PLOS WATER

RESEARCH ARTICLE

Atmospheric nitrate accumulation in headwater lakes and nutrient subsidy to downstream aquatic environments

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Citation: Grefe I, Mackay EB, Wynn PM, Grant HK, Pereira MG, Barker PA, et al. (2025) Atmospheric nitrate accumulation in headwater lakes and nutrient subsidy to downstream aquatic environments. PLOS Water 4(3): e0000342. https://doi.org/10.1371/journal.pwat.0000342

Editor: Peng Wu, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, CHINA

Received: August 22, 2024

Accepted: January 20, 2025

Published: March 10, 2025

Data availability statement: Data for this study is available from the UK Environmental Information Data Centre (EIDC): https://catalogue.ceh.ac.uk/documents/0d-6de9b6-1f80-4f78-b65a-503db8ba63cf

Funding: This research was funded by the Natural Environment Research Council, grant

Abstract

Atmospheric nitrate deposition and biogeochemical nitrogen cycling in headwaters have the potential to impact downstream ecosystem productivity, water chemistry and drinking water quality. However, not much is known about the fate of nitrogen in headwater lakes and during downstream transport through catchments. We used a multidisciplinary synoptic approach, including stable isotope analysis and nutrient limitation experiments, to investigate biogeochemical nitrogen transformations across hydrologically connected stream-lake headwater networks. Of particular interest were the contribution of atmospheric nitrate depositions to nitrogen budgets, as well as the response of primary producers to nitrogen supplies. In this study we show that some headwater lakes can act as sources of nitrate with stable isotope signatures suggesting accumulation of atmospheric depositions potentially contributing over 40% to the lentic nitrate pool. Despite nitrate accumulation in-lake, phytoplankton communities were frequently co-limited by both, phosphorus and nitrogen. Ammonium was undetectable in most water samples, suggesting rapid and preferred uptake over nitrate. Headwater streams were more closely connected to the catchment, and lake nitrogen signatures were rapidly overprinted. Overall, our data show that lakes can be important sources of bioavailable nitrogen with subsidies being rapidly turned over in downstream ecosystems.

Introduction

Headwater stream-lake networks provide unique habitats supporting biodiversity and ecosystem services, as well as socio-cultural and economic services [1–4]. They deliver suspended and dissolved materials to lowland catchments, and ultimately the coastal ocean [5–7]. Headwater stream-lake networks are often characterised by large catchment-to-waterbody area ratios, with narrow streams and small, shallow lakes relative to the total catchment area, and are therefore particularly vulnerable to nutrient pollution and climate change [8,9]. Impacts on these ecosystems therefore potentially affects water chemistry and -quality in downstream ecosystems. However, due to their remote settings and difficult sampling logistics, systematic

number NE/N006453/1 (BS, PW, PB) and NE/N00597X/1 (EM)), as well as the Natural Environment Research Council Life Sciences Mass Spectrometry Facility Analytical Support, grant number CEH_L_114_05_2018 (IG, BS, PW, PB). Fieldwork was supported by the Natural Environment Research Council award number NE/R016429/1 as part of the UK-SCaPE programme delivering National Capability (EM). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

information on nutrient sources and interactions with the aquatic ecosystem along the flow path of headwater stream-lake networks is lacking.

While freshwater networks in upland catchments frequently do not receive direct nutrient pollution such as sewage or agricultural runoff, atmospheric deposition can transport emissions over substantial distances and is an important source of nutrients to these aquatic ecosystems [10,11]. While the atmospheric deposition of phosphorus (P) is generally thought to be a less significant input that nitrogen (N), under certain circumstances it has been suggested that atmospheric inputs of P may warrant careful consideration [12]. In the past, acid rain was understood as the major stressor for upland waters [13]. However, since the reduction of sulphate emissions over recent decades, upland catchments in the UK have started to show signs of recovery from acidification [14]. In contrast to sulphate, N deposition remains high with reduced forms of N from agricultural sources becoming more important in some areas [15,16]. The impact of increased atmospheric N deposition rates since the beginning of the industrial revolution has led to noticeable shifts detected in the sediment records of mountain lakes, suggesting changes to total N concentrations and algal community composition [17,18]. In addition to atmospheric N deposition, a wide range of catchment characteristics, such as land cover, slope, catchment area-to-lake surface ratio and pH, determine the biogeochemical N cycle in upland freshwaters [19–21]. These combined factors result in substantial uncertainties regarding the response of these ecosystems to recent changes in atmospheric N depositions. In particular fluxes of bioavailable N through headwater stream-lake networks and their impact on downstream freshwater communities is currently under-researched [22,23].

Atmospheric depositions can potentially impact productivity in freshwaters, which is often assumed to be primarily limited by P [24]. However, recent research has shown the potential for N- or N and P co-limitation, especially in oligotrophic upland streams and lakes [25–27]. Nutrient limitation in these locations may also serve as an indicator for anthropogenic disturbance, where shifts from N- to P-limitation have been attributed to increased atmospheric N input [28–30]. Changes in algal biomass and community composition have also been reported across landscape gradients of N deposition; for example, increased phytoplankton biomass relative to total P concentration has been identified up to a deposition of ~4 kg N ha^{-1} yr^{-1} [31]. Furthermore, community changes can reflect species shifts associated with moderate nutrient enrichment [32–34]. Despite above efforts to better understand nitrogen dynamics in headwater lakes, important questions remain regarding the changes introduced by these lakes to stream networks in terms of nitrogen speciation, concentration, and subsidy, i.e. the provision of a critical resources from one habitat with the potential to increase productivity in a receiving ecosystem. Furthermore, specific contributions of atmospheric nitrate depositions to nutrient pools in headwater lakes warrant further investigation, as atmospheric nutrient pollution is increasingly being recognised as a stressor for these vulnerable ecosystems and important drinking water resources.

In the research presented here, we investigate sources of N and their biogeochemical cycling, as well as nutrient limitation in headwater stream-lake networks of the Lake District National Park (LDNP), UK. Our aims were to 1) identify sources and dominant processes driving N biogeochemistry between hydrologically connected streams and lakes over the course of the year, 2) determine whether oligotrophic lakes were sinks or sources of bioavailable N to downstream aquatic networks, and 3) identify interactions between water chemistry and nutrient limitation of primary producers in these upland ecosystems. Of particular interest was the importance of atmospheric N sources for these otherwise highly oligotrophic catchments.

Materials and methods

Sample site description

Seven oligotrophic mountain lakes in the central LDNP, as well as their in- and outflow streams were sampled (Table 1, Fig 1). All sites were sampled using publicly accessible routes and no permits were required prior to sampling. The lakes are small (10 ha or less, average 4.1 ha) and shallow (4.1 to 5.4 m mean depth). Dominant land cover for all catchments is either montane habitat or acid grassland (UK Lakes Portal, https://eip.ceh.ac.uk/apps/lakes/).

Three chains-of-lakes were sampled, Codale Tarn and Easedale Tarn (Codale-Easedale network), Blea Tarn and Watendlath Tarn (Blea-Watendlath network), Sprinkling Tarn and Styhead Tarn (Sprinkling-Styhead network). Angle Tarn was the sole lake within its sampled upland stream-lake network (Angle network).

Water sampling

The oligotrophic headwater stream-lake networks were sampled seasonally in March 2017 (spring 2017), August 2017 (summer), October 2017 (autumn), February 2018 (winter) and April 2018 (spring 2018). Samples were collected using a synoptic approach, representing near-instantaneous snapshots of the biogeochemistry in river-lake networks. This research focusses on the contemporaneous changes introduced by lakes to stream biogeochemistry by changing nutrient concentrations and compositions between upstream and downstream reaches. We did not aim to track a specific volume of water throughout the river-lake network and therefore did not apply a Lagrangian sampling scheme. Water chemistry samples for all networks were collected either on the same day or consecutive days. Bioassay samples were collected on consecutive days to water chemistry sampling in spring and summer 2017. In autumn and winter, bioassay samples were collected 19 and 6 days prior to water chemistry sampling, respectively. Grab samples were collected from lake shores and streambanks. During summer lake samples were collected using a small inflatable boat. Inflow and outflow streams were sampled close to the lakes, as well as near major confluences (Fig 1). Due to logistical restrictions the Angle network was only sampled in spring 2017, summer and autumn. The inflow to Watendlath Tarn was only accessible by boat and was sampled once in summer 2017. Water samples were directly filtered in the field using 0.45 μm cellulose acetate syringe filters for analysis of dissolved nutrient concentrations. 0.2 μm filters were used for stable isotope samples. Samples were kept on ice until return to the laboratory where stable isotope samples were frozen at -20°C; all other samples were stored at 4°C until analysis.

Table 1. Information on sampled lakes and their catchments. Dominant land cover based on CEH Landcover Map of Great Britain [35].

Lake	Lake surface area/ha	Mean depth/m	Catchment area/ha	Elevation/m above mean sea level	Catchment mean slope/°	Dominant land cover
Codale Tarn	1	5.1	39	468	17.22	Montane habitats (75%), acid grassland (14%)
Easedale Tarn	10	5.1	283	282	16.79	Acid grassland (45%), montane habitats (20%)
Sprinkling Tarn	2	5.4	17	598	17.4	Montane habitats (64%), acid grassland (20%)
Styhead Tarn	2	5.1	93	437	17.79	Acid grassland (67%), montane habitats (19%)
Blea Tarn	7	4.1	128	478	9.75	Acid grassland (34%), montane habitats (23%)
Watendlath Tarn	4	4.9	588	262	9.9	Acid grassland (48%), bog (16%)
Angle Tarn	3	5.2	53	568	21.69	Montane habitats (40%), acid grassland (23%)

https://doi.org/10.1371/journal.pwat.0000342.t001

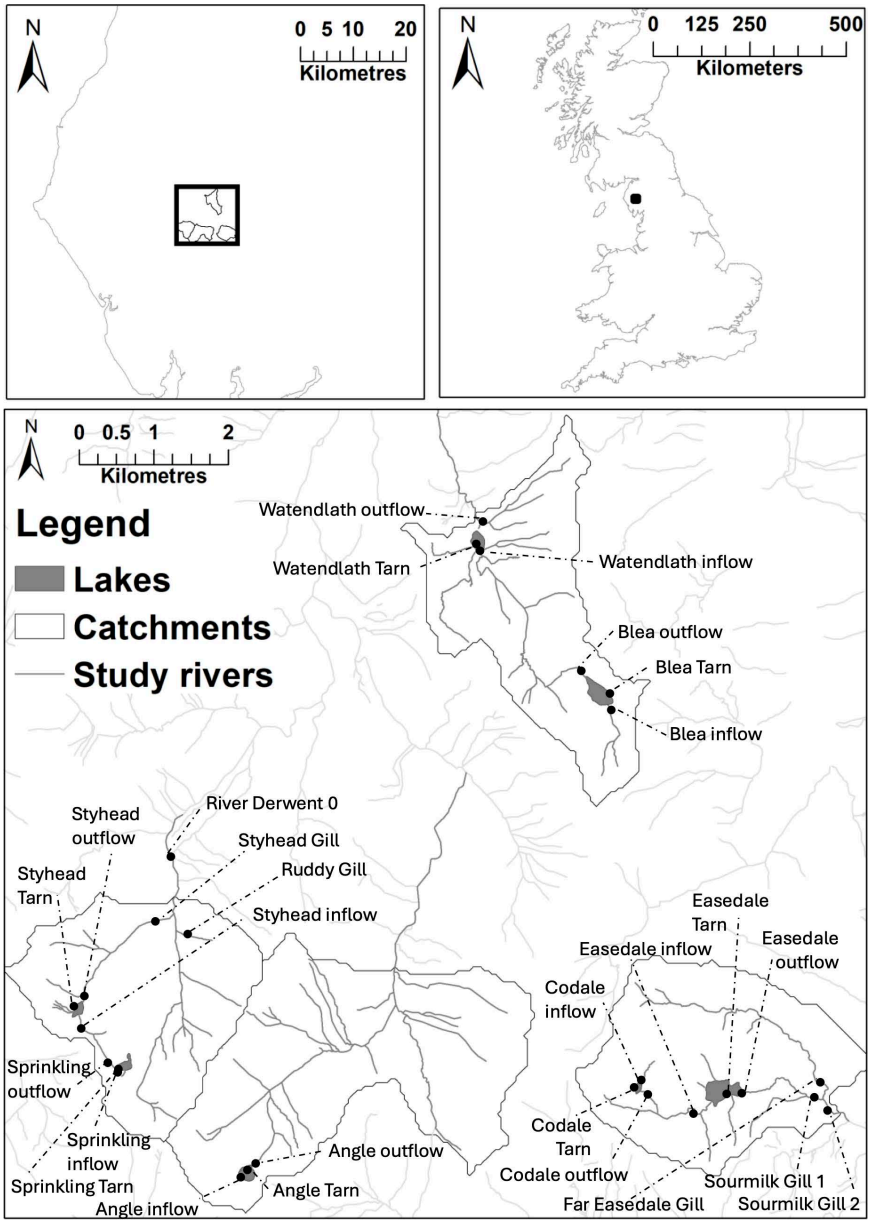

Fig 1. Stream-lake networks and sample locations. Sampled lakes, rivers and catchment boundaries are highlighted in dark grey. Sampling sites are indicated by black symbols. The inflow stream for Sprinkling shown in the map is ephemeral and smaller than an unmapped inflow stream which was sampled instead. Map base layer available from EEA https://www.eea.europa.eu/data-and-maps/data/eea-reference-grids-2/gis-files/great-britain-shapefile under CC BY 4.0 licence (https://www.eea.europa.eu/en/legal-noticehttps://creativecommons.org/licenses/by/4.0/).

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Nutrient concentration measurements and NO_3^- dual isotope analysis

Concentrations of dissolved inorganic nitrogen (DIN = nitrate (NO_3^-) + nitrite (NO_2^-) + total ammonia NH_4^+) and soluble reactive phosphorus (SRP) were analysed colourimetrically on an AQ2 Discrete Analyser (SEAL Analytical). Total dissolved N (TDN) was converted to NO_3^- using acidic persulfate digestion, followed by colourimetric analysis. Dissolved organic N (DON) was then calculated as the difference between TDN and DIN.

To quantify the impact of hydrological connectivity between streams and lakes on NO_3^- concentrations, $NO_{3\text{-diff}}$ was calculated as the percent difference between NO_3^- concentrations in-lake and in the respective inflow stream. Negative $NO_{3\text{-diff}}$ values indicate lower concentrations in-lake, and positive values indicate higher concentrations in-lake compared to the main inflow (potential NO_3^- retention and subsidy, respectively). The relationship between $NO_{3\text{-diff}}$ and NO_3^- concentrations within inflow streams for sampling sites closest to the lake was assessed using a generalised least squares (GLS) model with a power variance error structure, which accounted for patterns in the model residuals. Residuals were visually checked to assess conformity to assumptions of normality, homogeneity of variance and independence of observations. GLS models were fitted using the nlme package [36,37] in R version 4.2.2 [38].

NO_3^- dual isotopes $\delta^{15}N$ and $\delta^{18}O$ were measured using the denitrifier technique [39,40]. Depending on measured NO_3^- concentrations 10 or 20 nmol of sample NO_3^- were injected to bacterial vials with the resulting nitrous oxide being analysed on an Isoprime isotope ratio mass spectrometer coupled to a TraceGas preconcentrator unit and autosampler at UKCEH Lancaster, UK. Samples were calibrated against international standards USGS-34, USGS-35 and IAEA-NO-3, and an internal NO_3^- standard was used as quality control. Standard deviation for replicates were 0.2‰ or better for $\delta^{15}N$ and 0.5‰ or better for $\delta^{18}O$. All values are reported relative to AIR-N_2 ($\delta^{15}N$) and VSMOW ($\delta^{18}O$). Water ^{18}O values were determined for the calculation of expected nitrification $\delta^{18}O$ signatures. Unfiltered water samples were analysed by high temperature pyrolysis on a VarioPYROcube coupled to an Isoprime100 IRMS (Elementar) at Lancaster University, UK. All data was calibrated using international standards GISP and VSMOW2 and is reported relative to VSMOW ($\delta^{18}O$) with standard deviation for replicates < 0.3‰.

Bioassay experiments

Laboratory bioassay experiments to assess nutrient limitation of phytoplankton communities were carried out on water samples collected from Codale Tarn and Easedale Tarn in four consecutive seasons: spring, summer and autumn 2017 and winter 2018. Lake surface water dip samples were collected from shoreline locations and transported to the laboratory, with experiments starting within 24 hours of sampling. Water was initially screened using a 100 μm mesh to remove detritus and zooplankton, then 35 mL samples were incubated in 50 mL boiling tubes in triplicate nutrient treatments. Five treatments were used in each experiment with additions made at approximately Redfield ratio proportions (N at 90 μmol L^{-1} and P at 6 μmol L^{-1}): 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). An additional aliquot of screened water was used to determine initial chlorophyll *a* concentration following filtration onto a Whatman GF/C filter and freezing at −20°C. Incubations took place for 14 days at 20°C over a 18h light, 6h dark cycle, with a photon irradiance of 80–120 μmol m^{-2} s^{-1} (photosynthetically available radiation, Macam Q102). Tube contents were resuspended and filtered onto a Whatman GF/C filter following the incubation and frozen at -20°C. Initial chlorophyll *a* concentration and bioassay growth replicate filters were defrosted and chlorophyll *a* content extracted in hot methanol following Talling [41], with chlorophyll *a* concentration determined using equations in Ritchie [42]. The natural log response ratio [43] was used to assess the growth response of the different nutrient treatments relative to the control, with ratios calculated for each replicate and averaged per treatment. Assessment of nutrient limitation was done using a critical effect size threshold following Mackay et al. [44].

Results

Nutrient concentrations

NO_3^- concentrations in the sampled oligotrophic upland stream-lake networks ranged from below 0.01 mg NO_3-N L^{-1} to 0.49 mg NO_3-N L^{-1}, while DON concentrations of up to 0.38 mg N L^{-1} were observed. NH_4^+ concentrations were almost always below detection limit (0.02 mg NH_4-N L^{-1}), and SRP was only detectable during spring 2017 (S1 Fig). NO_2^- concentrations were below the detection limit of 0.01 mg NO_2-N L^{-1} throughout the year. Annual average NO_3^- concentrations for all sites within a stream-lake network were highest in the Sprinkling-Styhead and Angle networks (Fig 2a). NO_3^- concentrations for all surveyed networks were lowest in summer when DON concentrations were highest (Fig 2b). Over the course of the year, DON concentrations were highest in the Blea-Watendlath network, followed by Codale-Easedale, exhibiting inverse behaviour to NO_3^- concentrations (Spearman's correlation S_r = -0.81, $p < 0.01$, 95% CI -0.867, -0.712). There was a small but statistically significant negative correlation between NO_3^- concentrations and altitude along stream-lake networks (S_r = -0.25, $p < 0.01$, 95% CI -0.424, -0.066). DON contributed up to 96% of the TDN pool and was detectable in most samples with the exception of the Sprinkling-Styhead network in winter and spring 2018 (S2 Fig). However, there was no clear relationship between DON concentration and altitude, nor a clear pattern of DON retention or subsidy in lakes.

NO_3^- stable isotope signatures

NO_3^- dual isotope values ranged from -2.0 to +2.7‰ for $\delta^{15}N$ and from -1.8 to +21.6‰ for $\delta^{18}O$. Over the course of a year, the most isotopically depleted $\delta^{15}N$ values were recorded for Sprinkling-Styhead and Angle networks, and the most isotopically enriched values for the Blea-Watendlath network (Fig 3). Highest NO_3^- ^{18}O values were observed in the Angle network, while the Codale-Easedale network was most depleted in NO_3^- ^{18}O. On a seasonal basis, there was high variability between catchments with the only clear trend being significantly higher $\delta^{15}N$ and $\delta^{18}O$ values (enriched in heavy isotopes) in summer than for all other seasons (T-test, $p < 0.05$). Overall, there was high seasonal and between-network variability in stable isotope signatures of NO_3^-. A moderate Spearman's correlation between $\delta^{18}O$ and altitude was observed (S_r = 0.38, $p < 0.01$, 95% CI 0.198, 0.551) where NO_3^- became more enriched in ^{18}O with increasing altitude. $\delta^{15}N$ became more negative with increasing elevation, but the correlation was much smaller and statistically not significant (S_r = -1.6, $p < 0.05$, 95% CI -0.361, 0.043).

Impact of lakes on N dynamis in stream-lake networks

NO_3^- concentrations changed at times dramatically in lakes compared to concentrations in the inflow stream as expressed in the $NO_{3\text{-diff}}$ value. $NO_{3\text{-diff}}$ ranged from -69 to +306% with relative lake NO_3^- retention being highest in autumn and winter, and relative lake subsidy being highest in summer. Angle Tarn and Blea Tarn showed, on average, the highest relative subsidy of in-lake NO_3^- concentrations, while Sprinkling Tarn and Styhead Tarn most strongly retained NO_3^- (Fig 4). These average values mask, however, large variabilities between seasons, with lakes showing the largest change in concentration also having highest variabilities (Fig 2a).

A negative, non-linear relationship was found between $NO_{3\text{-diff}}$ and NO_3^- concentrations in the inflow (β = -203.23, SE = 67.35, F(1, 26) = 9.11, $p < 0.05$) with some of the highest in-lake increases in NO_3^- concentrations observed under conditions of inflow concentrations below 0.2 mg NO_3-N L^- (Fig 5).

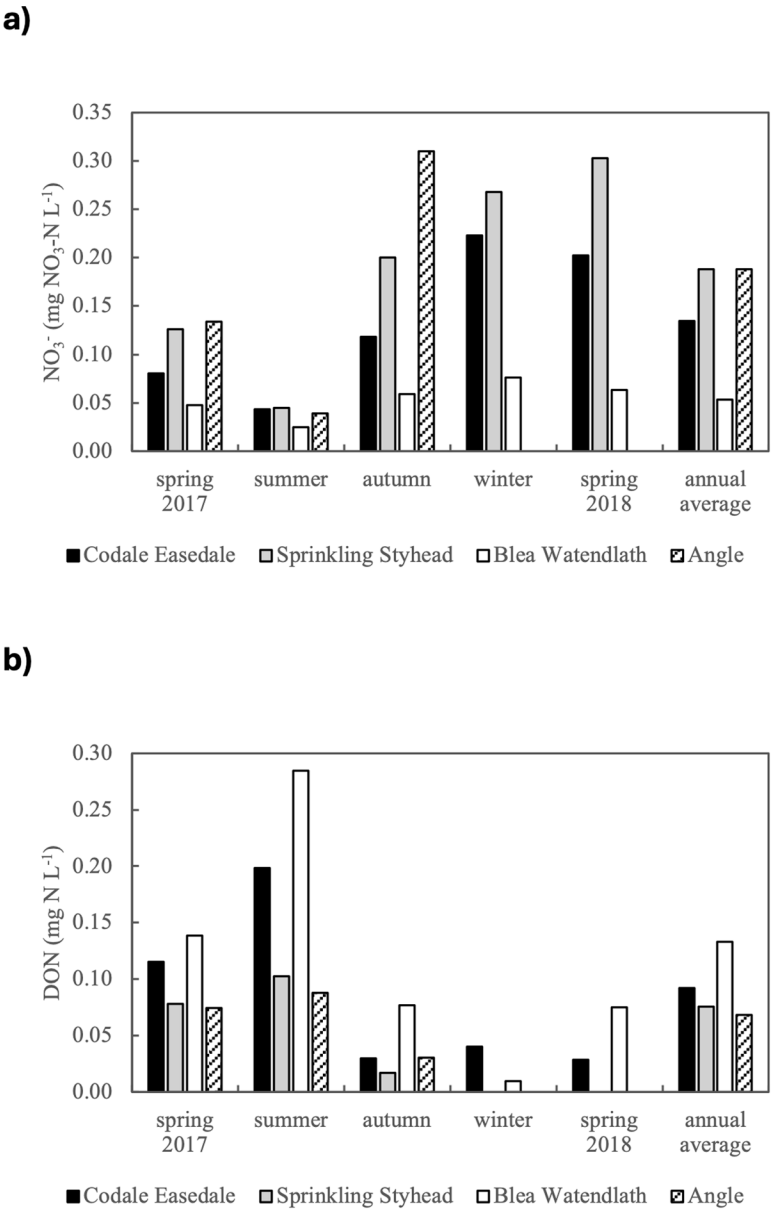

Fig 2. Seasonal and annual average values for a) NO_3^- and b) DON concentrations for the four sampled stream-lake networks. Note that the Angle network was only sampled in spring, summer and autumn 2017.

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Similar to NO_3^- concentrations, there was high variability in changes to $\delta^{15}N$ and $\delta^{18}O$ values in-lake relative to inflow streams. Seasonality did not appear to control fractionation. However, over the course of the year and across catchments there appeared to be a relationship between stable isotope signatures and relative NO_3^- retention and subsidy. In cases where lakes acted as a sink (negative $NO_{3\text{-diff}}$), residual NO_3^- was more enriched in ^{15}N relative to inflow values, while lakes where concentrations increased (positive $NO_{3\text{-diff}}$) tended towards more negative lentic $\delta^{15}N$ values relative to inflow values, albeit not in a statistically significant correlation (S_r = -0.36, $p < 0.05$, 95% CI -0.704, 0.056, Fig 6a). Most samples showed a net increase in $\delta^{18}O$ values relative to the inflow, even in lakes that acted as NO_3^- sources,

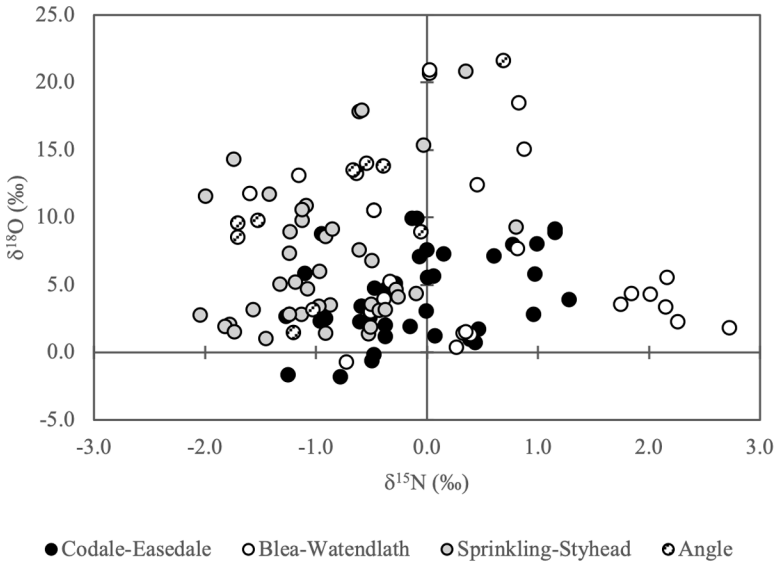

Fig 3. NO_3^- dual isotope composition within the four stream-lake networks.

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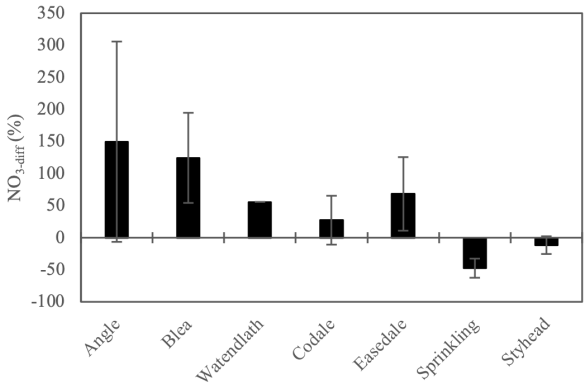

Fig 4. Annual average change of NO_3^- concentrations within lakes compared to inflow concentrations ($NO_{3\text{-diff}}$). Positive values indicated higher concentrations in lake (potential subsidy), negative values lower concentrations (potential retention). Error bars indicate standard deviation. Inflow concentrations for Watendlath Tarn could only be measured in summer 2017.

https://doi.org/10.1371/journal.pwat.0000342.g004

although there was no statistically significant correlation between NO_3^- drawdown $\delta^{18}O$ values (Fig 6b). Only six samples showed a relative depletion of ^{18}O in-lake, three of these samples were from Styhead Tarn, two from Angle Tarn and one from Blea Tarn.

In-lake changes to the bioavailable N pool can be exported into the outflow stream and impact downstream ecosystems. While intra-annual variability was at times high for the sampled stream-lake networks, some consistent patterns of downstream impacts from lake biogeochemistry were observed. Downstream of lakes, $\delta^{15}N$ values were variable and often did not show clear trends along the headwater stream transects. With the exception of the Blea-Watendlath network, the main focus for the stream-lake transects will be on $\delta^{18}O$ dynamics downstream of headwater lakes. The lakes Codale Tarn and Easedale Tarn were predominantly sources of NO_3^- with outflow concentrations often close to those within the lake (Fig 7a). Lake NO_3^- was more enriched in ^{18}O than in the inflow, with more positive isotope

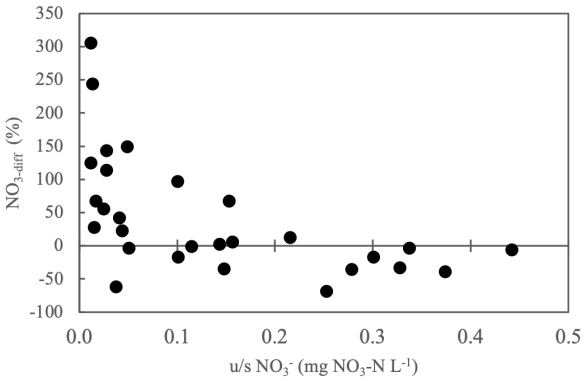

Fig 5. Relationship between change of NO_3^- concentrations within lakes relative to inflow stream concentrations ($NO_{3\text{-diff}}$) and upstream NO_3^- concentration values (u/s NO_3^-).

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values persisting in the outflow (Fig 7b). However, these lake-NO_3^- signatures were over-printed between the outflow of the first lake (Codale Tarn) and the inflow of the second lake (Easedale Tarn), as well as downstream of the Easedale Tarn outflow. NO_3^- concentrations increased downstream except for the spring seasons, while $\delta^{18}O$ values tended to approach the less enriched values of the Codale Tarn inflow. The confluence with the tributary stream Far Easedale Gill between sites Sourmilk Gill 1 and 2 caused an increase in NO_3-N concentrations and a decrease in $\delta^{18}O$ values, except for spring 2018 when concentrations decreased and NO_3^- became more enriched in ^{18}O.

In the Sprinkling-Styhead network, lakes acted predominantly as NO_3^- sinks (negative $NO_{3\text{-diff}}$) with lower concentrations persisting into the lake outflow. NO_3^- concentrations increased between the outflow of the first lake (Sprinkling Tarn) and the inflow of the second lake (Styhead Tarn), as well as downstream of the outflow of Styhead Tarn (Fig 7c). NO_3^- became more enriched in ^{18}O within Sprinkling Tarn, however, there was a trend towards depletion of ^{18}O in Styhead Tarn for most of the year (Fig 7d). Confluence with the tributary stream Ruddy Gill between sites Styhead Gill and River Derwent 0 did not cause substantial changes in NO_3^- concentrations or stable isotope signatures with the exception of summer 2017 where concentrations increased downstream of the confluence.

For the Blea-Watendlath network, NO_3^- concentrations within the first lake increased relative to the inflow throughout the year while outflow NO_3^- concentrations were similar to those in-lake (Fig 7e). $\delta^{18}O$ values became more enriched in the first lake (Blea Tarn) compared to the inflow with the exception of summer 2017 (Fig 7g). There was a substantial increase in NO_3^- concentrations between the two lakes; furthermore, NO_3^- in the second lake (Watendlath Tarn) was depleted in ^{15}N and enriched in ^{18}O for most of the year compared to the first lake (Fig 7f and g). However, due to limited data from the inflow site to Watendlath Tarn, it is not clear whether this change is driven by processes within lake or the inflow. The limited data for the Angle network did not show any consistent trends for the impact of lakes on NO_3^- concentrations or isotope signatures in stream-lake networks (S3 Fig).

Phytoplankton biomass and nutrient limitation

Nutrient limitation of phytoplankton communities was determined for Codale Tarn and Easedale Tarn. Chlorophyll *a* concentrations were low (< 2.5 μg L^{-1}) over the sampling period, confirming the oligotrophic status of both lakes according to the OECD classification [45]. Despite the low overall chlorophyll *a* concentration, both lakes exhibited a seasonal pattern

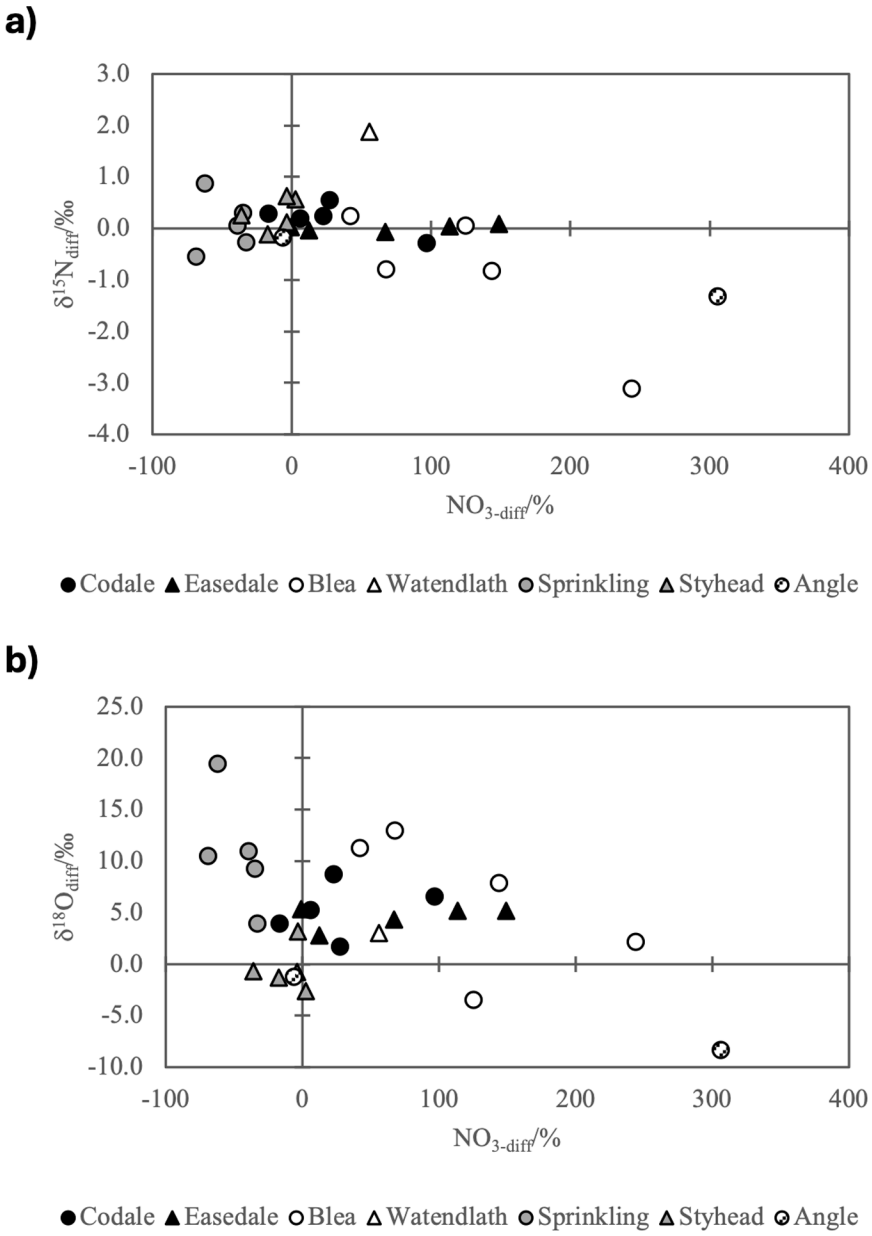

Fig 6. Change in a) $\delta^{15}N$ ($\delta^{15}N_{diff} = \delta^{15}N_{lake} - \delta^{15}N_{inflow}$) and b) $\delta^{18}O$ ($\delta^{18}O_{diff} = \delta^{18}O_{lake} - \delta^{18}O_{inflow}$) values in-lake relative to inflow stream values and change in NO_3^- concentrations ($NO_{3\text{-}diff}$) between lake and inflow stream. Positive values for $\delta^{15}N_{diff}$ and $\delta^{18}O_{diff}$ indicated NO_3^- in-lake is more enriched in heavy isotopes relative to the inflow; negative $\delta^{15}N_{diff}$ and $\delta^{18}O_{diff}$ indicate a depletion in heavy isotopes. Positive $NO_{3\text{-}diff}$ values indicate an increase in NO_3^- concentrations in-lake while negative $NO_{3\text{-}diff}$ values indicate lower concentrations in-lake.

https://doi.org/10.1371/journal.pwat.0000342.g006

of growth with the highest concentrations occurring during spring and summer and lowest in winter. The downstream lake Easedale Tarn generally had a higher phytoplankton biomass than upstream Codale Tarn. Nutrient limitation differed between the lakes in spring, with N and P co-limitation being observed for Codale Tarn and P limitation for the downstream Easedale Tarn. Both lakes were co-limited during summer and P limited during autumn, before deviating again in winter between P limitation for Codale Tarn and co-limitation for Easedale Tarn (Fig 8).

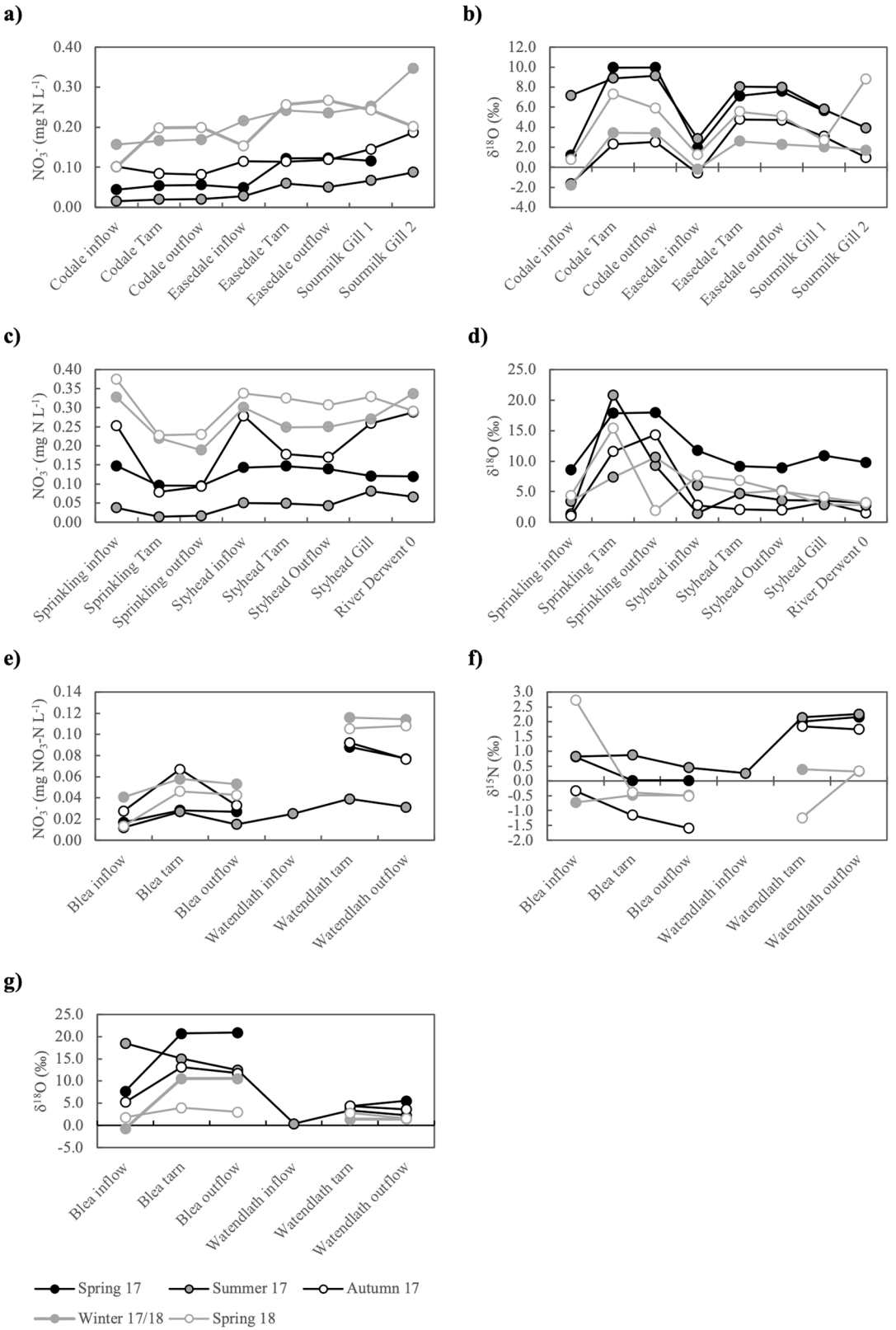

Fig 7. Transect data from the most upstream (left) to the most downstream (right) sampling sites. Seasonal data for stream-lake networks Codale Easedale a) NO_3^- concentrations and b) $\delta^{18}O$ values, Sprinkling-Styhead c) NO_3^- concentrations and d) $\delta^{18}O$ values, Blea-Watendlath e) NO_3^- concentrations, f) $\delta^{15}N$ and g) $\delta^{18}O$ values.

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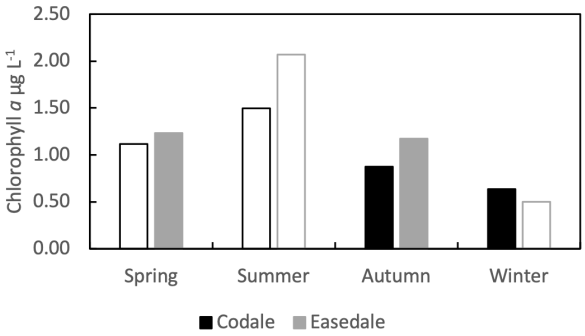

Fig 8. Initial chlorophyll *a* as indicator of biomass for seasonal bioassay experiments in Codale Tarn and Easedale Tarn. Filled symbols indicate P-limitation, open symbols P and N co-limitation.

https://doi.org/10.1371/journal.pwat.0000342.g008

Discussion

Nutrient availability in oligotrophic headwater stream-lake networks

The sampled stream-lake networks in the LDNP are located in upland catchments with headwaters close to summits and water flowpaths along steep slopes. There are no known point sources of nutrient pollution, and while some sheep grazing on unimproved grassland is practiced, there is mostly no direct input from agricultural fertilisers to the nutrient-poor catchment soils. Accordingly, concentrations for the major inorganic nutrients SRP, NO_3^- and NH_4^+ were low within surface waters. In particular NH_4^+ and SRP concentrations were below detection limits for most of the year. Only in spring 2017 was SRP detected consistently in all catchments (S1 Fig). This might have been due to a short-term increase of wet deposition during a substantial rainfall event at the time of sampling [46], potentially increasing concentrations in surface waters [47]. Sediment resuspension and release from pore water are other potential sources of SRP during the rainfall event. Stream-lake networks in the LDNP flush rapidly and the reduced contact time between dissolved nutrients in the water column and organisms in sediments and biofilms might have prevented substantial SRP uptake [48,49] during the rainfall event while sampling in spring 2017.

In addition to P, atmospheric deposition also delivers reduced and oxidised N to aquatic ecosystems. With approximately 4.8 kg NO_3-N ha^{-1} a^{-1} and 10.6 kg DIN-N ha^{-1} a^{-1} [50], the LDNP receives some of the highest rates of N deposition in the UK despite overall reductions of N emissions since 1990 [16,51]. In particular, agricultural emissions have led to a relative increase in reduced forms of N which are replacing oxidised forms as the dominant component of bioavailable atmospheric N [16,52,53]. While this N speciation shift in atmospheric deposition is changing stream water chemistry in some catchments, resulting in higher concentrations of dissolved NH_4^+ [54], NH_4^+ was only rarely detected in LDNP headwater stream-lake networks, despite expected atmospheric deposition rates of a similar magnitude to NO_3^- [16]. Furthermore, contrary to observed SRP concentrations, no corresponding increase in either NH_4^+ or NO_3^- concentrations was observed during the rainfall event in spring 2017, suggesting different behaviour of N and P in the catchments. In fact, average NO_3^- concentrations in the river-lake networks of all sampled catchments were lower during sampling events in spring 2017 than in spring 2018, suggesting dilution of N pools during the spring 2017 rainfall event, rather than additional inputs. Furthermore, demand for bioavailable N might be different to SRP in oligotrophic aquatic ecosystems as higher uptake rates for N than for P have been observed for bioassay experiments in some oligotrophic mountain streams [27].

NH_4^+ appears to be preferred over NO_3^- by some phytoplankton communities as a source of bioavailable N [55], potentially resulting in negligible dissolved aquatic concentrations in the sampled headwater networks throughout the year. NH_4^+ concentrations below detection limit were also observed in some alpine lakes, despite approximately equal sources of atmospheric NO_3^- and NH_4^+ [56]. The authors of the study suggested uptake and subsequent nitrification of atmospheric NH_4^+ occurred in soils, with excess N being flushed into lakes in the form of NO_3^-. Day and Hall [57] also observed rapid removal of NH_4^+ from mountain streams, but suggested assimilation and sorption, rather than nitrification as the dominant uptake process. On the other hand, Maberly et al. [26] observed only a small preference for NH_4^+ determining yields for oligotrophic lakes in UK mountain catchments, including some sites in the LDNP. Nevertheless, our data shows limited dissolved NH_4^+ in headwater stream-lake networks, despite high deposition rates, suggesting rapid and preferred uptake over NO_3^-, for example via phytoplankton and microbial assimilation, nitrification and adsorption to sediments and riverbanks, and warranting further investigations of uptake and transformation processes within surface waters and surrounding catchments.

DON was detected across stream-lake networks throughout the year, showing overall opposite behaviour to NO_3^- concentrations, i.e., DON concentrations were highest in the catchments and seasons where NO_3^- concentrations were lowest (Fig 2). DON can be an important part of the N-pool in freshwater systems, at times being more abundant than DIN in oligotrophic mountain freshwaters [58]. While parts of the DON pool may comprise recalcitrant plant material from the catchment, the statistically significant negative correlation with NO_3^- concentrations suggests that a proportion of the inorganic N pool may be converted to organic N through microbial activity. It has been observed that microbially produced non-humic DOM concentrations are elevated in alpine lakes during summer when primary production is also at its highest [59]. DON can be an important nutrient source in freshwaters, in particular when overall nutrient concentrations are low [44]. Furthermore, DON was much less prevalent and mostly limited to spring and summer in more eutrophic lowland stream-lake networks [60], directly downstream of the sites sampled in this study. The sharp decrease in DON concentrations between upland and lowland catchments could indicate bioavailability of this organic N resource, exporting N from DIN accumulation sites in headwater lakes to downstream ecosystems.

In-lake, catchment and atmospheric drivers of N dynamics in hydrologically connected stream-lake netoworks

In-lake processes. A variety of processes can drive N concentration and speciation changes within lakes, including abiotic factors such as mixing, dilution, water lag and retention, as well as phytoplankton assimilation and microbial cycling. Mixing of water masses, including lag and storage, was investigated comparing differences in chloride concentrations between main inflow stream and lake (S1 Table). Over 80% of sites showed differences within the ± 20% margin for hydrological balance suggested by Whitney et al. [61], suggesting that variations in lake inflow, for example during storm events, and subsequent storage of this "new" water are unlikely to be a dominant control on observed changes in N signatures between streams and lakes.

Lakes are often considered NO_3^- sinks, reducing concentrations through assimilation, sedimentation and denitrification during extended contact times with sediment and water column biota due to increased residence times relative to streams [52,62,63]. Contrary to these expectations, sampled mountain lakes in the LDNP frequently provided subsidies of NO_3^- to downstream ecosystems compared to inflow streams (positive $NO_{3\text{-diff}}$), rather than retaining

bioavailable N. In particular, lakes with low inflow concentrations of NO_3^- showed the highest lentic increases relative to riverine sites directly upstream (Fig 5). It should be noted that the focus of this study was on surface waters and nitrogen transport and transformations along the flowpath, and therefore did not investigate nutrient dynamics at depth. While we cannot completely rule out spatial heterogeneity with depth and increasing proximity to shore or sediments, the small size and exposure of lakes supports well mixed conditions.

Potential sources of NO_3^- in lakes are biological nitrogen fixation (BNF) and nitrification of atmospheric NH_4^+ depositions, as well as catchment sources [64,65]. However, there are substantial uncertainties about the relevance of BNF in freshwaters, especially for acidic systems [66,67]. The $\delta^{18}O$ of NO_3^- is an important tracer of microbial N cycling, as well as atmospheric N deposition [50,68] and was used to further investigate contributions of microbial processes to N cycling. For example, enrichment in ^{18}O can indicate denitrification in anoxic waters, or an atmospheric nitrate source with characteristically high $\delta^{18}O$ and low $\delta^{15}N$ values. Due to isotope fractionation and incorporation of oxygen atoms from water and dissolved oxygen, BNF, as well as aquatic nitrification, are expected to result in $\delta^{18}O$ values between +3.7 and -13.3‰ [69,70; S2 Table], whereas observed $\delta^{18}O$ values in this study were frequently more enriched, ranging from -1.8 to +21.6‰. This suggests BNF and water column nitrification are not dominant sources of NO_3^- to headwater lakes in the LDNP.

Catchment and atmospheric sources of lake NO_3^-. Other potential sources of NO_3^- to surface waters are soil nitrification, as opposed to aquatic nitrification, and atmospheric deposition. In absence of soil water isotope data for this study, we used monthly values for the nearby Scoat Tarn catchment from April, August, October and February, matching sampling months for our study, from Curtis et al. [19] to calculate theoretical $\delta^{18}O$ values for NO_3^- produced by soil nitrification (S2 Table). Soil characteristics in the Scoat Tarn catchment are similar to the catchments in our study. Furthermore, we used $\delta^{18}O$ values for atmospheric NO_3^- depositions from Curtis et al. [19] to account for the other presumed source of NO_3^- to our headwater systems (S2 Table). Using a simple mixing model [50], we estimate that, based on soil water- and atmospheric NO_3^- $\delta^{18}O$ signatures, between 9 and 46% of lake NO_3^- was from unprocessed atmospheric depositions. For comparison, the proportion of unprocessed atmospheric NO_3^- in Scoat Tarn ranged from 9 to 21% [50]. Atmospheric deposition is an important allochthonous source of bioavailable N for these high-altitude ecosystems [71–73], and could partially counteract loss of nutrients through flushing in headwater networks. In this study, the sampled stream-lake networks NO_3^- stable isotope signatures changed significantly with altitude. NO_3^- became depleted in ^{15}N and enriched in ^{18}O with increasing elevation, moving isotope values closer towards those of the expected atmospheric endmember. Furthermore, positive correlation between $\delta^{18}O$ and altitude, as well as a weaker, not significant negative correlation between $\delta^{15}N$ and altitude suggest increasing biogeochemical processing of atmospheric depositions along the downhill water flowpath, partially overprinting the atmospheric isotope signature. Furthermore, the stronger correlation between $\delta^{18}O$ and altitude compared to $\delta^{15}N$ suggests ^{18}O signatures are a better tracer of atmospheric depositions and were therefore used to estimate contributions of atmospheric NO_3^- to the overall pool. The reduced isotopic overprinting by processes such as assimilation and nitrification at sites furthest upstream suggests that these atmospheric NO_3^- depositions are partially in excess of microbial demands in these headwaters. In contrast to NO_3^-, NH_4^+ appears to be the preferred form of bioavailable nitrogen with atmospheric depositions being rapidly removed from the aquatic environment throughout the stream-lake network.

For most headwater lakes in our study, the proportion of atmospheric NO_3^- in-lake increased relative to the value calculated for the respective inflow stream (Fig 9). These results

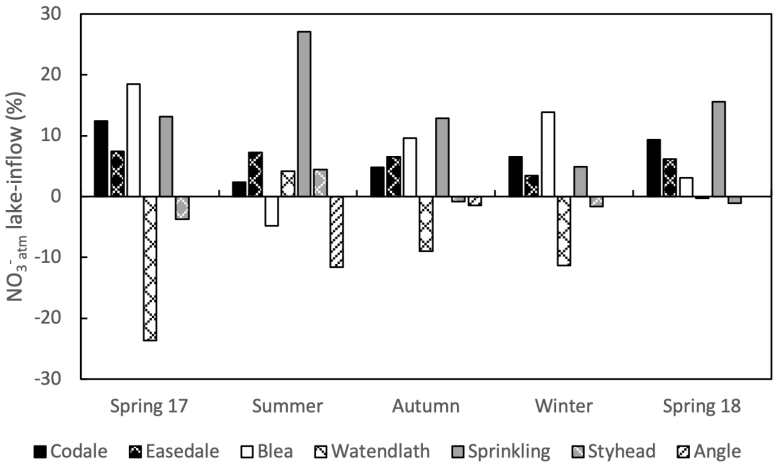

Fig 9. Change of atmospheric NO_3^- proportion ($NO_{3\ atm}^-$) to the NO_3^- pool between inflow stream and respective lake between spring 2017 and spring 2018. Positive values indicate a higher proportion of atmospheric NO_3^- in-lake, negative values indicate a lower proportion in-lake.

https://doi.org/10.1371/journal.pwat.0000342.g009

suggests that some headwater lakes in the LDNP may accumulate unprocessed atmospheric N depositions due to longer water residence times compared to streams. Furthermore, due to the larger water volume in lakes, there is reduced contact area between water and the sediments, reducing the relative contribution of soil nitrification to the lake NO_3^- pool. The accumulated lake NO_3^- can then subsequently be exported to downstream ecosystems. Notable exceptions to this behaviour are Watendlath Tarn and Styhead Tarn, highlighting the importance of catchment variability as discussed below.

For Blea Tarn, an estimated average of 26 to 32% of lake NO_3^- was from atmospheric sources throughout the year. Together with very low absolute NO_3^- concentrations near or below 0.1 mg DIN-N L^{-1}, accumulation of atmospheric NO_3^- is likely to contribute to lake N subsidy as discussed above. The downstream Watendlath Tarn had substantially higher NO_3^- concentrations than Blea Tarn for most of the year, but since inflow samples could only be collected during summer 2017, it is not clear if this pattern is due to in-lake or catchment processes. Summer data suggest a combination of processes with NO_3^- concentrations already increasing in the Watendlath inflow sample, as well as a step change for $\delta^{18}O$ values in the inflow and for $\delta^{15}N$ in-lake (Fig 7e–g). Watendlath Tarn is surrounded by farmland, and agricultural runoff could explain the substantial increase of in-lake NO_3^- concentrations. Furthermore, more positive $\delta^{15}N$ values and more negative $\delta^{18}O$ values compared to upstream samples suggest a potential shift from an atmospheric source (depleted in ^{15}N and enriched in ^{18}O) to manure NO_3^- either from livestock or fertiliser [68]. While the exact isotopic endmember composition of local manure, slurry or fertiliser is not known, $\delta^{15}N$ values tend to be more enriched than for atmospheric NO_3^-, and in particular $\delta^{18}O$ values are much lower in comparison [74]. Estimated atmospheric NO_3^- contributions for Watendlath Tarn are the lowest for all sampled lakes (13 to 20% annual average). Arable land cover and improved grassland together only make up < 2% of the land cover in the Watendlath Tarn catchment. However, our data shows that even these small sources can make a substantial difference to N availability and shift lake ecosystems from a predominantly atmospheric control upstream to mainly anthropogenic influences downstream. In light of the proximity of the farmland to the lake, this drastic change of N dynamics can be important for land management and restoration considerations.

While the Codale-Easedale and Blea-Watendlath chains-of-lakes were predominantly NO_3^- sources, Sprinkling Tarn and Styhead Tarn were NO_3^- sinks for most of the year (Fig 4). Nevertheless, absolute NO_3^- concentrations in Sprinkling Tarn were comparable to those in Codale Tarn (Figs 7a and c). However, there appear to be different biogeochemical processes driving NO_3^- concentrations in Sprinkling Tarn and Styhead Tarn as indicated by the stable isotope data. Residual NO_3^- within Sprinkling Tarn was more enriched in ^{18}O, as well as ^{15}N on most occasions, relative to inflow values (Fig 6). As the lake was fully oxygenated even in summer, denitrification is not likely to be a dominant control on NO_3^- concentrations and stable isotope fractionation. Assimilation, on the other hand, would explain decreasing NO_3^- concentrations, as well as enrichment in ^{18}O and ^{15}N of lake NO_3. The second lake, Styhead Tarn, was also a NO_3^- sink for most of the year, but $\delta^{18}O$ values became more negative relative to the inflow stream (Fig 6c). Nitrification would result in lower $\delta^{18}O$ values, approaching the expected range of +3.7 to -13.3‰. Together, these changes in concentrations and isotope values suggest that remineralisation and nitrification may be more important than atmospheric deposition in controlling the NO_3^- pools of Sprinkling Tarn and Styhead Tarn, as well as partially overprinting isotope signatures of assimilation in the case of downstream Styhead Tarn, resulting in apparent NO_3^- retention in-lake. While small scale variability can be expected for atmospheric depositions, we are not aware of any processes that might substantially reduce input for the Sprinkling-Styhead catchment. Microbial communities in streams and lakes may differ between catchments to some extent and could potentially increase N turnover rates at some sites. However, the relative geographic proximity of the different stream-lake networks considered in this study suggest that assemblage differences are unlikely to be the dominant cause of the differences in the NO_3^- data. Lake depth and volume, catchment slope or land cover for Sprinkling Tarn and Styhead Tarn are within the range of other sampled lakes (Table 1). However, the catchment-to-lake area ratio for upstream Sprinkling Tarn is with a value of 7.1 by far the lowest within the data set presented here, possibly reducing catchment sources such as soil nitrification to the chain of lakes. With less NO_3^- produced by soil nitrification leaching into the Sprinkling Tarn, lake phytoplankton may rely to a higher degree on atmospheric sources, resulting in concentration drawdowns accompanied by stable isotope enrichment and overprinting in the residual NO_3^- pool. For the downstream Styhead Tarn, NO_3^- produced by internal N cycling and leaching from a larger catchment area could provide substrate for phytoplankton. Increased contribution from soil nitrification would result in more depleted ^{18}O values as observed in our data, partially compensating for fractionations introduced during assimilation.

Nutrient limitation in headwater lakes. While NO_3^- concentrations tended to increase relative to inflow concentrations in most sampled lakes, bioassays for Codale Tarn and Easedale Tarn showed co-limitation by both N and P at times over the course of a year. Upstream Codale Tarn was co-limited in spring, downstream Easedale Tarn was co-limited in winter, and both lakes were co-limited in summer. During summer, as well as during spring and autumn, NO_3^- concentrations in Codale Tarn were very low and approaching the value considered limiting to phytoplankton production (0.1 mg DIN-N L^{-1}). In contrast, winter NO_3^- concentrations were in excess of the limiting threshold and $\delta^{18}O$ values in the second lake were less enriched in heavy isotopes, suggesting higher contributions from internal N cycling, presumably nitrification, to the NO_3^- pool. Winter rainfall may increase the contribution of catchment sources to the lakes, with increased flushing due to higher precipitation reducing water residence times and therefore time for the lake phytoplankton community to assimilate nutrients. Therefore, N co-limitation in these lakes appears to be driven by different processes throughout the year. During spring and summer, low overall NO_3^- concentrations can result in co-limitation, while increased flushing during winter

may reduce interactions between phytoplankton and the nutrient pool. For all lakes, $\delta^{15}N$ and $\delta^{18}O$ values in the residual NO_3^- pool were most enriched in summer, suggesting phytoplankton assimilation as the dominant process reducing overall concentrations at this time of year. While no difference in bioassay response was observed for NH_4^+ and NO_3^- additions only, there might be an overall preference for NH_4^+ over NO_3^-, resulting in rapid drawdown of NH_4^+ concentrations below detection limit throughout the sampled catchments. Since N limitation alone was not found in the sampled lakes, combined bioassays testing for response to P+NH_4^+ and P+NO_3^- are required to better understand N dynamics in upland waters.

Conclusions

Seasonality, catchment characteristics and altitude all control concentrations of bioavailable N in headwater stream-lake networks. In this study we used a combination of bioassays and water chemistry data to investigate the impact of hydrological connectivity between streams and lakes on nutrient limitation along an altitude transect. Dual isotope signatures of NO_3^- revealed dominant microbial processes, as well as atmospheric sources controlling the bioavailable N pool, illustrating the impact of water chemistry on nutrient limitation of primary producers and the wider aquatic ecosystem. While atmospheric NO_3^-, but not NH_4^+, depositions accumulated in the majority of lakes, catchment processes rapidly overprinted N signatures within outflow streams. Our observations highlight the importance of oligotrophic upland freshwater habitats on nutrient delivery to downstream environments, as well as ecosystem productivity, and the need for more research, combining ecological approaches and geochemical observations, to fully understand the controls on headwater processes and their impact on downstream water quality and ecosystem services.

Supporting information

S1 Fig. SRP concentrations in spring 2017 a) Codale-Easedale network, b) Blea-Watendlath network, c) Sprinkling-Styhead network, d) Angle network.
(TIFF)

S2 Fig. Concentrations of dissolved organic nitrogen DON from spring 2017 to spring 2018 for a) Codale-Easedale network, b) Blea-Watendlath network, c) Sprinkling-Styhead network, d) Angle network.
(TIFF)

S3 Fig. a) Nitrate concentrations, b) $\delta^{15}N$ and c) $\delta^{18}O$ along stream-lake network of Angle Tarn.
(TIFF)

S1 Table. Percent difference in chloride concentrations between main inflow stream and lake.
(DOCX)

S2 Table. $\delta^{18}O$ values for biological nitrogen fixation (BNF) and aquatic nitrification based on measured $\delta^{18}O$-H_2O values and calculations in [69,70]. Atmospheric deposition $\delta^{18}O$ values from Curtis et al. [19] for the months of April, August, October and February. Soil nitrification calculated following [69,70] using soil water isotope data from Curtis et al. [19] for the months of April, August, October and February.
(DOCX)

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