12–24 hr) for gut evacuation. Zooplankton were then sorted and pooled into groups of cladocerans and calanoid/cyclopoid copepods, respectively, and then analysed for C, N and P content. For analyses of C and N, samples from each group were freeze dried, homogenised and weighed into tin capsules. Samples were then analysed using a continuous-flow isotope ratio mass spectrometer (PDZ Europa 20–20; MedWOW Ltd.) at the Swedish University of Agricultural Sciences in Umeå. For P, each zooplankton group was transferred onto preweighed and acid-washed (1.2 M HCl) Whatman GF/C filters (Whatman Arbor Technologies), dried overnight (60°C), and then weighed again (Mettler Toledo MT5; resolution ±1 μg; Mettler-Toledo AB) to determine dry mass. The P content was analysed according to Swedish standard (ISO 15681-1, rev 4) using a FIAstar 5000 (FOSS Inc.) as described above. Relative standard deviations were $<2\%$ for zooplankton C and N, and 1.1% for P. The zooplankton C, N, and P content were reported as percent DW (for example: % C per DW = μg C per μg DW in zooplankton).

2.4 | Nutrient imbalances and N:P recycling ratios of zooplankton

The ecological stoichiometric models by Sterner (1990) and Hassett et al. (1997) were used to estimate the molar N:P imbalances between resources (seston) and consumers (zooplankton) and the molar N:P ratio of nutrients recycled by zooplankton. We judged that ecological stoichiometric models were suitable for estimating the N:P recycling ratio of zooplankton on this small body mass scale of the food chain, although evidence comparing organism groups across a larger scale suggests that differences in N:P recycling ratios and excretion rates are related to body mass (Allgeier et al., 2015). In the ecological stoichiometric models, a positive nutrient imbalance ($N{:}P_{seston} > N{:}P_{consumer}$) indicates that zooplankton are feeding on resources with suboptimal P content relative to their nutrient demand, and the N:P recycling ratio of zooplankton is calculated as: $([N{:}P_{seston} - N{:}P_{zooplankton}] \times$ accumulation efficiency $[AE])/(1 - AE)$. A negative imbalance ($N{:}P_{seston} < N{:}P_{consumer}$) indicates that zooplankton are feeding on resources with suboptimal N content relative to their nutrient demand. The N:P recycling ratio of zooplankton is then calculated as: $(N{:}P_{seston} \times [1 - AE])/(1 - [AE \times N{:}P_{seston}/N{:}P_{zooplankton}])$ (Hassett et al., 1997). The N:P recycling ratio of zooplankton was estimated by assuming an identical AE of 0.75 for both N and P, and we also performed a sensitivity analysis by calculating the N:P recycling ratio of zooplankton using AE equal to 0.5 and 0.9 (Sterner, 1990). The N:P elemental imbalances and recycling ratios were calculated for all zooplankton combined (weighted means), using the respective measured N:P stoichiometry of calanoids, cyclopoids, and cladocerans multiplied by their relative proportions of the total zooplankton biomass (as in Bergström et al., 2015, 2018). We then assessed how the N:P recycling ratio of zooplankton changed with lake water DIN:TP and DOC among regions.

2.5 | Data analysis

Linear regressions were used to determine if there were temporal trends in N deposition (estimated for absolute and Z-standardised values using the long-term monitoring data) that might influence lake DIN concentrations in each study site (see Bergström et al., 2020).

For all parameters and tests, we first calculated seasonal averages for each study lake, and then used the seasonal averages for comparisons among study sites, as the lake water chemistry showed much larger variations between study sites than between seasons (Lau et al., 2021). Comparisons were made between the north (Abisko [subarctic-north] and Västerbotten [boreal-north]) and the south (Jämtland [subarctic-south] and Värmland [boreal-south]) to assess the effects of low (north) versus high N deposition (south) on the lakes. All C:N:P stoichiometric calculations were expressed in molar ratios. We used the Chl-*a*:TP ratio as an indicator of the DIN fertilisation effect on phytoplankton biomass per unit of lake water TP (Bergström & Jansson, 2006). Lake water DIN:TP, Chl-*a*:TP, and seston and zooplankton C:N:P stoichiometry were $\log_{10}$-transformed for normal-distribution approximation and/or variance homoscedasticity before the seasonal averaging (Isles, 2020).

We compared relationships and differences among all four study sites or between the north (Abisko and Västerbotten) and the south (Jämtland and Värmland), of lake water characteristics (temperature, k_d, DOC, DIN, TDN, TP, DIN:TP, Chl-*a*, Chl-*a*:TP), seston C:N:P stoichiometry, and zooplankton biomass using Pearson correlation, linear regression, one-way ANOVA and post hoc Tukey's test (or Kruskal–Wallis test and Dunn's test for non-normally distributed data), or two-tailed t-test (or Mann–Whitney test for non-normally distributed data; for predictions 1, 2, 3, and 4).

Nonlinear regression (Gaussian peak) analyses were used to test if Chl-*a* was unimodally distributed with lake DOC and k_d (see Bergström & Karlsson, 2019; Isles et al., 2021). We compared these nonlinear models with the linear and null models, and the model with the lowest corrected Akaike information criterion (AICc) was selected as the best model (for prediction 2).

Non-metric multidimensional scaling (NMDS) was used to compare the zooplankton community composition, which was estimated by the biomass proportions of calanoid/cyclopoid copepods and cladocerans (see Table S3), among the four study sites. NMDS was based on Bray–Curtis dissimilarity and two dimensions. Differences in zooplankton community composition among study sites were further tested using permutational multivariate analysis of variance (PERMANOVA). To determine whether the zooplankton community composition was related to lake $\log_{10}$(DIN:TP) and DOC (for prediction 5), we fitted these environmental data on the NMDS ordination and tested their statistical significance with random permutations. One-way ANOVAs with post hoc Tukey's test were used to assess differences in C:N:P stoichiometry in calanoid/cyclopoid copepods and cladocerans, and in all zooplankton (weighted means; for prediction 5).

Analysis of covariance (ANCOVA) was used to test whether lake DIN:TP and the N:P recycling ratios of zooplankton changed with lake DOC, and whether these relationships differed between the northern versus the southern regions, i.e., if there was an interaction between region (that is north and south) and lake DOC (for predictions 1 and 6). Region was used as the fixed factor, with lake DOC as the covariate. When ANCOVA indicated significant effects of region, we used Student's *t* tests for post hoc comparisons.

Non-metric multidimensional scaling, PERMANOVA and environmental fitting were run using the vegan package in R (Oksanen et al., 2020; R Core Team, 2020). All other statistical analyses were conducted using Sigma Plot 14.0 (Systat Software Inc.) and JMP® version 14.01 (JMP, Cary, U.S.A.). Statistical significance was defined at $p < 0.05$.

3 | RESULTS

3.1 | Long-term trends in N-deposition

There was a declining trend in N deposition in the long-term monitoring data in both regions, with steeper rates of declines in the boreal sites (slope = −16.2 kg N ha^{-1} year^{-1} in Värmland and − 10.6 kg N ha^{-1} year^{-1} in Västerbotten) than in the subarctic sites (slope = −8.4 kg N ha^{-1} year^{-1} in Abisko and − 7 kg N ha^{-1} year^{-1} in Jämtland). N deposition in Värmland (boreal-south) was higher than in Västerbotten (boreal-north), Jämtland (subarctic-south) and Abisko (subarctic-north; Kruskal–Wallis test and Dunn's test, $p < 0.001$), decreasing from approximately: 655 to 433 kg ha^{-1} year^{-1} (−34%) in Värmland, 327 to 223 kg ha^{-1} year^{-1} (−32%) in Västerbotten, 289 to 147 kg ha^{-1} year^{-1} (−49%) in Jämtland and 168 to 123 kg ha^{-1} year^{-1} (−27%) in Abisko (Figure 1).

3.2 | Physico-chemical characteristics of lake water

Surface water temperatures over the summer ranged between 10.9 and 19.2°C with significant lower temperatures in Abisko (subarctic-north; 11.7°C) than in Jämtland (subarctic-south; 14.0°C), Värmland (boreal-south; 16.0°C) and Västerbotten (boreal-north; 18.4°C; one-way ANOVA and Tukey's test, $p < 0.001$). Most lakes in Abisko were unstratified over the sampling period (Isles et al., 2020). Log_{10}DIN:TP ratios were lower in the northern (Abisko and Västerbotten) than in the southern (Jämtland and Värmland) lakes. Log_{10}DIN:TP ratios were also lower in lakes with higher DOC (Tables 1-2; Figure 2a) and poorer light conditions (with increased k_d; $r = -0.55$, $p < 0.001$; Table S1). Based on the ANCOVA, there was no interaction between region (that is north and south) and lake DOC on $\log_{10}$DIN:TP (Table 2; Figure 2a).

Lake DIN concentrations were lower in the northern lakes (Abisko and Västerbotten; 4.2 μg/L) compared to the southern lakes (Jämtland and Värmland; 10.6 μg/L; two-tailed t-test, $p < 0.001$), whereas lake TDN concentrations did not differ between the northern (300 μg/L) and southern (287 μg/L) lakes (two-tailed t-test, p = 0.78). Lake TP was higher in Västerbotten (boreal-north; 14.1 μg/L) than in Abisko (subarctic-north) and Jämtland (subarctic-south; 3.6 and 4.7 μg/L) but did not differ from lake TP in Värmland (boreal-south; 5.5 μg/L; Kruskal–Wallis test and Dunn's test, $p < 0.001$). Lake DOC in boreal Västerbotten and Värmland (13 and 11.3 mg/L) were significantly higher than in subarctic Jämtland and Abisko (5.2 mg/L for both; one-way ANOVA and Tukey's test, $p < 0.001$). Abisko lakes (subarctic-north) had significantly lower k_d (0.6 m^{-1}) compared to lakes in Jämtland (subarctic-south; 1.0 m^{-1}), Värmland (boreal-south; 2.0 m^{-1}) and Västerbotten (boreal-north; 2.2 m^{-1}; Kruskal–Wallis test and Dunn's test, $p < 0.001$). For all lakes combined, lake DOC was directly related to lake TP ($r = 0.67$; $p < 0.05$) and k_d ($r = 0.93$; $p < 0.001$; Table S1).

3.3 | Chl-a and seston C:N:P stoichiometry

The epilimnetic Chl-*a* ranged between 0.5 and 3.6 μg/L (Table S2) and did not differ between the northern (Abisko and Västerbotten;

TABLE 1 Linear regressions of lake dissolved inorganic nitrogen to total phosphorus ratio ($\log_{10}$DIN:TP; molar) with lake dissolved organic carbon concentrations (DOC; mg/L); $\log_{10}$Seston C:P (molar) and $\log_{10}$Seston N:P (molar) with $\log_{10}$DIN:TP, DOC, and k_d; and $\log_{10}$Zooplankton N:P (molar) with $\log_{10}$DIN:TP and DOC. Regression analyses for all lakes combined with the exception for the regressions of $\log_{10}$DIN:TP with lake DOC where North and South denotes analyses for Abisko and Västerbotten (subarctic- and boreal-north), and Jämtland and Värmland (subarctic- and boreal-south) lakes combined, respectively

Variable Y	Linear regression	*p*	r^2	*df*	*F*
$\log_{10}$DIN:TP (North)	0.659 – (0.053 × DOC)	**<0.05**	0.41	16,1	10.27
$\log_{10}$DIN:TP (South)	1.139 – (0.054 × DOC)	**<0.001**	0.57	15,1.	18.27
$\log_{10}$Seston C:P	2.568 + (0.145 × $\log_{10}$DIN:TP)	**<0.001**	0.32	32,1	14.64
$\log_{10}$Seston N:P	1.529 + (0.145 × $\log_{10}$DIN:TP)	**<0.001**	0.36	32,1	17.06
$\log_{10}$Seston C:P	2.696 – (0.0077 × DOC)	0.07	0.11	32,1	3.62
$\log_{10}$Seston N:P	1.661 – (0.0081 × DOC)	**<0.05**	0.13	32,1	4.65
$\log_{10}$Seston C:P	2.678 – (0.032 × k_d)	0.10	0.08	32,1	2.86
$\log_{10}$Seston N:P	1.647 – (0.037 × k_d)	**<0.05**	0.125	32,1	4.42
$\log_{10}$Zooplankton N:P	1.605 + (0.151 × $\log_{10}$DIN:TP)	**<0.05**	0.18	32,1	6.64
$\log_{10}$Zooplankton N:P	1.547 – (0.0005 × DOC)	0.94	0.00	32,1	0.00

Note: *p* values <0.05 are boldfaced.

TABLE 2 ANCOVA comparisons of lake dissolved inorganic nitrogen to total phosphorus ratio (DIN:TP; $\log_{10}$-transformed) and the N:P recycling ratio ($\log_{10}$-transformed) of zooplankton between region south (Jämtland and Värmland) and region north (Abisko and Västerbotten), and across the gradient in lake dissolved organic carbon (DOC) concentration

Variable	Sum of squares	$F_{1,29}$	p	Student's t	Model r^2
$\log_{10}$DIN:TP					
Region	1.796	20.413	**<0.001**	South > north	0.585
DOC	2.231	25.367	**<0.001**		
Interaction	<0.001	<0.001	0.996		
$\log_{10}$N:P recycling ratio					
Region	1.691	37.721	**<0.001**	South > north	0.567
DOC	0.249	5.557	**0.025**		
Interaction	0.003	0.068	0.796		

Note: p values <0.05 are boldfaced. Post hoc Student's t tests were used to compare the effects between region south and region north.

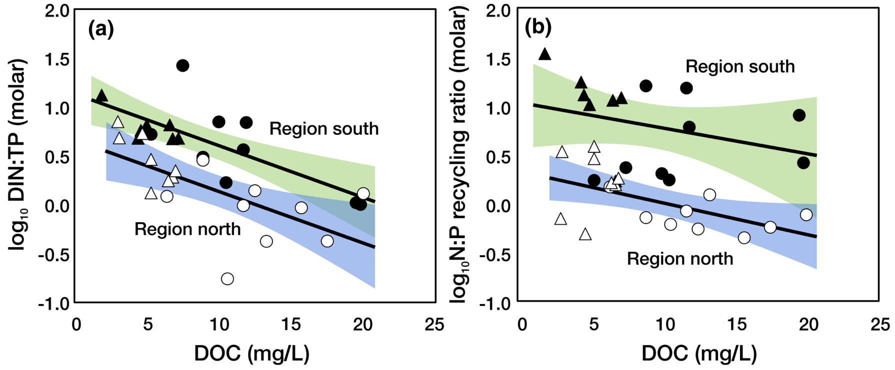


FIGURE 2 (a) Lake dissolved inorganic nitrogen to total phosphorus ratio (DIN:TP; $\log_{10}$-transformed) and (b) the N:P recycling ratio ($\log_{10}$-transformed) of zooplankton against dissolved organic carbon (DOC) concentration in the study lakes. Data are averages over summer for each lake. Filled circles = Värmland lakes (boreal-south), open circles = Västerbotten lakes (boreal-north), filled triangles = Jämtland lakes (subarctic-south), and open triangles = Abisko lakes (subarctic-north). Lines of fit with 95% CI (shaded areas) are indicated for lakes in region south (green) and region north (blue), respectively. See Tables 2 for statistical results

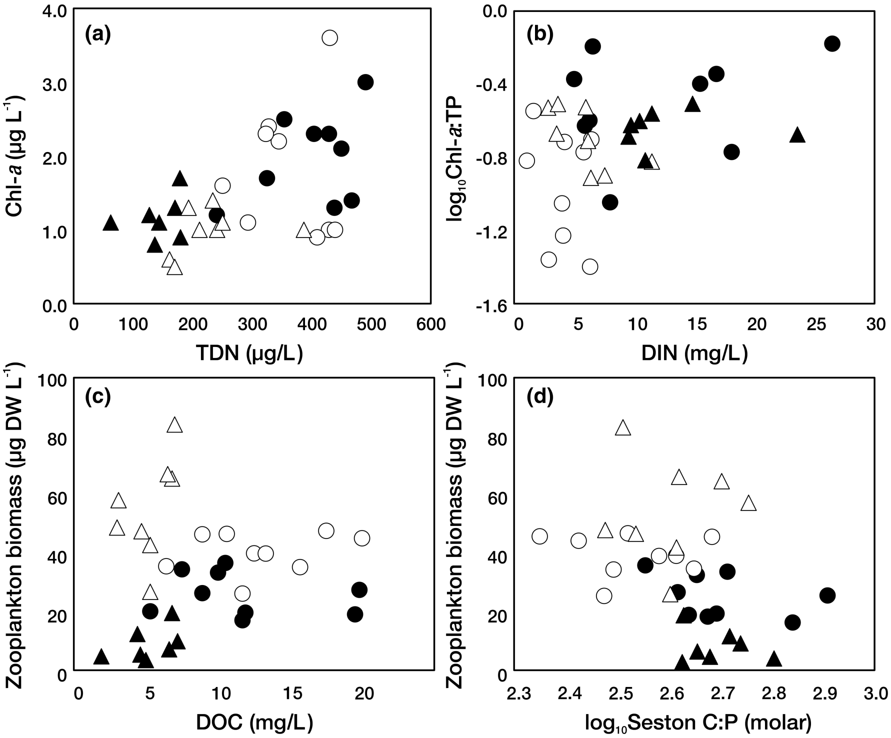
1.10 μg/L) and southern (Jämtland and Värmland; 1.35 μg/L) lakes (two-tailed t-test; $p = 0.16$). For all lakes combined, lake Chl-*a* tended to increase with increased lake TDN ($r = 0.53$, $p < 0.05$; Figure 3a), but not with increased lake TP ($r = 0.1$, $p = 0.57$). Lake Chl-*a* was unimodally related with both lake DOC and k_d (Gaussian peak model with DOC: $r^2 = 0.45$, $p < 0.05$, AICc = 60.80; with k_d: $r^2 = 0.44$, $p < 0.05$, AICc = 61.27; Figure S1). These nonlinear models had lower AICc compared to the linear and null models whose AICc ranged from 70.66 to 75.29. The $\log_{10}$Chl-*a*:TP ratios (Table S2) were lower in northern than in southern lakes (two-tailed t-test; $p < 0.05$). Thus, higher lake DIN concentrations in the south (Jämtland and Värmland) tended to promote higher $\log_{10}$Chl-*a*:TP ratios, with more pronounced differences in lakes between boreal-north and -south (Västerbotten and Värmland) compared to the subarctic-north and -south (Abisko and Jämtland; Figure 3b).

Seston C and N (means for Abisko, Jämtland, Västerbotten and Värmland for C: 389, 425, 564, and 488 μg/L; and for N: 42, 48, 57, and 48 μg/L) did not differ between northern and southern lakes (one-way ANOVAs, $p = 0.08$ and 0.15), but Västerbotten (boreal-north) had higher seston P (4.3 μg/L) than the other study sites (2.2, 2.6, and 2.6 μg/L; one-way ANOVA and Tukey's test, $p < 0.001$). The $\log_{10}$Seston C:P and $\log_{10}$Seston N:P ranged between 2.42–2.91 (non-logged values within parentheses; 263–812) and 1.34–1.80 (22–63), respectively (Table S2). The $\log_{10}$Seston C:P and $\log_{10}$Seston N:P were significantly lower in the north (Västerbotten and Abisko: 2.56 [363] and 1.51 [32]) compared to in the south (Värmland and Jämtland: 2.69 [489] and 1.66 [46]; two-tailed *t*-test; $p < 0.001$ for both). For all lakes combined, $\log_{10}$Seston C:P and $\log_{10}$Seston N:P tended to increase with increasing lake DIN:TP and decline with increasing lake DOC and k_d (Table 1).

3.4 | Zooplankton biomass, community composition, and C:N:P stoichiometry

Zooplankton biomass ranged between 3.5 and 84 μg DW/L (Table S3) and was significantly higher in Abisko (subarctic-north: 53.5 μg DW/L) than in Värmland (boreal-south) and Jämtland (subarctic-south; 26.4 and 7.2 μg DW/L) but did not differ from Västerbotten (boreal-north; 40 μg DW/L; Kruskal–Wallis test and Dunn's test, $p < 0.001$; Figure 3c). Zooplankton biomass was not related to Chl-*a* ($p = 0.39$). However, zooplankton biomass, and cladoceran biomass specifically, were negatively related to $\log_{10}$Seston N:P ($r = -0.45$ and -0.54; $p < 0.05$ for both) and $\log_{10}$Seston C:P ($r = -0.40$

FIGURE 3 Chlorophyll-*a* (Chl-*a*) concentration against (a) total dissolved nitrogen concentration (TDN) and (b) Chl-*a* concentration per total phosphorus concentration (Chl-*a*:TP; $\log_{10}$-transformed) against dissolved inorganic nitrogen (DIN) concentration in the study lakes. Zooplankton biomass against (c) dissolved organic carbon (DOC) concentration and (d) seston C:P ($\log_{10}$-transformed) in the study lakes. Mean concentration over summer for each lake. Filled circles = Värmland lakes (boreal-south), open circles = Västerbotten lakes (boreal-north), filled triangles = Jämtland lakes (subarctic-south), and open triangles = Abisko lakes (subarctic-north). For statistical relationships, see Results



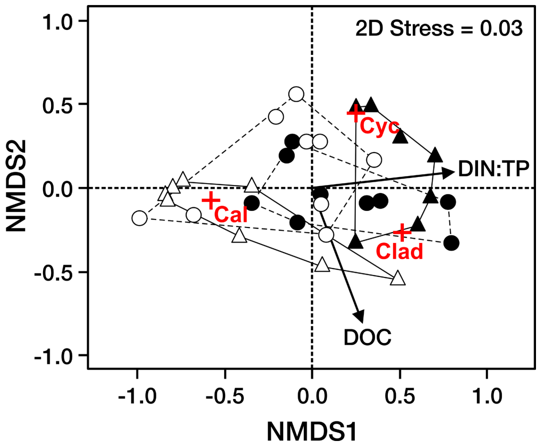


FIGURE 4 Non-metric multidimensional scaling (NMDS) results of the lake zooplankton communities based on Bray–Curtis dissimilarity. Solid and broken lines indicate convex hulls of individual subarctic and boreal study sites, respectively. Data of lake dissolved inorganic nitrogen to total phosphorus ratio (DIN:TP; $\log_{10}$-transformed) and lake dissolved organic carbon (DOC) concentration were fitted on the NMDS ordination, but they were not statistically significant based on permutation tests ($p > 0.05$). Filled circles = Värmland lakes (boreal-south), open circles = Västerbotten lakes (boreal-north), filled triangles = Jämtland lakes (subarctic-south), and open triangles = Abisko lakes (subarctic-north). Taxon abbreviations: Clad, cladocerans; Cal, calanoids; Cyc, cyclopoids

and −0.5; $p < 0.05$ for both; Figure 3d), whereas cyclopoid copepod biomass was negatively related to $\log_{10}$Seston N:P only ($r = -0.42$, $p < 0.05$). Calanoid copepod biomass was not related to $\log_{10}$Seston C:P or $\log_{10}$Seston N:P.

Zooplankton community composition also differed among study sites based on NMDS and PERMANOVA ($F_{3,29} = 6.01$, $p = 0.002$; Figure 4; Table S3). The NMDS ordination had a stress value of 0.03, which indicates an excellent configuration with a very low risk of false inference (Clarke, 1993). Calanoid copepods, which correlated well with the first NMDS axis, were the major contributor of the differences. There was thus a north-to-south separation in zooplankton communities, with a dominance of calanoid copepods in the north (Abisko and Västerbotten) and a dominance of cladocerans in the south (Jämtland and Värmland). Also, greater differences were found between subarctic study sites (Abisko and Jämtland) than between boreal study sites (Västerbotten and Värmland; Table S3; Figure 4). The north-to-south differences in zooplankton communities tended to be related to the increasing DIN:TP ratios toward the south (Figure 4), although this relationship was not significant based on permutation tests ($p > 0.05$). For lakes in the south, there was also a tendency of increasing cladoceran dominance and decreasing cyclopoid copepods with increasing lake DOC (Figure 4), but this relationship was also not significant (permutation tests: $p > 0.05$). Overall, we found differences in zooplankton communities among our study sites that could not be explained by individual gradients in lake DIN:TP and DOC. The larger dissimilarities between subarctic study sites than between boreal study sites suggest that increases in lake DOC would reduce the differences in zooplankton community composition caused by declines in lake DIN:TP.

The C content (Table S4) of zooplankton (Värmland: 53.8%, Västerbotten: 54.2%; Jämtland: 52; Abisko: 54.4) was significantly lower in Jämtland (subarctic-south) compared to the other study sites (one-way ANOVA and Tukey's tests, $p < 0.05$), a difference that was primarily driven by significantly lower C content among cladocerans in Jämtland (50.2%) compared to other study sites (Värmland: 52.6%, Västerbotten: 55.3%; Abisko 54%). The N content (Värmland: 10.3%, Västerbotten: 10.7%; Jämtland: 10.4%; Abisko: 10.6%) did not differ among study sites, and calanoid and cyclopoid copepods (10.1–11.3%) were overall richer in N compared to cladocerans (8.6–9.6%). The zooplankton P content (Värmland: 0.81%, Västerbotten: 0.64%; Jämtland: 1.01%; Abisko: 0.54%) was significantly higher in Jämtland (subarctic-south) than in Abisko (subarctic-north) and Västerbotten (boreal-north), and higher in Värmland (boreal-south) than in Abisko (one-way ANOVA and Tukey's test, $p < 0.001$). Higher P content in zooplankton in the south was an effect of high proportions of P-rich cyclopoid copepods and cladocerans (Tables S3, S4).

There were some differences in zooplankton N:P stoichiometry among study sites. The calanoid copepod log$_{10}$N:P did not differ among study sites (one-way ANOVA, $p = 0.36$), whereas the cyclopoid copepod log$_{10}$N:P in Abisko (subarctic-north: 1.56) was higher than in Jämtland (subarctic-south: 1.33; one-way ANOVA and Tukey's test, $p < 0.05$), but not different from boreal-south or -north (Värmland and Västerbotten: 1.47, 1.46; Tukey's test, $p > 0.05$; Table S4). The cladoceran log$_{10}$N:P was significantly higher in the subarctic-north (Abisko: 1.61) than in the south (Värmland and Jämtland: 1.38 for both; one-way ANOVA and Tukey's test, $p < 0.05$), but not different from the boreal-north (Västerbotten 1.52; Tukey's test, $p > 0.05$; Table S4). As a result, the log$_{10}$ZooplanktonN:P ratio (weighted means) was higher in the north (Västerbotten and Abisko: 1.60 and 1.69) than in the south (Värmland and Jämtland: 1.49 and 1.37; t-test, $p < 0.001$; Table S4). For all lakes combined, log$_{10}$ZooplanktonN:P was negatively related to log$_{10}$DIN:TP ratios ($r = -0.42$, $p < 0.05$; Figure 5c), but not related to DOC (Figure 5d). Overall, the difference between the north and the south in log$_{10}$Zooplankton N:P ratio was primarily the result of shifts in community composition from high proportions of calanoid copepods of high N:P in the north to high proportions of cladocerans of low N:P in the south (Figure 4), rather than the result of changes in N:P stoichiometry within individual zooplankton taxa across different regions (Tables S3, S4).

3.5 | Nutrient imbalances and N:P recycling ratios of zooplankton

The differences in seston N:P and zooplankton N:P (Figure 5) resulted in significantly lower nutrient imbalances (i.e., seston N:P – zooplankton N:P) in the north (Abisko, Västerbotten: −12.3 and − 10.4) than in the south (Jämtland, Värmland: 25.5 and 13; one-way ANOVA and Tukey's test, $p < 0.001$; Table S4). Due to these differences, the log$_{10}$N:P recycling ratios of zooplankton were higher in the south (Värmland, Jämtland: 1.57 and 1.82) than in the north (Västerbotten, Abisko: 1.12 and 1.33; one-way ANOVA and Tukey's test, $p < 0.001$; Table S4). Results from ANCOVA showed that the log$_{10}$N:P recycling ratios of zooplankton were lower in the north and with higher lake DOC, but there was no interaction between region (that is north vs. south) and lake DOC on zooplankton N:P recycling ratios (Table 2; Figure 2b). Based on our sensitivity analyses, the effects of region (north vs. south) and lake DOC were only significant when the AE was higher than 0.5 in the stoichiometric models (Table S5; Figure S2).

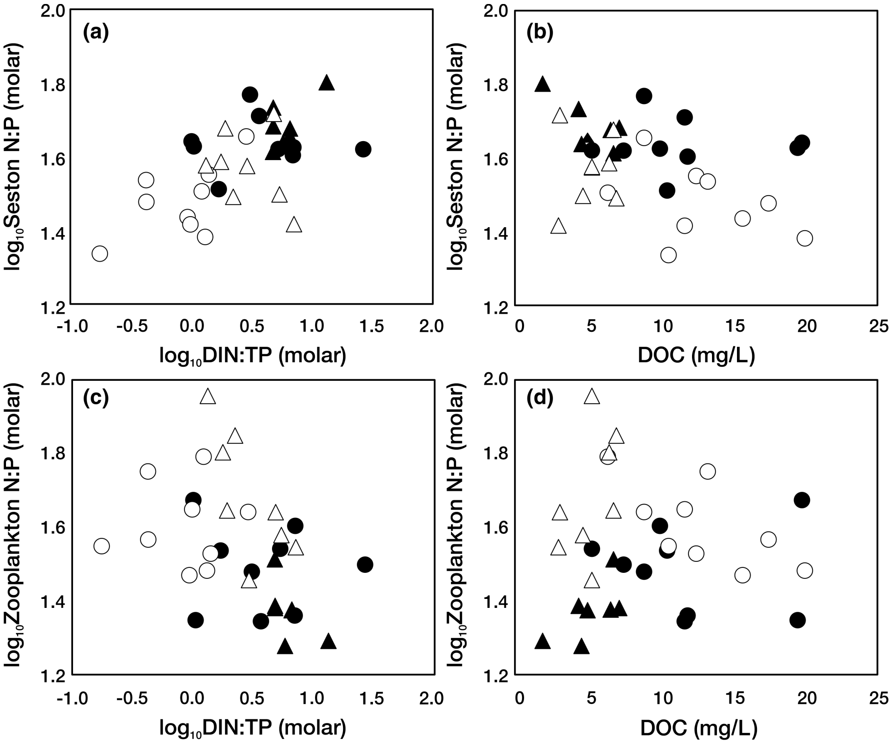


FIGURE 5 Lake dissolved inorganic nitrogen to total phosphorus ratio (DIN:TP; log$_{10}$-transformed) and lake dissolved organic carbon (DOC) concentrations against (a, b) seston N:P (log$_{10}$-transformed) and (c, d) zooplankton N:P (log$_{10}$-transformed) in the study lakes. Mean concentration over summer for each lake. Filled circles = Värmland lakes (boreal-south), open circles = Västerbotten lakes (boreal-north), filled triangles = Jämtland lakes (subarctic-south), and open triangles = Abisko lakes (subarctic-north). For statistical relationships, see Results

4 | DISCUSSION

We found that, while N deposition was declining in all study sites, there remained an N fertilisation effect in terms of enhanced lake DIN:TP in southern boreal and subarctic lakes (Värmland, Jämtland) compared to northern ones (Abisko, Västerbotten). For all lakes, there were similar declining rates in $\log_{10}$DIN:TP per mg/L increase of DOC (regression slopes were −0.054 and −0.053 for the southern and northern lakes, respectively), but with a higher intercept for lakes in the south (y-intercept at $\log_{10}$DIN:TP of 1.14) than in the north (y-intercept at $\log_{10}$DIN:TP of 0.66; support for prediction 1). These similar declining rates support the fact that N deposition accounts for most of the DIN inputs in small lakes (Isles et al., 2018), whereas lake TP to a large extent is related to catchment vegetation cover and the delivery of P associated with terrestrial dissolved organic matter (Hessen, 2013; Isles et al., 2020). These processes in combination set the levels and the declining rates in lake DIN:TP with increasing lake DOC (Bergström et al., 2018; Isles et al., 2018, 2020).

Lake Chl-*a* tended to increase with increased lake TDN and be unimodally related with lake DOC and k_d, in support of previous studies that recognised the widespread N- to co-NP limitation of phytoplankton in Swedish lakes (Bergström et al., 2008;Bergström et al., 2020; Isles et al., 2020) and the trade-offs between light and nutrient availability with increased lake DOC on shaping lake primary production (Ask et al., 2009; Kelly et al., 2018; Olson et al., 2020; Vasconcelos et al., 2019) and phytoplankton biomass (Bergström & Karlsson, 2019; Isles et al., 2021). Although lake Chl-*a* concentrations did not differ between lakes in the north and in the south, lake Chl-*a*:TP ratios were higher in the south than in the north, suggesting a DIN fertilisation effect from higher N deposition on phytoplankton biomass per unit of P (Bergström et al., 2020; Bergström & Jansson, 2006) (support for prediction 2).

The N fertilisation effect induced by higher N deposition was related to seston mineral quality (support for prediction 3). Lake DIN:TP ratio clearly impacted seston mineral quality, which was lower (that is higher C:P and N:P) in both subarctic and boreal southern lakes (Jämtland, Värmland). The ongoing N deposition declines, which ranged from −27 to −49% over the past 20 years (from 1997 to 2017) and reflected a reverting of lakes closer to their natural state (cf. Bergström & Jansson, 2006), will probably be an important determinant of future declines in the lake DIN:TP ratio and N limitation for phytoplankton in these lakes (see Isles et al., 2018, 2020). Since lake DIN:TP was directly related to seston C:P and N:P, increases in lake DOC, which at least partly are a consequence of climate change (Creed et al., 2018), are mitigating the reduction in seston mineral quality in these lakes. However, based on the slopes in the regression analyses, the antagonistic effect of increased lake DOC (slopes: −0.0077 [seston C:P[, −0.0082 [seston N:P]) on the reduction in seston mineral quality is much smaller than the enhancing effect of decreased lake DIN:TP (slopes: −0.145 for both).

In contrast to other studies (McCauley & Kalff, 1981) and our prediction 4, zooplankton biomass was not related to phytoplankton biomass, which might be due to preferential grazing of zooplankton for certain phytoplankton in the lakes (Hessen et al., 1995). Phytoplankton biomass (Chl-*a* ranged from 0.5 to 3.6 μg/L, which is about 25–180 μg C/L; see Ahlgren, 1983), was below to well above the estimated threshold of 50 μg C/L for maintenance metabolism for some zooplankton (i.e., *Daphnia* and *Eudiaptomus*) (Andersen, 1997; Lampert, 1977; Sterner, 1997); yet, zooplankton biomass, and specifically the biomass of cladocerans and cyclopoid copepods, increased with increasing seston mineral quality (that is decreasing seston C:P and N:P), and were lower in southern lakes (Jämtland and Värmland) with higher DIN:TP (support for prediction 4). In the southern lakes, seston C:P reached levels well above threshold elemental ratios for P-limitation in cladocerans (*Daphnia* 200–300; Sterner & Elser, 2002) and for some calanoid copepods (*Eudiaptomus* >300; Kibby, 1971; Hessen & Lyche, 1991), implying reduced growth rates in zooplankton due to poor mineral quality. Growth rates of *Daphnia* and calanoid copepods have been shown to be negatively related with seston C:P (Deininger et al., 2017b; DeMott & Gulati, 1999; Sterner et al., 1997; Vrede et al., 2002), although zooplankton growth is also influenced by dietary polyunsaturated fatty acid availability (Müller-Navarra, 1995; Müller-Navarra et al., 2000) and by fish predation (Hessen et al., 1995). Fish were present in most study lakes except for one lake in Västerbotten (boreal-north) and three lakes in Abisko (subarctic-north). Still, our results are in line with Hessen (2013), suggesting that a lower seston C:P resulting from ongoing declines in N deposition will mitigate the negative impact of P limitation on zooplankton and higher trophic levels.

There was no relationship between zooplankton community composition and their somatic N:P with lake DOC (lack of support for part of prediction 5). Instead, $\log_{10}$ZooplanktonN:P ratios were negatively related with increasing $\log_{10}$DIN:TP (support for part of prediction 5). This result was due to community shifts in zooplankton, with calanoid copepods of high somatic N:P dominating in the north (Abisko and Västerbotten) and cladocerans and perhaps cyclopoid copepods of low somatic N:P dominating in the south (Jämtland and Värmland). Thus, differences in zooplankton N:P stoichiometry among the study sites were not related to plasticity or within-group differences, which were generally low (in support of prediction 5), although cladocerans in Abisko in the subarctic-north had deviating values. It is possible that lower water temperatures in the Abisko lakes caused low growth rates and low requirement of ribosomal RNA (Bullejos et al., 2014; Elser et al., 2000; Vrede et al., 2004), or reduced ingestion rates due to increased water viscosity (Loiterton et al., 2004), causing lower P content in cladocerans in Abisko compared to cladocerans in the southern lakes (Jämtland and Värmland). Still, within-group variability in N:P stoichiometry was only noted for cladocerans, and only for the Abisko lakes, which had very low proportions of cladocerans. Therefore, variation in zooplankton N:P among study sites was driven by their community composition changes.

The reduction of calanoid copepods toward the south might be related to differences in feeding modes among zooplankton.

Calanoid copepods have a stronger preference for phytoplankton over terrestrial organic particles and bacteria than do cladocerans, and for phytoplankton over microplankton (such as flagellates) than do cyclopoid copepods (Berggren et al., 2015). Thus, it is possible that when lake DIN:TP is high and lakes are more N-rich, the raptorial feeding mode of cyclopoid copepods allows them to outcompete calanoid copepods for phytoplankton prey by additionally feeding on bacterivorous microplankton fuelled by terrestrial organic matter (Berggren et al., 2015), thereby suppressing calanoid copepod abundance and indirectly increasing the relative abundance of cladocerans. In this sense, lake DOC concentrations and lake DIN:TP ratios seem to interact in affecting zooplankton community composition and their N:P stoichiometry. To some extent, this is in line with other studies showing declining trends of calanoid copepods with increased nutrient and DOC concentrations (Pace, 1986; Patoine et al., 2000).

Changes in seston N:P and zooplankton N:P with higher lake DIN:TP coincided with higher N:P recycling ratios in the south (Jämtland and Värmland; 1.82 and 1.57; unlogged 66 and 37) compared to in the north (Abisko and Västerbotten; 1.33 and 1.12; unlogged 21 and 14; in line with prediction 6). The predicted nutrient imbalances were therefore positive in lakes in the south and negative in the north. This suggests that zooplankton in the south were feeding on food resources with suboptimal P content relative to their nutrient demand and were therefore P limited, and zooplankton in the north were feeding on food resources of suboptimal N content relative to their nutrient demand and were therefore N limited. Zooplankton feeding on the available food resources in each study site might feedback and sustain dual NP- to P-limitation in phytoplankton in the south (high N:P recycling ratio), and N limitation in phytoplankton in the north (low N:P recycling ratio) (Bergström et al., 2020; Isles et al., 2020).

However, the impact of these changes in zooplankton N:P recycling ratios on phytoplankton nutrient limitation will depend on the magnitude in N:P recycling *rates* of zooplankton relative to other nutrient supplies, such as external nutrient loadings (Bergström et al., 2015) and internal sediment nutrient release (Levine & Schindler, 1992). The N:P recycling rates of zooplankton will increase with increasing body size (Allgeier et al., 2015), biomass (Elser et al., 1988), and temperature (Allen & Gillooly, 2009). Since we picked primarily large specimens, the differences in body size among zooplankton taxa should be of lesser importance compared to differences in total zooplankton biomass among study sites. Zooplankton biomass was particularly low in Jämtland (subarctic-south), suggesting lower N:P recycling rates of zooplankton and lower impacts on phytoplankton nutrient limitation regimes in Jämtland relative to the other study sites, especially in Abisko (subarctic-north), which had the highest zooplankton biomass. However, lower water temperature in Abisko implies lower zooplankton metabolic rates, which could cause lower excretion rates of N and P (Alcaraz et al., 2013) relative to warmer, higher DOC lakes. This has been shown in laboratory experiments, where the metabolism of zooplankton individuals of similar body mass increased exponentially with increasing temperature, leading to increased excretion rates of N and P, with a declining trend in the N:P recycling ratios (Alcaraz et al., 2013). In addition, our results based on ecological stoichiometry models showed no interaction between lake region (i.e., north vs. south) and lake browning on the N:P recycling ratio of zooplankton.

In conclusion, our results suggest that ongoing declines in N deposition combined with increases in lake DOC are likely to reduce the N:P recycling ratios of zooplankton in both subarctic and boreal lakes, by lowering lake DIN:TP, improving phytoplankton mineral quality and zooplankton biomass, and shifting the zooplankton community toward greater dominance by calanoids, thereby potentially improving pelagic food web efficiencies.

AUTHOR CONTRIBUTIONS

A-K.B., D.C.P, P.D.F., and I.C.F. conceived the ideas, designed the research, and analysed the data. A-K.B., P.D.F., and A.J. collected samples and performed the field work. A-K.B. led the writing, with assistance from D.C.P, P.D.F., A.J., and I.C.F.

ACKNOWLEDGMENTS

This study was funded in part by the Swedish Research council's VR (dnr:621-2014-5909) and Knut och Alice Wallenbergs Stiftelse (dnr: 2016.0083). We also acknowledge support for our laboratory facility BAF at the department of Ecology and Environmental Science from the Chemical Biological Centre (KBC-group) at UmU.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENTS

Data are contained in the supporting information or available from the authors upon reasonable request.

ORCID

Ann-Kristin Bergström https://orcid.org/0000-0001-5102-4289
Danny C. P. Lau https://orcid.org/0000-0002-3246-7508
Irena F. Creed https://orcid.org/0000-0001-8199-1472

REFERENCES

Ahlgren, G. (1983). Comparison of methods for estimation of phytoplankton carbon. *Archiv für Hydrobiologie*, *98*, 489–508.

Alcaraz, M., Almeda, R., Saiz, E., Calbet, A., Duarte, D. M., Augusti, S., … Alonso, A. (2013). Effects of temperature on the metabolic stoichiometry of Arctic zooplankton. *Biogeosciences*, *10*, 689–697.

Allen, A. P., & Gillooly, J. F. (2009). Towards an integration of ecological stoichiometry and the metabolic theory of ecology to better understand nutrient cycling. *Ecology Letters*, *12*, 369–384.

Allgeier, J. E., Wenger, S. J., Rosemond, A. D., Schindler, D. E., & Layman, C. A. (2015). Metabolic theory and taxonomic identity predict nutrient recycling in a diverse food web. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(20), E2640–E2647.

Andersen, T. (1997). *Herbivores as sources and sinks for nutrients*. *Ecological Series 129*. Springer.

Andersen, T., & Hessen, D. O. (1991). Carbon, nitrogen, and phosphorus content of freshwater zooplankton. *Limnology and Oceanography*, *36*, 807–814.

Ask, J., Karlsson, J., Persson, L., Ask, P., Byström, P., & Jansson, M. (2009). Terrestrial organic matter and light penetration effects on bacterial and primary production in lakes. *Limnology and Oceanography*, *54*, 2034–2040.

Berggren, M., Bergström, A.-K., & Karlsson, J. (2015). Intraspecific autochthonous and allochthonous resource use by zooplankton in a humic lake during the transitions between winter, summer, and fall. *PLoS One*, *10*, 1–14.

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.

Bergström, A.-K., & Jansson, M. (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., & Karlsson, J. (2019). Light and nutrient control phytoplankton biomass responses to global change in northern lakes. *Global Change Biology*, *25*, 2021–2029.

Bergström, A.-K., Jonsson, A., & Jansson, M. (2008). Phytoplankton responses to nitrogen and phosphorus enrichment in unproductive Swedish lakes along a gradient of atmospheric nitrogen deposition. *Aquatic Biology*, *4*, 55–64.

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

Bergström, A.-K., Karlsson, D., Karlsson, J., & Vrede, T. (2015). N-limited consumer growth and low nutrient regeneration N:P ratios in lakes with low N deposition. *Ecosphere*, *6*, Article 9, 1, 13.

Bergström, A.-K., Karlsson, J., Karlsson, D., & Vrede, T. (2018). Contrasting plankton stoichiometry and nutrient regeneration in northern arctic and boreal lakes. *Aquatic Sciences*, *80*, 24.

Bergström, A.-K., Jonsson, A., Isles, P. D. F., Creed, I. F., & Lau, D. C. P. (2020). Changes in nutritional quality and nutrient limitation regimes of phytoplankton in response to declining N deposition in mountain lakes. *Aquatic Sciences*, *82*, 31.

Bergström, A.-K., Deininger, A., Jonsson, A., Karlsson, J., & Vrede, T. (2021). Effects of nitrogen enrichment on zooplankton biomass and N:P recycling ratios across a DOC gradient in northernlatitude lakes. *Hydrobiologia*, *848*, 4991–4510.

Bottrell, H. H., Duncan, A., Gliwicz, Z. M., Grygierek, E., Herzig, A., Hillbrichtilkowska, A., … Weglenska, T. (1976). Review of some problems in zooplankton production studies. *Norwegian Journal of Zoology*, *24*, 419–456.

Brett, M. T., Kainz, M. J., Taipale, S. J., & Seshan, H. (2009). Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 21197–21201.

Bullejos, F. J., Carillo, P., Gorokhova, E., Medina-Sanchez, J. M., & Villar-Argaiz, M. (2014). Nucleic acid content in crustacean zooplankton: bridging metabolic and stoichiometric predictions. *PLoS One*, *9*(1), e86493.

Canham, C. D., Pace, M. L., Weathers, K. C., McNeil, E. W., Bedford, B. L., Murphy, L., & Quinn, S. (2012). Nitrogen deposition and lake nitrogen concentrations: A regional analysis of terrestrial controls and aquatic linkages. *Ecosphere*, *3*, art66.

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, *18*(1), 117–143.

Creed, I. F., Bergström, A.-K., Trick, C. G., Grimm, N. N., Hessen, D. O., Kidd, K. A., … Weyhenmeyer, G. A. (2018). Global change-driven effects on dissolved organic matter composition: Implications for food webs of northern lakes. *Global Change Biology*, *24*, 3692–3714.

Deininger, A., Faithfull, C. L., & Bergström, A.-K. (2017a). Phytoplankton response to whole lake inorganic N fertilization along a gradient in dissolved organic carbon. *Ecology*, *98*, 982–994.

Deininger, A., Faithfull, C. L., & Bergström, A.-K. (2017b). Nitrogen effects on the pelagic food web are modified by dissolved organic carbon. *Oecologia*, *184*, 901–916.

DeMott, W. R., & Gulati, R. D. (1999). Phosphorus limitation in Daphnia: Evidence from a long term study of three hypereutrophic Dutch lakes. *Limnology and Oceanography*, *44*, 1557–1564.

Diehl, S. (2007). Paradoxes of enrichment: Effects of increased light versus nutrient supply on pelagic producer-grazer systems. *American Naturalist*, *169*, 173–191.

de Wit, H. A., Valinia, S., Weyhenmeyer, G. A., Futter, M. N., Kortelainen, P., Austnes, K., … Vuorenmaa, J. (2016). Current browning of surface waters will be further promoted by wetter climate. *Environmental Science and Technology Letters*, *3*, 430–435.

Eimers, M. C., Watmough, S. A., Paterson, A. M., Dillon, P. J., & Yao, H. (2009). Long-term declines in phosphorus export from forested catchments in south-central Ontario. *Canadian Journal of Fisheries and Aquatic Sciences*, *66*, 1682–1692.

Elser, J. J., Elser, M. M., MacKay, N. A., & Carpenter, C. R. (1988). Zooplankton-mediated transitions between N- and P-limited algal growth. *Limnology and Oceanography*, *33*, 1–14.

Elser, J. J., O'Brien, W. J., Dobberfuhl, D. R., & Dowling, T. E. (2000). The evolution of ecosystem processes: Growth rate and elemental stoichiometry of a key herbivore in temperate and arctic habitats. *Journal of Evolutionary Biology*, *13*, 845–853.

Elser, J. J., Andersen, T., Baron, J. S., Bergström, A.-K., Jansson, M., Kyle, M., … Hessen, D. O. (2009). Shifts in lake N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. *Science*, *326*, 835–837.

Finstad, A. G., Andersen, T., Larsen, S., Tominaga, K., Blumentrath, S., de Wit, H. A., … Hessen, D. O. (2016). From greening to browning: Catchment vegetation development and reduced S-deposition promote organic carbon load on decadal time scales in Nordic lakes. *Scientific Reports*, *6*, 31944.

Hassett, R. P., Cardinale, B., Stabler, L. B., & Elser, J. J. (1997). Ecological stoichiometry of N and P in pelagic ecosystems: Comparison of lakes and oceans with emphasis on the zooplankton-phytoplankton interaction. *Limnology and Oceanography*, *42*, 648–662.

Hessen, D. O. (2013). Inorganic nitrogen deposition and its impacts on N:P ratios and lake productivity. *Water*, *5*, 327–341.

Hessen, D. O., & Lyche, A. (1991). Interspecific and intraspecific variations in zooplankton elemental composition. *Archiv fürHydrobiologie*, *121*, 343–353.

Hessen, D. O., Faafeng, B. A., & Andersen, T. (1995). Replacement of herbivore zooplankton species along gradients of ecosystem productivity and fish predation pressure. *Canadian Journal of Fisheries and Aquatic Sciences*, *52*, 433–742.

Hood, J. M., & Sterner, R. W. (2010). Diet mixing: Do animals integrate growth or resources across temporal heterogeneity? *American Naturalist*, *176*, 651–663.

Huser, B. J., Futter, M. N., Wang, R., & Fölster, J. (2018). Persistent and widespread long-term phosphorus declines in Boreal lakes in Sweden. *Science of the Total Environment*, *613-614*, 240–249.

Isles, P. D. F. (2020). The misuse of ratios in ecological stoichiometry. *Ecology*, *101*(11), e03153.

Isles, P. D. F., Creed, I. F., & Bergström, A.-K. (2018). Recent synchronous declines in DIN:TP in Swedish lakes. *Global Biogeochemical Cycles*, *32*, 208–225.

Isles, P. D. F., Jonsson, A., Creed, I. F., & Bergström, A.-K. (2020). Does browning affect the identity of limiting nutrients in lakes? *Aquatic Sciences*, *82*, 45.

Isles, P. D. F., Creed, I. F., Jonsson, A., & Bergström, A.-K. (2021). Trade-offs between light and nutrient availability across gradients of dissolved organic carbon lead to spatially and temporally variable responses of lake phytoplankton biomass to browning. *Ecosystems*, *24*, 1837–1852. https://doi.org/10.1007/s10021-021-00619-7

Kelly, P. T., Solomon, C. T., Zwart, J. A., & Jones, S. E. (2018). A framework for understanding variation in pelagic gross primary production of lake ecosystems. *Ecosystems*, *21*, 1364–1376.

Kibby, H. V. (1971). Energetics and population dynamics of *Diaptomus gracilis*. *Ecological Monographs*, *41*, 311–327.

Lampert, W. (1977). Studies on the carbon balance in *Daphnia pulex* de Geer as related to environmental conditions. *I-IV Archiv für Hydrobiologie*, *48*, 287–368.

Lau, D. C. P., Jonsson, A., Isles, P. D., Creed, I. F., & Bergström, A.-K. (2021). Lowered nutritional quality of plankton caused by global environmental changes. *Global Change Biology*, *27*, 6294–6306.

Levine, S. N., & Schindler, D. W. (1992). Modification of the N:P ratio in lakes by in situ processes. *Limnology and Oceanography*, *37*, 917–935.

Loiterton, B., Sundbom, M., & Vrede, T. (2004). Separating physical and physiological effects of temperature on zooplankton feeding rate. *Aquatic Sciences*, *66*, 123–129.

McCauley, E., & Kalff, J. (1981). Empirical relationships between phytoplankton and zooplankton biomass in lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, *38*, 458–463.

Monteith, D. T., Stoddard, J. L., Evans, C. D., de Wit, H. A., Forsius, M., Høga, T., … Vesely, J. (2007). Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature*, *450*, 537–540.

Müller-Navarra, D. C. (1995). Biochemical versus mineral limitation in *Daphnia*. *Limnology and Oceanography*, *40*(7), 1209–1214.

Müller-Navarra, D. C., Brett, M. T., Liston, A. M., & Goldman, C. R. (2000). A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature*, *403*(6), 74–77.

Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Wagner. H. (2020). vegan: Community Ecology Package. R package version 2.5–7. https://CRAN.R-project.org/package=vegan.

Olson, C. R., Solomon, C. T., & Jones, S. E. (2020). Shifting limitation of primary production: Experimental support for a new model in lake ecosystems. *Ecology Letters*, *23*, 1800–1808.

Pace, M. L. (1986). An empirical analysis of zooplankton community size structure across lake trophic gradients. *Limnology and Oceanography*, *31*, 45–55.

Patoine, A., Pinel-Alloul, B., Prepas, E. E., & Carignan, R. (2000). Do logging and forest fires influence zooplankton biomass in Canadian Boreal Shield lakes? *Canadian Journal of Fisheries and Aquatic Sciences*, *57*, 155–164.

Persson, J., Brett, M. T., Vrede, T., & Ravet, J. L. (2007). Food quantity and quality regulation of trophic transfer between primary producers and a keystone grazer (*Daphnia*) in pelagic freshwater food webs. *Oikos*, *116*, 1152–1163.

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Solomon, C. T., Jones, S. E., Weidel, B. C., Buffam, I., Fork, M. L., Karlsson, J., … Saros, J. E. (2015). Ecosystem consequences of changing inputs of terrestrial dissolved organic matter to lakes: Current knowledge and future challenges. *Ecosystems*, *18*, 376–389.

Sterner, R. W. (1990). The ratio of nitrogen to phosphorus resupplied by herbivores: Zooplankton and the algal competitive arena. *American Naturalist*, *136*, 209–229.

Sterner, R. W. (1997). Modeling interactions of food quality and quantity in homeostatic consumers. *Freshwater Biology*, *38*, 473–481.

Sterner, R. W., & Hessen, D. O. (1994). Algal nutrient limitation and the nutrition of aquatic herbivores. *Annual Review of Ecology and Systematics*, *25*, 1–29.

Sterner, R. W., & Elser, J. J. (2002). *Ecological Stoichiometry: The biology of elements from molecules to the biosphere*. Princeton University Press.

Sterner, R. W., Elser, J. J., Fee, E. J., Guildford, S. J., & Chrzanowski, T. H. (1997). The light:nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process. *American Naturalist*, *150*, 663–684.

Taipale, S., Strandberg, U., Peltomaa, E., Galloway, A. W. E., Ojala, A., & Brett, M. T. (2013). Fatty acid composition as biomarkers of freshwater microalgae: Analysis of 37 strains of microalgae in 22 genera and in seven classes. *Aquatic Microbial Ecology*, *71*, 165–178.

Vasconcelos, F. R., Diehl, S., Rodríguez, P., Hedström, P., Karlsson, J., & Byström, P. (2019). Bottom-up and top-down effects of browning and warming on shallow lake food webs. *Global Change Biology*, *25*, 504–521.

Ventura, M., & Catalan, J. (2005). Reproduction as one of the main causes of temporal variability in the elemental composition of zooplankton. *Limnology and Oceanography*, *50*, 2043–2056.

Villar-Argaiz, M., Medina-Sanchez, J. M., & Carrillo, P. (2002). Linking life history strategies and ontogeny in crustacean zooplankton: Implications for homeostasis. *Ecology*, *83*, 1899–1914.

Vrede, T., Persson, J., & Aronsen, G. (2002). The influence of food quality (P:C ratio) on RNA:DNA ratio and somatic growth rate of *Daphnia*. *Limnology and Oceanography*, *47*, 487–494.

Vrede, T., Dobberfuhl, D. R., Kooijman, A. L. M., & Elser, J. J. (2004). Fundamental connections among organisms C:N:P stoichiometry, macromolecular composition, and growth. *Ecology*, *85*, 1217–1229.

Wenzel, A., Vrede, T., Jansson, M., & Bergström, A.-K. (2021). Daphnia performance on diets containing different combinations of high-quality algea, heterotrophic bacteria, and allochthonous particulate organic matter. *Freshwater Biology*, *66*, 157–168.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Bergström, A-K, Lau, D. C. P., Isles, P. D. F., Jonsson, A., & Creed, I. F. (2022). Biomass, community composition and N:P recycling ratios of zooplankton in northern high-latitude lakes with contrasting levels of N deposition and dissolved organic carbon. *Freshwater Biology*, *67*, 1508–1520. https://doi.org/10.1111/fwb.13956