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The impact of lake discontinuities on nitrogen biogeochemistry in river networks

Imke Grefe^{a*}, Eleanor B. Mackay^b, Peter M. Wynn^a, Phil A. Barker^a, Helen K. Grant^{b1}, M. Gloria Pereira^b, Stephen C. Maberly^b and Ben W. J. Surridge^a

^aLancaster Environment Centre, Lancaster University, Lancaster, UK; ^bUK Centre for Ecology & Hydrology, Lancaster, UK

*corresponding author i.grefe@lancaster.ac.uk

¹now at Lancaster Environment Centre, Lancaster University, Lancaster, UK

ORCID IDs:

I. Grefe: 0000-0001-6211-4976

E. B. Mackay: 0000-0001-5697-7062

P. M. Wynn: 0000-0002-1221-5530

S. C. Maberly: 0000-0003-3541-5903

River networks connect terrestrial and marine ecosystems through transport of pollutants and nutrients. Lakes represent discontinuities within these river networks, which can be important biogeochemical hotspots, introducing substantial changes to the aquatic environment. Nitrogen is a key macronutrient that can potentially limit or co-limit primary production, but the processes that determine the fate of nitrogen during transport through river-lake networks are

poorly understood. We studied three river systems and their lake discontinuities, spanning a range of trophic states and average water residence times, to understand the changes introduced to riverine nitrogen biogeochemistry by lake discontinuities. In-lake processes noticeably altered the concentration and speciation of nitrogen. Annually, lakes reduced up to 44% of nitrate compared to main inflow concentrations, while there was large variability in nitrate dynamics seasonally. The drawdown in surface nitrate concentrations resulted at times in phytoplankton co-limitation by nitrogen in-lake, as well as in the downstream river, where altered nitrogen patterns could persist for several kilometers. However, lakes occasionally subsidized N to downstream rivers as ammonium or dissolved organic nitrogen. Assimilation of nitrate in lake surface waters was one of the dominant processes impacting nitrogen availability; however, stable isotope data revealed an unexpected contribution of nitrification on nitrogen cycling in the epilimnion throughout the year and across trophic gradients. These changes in nitrogen concentration, as well as speciation introduced by lake discontinuities have potentially important consequences for the composition and metabolism of communities in downstream rivers and contribute to our fundamental understanding of freshwater processes.

Keywords: river-lake networks; nitrogen cycle; nitrate stable isotopes; nutrient limitation; ammonium; nutrient biogeochemistry

Introduction

Freshwater ecosystems represent critical interfaces between terrestrial and marine systems (Bouwman et al. 2013; Beusen et al. 2016). Rather than conservatively transporting allochthonous inputs from terrestrial ecosystems towards estuarine and marine environments, many freshwaters are characterized by high biogeochemical process rates (e.g. Cheng and Basu 2017; Marcé et al. 2018). This has led to development of a reactive pipe framework for freshwaters, for example focussed on carbon (C) (Cole et al. 2007; Evans et al. 2017). Among the other key macronutrients, nitrogen (N) remains poorly constrained and there is a pressing requirement to better

understand the processes and controlling factors that determine the fate of N during transport across the freshwater interface (Maranger et al. 2018). Nitrogen speciation and concentrations are critical controls on multiple biogeochemical processes within freshwaters and their downstream ecosystems. For example, growing evidence demonstrates the role for N in limitation or co-limitation of primary production (Elser et al. 2007; Yan et al. 2016; Dodds and Smith 2016), and in the control of potentially harmful algal blooms in both freshwater and marine ecosystems (e.g. Smith 2003; Paerl et al. 2016). Furthermore, anthropogenic perturbations of the N cycle are significant and geographically extensive, exceeding safe planetary boundaries and thereby increasing the potential for eutrophication, hypoxia and loss of biodiversity in many aquatic ecosystems (Gruber and Galloway 2008; Steffen et al. 2015).

In many freshwaters, lentic and lotic systems are not isolated from each other but are instead hydrologically connected within integrated river-lake networks. Lakes potentially introduce significant physical, biological and chemical changes to the continuum of a river network, resulting in differences between parameters and processes upstream and downstream of this discontinuity (Jones 2010 and references therein). However, relatively little empirical research has adopted a network perspective to examine how natural lake discontinuities control the fate of N within these interconnected freshwaters. Processes in the biogeochemically active transition zone where inflowing streams first enter lentic systems have been shown to drive significant changes in the availability of nutrients, cascading to broader impacts on nutrient availability within other areas of lentic systems (e.g. Mackay et al. 2011; Larson et al. 2019, 2020). However, this research has not considered how processes within lake ecosystems ultimately determine N availability and speciation in outflowing streams. Other research has examined the impacts of reservoirs on the transport of N to

downstream ecosystems, often revealing significant retention of N within these anthropogenic systems. N drawdown within reservoirs has been ascribed to burial within sediments, driven by high N loading rates, high ratios of catchment surface area to reservoir surface area, and high N settling velocities, alongside denitrification in anoxic sediments or anoxic zones of the reservoir water column (e.g. Harrison et al. 2009; Clow et al. 2015). However, reservoirs and natural lakes differ significantly in catchment, waterbody and management characteristics (Hayes et al. 2017). Therefore, processes determining the fate of N within reservoirs cannot be assumed to translate directly to natural lakes. Some research has begun to examine how upstream lakes can drive variations in N : P stoichiometry and biological demand for fractions of the dissolved inorganic N pool within streams (e.g. Arp and Baker 2007), changes in absolute and relative availability of dissolved and particulate forms of N within streams (e.g. Kling et al. 2000), or changes in the inorganic and organic forms of N present in streams (e.g. Brown et al. 2008). However, the fundamental biogeochemical processes that determine the role of lake discontinuities in N transport within river-lake networks remain uncertain, as do the controls exerted by key factors such as lake trophic status or water residence time on the fate of N. This is particularly true of river-lake networks in temperate regions where seasonal controls are less tightly determined by meltwater-associated runoff, and more heavily associated with year-round variation in factors such as temperature and precipitation.

The research reported here aimed to determine how natural lake discontinuities influence the transport of N through river-lake networks. We hypothesized that water residence time within natural lakes will exert significant control over the fate of N, primarily by determining the opportunity for biogeochemical processes to impact the N cycle within lakes (e.g. Saunders and Kalff 2001; Maranger et al. 2018). We also

hypothesized that the trophic status of natural lakes would determine biological demand for N (e.g. Hall and Tank 2003; Wollheim et al. 2006), and therefore that lake trophic status will be an important control on N export from natural lakes to downstream rivers. In addition to net-effects on N export, we were particularly interested in unravelling the nature of the underlying biogeochemical processes driving concentration and speciation of N in river-lake networks. We developed a regional-scale approach to test these hypotheses across a series of river-lake networks that differ in water residence time and trophic status. A seasonal, synoptic sampling strategy was used to examine how intra-annual variations in weather, catchment and freshwater ecosystem states influenced the fate of N across these river-lake networks.

Materials and methods

Study site descriptions

Three river-lake networks in the English Lake District (ELD), Cumbria, UK were selected for this study, hereafter called Esthwaite network, Grasmere-Rydal network, and Derwent-Bassenthwaite network (Fig. 1, Table 1). These networks comprised sampling sites located across main inflow and outflow rivers, as well as within five natural lakes, with each river-lake network containing either one or two natural lake discontinuities. A synoptic sampling approach was used in the research reported here. This approach was designed to generate near-instantaneous snapshots of biogeochemical conditions within river-lake networks, focussed on understanding how lakes drive contemporaneous changes in river biogeochemistry between reaches that are upstream and downstream of lakes. A Lagrangian approach to sampling was not deployed in the research, meaning that specific volumes of water were not sampled as they moved through each river-lake network. Lake surface water samples were

collected at the location of the deepest point and away from major inflows. In common with other research and with regular monitoring approaches, these pelagic samples were deemed to be representative of conditions within the wider lake ecosystem. To reflect the hypotheses underpinning this research, the five lakes (Esthwaite Water, Grasmere, Rydal Water, Derwent Water, Bassenthwaite Lake) were selected to span a range of average water residence times, trophic states, and broader lake and catchment characteristics (Table 1). All catchments are affected by anthropogenic activities such as sewage discharge and septic tanks, agriculture and high numbers of visitors to the catchment, for example during busy tourist periods.

Esthwaite Water is located in the southeast of the ELD and is one of the most productive lakes in the region. The lake has three sub-basins separated by shallow sills (north, middle and south). In addition to Esthwaite Water itself, two sampling sites were located on the main inflow river to the lake (Black Beck), and three sites on the outflow river (Cunsey Beck). Final treated wastewater is discharged from Hawkshead wastewater treatment works (WwTW) upstream of Esthwaite Water between sites Black Beck 1 and Black Beck 2, and from Near Sawrey WwTW into the outflow river between sites Cunsey Beck 1 and Cunsey Beck 2.

The Grasmere-Rydal network includes a chain of two lakes located to the north of the Esthwaite network. Grasmere is a relatively productive lake within the ELD, on the mesotrophic-eutrophic boundary with higher average total phosphorus but lower average chlorophyll-*a* than Esthwaite Water (Table 1). The River Rothay is the major inflow to Grasmere, with the river being fed by outflow from Grasmere before flowing a short distance downstream into Rydal Water. Sampling sites were located on the River Rothay upstream of Grasmere (River Rothay 1) and at two sites on the reach of the river between Grasmere and Rydal Water (River Rothay 2 and 3). Rydal Water is a small

mesotrophic lake located 1 km downstream of Grasmere, with the shortest water retention time of all the lakes sampled. Inflows to Rydal Water are dominated by the River Rothay, and therefore by the outflow from Grasmere. The River Rothay is subsequently fed by outflow from Rydal Water, with three additional sampling sites distributed downstream of Rydal Water (River Rothay 4, 5 and 6). Final treated wastewater from Ambleside WwTW discharges between sites 5 and 6 on the River Rothay.

The Derwent-Bassenthwaite network includes a chain of two lakes draining to the north of the ELD. Derwent Water is the most nutrient-poor of the lakes included in the research reported here, with average total phosphorus and phytoplankton chlorophyll-*a* concentrations suggesting that the lake is at the mesotrophic-oligotrophic boundary. The River Derwent is the major inflow discharging into Derwent Water and was sampled at two sites upstream of Derwent Water (River Derwent 1 and 2). The River Derwent was also sampled at two locations downstream of Derwent Water (River Derwent 3 and 4). Keswick WwTW discharges treated wastewater to the River Derwent between these two sampling locations. Bassenthwaite Lake is mesotrophic and feeding the outflowing River Derwent which was sampled at two locations downstream of the lake (River Derwent 5 and 6).

Sample collection

Samples for a range of water quality analyses were collected from the river-lake networks described in the previous section on a seasonal basis in April 2017 (spring), August 2017 (summer), November 2017 (autumn), January/February 2018 (winter) and April 2018 (spring). This sampling strategy was designed to capture variation in the impacts of natural lakes on N biogeochemistry within freshwater networks driven by

seasonal changes in key controlling factors within lakes, including phytoplankton community composition, rates of primary production, and extent of stratification.

Phytoplankton bioassay experiments were conducted seasonally throughout 2017 for the five lakes, as well as for sites on the main inflow (Black Beck 2) and outflow (Cunsey Beck 1) rivers of Esthwaite Water.

Samples from rivers and lakes for water quality analyses were collected from sites within each river-lake network on the same day whenever possible. The three networks were usually sampled on consecutive days depending on weather conditions and logistical constraints. Rivers were sampled from road bridges or riverbanks using a custom built off-bridge sampler to collect water from well-mixed areas of the flow. Temperature and dissolved oxygen depth profiles were measured at the deepest point of each lake using a dissolved oxygen probe and handheld meter (HQ30dHach), and lake water samples were collected from the epilimnion. Water temperature, pH and electrical conductivity were measured on unfiltered samples immediately after collection in the field using handheld probes and meters (WTW 3420 and WTW 340i). Water samples for analysis of dissolved inorganic N (DIN, the sum of nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+)), total dissolved N (TDN) and soluble reactive phosphorus (SRP) were then immediately filtered in the field using 0.45 μm cellulose acetate syringe filters. Samples for NO_3^- stable isotope analysis were filtered immediately in the field to 0.2 μm using cellulose acetate syringe filters.

Nutrient concentration and stable isotope measurements

NO_2^- , NO_3^- , NH_4^+ , TDN and SRP concentrations were analyzed colorimetrically on an AQ2 Discrete Analyser (SEAL Analytical). Analysis of NO_2^- was based on USEPA method 353.2, the reaction of NO_2^- with sulfanilamide. NO_3^- was analyzed with the

same method after reduction to NO_2^- using a copperized cadmium coil. Concentrations were calculated as the difference between NO_2^- measurements with and without cadmium reduction. TDN was measured by acidic persulphate digest to NO_3^- followed by cadmium reduction and reaction with sulfanilamide as described above. DON concentrations were calculated as the difference between TDN and DIN. NH_4^+ was determined following USEPA Method 350.1, in which ammonia reacts with hypochlorite and salicylate at alkaline pH. Finally, analysis of SRP was equivalent to USEPA Method 365.1 in which phosphate ions react with an acidic molybdate reagent to form an antimony phospho-molybdate complex, which is chemically reduced by ascorbic acid.

The dual isotope signature of NO_3^- ($\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$) was measured using the denitrifier method (Sigman et al. 2001; Casciotti et al. 2002). Samples containing NO_2^- were treated with sulfamic acid (Granger and Sigman 2009) prior to analysis in order to remove isotopic interference. Depending on NO_3^- concentrations in water samples, 20 or 10 nmol of NO_3^- were converted to nitrous oxide. Isotopic composition was analysed on an Isoprime TraceGas preconcentrator inlet and autosampler coupled to an Isoprime isotope ratio mass spectrometer at the NERC National Environmental Isotope Facility (NEIF) at UKCEH Lancaster, UK. $\delta^{15}\text{N}-\text{NO}_3^-$ values were calibrated using international standards USGS-34 and IAEA-NO-3 (-1.8‰ and +4.7‰, respectively). $\delta^{18}\text{O}-\text{NO}_3^-$ values were calibrated using USGS-34, USGS-35 and IAEA-NO-3 (-27.9‰, +57.5‰ and +25.6‰). All international standards were run in triplicate, as well as an internal NO_3^- standard. At least one environmental sample in each run was also analyzed in triplicate. Standard deviation for international and internal standard replicates, as well as environmental sample replicates was 0.2‰ or

better for $\delta^{15}\text{N-NO}_3$ and 0.5‰ or better for $\delta^{18}\text{O-NO}_3$. $\delta^{15}\text{N-NO}_3$ is reported relative to AIR-N₂, $\delta^{18}\text{O-NO}_3$ relative to VSMOW throughout.

Phytoplankton bioassay experiments

Laboratory bioassay experiments were undertaken to examine how growth of lake phytoplankton communities within the river-lake networks was limited by the availability of inorganic N and P. Samples were usually collected on the same day or within a couple of days of samples for water quality analyses described in the previous section. Bioassays were performed within 24 hours of sample collection following Maberly et al. (2002). Lake water from surface samples was initially filtered through a 100 μm mesh to remove large particles and zooplankton and 35 mL of sample was added to 50 mL boiling tubes. An additional sub-sample was analysed for initial chlorophyll-*a* concentration after filtration onto Whatman GF/C filters (nominal pore size 1.2 μm) and freezing at -20°C (see below). Five treatments were performed in triplicate for each sample in the bioassays: control (no nutrient addition), inorganic phosphorus (sodium phosphate), NO_3^- (sodium nitrate), NH_4^+ (ammonium chloride), and inorganic P and N (sodium phosphate + ammonium nitrate). Nutrient additions were made at approximately Redfield ratio proportions, N at $90 \mu\text{mol L}^{-1}$ and P at $6 \mu\text{mol L}^{-1}$. Following the nutrient additions, tubes were stoppered with foam bungs to allow gas exchange and incubated in a constant temperature room at 20°C and a photon irradiance of $80 - 120 \mu\text{mol m}^{-2} \text{s}^{-1}$ (photosynthetically available radiation, Macam Q102) with an 18 h light, 6 h dark cycle for 14 days. After the incubation period, the tube contents were resuspended by scraping and the use of a vortex mixer and filtered onto a Whatman GF/C glass fibre filter, then frozen at -20°C . Defrosted filters for both initial chlorophyll-*a* content and bioassay growth responses were extracted in hot

methanol and optical density measured using a spectrophotometer following Talling (1974) and chlorophyll-*a* concentration calculated using equations in Ritchie (2008).

Growth response of the bioassays was assessed using a natural log response ratio following Elser et al. (2007):

$$NR_x = \ln\left(\frac{T_x}{C_x}\right)$$

NR_x is the nutrient response ratio for nutrient addition x , T_x is the chlorophyll-*a* concentration of the nutrient treatment and C_x is the chlorophyll-*a* concentration of the control treatment. Response ratios were calculated for each of the treatment replicates and then averaged. The assessment of nutrient limitation status was determined following Mackay et al. (2020) based on a critical effect size threshold from Harpole et al. (2011). This threshold is used to identify whether a growth response is greater or less than the control and avoids Type II errors associated with low replication that can limit interpretation of traditional analyses. A linear mixed effect model was used to assess the relationship between relative NO_3^- concentration changes in-lake, nutrient limitation and chlorophyll-*a* concentration. The model had a Gaussian error distribution, with NO_3^- drawdown and nutrient limitation as fixed effects, it included a random intercept term for season and the natural log of chlorophyll-*a* concentration. It was fitted using the lme4 package in R (Bates et al. 2014), using likelihood ratio tests to assess different random and fixed effects terms following the model selection approach outlined in Zuur et al. (2009). Visual assessment of model residuals was carried out to assess normality, homogeneity of variance and independence of observations. All analyses were carried out in R version 4.2.1 (R Development Core Team 2011).

Results

Changes in dissolved N pools driven by lake discontinuities

In rivers immediately upstream of lake discontinuities, DIN and TDN pools were dominated by NO_3^- throughout the year, reaching maximum concentrations between $0.42 \text{ mg NO}_3\text{-N L}^{-1}$ and $0.92 \text{ mg NO}_3\text{-N L}^{-1}$. Concentrations of NO_3^- within lakes differed often substantially compared to main river sites immediately upstream. At an annual average scale, lakes acted as sinks for NO_3^- , with concentration decreases ranging from 1 to 44% of the concentration within major inflow rivers (Table 2). The magnitude of the annual average change in NO_3^- concentration scaled consistently with annual average water residence time within lakes, meaning that a greater magnitude of NO_3^- drawdown compared to upstream rivers was observed for lakes with longer water residence times, such as Esthwaite Water and Derwent Water, compared to lakes with shorter water residence times, such as Rydal Water and Grasmere. Another potential control on NO_3^- retention in-lake is the hydraulic load, calculated as average lake depth divided by annual average water residence time. Annual average NO_3^- retention also declined with increasing average hydraulic load, reflecting the inverse relationship between average hydraulic load and average water residence time (Table 1 and 2). In addition to these annual average patterns, there was considerable seasonal variation in the impact of lake discontinuities on NO_3^- concentrations (Fig. 2). The nature of this seasonal response varied for lakes with different annual average water residence times. For both Esthwaite Water and Derwent Water, the magnitude of NO_3^- drawdown was greatest in summer 2017, at which point NO_3^- concentrations fell to below the detection limit of $0.012 \text{ mg NO}_3\text{-N L}^{-1}$ in the three sub-basins of Esthwaite Water and to only $0.03 \text{ mg NO}_3\text{-N L}^{-1}$ in Derwent Water. The decrease in NO_3^- concentration within these lakes was less pronounced in autumn compared to summer 2017, but still resulted in

concentration decreases of $\geq 50\%$ compared to the main inflow rivers. Lakes with shorter annual average water residence times showed lower seasonal NO_3^- drawdown (Bassenthwaite Lake), or fluctuated between sinks and sources of NO_3^- (Grasmere and Rydal Water).

In addition to NO_3^- concentrations, $\delta^{15}\text{N}-\text{NO}_3$ and $\delta^{18}\text{O}-\text{NO}_3$ values also showed substantial changes within lakes compared to major inflow rivers. Differences in $\delta^{15}\text{N}-\text{NO}_3$ ($\delta^{15}\text{N}-\text{NO}_3_{\text{diff}}$) and $\delta^{18}\text{O}-\text{NO}_3$ ($\delta^{18}\text{O}-\text{NO}_3_{\text{diff}}$) are expressed as $\delta^{15}\text{N}-\text{NO}_3_{\text{lake}} - \delta^{15}\text{N}-\text{NO}_3_{\text{inflow}}$ and $\delta^{18}\text{O}-\text{NO}_3_{\text{lake}} - \delta^{18}\text{O}-\text{NO}_3_{\text{inflow}}$, respectively (Fig. 3). However, unlike changes in NO_3^- concentrations, the magnitude of the change in nitrate stable isotope composition did not vary consistently with water residence time, or with season (data not shown). The direction of change (relative isotopic enrichment or depletion in lakes compared to upstream rivers) was also less variable than for NO_3^- concentrations. Overall, lake discontinuities were associated with a relative isotopic depletion in ^{15}N (values becoming more negative) and an enrichment (values becoming more positive) in ^{18}O for NO_3^- , compared to the main inflow river. The difference between lake and main inflow river averaged -0.4‰ (range -1.5‰ to 0.2‰) for $\delta^{15}\text{N}-\text{NO}_3$ and $+1.0\text{‰}$ (range -0.2‰ to $+4.4\text{‰}$) for $\delta^{18}\text{O}-\text{NO}_3$.

In-lake changes to N fractions other than NO_3^- were less consistent across the river-lake networks. However, seasonal sampling did reveal substantial changes for individual fractions of the TDN pool introduced by Esthwaite Water. During summer, when concentrations of NO_3^- decreased to below detection in Esthwaite Water, $>90\%$ of the TDN pool within the lake epilimnion was comprised of DON, reaching concentrations between $0.2 - 0.3 \text{ mg N L}^{-1}$ across the three sub-basins within the lake (SI Fig. 1a). Furthermore, during autumn, a substantial shift in N speciation from NO_3^- to NH_4^+ was associated with Esthwaite Water (SI Fig. 1b). Coupled with significant

drawdown of NO_3^- concentrations within the lake, the relative importance of NH_4^+ grew from $\leq 6\%$ of the DIN pool in the upstream river to c.50% within the lake during this sampling event.

Nutrient limitation of phytoplankton communities

Phytoplankton growth across the lakes was either co-limited (nine out of 20 bioassays) or P-limited (11 out of 20 bioassays), with co-limitation more frequent in spring and summer (seven out of nine co-limited bioassay results) and P-limitation more common in autumn and winter (eight out of 11 P-limited bioassay results). Initial chlorophyll-*a* concentrations varied between 0.6 – 23 $\mu\text{g L}^{-1}$ across sites and seasons, with the highest values associated with spring and summer 2017 samples from Esthwaite Water and lowest values in winter samples from Grasmere and Rydal Water. The linear mixed effect model showed that both nutrient limitation ($F(1,15.3) = 4.8, p < 0.05$) and NO_3^- drawdown ($\beta = -0.009, \text{SE} = 0.003, F(1, 15.0) = 6.6, p < 0.05$) were significantly related to chlorophyll-*a* concentration as individual effects, but that the interaction between the two was not significant, possibly because of the limited number of observations. Greater NO_3^- drawdown within lakes was associated with higher chlorophyll-*a* concentrations, and chlorophyll-*a* was higher when sites were co-limited compared to P-limited (Fig. 4).

Within the Esthwaite network, bioassays were also conducted in the rivers upstream (Black Beck 2) and downstream (Cunsey Beck 1) of the lake (Fig. 1).

Phytoplankton nutrient limitation varied between seasons and between each river site (Fig. 5). In particular, during summer 2017, phytoplankton in the inflow river and all three lake sub-basins (north, middle and south) were co-limited by N and P. However, in the outflow river downstream of Esthwaite Water the phytoplankton community showed evidence of N-limitation. During all other seasons between spring 2017 and

winter 2018, the Esthwaite network was predominantly P-limited, both within the inflow river, all lake sub-basins, as well as the outflow river.

Downstream propagation of altered lake N signatures

Along the 2.6 km of the outflow river sampled downstream of Esthwaite Water (Cunsey Beck 1 to 3, Fig. 1), average concentrations of NO_3^- remained significantly below those upstream of the lake in the main inflow river (Black Beck 1 and 2). This pattern was observed consistently during each season between April 2017 and April 2018. The magnitude of the difference in average river NO_3^- concentrations between sites upstream and downstream of Esthwaite Water was strongly and positively correlated with the extent of NO_3^- drawdown in the lake (Fig. 6) (Spearman's correlation, $r_s = 0.90$, $p = 0.037$), with the largest differences in average concentrations upstream and downstream of the lake observed in summer ($0.49 \text{ mg NO}_3^- \text{-N L}^{-1}$ between sites). Changes in average NO_3^- concentration between the main inflow (River Rothay 1) and outflow river (River Rothay 2 and 3) for Grasmere were also consistent with the control exerted on NO_3^- concentrations by the lake itself (Spearman's correlation, $r_s = 0.983$, $p = 0.003$). However, unlike Esthwaite Water, Grasmere frequently switched between a net source and a net sink of NO_3^- compared to concentrations within the upstream river. Therefore, the downstream effect of Grasmere within the River Rothay network varied between an increase (winter) and a decrease (autumn) of up to $0.13 \text{ mg NO}_3^- \text{-N L}^{-1}$ on average, compared to the river upstream of the lake (Fig. 6). For Rydal Water, average changes in NO_3^- concentration within the main inflow river (River Rothay 2 and 3) compared to the outflow river (River Rothay 4 to 6) were small, only ever reaching a maximum of $0.08 \text{ mg NO}_3^- \text{-N L}^{-1}$ and were not significantly correlated with the relatively small change in NO_3^- concentration observed in the lake compared to upstream river sites. Changes in NO_3^- concentration in the river downstream of Derwent

Water and Bassenthwaite Lake did not exceed $0.07 \text{ mg NO}_3^- \text{-N L}^{-1}$ compared to the river upstream of both lakes and were not correlated with patterns of NO_3^- drawdown observed in either lake.

Within the Esthwaite network, seasonal subsidies of N from the lake to the downstream river were observed in the form of both NH_4^+ and DON (SI Fig. 1). During the summer 2017 sampling event, the relatively high absolute concentrations of DON and the dominant contribution made by DON to the TDN pool within Esthwaite Water were exported downstream, influencing all three sites along the outflow river. High absolute and relative concentrations of NH_4^+ from Esthwaite Water in autumn 2017 impacted the first downstream river site, and although the absolute concentration of NO_3^- and the relative importance of NO_3^- to the DIN pool increased with distance downstream from the lake in Cunsey Beck, these parameters did not recover sufficiently to reach the levels observed upstream of the lake by the most downstream sampling point at Cunsey Beck 3.

Evidence of a similar increase in downstream river NH_4^+ concentrations introduced by a lake was also observed in the Grasmere network during autumn 2017 (SI Fig. 1c). However, concentrations of NH_4^+ in the Grasmere-River Rothay network were lower than those within the Esthwaite network, with NH_4^+ concentrations in Grasmere only increasing to 0.07 mg N L^{-1} and representing only c.17% of the DIN pool. Evidence of increases in DON concentrations within lakes and export of elevated DON concentrations to rivers downstream of lakes was also observed in other networks during some seasonal sampling events, including Rydal Water in spring 2017, Grasmere in spring 2018, Derwent Water and Bassenthwaite Lake in spring, summer and autumn 2017 and winter 2018 (SI Fig. 1d to i).

Discussion

Biogeochemical processes driving changes to N pools in lake discontinuities

Our results demonstrate significant changes in the concentration and speciation of N within lake discontinuities, which are subsequently exported to downstream river networks. The DIN pool within the main inflow rivers of all three river-lake networks was dominated by NO_3^- , with NO_2^- and NH_4^+ making only minor contributions, similar to other fresh waters in the UK (Lloyd et al. 2019; Yates et al. 2019). Biogeochemical processes operating within the five lakes in these networks resulted in net decreases in NO_3^- concentration, on an annual average basis reaching between 1 and 44% of the concentration within main inflow rivers. For comparison, Cheng and Basu (2017) estimated average NO_3^- removal by lakes as $59.4 \pm 28.2\%$, based on a global dataset, suggesting that lakes in the ELD decrease NO_3^- at lower rates compared to these global averages, at least at annual scale. Annual average NO_3^- retention efficiencies for reservoirs range between 4 and 58% (David et al. 2006; Kong et al. 2019), suggesting that the natural lakes in the ELD may have a similar impact on net N drawdown compared to these anthropogenically managed water bodies.

However, substantial differences in the annual average NO_3^- drawdown were observed among individual lakes (Table 2). These differences are not significantly related to measures of lake productivity, including the long-term average concentrations of total P or phytoplankton chlorophyll-*a*. A qualitative relationship was observed between annual average lake NO_3^- drawdown and annual average lake water residence time, in which longer average water residence times were generally associated with greater NO_3^- drawdown in lakes relative to main inflow rivers. Furthermore, increasing NO_3^- retention in-lake is associated with decreasing hydraulic load (Seitzinger et al. 2002; David et al. 2006; Whitney et al. 2023), which appears to be driven by water

retention time, rather than average lake depth. Average water depth appears to be less important for NO_3^- drawdown, at least for the depth range of sampled lakes in the ELD (5.3 to 7.7 m, Table 1). These findings confirm one of the key hypotheses proposed in this paper that water residence time in lakes will impact the fate of N, and are consistent with other research that has highlighted the importance of water residence time for biogeochemical processes within lentic systems (e.g. Saunders and Kalff 2001; Fairchild and Velinsky 2006; Kong et al. 2019). The nature of these processes is further discussed below.

Our research also highlights that a focus solely on annual average changes in NO_3^- concentration driven by lake discontinuities masks significant seasonal variation. The amplitude of this seasonal variation differed among lakes and appeared to be dependent on lake water residence time, in which lakes characterized by longer average water residence times were generally associated with greater seasonal variation in their effect on NO_3^- concentrations compared to major inflow rivers (Fig. 2). Lakes with a shorter average water residence time oscillated between source and sink behavior with respect to NO_3^- concentrations, without any clear seasonal patterns. It is possible that these river-lake networks are more dynamic and responsive to short-term variations in point discharge and catchment sources of NO_3^- , meaning that the synoptic sampling approach used in our research capturing snapshots of highly variable ecosystem states.

Furthermore, substantial changes in NO_3^- concentrations within lake discontinuities were also observed in winter, highlighting the potential importance of winter as an important season, both from geochemical and ecological perspectives (Powers and Hampton 2016; Snell et al. 2019).

During the period of transient storage within lakes that are part of river-lake networks, multiple processes may drive changes within N pools. These include mixing

of different water sources, dilution of substances delivered via inflow rivers, and a range of biogeochemical processes acting on substances within lakes. We believe that simple dilution of NO_3^- concentrations of water discharging from rivers into lakes is unlikely to explain the observations reported here. Changes in NO_3^- stable isotope signatures, alongside concentration changes, suggest that biogeochemical processes play an important role driving the changes in NO_3^- concentrations in lake discontinuities (e.g. Kendall et al. 2007; Botrel et al. 2017). Increased NO_3^- retention within lakes compared to upstream rivers was positively associated with depletion of ^{15}N (values becoming more negative, loss of heavy ^{15}N isotopes in the residual NO_3^- pool) and enrichment of ^{18}O (values becoming more positive, accumulation of heavy ^{18}O isotopes in the residual NO_3^- pool) within the residual NO_3^- remaining in lake water (Fig. 3). While it was not possible to sample every individual inflow stream to each lake included in the research reported here, simple mixing of water and NO_3^- from different tributaries within lake is unlikely to be able to explain the changes in NO_3^- isotope composition we report. As NO_3^- concentrations in lake samples were often lower than within major inflow rivers, mixing of NO_3^- sources alone would require other endmembers contributing NO_3^- to a lake to be associated with highly depleted values of ^{15}N and enriched values of ^{18}O . While atmospheric NO_3^- deposition is a potential source of depleted $\delta^{15}\text{N}$ and enriched $\delta^{18}\text{O}$ (Kendall et al. 2007), the catchments are strongly influenced by anthropogenic activities which are expected to overprint atmospheric signatures. Furthermore, minor inflows to each lake are likely to drain sub-catchments with very similar N sources, due to similar landuse and atmospheric deposition patterns, compared to the major inflow rivers sampled in the research reported here. Therefore, we believe it is unlikely that any NO_3^- within these minor inflow streams would differ sufficiently in isotopic composition and concentration to explain our observations based solely on mixing of

unconstrained inputs of NO_3^- to the lakes. To further investigate mixing, as well as lag and storage, as potential explanations for the data we report, chloride concentrations were compared within inflow and lake surface water samples (data not shown). For over 70% of this dataset, differences between inflow and lake epilimnion samples were within the $\pm 20\%$ margin for hydrological balance as suggested by Whitney et al. (2023). Whilst the storage of seasonally varying inflow waters to lakes, and the associated lag time between upstream and downstream transfer of volumes of water through river-lake networks, are likely to be important factors, our data indicate that, by themselves, these factors are unlikely to explain the data reported here, particularly in terms of the stable isotope composition of nitrate.

Assimilation and denitrification are frequently identified as the main drivers of NO_3^- retention in lakes (Bennion and Smith 2000; Saunders and Kalff 2001; Fairchild and Velinsky 2006), with both processes resulting in fractionation towards heavier isotopes in the residual NO_3^- pool (e.g. Sigman et al. 1999; Granger et al. 2004, 2008). However, denitrification is inhibited by oxygen (Tiedje 1988) and is not likely to occur in oxygenated lake epilimnion samples in the research reported here. During stratification, denitrification takes place in anoxic bottom waters which can be entrained into the epilimnion during the breakdown of stratification in autumn, or through eddy diffusion across the oxycline in summer. However, NO_3^- drawdown was observed throughout the year, including in lakes where the hypolimnion remained oxygenated at all times. Therefore, we believe that assimilation rather than denitrification was more likely to be a major NO_3^- removal process in the lake epilimnion.

While assimilation fractionates towards heavier isotopes in the residual NO_3^- pool along a 1:1 trajectory (Granger et al. 2010), our data indicate that epilimnion NO_3^- becomes relatively depleted in ^{15}N within lakes, rather than enriched, compared to

major inflow rivers. Therefore, fractionation of ^{15}N and ^{18}O appears to be decoupled and falls above a 1:1 trajectory (Fig. 3). Consistent patterns of depletion in ^{15}N and enrichment in ^{18}O of epilimnion NO_3^- across all five lakes suggest a common underlying biogeochemical explanation. Biological nitrogen fixation (BNF) and nitrification both result in lighter $\delta^{15}\text{N}\text{-NO}_3$ signatures in produced NO_3^- (Heaton 1986; Casciotti 2009). However, little is known about the extent of BNF in river-lake networks (Marcarelli et al. 2022) and while some heterocysts were observed during summer in the epilimnion of Esthwaite Water, suggesting the presence of diazotrophs, their numbers were low. While a contribution from BNF to the NO_3^- pool in lake surface waters cannot be excluded, there is no strong evidence that the significant depletion in ^{15}N of epilimnion NO_3^- observed throughout the year and across all five lakes can be explained by BNF. Nitrification may also lead to depletion of ^{15}N in generated NO_3^- . Nitrification was initially assumed to be an aphotic process with apparent inhibition of nitrifying bacteria by sunlight (Olson 1981). However, more recent research suggests that this process can also occur within the euphotic zone (Ward 2005; Wankel et al. 2007; Botrel et al. 2017). With decoupled fractionation of ^{15}N and ^{18}O leading to a positive deviation from the 1:1 trajectory, our observations may reflect the net effects of phytoplankton assimilation of NO_3^- alongside concurrent nitrification in the epilimnion of lakes. Assimilation and concurrent nitrification, resulting in decoupled fractionation of ^{15}N and ^{18}O , appear to occur widely in marine systems (e.g. Yool et al. 2007; Granger and Wankel 2016) and have been observed on a seasonal basis for freshwaters (Botrel et al. 2017). Our isotope data suggest some contribution from nitrification to the NO_3^- pool may occur throughout the year in all five lakes that were sampled in the research reported here. Therefore, nitrification in the epilimnion may be more important for N biogeochemistry in freshwater systems than previously

reported, replenishing the NO_3^- pool in lakes and meeting at least some of the metabolic demand for N to support production.

Causes of phytoplankton nutrient limitation in lake discontinuities

Bioassay data show a significant positive relationship between biomass, as indicated by chlorophyll-*a* concentration, and NO_3^- drawdown within lakes on a seasonal basis.

These observations provide further support to the argument that phytoplankton assimilation is an important NO_3^- uptake mechanism in these lakes, partially supporting the initial hypothesis of trophic control being exerted on N demand within lakes.

Interestingly, there was no correlation between long-term lake trophic state, as described by annual average chlorophyll-*a* concentrations or total P concentrations, and annual average NO_3^- drawdown, highlighting the impact of seasonal variability on production and nutrient uptake. Highest NO_3^- drawdown within lakes was associated with increased chlorophyll-*a*, suggesting that at least some of the N delivered to lake epilimnion from main inflow rivers was assimilated and incorporated into phytoplankton biomass. Higher chlorophyll-*a* was also associated with co-limitation of phytoplankton growth by N and P, particularly in spring and summer for Esthwaite Water, suggesting that inflow N sources at this time of year may be important for promoting or sustaining phytoplankton growth in these lakes. The association between co-limitation and chlorophyll-*a* is potentially indicative of the phytoplankton community maximizing growth through efficient nutrient utilization by a more diverse assemblage of species found in the summer for these types of lakes (Maberly et al. 2002). Higher occurrences of co-limitation in summer appear common in upland and lowland temperate lakes (Maberly et al. 2002; Kolzau et al. 2014) and further suggest assimilation as a key driver of NO_3^- drawdown in the epilimnion of sampled lakes.

However, high NO_3^- drawdown also occurred in P-limited systems with lower chlorophyll-*a* concentrations, suggesting that processes other than assimilation into phytoplankton biomass may be important for N retention in lakes, including denitrification, assimilation by heterotrophic bacteria or sedimentation. Denitrification is unlikely to be a key process in surface waters, with the exception of stratification breakdown in autumn and a potentially small contribution from diffusion across the oxycline from anoxic bottom waters. Nitrate uptake by heterotrophic bacteria has the potential to control NO_3^- drawdown without contributing to chlorophyll-*a* concentrations. There is some evidence that NO_3^- assimilation by heterotrophic bacteria can be relevant in the environment (Middelburg and Nieuwenhuize 2000; Taylor and Townsend 2010; Möbius and Dähnke 2015). Nitrate assimilation by a heterotrophic α -proteobacterium strain was shown to introduce isotope fractionation in the residual NO_3^- pool with ^{18}O to ^{15}N trajectories being approximately two, noticeably diverging from the usual 1:1 ratio associated with cyanobacterial and prokaryotic NO_3^- assimilation (Granger et al. 2010). However, our stable isotope data do not show the expected ^{18}O to ^{15}N trajectory of two associated with this process (Fig. 3), suggesting heterotrophic NO_3^- uptake was not a significant process. Finally, sedimentation of biomass could reduce NO_3^- concentration via assimilation followed by removal of biomass from the epilimnion through burial in sediments. However, NO_3^- stable isotope data indicate active nitrification in the epilimnion year-round, suggesting that remineralization of biomass at least partially replenished surface NO_3^- pools in the water column and may have reduced the impacts of sedimentation. While sedimentation appears the most likely process driving NO_3^- drawdown in P-limited, low chlorophyll-*a* lakes, there is some uncertainty about the magnitude of this process. Further investigation is warranted into

the factors driving seasonal changes in nutrient limitation and their impact on phytoplankton biomass.

Our data also demonstrate that NO_3^- drawdown in more productive lakes, such as Esthwaite Water, may reduce the DIN to SRP ratio sufficiently to increase the potential for N-limitation of phytoplankton growth (Fig. 5). These spatial changes in nutrient limitation over relatively short distances (< 3 km) are indicative of the role of lake discontinuities as potential 'hot spots' for biogeochemical alterations to nutrient conditions within river networks. Changing conditions within a lake are likely to affect the timing and type of nutrients exported into a downstream river network (Wurtsbaugh et al. 2005; Brown et al. 2008; Wu et al. 2022), with potentially significant impacts on downstream nutrient limitation for primary producer communities.

Propagation of altered lake N pools to downstream river networks

Our research demonstrates that lake discontinuities can substantially alter the form and availability of N within river networks and that this change can be maintained in rivers for several kilometers downstream of natural lakes. While river tributaries and WwTWs may modify the downstream propagation of lake signals within river networks (Benda et al. 2004), average concentrations of NO_3^- within rivers downstream of Esthwaite Water in our research were consistently lower than those upstream of the lake, despite the discharge of wastewater treatment work effluent to the river downstream of the lake. These findings illustrate that lake discontinuities have the potential to significantly reduce N loads within downstream river networks. Furthermore, our data also reveal that lakes can periodically deliver N subsidies to downstream rivers. Observations from Grasmere and, in particular, Esthwaite Water in autumn evidence substantial increases in NH_4^+ concentration within the lake epilimnion, which are subsequently exported to river networks downstream of the lake. Despite downstream attenuation of NH_4^+

concentrations, potentially due to in-stream nitrification, concentrations of NH_4^+ remain higher than inflow concentrations, suggesting lentic subsidies persist and impact ecosystems for several km downstream of the lake. Anoxic conditions in the hypolimnion of both lakes during summer inhibit nitrification and result in accumulation of NH_4^+ , which is entrained into the epilimnion during breakdown of lake stratification in autumn (SI Fig. 2). Given the prevalence of thermal stratification under current conditions in many lakes globally, alongside predictions of significant increases in the occurrence and duration of the stratified period under future climate scenarios (Woolway et al. 2021), the potential for seasonal NH_4^+ subsidies to downstream rivers and subsequent impacts on the ecosystem should be a priority for future research (Carey et al. 2022).

DON subsidies to downstream rivers were observed in at least some samples from all lakes throughout the year, with increased concentrations of DON observed in many lentic waters in spring or summer seasons (e.g. Fairchild and Velinsky 2006; Brown et al. 2008). For lakes in which DIN fell to extremely low concentrations due to NO_3^- drawdown, including both Esthwaite Water and Derwent Water in summer, DON became the dominant species of N contributing to the TDN pool. Under these circumstances, not only did the lake discontinuity provide a subsidy of potentially bioavailable DON to downstream river ecosystems, but they also drove a significant changes in the speciation of the TDN pool away from inorganic and towards organic nutrient compounds (e.g. Lloyd et al. 2019; Yates et al. 2019). Despite the at times substantial subsidies of NH_4^+ and DON exported from lakes to downstream rivers, it should be noted that these lakes act as net TDN sinks for most of the year, with NO_3^- retention frequently offsetting NH_4^+ and DON export (SI Table 1).

Changes in the concentration and the speciation of N within the riverine TDN pool, due to processes occurring within lake discontinuities may result in significant changes in community composition and metabolic processes within downstream river networks. Changes in the absolute concentration of NH_4^+ in rivers due to subsidy from upstream lakes, alongside changes in the ratio of NH_4^+ to NO_3^- may have significant effects on lentic and lotic algal and bacterial communities. For example, increased availability of NH_4^+ in phosphorus-rich environments may favor cyanobacteria, due to their superior uptake kinetics for this molecule compared to eukaryotic algae such as diatoms (e.g. Donald et al. 2011). Furthermore, some cyanobacteria may be able to outcompete nitrifying bacteria for NH_4^+ (Hampel et al. 2018), impacting the internal N cycle and concentrations of NO_3^- produce by remineralization.

It has been reported that higher concentrations of DIN, for example from human activities, can shift an aquatic system towards lower DON concentrations (Wymore et al. 2021). Some of the lakes in this study showed at times signs of reversing these trends by decreasing DIN concentrations and increasing DON. Subsidies of DON provided by these lakes to downstream rivers, particularly where DON becomes the dominant component of the TDN pool, may select for organisms that are able to access these organic compounds to support their metabolism. Both low and high molecular weight DON compounds have been shown to be bioavailable to phytoplankton (e.g. Glibert et al. 2004; Bronk et al. 2007) and while the energetic cost of accessing DON compounds, for example associated with the synthesis and excretion of extracellular enzymes, may suggest that these compounds would only be used to support metabolism under conditions of N limitation, recent research has demonstrated positive effects on phytoplankton growth of DON even within nutrient-enriched fresh waters (Mackay et al. 2020). Therefore, the impacts of DON, as well as NH_4^+ , subsidies from lakes on

downstream river ecosystems may be an important concern under a wide range of freshwater trophic condition.

Conclusions

Lake discontinuities have a substantial impact on N biogeochemistry within river networks. While lakes are often sites associated with NO_3^- drawdown, subsidies of N in the form of both NH_4^+ and DON may also be exported to downstream rivers. Therefore, lake discontinuities not only modify the absolute amount, but also the speciation, of bioavailable N within rivers, with potential consequences for both the biogeochemical and ecological properties of river-lake networks. Beyond nutrient concentrations, the nature of nutrient limitation for primary production may also be altered by lake discontinuities, including shifts towards N-limitation in highly productive river-lake networks which can be exported to downstream phytoplankton communities. While the natural lakes examined in the research reported here frequently decreased NO_3^- concentrations in surface waters, stable isotope data suggest an active internal N cycle, replenishing the NO_3^- pool via nitrification in the epilimnion year-round and within lakes that span a range of water residence times and trophic states. Concurrent phytoplankton assimilation and nitrification may be more important mechanisms driving modifications to N biogeochemistry in natural lakes than previously assumed and future research needs to further investigate these processes, for example through rate measurements, as part of work to predict the consequences of contemporary and future scenarios for the biogeochemistry and ecology of river-lake networks.

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Table 1. Lake and associated catchment characteristics for river-lake networks (Maberly et al. 2015, UK Lakes Portal).

Lakes	Esthwaite Water	Grasmere	Rydal Water	Derwent Water	Bassenthwaite Lake
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Main inflow river (% catchment drainage to lake)	Black Beck (55)	River Rothay (86)	River Rothay (92)	River Derwent (65)	River Derwent (60)
Main outflow river	Cunsey Beck	River Rothay	River Rothay	River Derwent	River Derwent
Lake trophic state	Eutrophic - mesotrophic	Eutrophic - mesotrophic	Mesotrophic	Mesotrophic-oligotrophic	Mesotrophic
Mean annual chl-<i>a</i> (mg m³)	14	8	6	5	7
Mean annual TP (mg m³)	17.5	21.5	13.5	9.5	11
Average water retention time (d)	100	25	9	55	30
Mean depth (m)	6.4	7.7	5.3	5.5	5.4
Lake surface area (ha)	96	61	30	529	524
Thermocline depth					
Spring 2017	Weak stratification at 5 m	8.5 m	5 m	No stratification	No stratification
Summer 2017	6 m	6 m	6.5 m	Weak stratification	Weak stratification
Autumn 2017	14 m	Weak stratification at 18 m	15 m	Weak stratification at 10 m	Weak stratification at 14 m
Winter 2018	No stratification	No stratification	No stratification	No stratification	No stratification
Spring 2018	Weak stratification at 6.5 m	Weak stratification at 7 m	No depth profile	No stratification	No stratification
				No stratification	No stratification

Land cover %	43.8	15.1	16.8	15.5	16.2
Woodland	48.3	71.2	70.1	53.9	55.2
Grassland	0.2	1.9	1.7	1.4	3.6
Arable	1.7	0.8	0.7	0.6	0.9
Urban					

Table 2. Water residence time (Maberly et al. 2016), hydraulic load calculated as average depth/average annual water residence time, and decrease in NO₃⁻ concentrations in lakes compared to main inflow rivers (NO₃⁻ retention = (NO₃⁻_{inflow} - NO₃⁻_{lake}) / NO₃⁻_{inflow} * 100). All values are for annual average, spring 2017 to spring 2018.

Lake	Esthwaite Water	Derwent Water	Bassenthwaite Lake	Grasmere	Rydal Water
Average annual water residence time (days)	100	55	30	25	9
Average annual hydraulic load (m/d)	0.06	0.10	0.18	0.31	0.59
Average annual NO ₃ ⁻ retention (%) in-lake relative to inflow	42	44	6	1	1

Figure 1. Location of the three river-lake networks and sampling sites. a) Overview of the English Lake District, UK. Sampled river-lake networks outlined in black, all others in grey; b) Esthwaite network with sampling locations along the main inflow Black Beck, in the three sub-basins Esthwaite Water north (deep point), middle and south, and along the outflow Cunsey Beck; c) Grasmere-Rydal network with sampling locations along the main in- and outflow River Rothay, in Grasmere and Rydal Water; d)

Derwent-Bassenthwaite network with sampling locations along the main in- and outflow River Derwent and in Derwent Water and Bassenthwaite Lake.

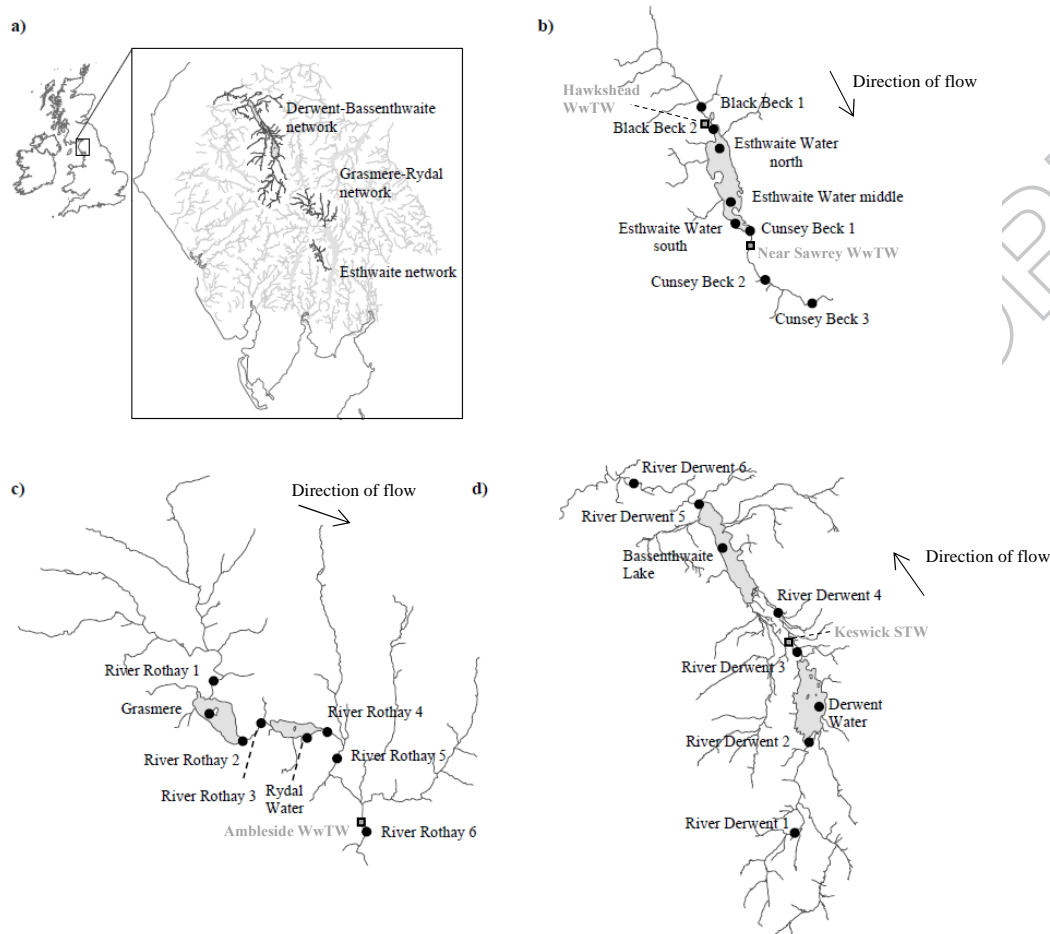


Figure 2. Seasonal and annual average percentage change in NO_3^- concentrations. Negative values indicate relative NO_3^- retention within a lake, positive values indicate relative NO_3^- subsidies within a lake compared to the main inflow river.

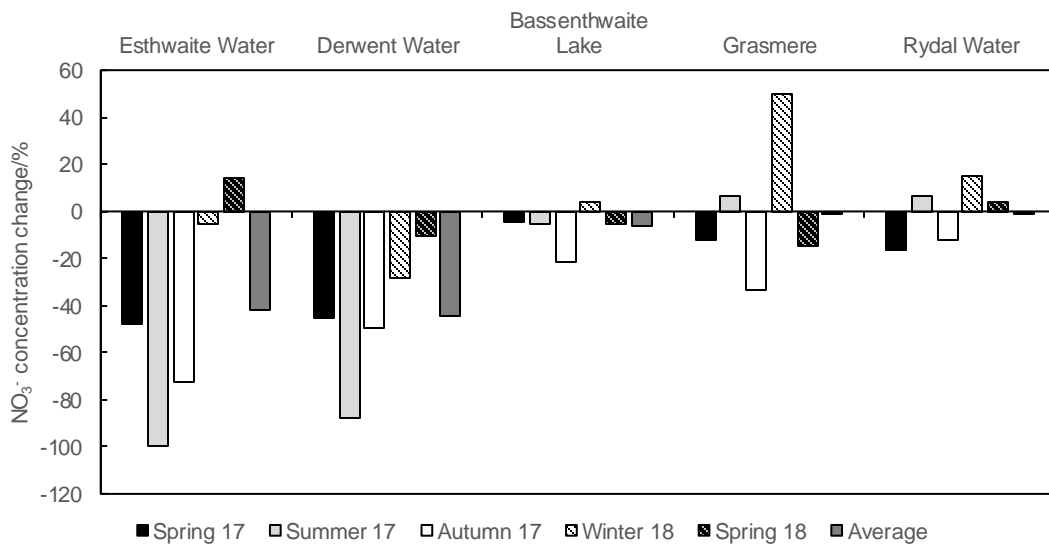


Figure 3. Difference in NO_3^- isotope signatures between lake sites and main inflow rivers. $\delta^{15}\text{N}-\text{NO}_3 \text{ diff} = \delta^{15}\text{N}-\text{NO}_3 \text{ lake} - \delta^{15}\text{N}-\text{NO}_3 \text{ inflow}$ and $\delta^{18}\text{O}-\text{NO}_3 \text{ diff} = \delta^{18}\text{O}-\text{NO}_3 \text{ lake} - \delta^{18}\text{O}-\text{NO}_3 \text{ inflow}$. Dashed line indicates theoretical 1:1 trajectory of coupled ^{15}N and ^{18}O fractionation.

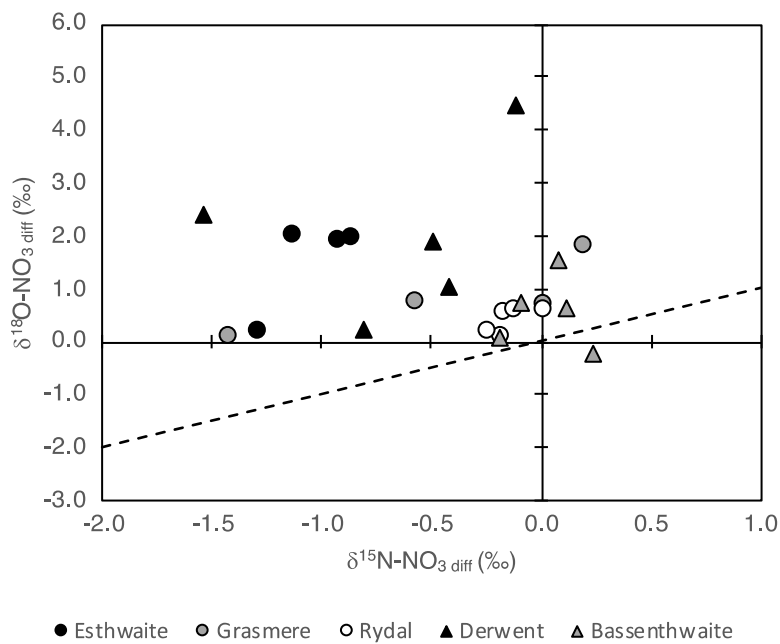


Figure 4. Lake chlorophyll-*a* concentrations relative to NO_3^- concentration change between lakes and main inflow rivers. Negative values of NO_3^- concentration change indicate relative NO_3^- retention within a lake, positive values of NO_3^- concentration change indicate relative NO_3^- subsidy. Filled symbols represent lakes with N and P co-limitation as determined by bioassays, open symbols represent lakes with P-limitation only.

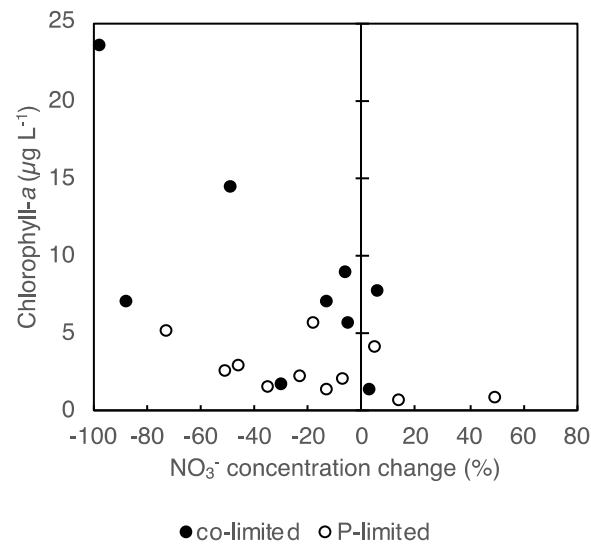


Figure 5. Changes in phytoplankton nutrient limitation along the Black Beck - Esthwaite Water (EW) - Cunsey Beck transect across spring (circles), summer (squares), autumn (diamonds) and winter (triangles). Open symbols indicate P-limitation, filled symbols indicate co-limitation by N and P in black, and N-limitation in gray. Nutrient limitation data shown relative to a) DIN and b) SRP concentrations.

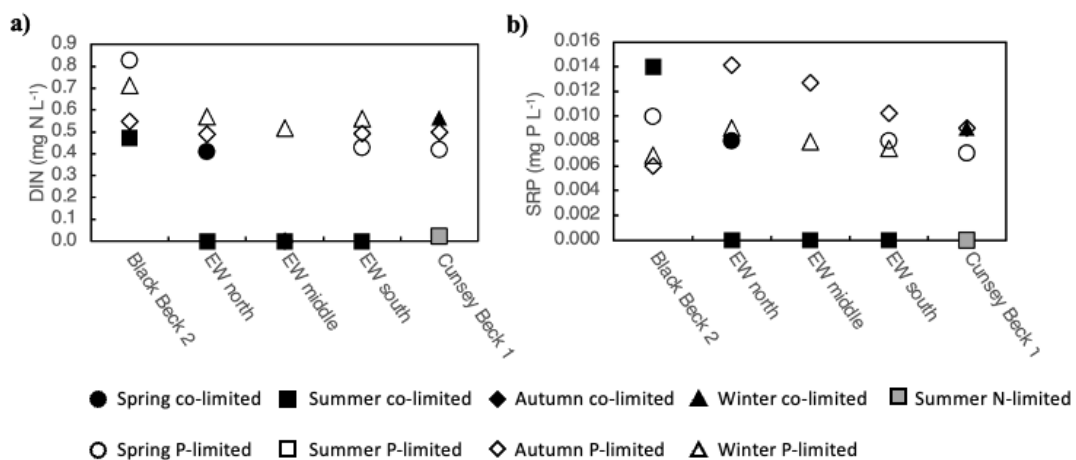
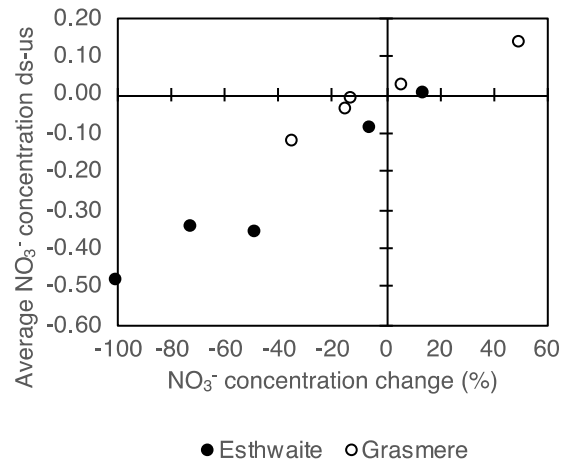


Figure 6. Impact of NO_3^- concentration changes within lakes on outflow river concentrations for the Esthwaite network (filled symbols) and Grasmere network (open symbols). In-lake changes calculated as percentage relative to the main inflow river. Changes in outflow rivers calculated as average NO_3^- concentrations in the outflow river (ds) minus average concentrations in the main inflow river upstream (us).



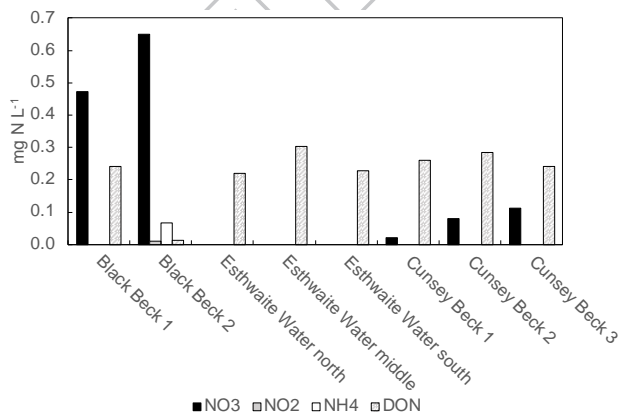
Appendix: Supplementary tables and figures

SI Table 1 Net TDN drawdown in lakes calculated as difference between inflow and lake epilimnion concentrations of NO_3^- , NH_4^+ , NO_2^- and DON. Negative values indicate lower concentrations in-lake (N retention) and positive values indicate higher concentrations in-lake (N-subsidy). Values in brackets show breakdown in DIN and DON drawdown. Bold TDN drawdown values indicate that DIN retention in-lake was offset by DON subsidy.

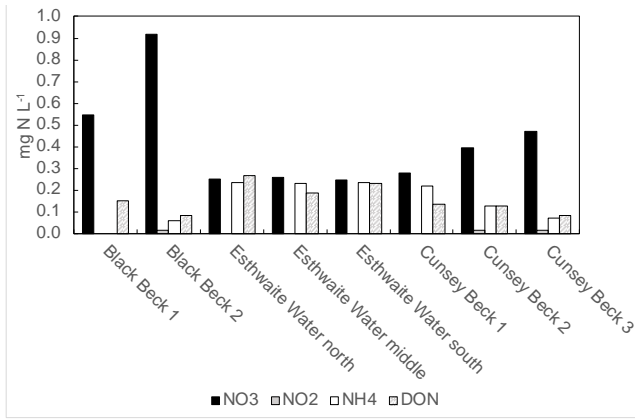
	Spring 2017	Summer	Autumn	Winter	Spring 2018
Esthwaite Water	-0.355	-0.543	-0.325	-0.203	-0.063
	(-0.434, 0.079)	(-0.729, 0.187)	(-0.506, 0.181)	(-0.054, 0.149)	(-0.036, 0.027)

Derwent Water	-0.027 (-0.119, 0.092)	-0.071 (-0.180, 0.110)	-0.096 (-0.152, 0.056)	-0.059 (-0.112, 0.053)	-0.042 (-0.042, 0.000)
Bassenthwaite Lake	0.052 (-0.046, 0.098)	0.025 (-0.040, 0.065)	-0.039 (-0.070, 0.031)	0.075 (0.014, 0.062)	-0.111 (-0.111, 0.000)
Grasmere	-0.127 (-0.042, -0.085)	0.006 (-0.009, 0.015)	-0.101 (-0.100, -0.002)	0.154 (0.198, -0.043)	0.088 (-0.065, 0.153)
Rydal Water	-0.004 (-0.058, 0.054)	0.004 (0.016, -0.012)	-0.064 (-0.064, 0.000)	0.097 (0.080, 0.017)	0.002 (0.002, 0.000)

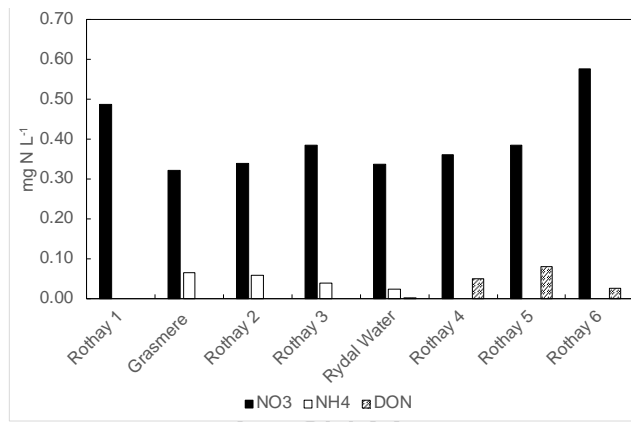
a)



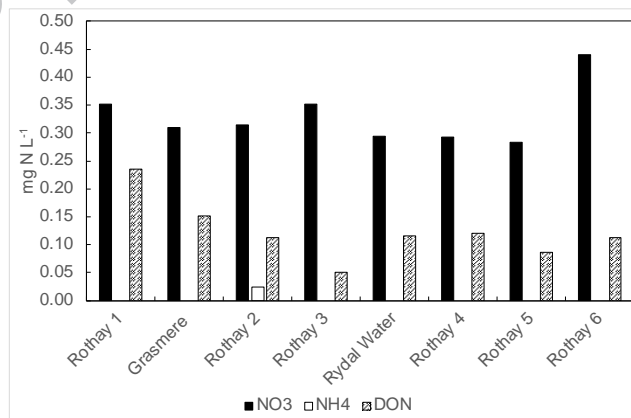
b)



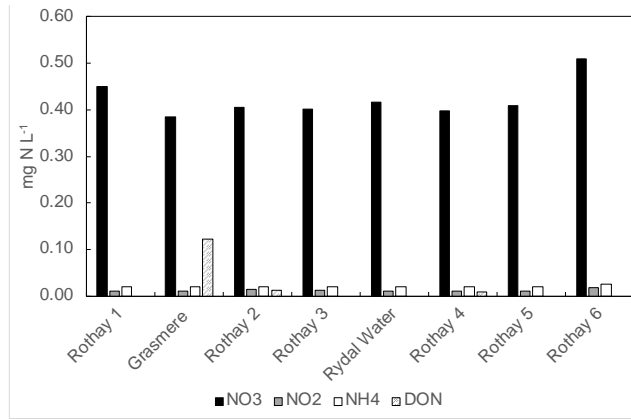
c)



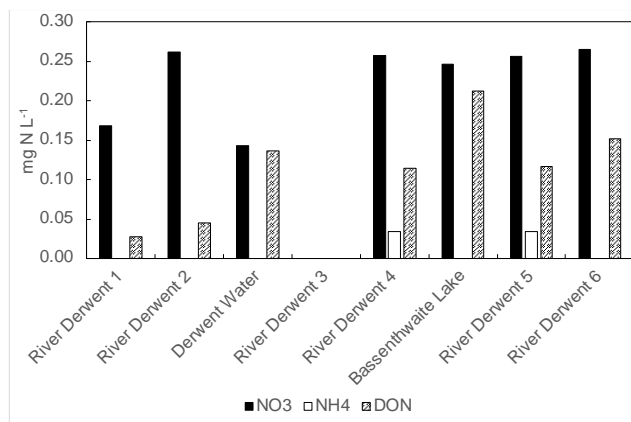
d)



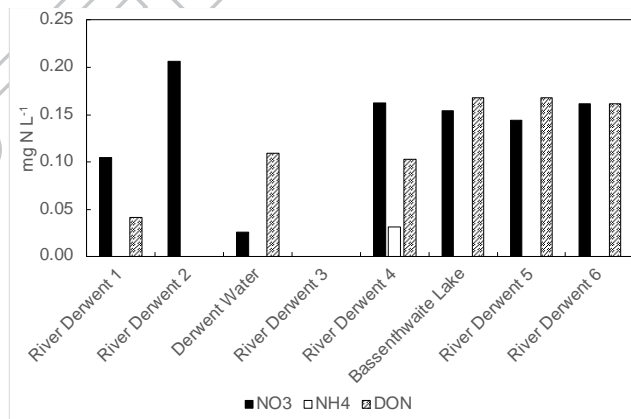
e)



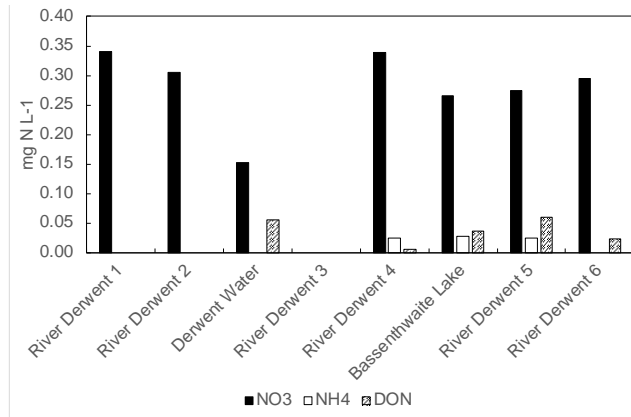
f)



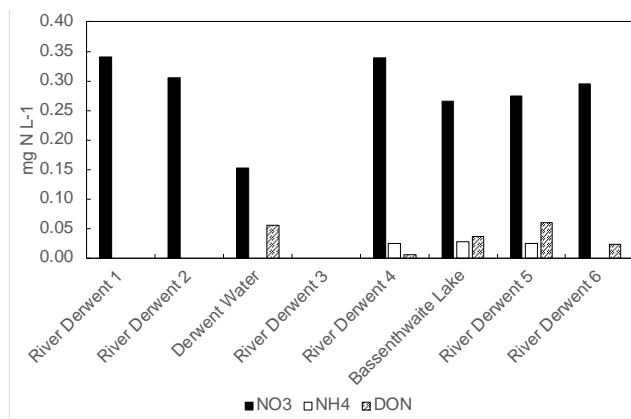
g)



h)

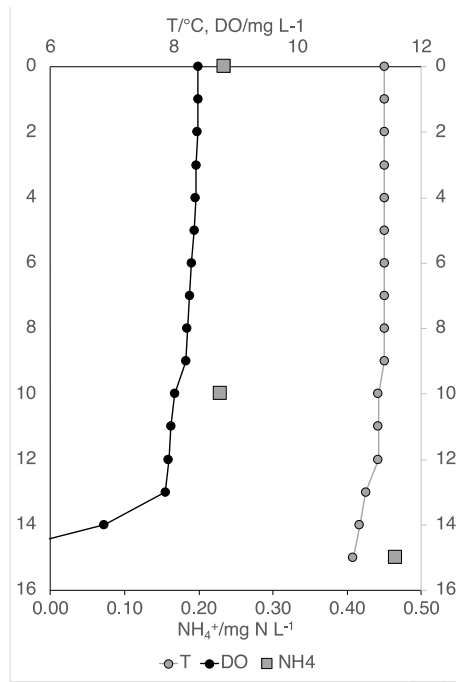


i)

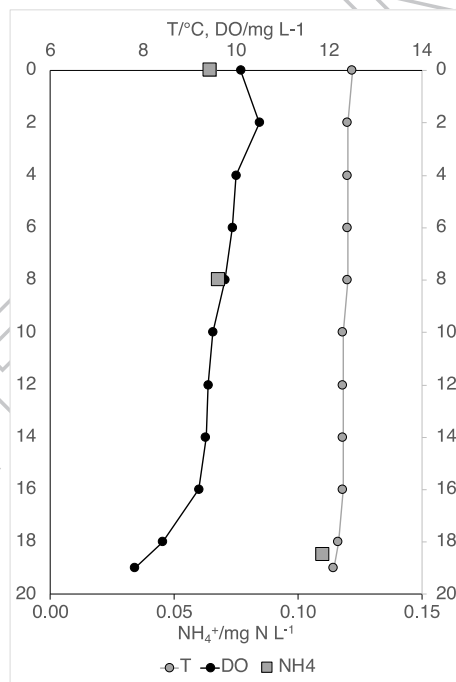


Supplementary Figure 1. Dissolved N fractions for river-lake networks. a) Esthwaite network summer 2017 b) Estwaite network autumn 2017 c) Grasmere-Rydal network autumn 2017 d) Grasmere-Rydal network, spring 2017 e) Grasmere-Rydal network, spring 2018 f) Derwent-Bassenthwaite network spring 2017 g) Derwent-Bassenthwaite network summer 2017 h) Derwent-Bassenthwaite network autumn 2017 i) Derwent-Bassenthwaite network winter 2018

a)



b)



Supplementary Figure 2. Lake T and DO depth profile during autumn 2017 showing high NH₄⁺ concentrations in the hypolimnion and entrainment into the epilimnion during erosion of stratification. a) Esthwaite Water b) Grasmere