Dominance of biologically produced nitrate in upland waters of Great Britain indicated by stable isotopes

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Abstract

Atmospheric deposition of nitrogen (N) compounds is the major source of anthropogenic N to most upland ecosystems, where leaching of nitrate (NO₃⁻) into surface waters contributes to eutrophication and acidification as well as indicating an excess of N in the terrestrial catchment ecosystems. Natural abundance stable isotopes ratios, ¹⁵N/¹⁴N and ¹⁸O/¹⁶O, (the “dual isotope” technique) have previously been used in biogeochemical studies of alpine and forested ecosystems to demonstrate that most of the NO₃⁻ in upland surface waters has been microbially produced. Here we present an application of the technique to four moorland catchments in the British uplands including a comparison of lakes and their stream inflows at two sites. The NO₃⁻ concentrations of bulk deposition and surface waters at three sites are very similar. While noting the constraints imposed by uncertainty in the precise δ¹⁸O value for microbial NO₃⁻, however, we estimate that 79-98% of the annual mean NO₃⁻ has been microbially produced. Direct leaching of atmospheric NO₃⁻ is a minor component of catchment NO₃⁻ export, although greater than in many similar studies in forested watersheds. A greater proportion of atmospheric NO₃⁻ is seen in the two lake sites relative to their inflow streams, demonstrating the importance of direct NO₃⁻ deposition to lake surfaces in catchments where terrestrial ecosystems...
intercept a large proportion of deposited N. The dominance of microbial sources of NO$_3^-$ in upland waters suggests that reduced and oxidised N deposition may have similar implications in terms of contributing to NO$_3^-$ leaching.

**Keywords**

Nitrate leaching, nitrification, $^{18}$O, $^{15}$N, uplands, lakes, N deposition

**Running title:**

Dominance of biological nitrate in upland waters

**Introduction**

The environmental problems associated with anthropogenic nitrogen (N) deposition to semi-natural, upland catchments include surface water acidification and eutrophication of both aquatic and terrestrial ecosystems (Curtis et al. 2005a; Maberly et al. 2002; Galloway et al. 2003; Bergström and Jansson 2006; Emmett 2007; Elser et al. 2009). However, despite many biogeochemical studies of N in upland catchments over the last 10-15 years, the mechanistic links between N deposition and nitrate (NO$_3^-$) leaching are still not well understood. A key factor is that inorganic forms of N are readily available for biological uptake in N-limited terrestrial upland ecosystems, so that only a small proportion of deposition inputs ever reaches surface waters. Simple empirical observations of NO$_3^-$ outputs versus N deposition inputs show that there may be a deposition threshold below which NO$_3^-$ leaching rarely occurs, but above which there may be a wide range of responses from very low to very high NO$_3^-$ leaching (Dise et al. 1998). Several factors related to cumulative N deposition effects
have been proposed to explain these patterns, including soil C:N ratios (e.g. Dise and Wright 1995; Gundersen et al. 1998; MacDonald et al. 2002; van der Salm et al. 2007) and total catchment carbon pools or proportion of organic soils (Evans et al. 2006; Helliwell et al. 2007). Spatial variations in catchment sensitivity to N deposition and associated NO$_3^-$ leaching have also been linked to vegetation type (Rowe et al. 2006) and certain physical catchment attributes such as altitude, presence of bare rock and steep slopes (Kopácek et al. 2005; Helliwell et al. 2007).

A simple, initial hypothesis is that, since ammonium concentrations in upland waters are generally negligible, and elevated NO$_3^-$ concentrations only occur where there are high deposition loads of oxidized N (NOx), the NO$_3^-$ observed in impacted upland waters must derive directly from NO$_3^-$ in precipitation (cf. NEGTAP 2001; Durka et al. 1994; Emmett 2007). Ammonium in soilwaters is subject to cation exchange and is less mobile than NO$_3^-$, with a longer residence time in soils and hence greater potential for microbial or vegetation uptake, so NO$_3^-$ is more likely to bypass these biological sinks for N to be leached into surface waters. This hypothesis is superficially supported by the observation that steeper, rockier catchments may leach more NO$_3^-$, leading to the usage of the term “hydrological NO$_3^-$” (e.g. Moldan et al. 1995; Curtis et al. 2005a, b) and the idea of the “Teflon basin” in early studies of alpine lakes in North America, whereby rapidly flushed NO$_3^-$ is not retained in alpine basins (later questioned by Campbell et al. 2002). Such observations have resulted in a common perception that enhanced NO$_3^-$ leaching is associated primarily with NOx deposition in upland catchments.
Several studies, primarily from North America, have more recently challenged the perception that leached NO$_3^-$ is of direct, atmospheric origin, through the use of stable isotope techniques to identify the source of NO$_3^-$ isolated from surface water samples (e.g. Durka et al. 1994; Williard et al. 2001; Spoelstra et al. 2001; Campbell et al. 2002, 2006; Hales et al. 2007; Sebestyen et al. 2008). Many of these studies have demonstrated that even in alpine catchments, a very large proportion of leached NO$_3^-$ is of microbial origin, as indicated by large differences in the abundance of the naturally occurring heavy isotope of oxygen, $^{18}$O, between NO$_3^-$ collected from precipitation and surface water samples. The direct implication is that leaching of unaltered, atmospheric NO$_3^-$ is a minor source of surface water NO$_3^-$ in the uplands and that other factors linked to the nitrification of ammonium are much more important.

The aim of the present study is to determine the relative importance of atmospheric versus microbial sources of NO$_3^-$ across four semi-natural, non-forest upland catchments in the UK with differing levels of NO$_3^-$ leaching, deposition loads and different land cover / physical attributes. The UK uplands experience very high nitrogen deposition loads compared with most of the semi-natural watersheds in North America where dual isotope studies have been carried out; total wet + dry N deposition in 2006 exceeded 24 kgN ha$^{-1}$ yr$^{-1}$ across many upland regions (RoTAP in press). Furthermore, many upland waters in these areas experience chronic NO$_3^-$ leaching for much or all of the year (Curtis et al. 2005a). The hypothesis that leached NO$_3^-$ is primarily of atmospheric origin suggests that policy measures to control NOx emissions should be the main priority to protect upland waters from the adverse effects of enhanced NO$_3^-$ leaching. If leached NO$_3^-$ is mainly microbially produced,
then all external sources of inorganic N are likely to contribute to observed patterns of NO₃⁻ leaching.

Site description

Four study sites were selected on the basis of the availability of co-located long-term water chemistry data from the UK Acid Waters Monitoring Network (AWMN: Monteith and Shilland 2007) and bulk deposition chemistry from the UK Acid Deposition Monitoring Network (ADMN) since 1999 when the sites were instrumented (Lawrence et al. 2008; Table 1). All four sites occupy semi-natural headwater catchments (Fig. 1) with atmospheric deposition as the only major source of anthropogenic pollution. The Afon Gwy and Scoat Tarn catchments comprise mainly acid grassland with sheep grazing. The River Etherow catchment is mainly Calluna vulgaris dominated moorland on blanket peat, which is managed as a grouse moor by burning, with some sheep grazing. The blanket bog is affected by gully erosion and historical loss of Sphagnum moss cover, which has been attributed to acid deposition (Tallis 1987). The catchment of Lochnagar comprises alpine/subalpine heath with a large proportion of bare rock and scree. The Afon Gwy and River Etherow are streams, while Scoat Tarn and Lochnagar are headwater lakes with smaller catchments (Fig. 1, Table 1). All four sites are acidified, showing exceedance of critical loads for acidity (Curtis et al. 2005a), with moderate NO₃⁻ leaching at the Afon Gwy, high NO₃⁻ leaching at Scoat Tarn and Lochnagar and extremely high NO₃⁻ (for a semi-natural upland site) in the River Etherow (Table 1). These patterns reflect NO₃⁻ concentrations in bulk deposition, with comparable concentrations between surface waters and bulk deposition at all sites except the River Etherow, where mean NO₃⁻ concentrations are much higher in the stream (Table 1). This observation
appears to support the hypothesis that surface water NO$_3^-$ may be largely dictated by concentrations in rainfall, with some additional source of NO$_3^-$ at the Etherow.

Methods

A pilot study to assess the feasibility of the dual isotope ($^{15}$N/$^{14}$N and $^{18}$O/$^{16}$O) method was first carried out at the Afon Gwy from September 2004 to August 2005, on the basis of previous studies at the site and the development of a conceptual model of NO$_3^-$ leaching zones (Evans et al. 2004). A high volume bulk deposition collector with a small collecting roof (c. 0.5m$^2$) was installed adjacent to the streamwater sampling point. Three zero-tension tray lysimeters were installed in an exposed hillslope cutting approximately 700m from the bulk deposition collector on the adjacent hillslope (grid ref. SN81967, 86100), at depths of c. 10cm (bottom of rooting zone), 20cm (base of O horizon) and 50cm (B horizon, above an impermeable ironpan). Streamwaters, bulk deposition and tray lysimeters were all sampled monthly for isotopes. Additional streamwater samples were obtained from a number of subcatchments on a quarterly basis for comparison with the main channel, with subcatchments selected on the basis of dominant soils and previous water chemistry data from Evans et al. (2004). Only data from tributary LB7a are presented here.

Following successful application of the techniques at the Afon Gwy, monthly isotopic sampling commenced at the other three sites in August 2005, ending in July 2006 (Table 1). At these sites, bulk deposition collectors were located close to surface water
sample points and two tray lysimeters were deployed nearby in deeper organic
horizons at c. 10cm and 20cm depths. The high-volume bulk deposition collectors at
the three new sites comprised a large diameter HDPE funnel (c. 32cm diameter)
attached to a pole about 2m above ground level and connected via a length of
neoprene tubing to a sealed 5L LDPE carboy buried under c. 10cm of soil. At the
River Etherow, streamwater samples were obtained from two tributary streams (Rose
Clough and Swan Clough) as well as the main river channel at the weir (Table 1; Fig.
1). At Scoat Tarn, the two major inflow streams were sampled as well as the lake
outflow, while at Lochnagar a spring-fed inflow stream was sampled as well as the
lake outflow.

Sample collection and analysis

Surface water and bulk deposition sampling and analysis for isotopes
For isotopic analysis, monthly surface water and bulk deposition samples were
collected and a subsample analysed in the field using a portable spectrophotometer
(Hach DR/2400) for concentrations of the acid anions NO$_3^-$, sulphate and chloride to
provide approximate estimates of required sample volumes for isotopic analysis
(target was at least 100 µeq NO$_3^-$). All samples were collected in acid-washed, DIW-
rinsed LDPE carboys. For surface waters at least one 20L carboy was filled after
rinsing three times with sample (up to 40L in two carboys in summer when low NO$_3^-$
concentrations <5 µeq l$^{-1}$ were found). Bulk deposition samples were collected in the
original 5L LDPE carboys which were then replaced with clean carboys following
rinsing of the funnel and tubing with DIW.
Streamwater and bulk deposition NO$_3^-$ samples were then filtered to 0.45 μm using high capacity groundwater filtration cartridges or 142mm diameter disc filters (Whatman GF/F 0.7μm pre-filter and Pall Supor-450 0.45μm membrane filter) and pre-treated through cation exchange resins (AG50W-X8) prior to collection on anion exchange resins (Dowex AG1-X8) according to the method of Chang et al. (1999). Where possible, at least 100 μeq of NO$_3^-$ was passed through the exchange columns but care had to be taken not to exceed the exchange capacity of the anion resins (6000 μeq) to avoid possible isotope fractionation of the collected NO$_3^-$ sample. In practice, it was not always possible to collect sufficient NO$_3^-$ for isotopic analysis due to very low concentrations in streamwaters relative to other anions, especially chloride. The anion resins were refrigerated prior to being transported to the NERC Isotope Geosciences Laboratory, Keyworth, where the NO$_3^-$ was converted to silver nitrate (Silva et al., 2000; Heaton et al., 2004). $^{15}$N/$^{14}$N and $^{18}$O/$^{16}$O ratios were analysed by combustion to N$_2$ in a Flash EA Elemental Analyzer, or thermal conversion to CO in a TC-EA, respectively, with gases passed on-line to a Delta Plus XL mass spectrometer (all ThermoFinnigan, Bremen, Germany). Sample purity and freedom from organic contamination was checked by ensuring that N/O ratios were close to those of pure NO$_3^-$ standards (Heaton et al. 2004). Nitrate isotope ratios were calculated as δ$^{15}$N values versus air (atmospheric N$_2$) and δ$^{18}$O versus VSMOW by comparison with standards IAEA N-1 and N-2, and USGS 34 and 35. Water $^{18}$O/$^{16}$O ratios were determined following equilibration with CO$_2$ in an Isoprep-18 on-line to a SIRA II mass spectrometer (VG Isotopes, Middlewich, England), and calculated as δ$^{18}$O values versus VSMOW by comparison to IAEA standards VSMOW and SLAP.
Soilwater sampling and analysis

Monthly tray lysimeter soilwater samples were filtered and analysed for acid anions in the field using the methods described above. Where sufficient NO$_3^-$ was measured, the sample was loaded onto ion-exchange resin columns for dual isotope analysis. Where sample volumes were insufficient for isotopic analysis, samples were kept refrigerated and bulked with subsequent months until sufficient sample volume was obtained.

Water chemistry sampling programmes

Water chemistry data were obtained from separate ongoing sampling programmes at the study sites. Separate surface water and bulk deposition samples were obtained at the same frequency (or greater) as isotope samples (except at Scoat Tarn) and analysed for water chemistry in the laboratory according to the established protocols of the AWMN for surface waters (Monteith and Shilland 2007) and ADMN for bulk deposition (Lawrence et al. 2008), using ion chromatography with detection limits of c. 1 µeq l$^{-1}$ for NO$_3^-$ and NH$_4^+$ and ion-balance checks as part of routine analytical qualitative control. Surface water samples were taken weekly from the Afon Gwy, 2-weekly from Lochnagar, monthly at the River Etherow and quarterly at Scoat Tarn.

Bulk deposition sampling for water chemistry was carried out 2-weekly at all sites using collectors following the design of Hall (1986). Only these chemistry data are reported here; monthly field results obtained using the portable spectrophotometer are considered to be approximate only.

Data analysis
Calculation of the theoretical $\delta^{18}O$-NO$_3^-$ of bacterial NO$_3^-$

Determination of the proportions of atmospheric and microbial NO$_3^-$ using $\delta^{18}O$ relies on the measurement of atmospheric $\delta^{18}O$-NO$_3^-$ in bulk deposition, and the theoretical calculation of microbial $\delta^{18}O$-NO$_3^-$ based on measurement of soilwater $\delta^{18}O$-H$_2$O. This calculation has traditionally been based on the assumption that autotrophic microbial NO$_3^-$ derives one part of its oxygen from atmospheric O$_2$ ($\delta^{18}O = +23\%$) and two parts from soilwater H$_2$O, which is measured directly (Amberger and Schmidt 1987; Kendall 1998):

$$\delta^{18}O$-NO$_3^-$ = (2/3 $\delta^{18}O$-H$_2$O soilwater) + (1/3 $\delta^{18}O$-O$_2$ atmosphere) \quad (1)$$

This theoretical calculation makes a number of assumptions which may be valid for many, but not all environments (Mayer et al. 2001; Kendall et al. 2007; Spoelstra et al. 2007; Snider et al. 2010). Thus, from experimental results Mayer et al. (2001) suggested that there may be some circumstances under which heterotrophic nitrification might yield NO$_3^-$ oxygen derived from two parts atmospheric oxygen and only one part soilwater oxygen. In contrast, recent incubation experiments with $^{18}O$-labelled waters have suggested that in some cases over 90% of the NO$_3^-$ oxygen might be derived from soil water (Snider et al. 2010). Here we calculate the soil microbial NO$_3^-$ end-member using the commonly used equation above, assuming the ratio of atmospheric-O to soilwater-O equals 1 to 2; but comment later on the validity of this. Future isotope studies may be able to overcome these uncertainties by using techniques for measuring NO$_3^-$ $\delta^{17}O$ in addition to $\delta^{18}O$ (Michalski et al. 2004; Curtis et al. 2011).
Statistical analysis

A series of linear models fitted by ordinary least squares was used to formally address a set of key hypotheses; i) there are differences between study sites in the isotopic signature of deposited NO$_3^-$ (i.e. both $\delta^{18}$O-NO$_3^-$ and $\delta^{15}$N-NO$_3^-$), ii) there are differences between sites in $\delta^{18}$O-H$_2$O of soilwaters, used in the derivation of theoretical microbial $\delta^{18}$O-NO$_3^-$, iii) there are isotopic differences between deposition NO$_3^-$ and surface water NO$_3^-$ within study sites (for both $\delta^{18}$O-NO$_3^-$ and $\delta^{15}$N-NO$_3^-$), iv) there are within-site differences in both $\delta^{15}$N-NO$_3^-$ and $\delta^{18}$O-NO$_3^-$ between inflow streams and lake outflows indicating differences in the contribution of atmospheric NO$_3^-$ leaching, v) there are differences between sites in $\delta^{18}$O-NO$_3^-$ of streamwaters indicative of differing levels of atmospheric NO$_3^-$ leaching. The assumptions of ordinary least squares were checked via exploratory plotting of data and via Fligner-Kileen tests for homogeneity of variances. Where the homogeneity of variances assumption was not met by the data, a sandwich estimator of the standard errors of model parameters was used, providing heteroscedastic-consistent standard errors.

Post-hoc pair-wise comparisons were conducted according to the method of Tukey's Honest Significance Differences (Tukey's range test) at a 95% family-wise confidence level (Bretz et al. 2010). All analyses were performed using R (version 2.11-patched R Core Development Team, 2010) with the multcomp (version 1.2-2, Hothorn et al. 2008) and sandwich (2.2-6, Zeileis 2004) packages.

Results

Deposition and surface water chemistry
Concentrations of NO$_3^-$ and NH$_4^+$ in bulk deposition were significantly related (ANCOVA: likelihood ratio 109.9045, 4 d.f., p < 0.0001; Fig. 2). The highest concentrations in precipitation occurred at the River Etherow, regularly exceeding 50 µeq l$^{-1}$ for both NO$_3^-$ and NH$_4^+$, while lower peaks in the range 20-30 µeq l$^{-1}$ occurred at the Afon Gwy. At Lochnagar, concentrations of NO$_3^-$ in precipitation were generally higher than NH$_4^+$ while at other sites NH$_4^+$ concentrations regularly exceeded those of NO$_3^-$.

Mean concentrations and deposition fluxes of NO$_3^-$, ammonium and dissolved inorganic N (DIN) are presented in Table 2. While concentrations of all ions in bulk deposition were greatest at the River Etherow site, the greatest deposition fluxes occurred at Scoat Tarn, where rainfall was more than double that at the Etherow. Furthermore, while mean concentrations of NO$_3^-$ and NH$_4^+$ were lowest at the Afon Gwy, much lower rainfall at Lochnagar meant that deposition fluxes were lowest at the latter site.

Concentrations of NO$_3^-$ in bulk deposition were generally higher than or similar to those in surface waters for most of the year except at the River Etherow, where NO$_3^-$ concentrations in the stream exceeded those in precipitation on about 50% of sampling occasions (Fig. 2). Surface water concentrations of NH$_4^+$ were negligible at all sites except for occasional measurable levels at the River Etherow and are not considered further.

Surface water NO$_3^-$ concentrations over the study period were very similar to the longer-term means in Table 1 and showed the same pattern relative to bulk deposition.
Mean NO$_3^-$ concentrations were similar in the stream and bulk deposition at the River Etherow while at the other three sites, mean concentrations in surface waters were slightly lower than in bulk deposition. Although flow was not measured at the River Etherow or Scoat Tarn, flow data for the Afon Gwy were used to calculate a flow-weighted mean concentration of 6.8 µeq l$^{-1}$ which is slightly higher than the unweighted mean of 6.0 µeq l$^{-1}$ and closer to the volume weighted mean of 10.1 µeq l$^{-1}$ in bulk deposition. At Lochnagar, monthly scaling factors based on historical flow data were used to calculate a flow-weighted mean NO$_3^-$ concentration of 15.2 µeq l$^{-1}$, which is slightly lower than the unweighted mean of 15.8 µeq l$^{-1}$ but close to the volume-weighted mean value in bulk deposition of 17.7 µeq l$^{-1}$. In the absence of standard flow data for all four sites, catchment leaching fluxes of NO$_3^-$ were estimated from modelled runoff data. Runoff estimates were provided by the Centre for Ecology and Hydrology, Wallingford, and were derived from interpolated long-term mean rainfall measurements (1941-70) and modelled evapotranspiration on a 1km grid for the UK, based on meteorological data obtained from the UK Met Office.

Dual isotope analysis

All isotope data are summarised in the Supplementary Tables S2 to S5.

Summary box plots of the monthly dual isotope analysis of bulk deposition NO$_3^-$ at the four study sites are shown in Figs. 3-4. The range of δ$^{15}$N-NO$_3^-$ varied from -2.3 to +3.0‰ (n=9, mean= -0.3‰, SD= 1.6‰) at the Afon Gwy, -4.5 to +3.1‰ (n=12, mean= +0.5‰, SD= 2.3‰) at the River Etherow, -4.3 to +1.0‰ (n=11, mean= -
1.5‰, SD= 1.5‰) at Scoat Tarn and -5.7 to +1.0‰ (n=11, mean= -1.8‰, SD= 1.8‰) at Lochnagar.

Analysis of variance indicated that there were significant differences in bulk deposition $\delta^{15}$N-NO$_3^-$ between some sites ($F(3,38)=3.41, p=0.027$; Fig.3). Post hoc comparison of pairwise differences of means showed a significant difference in $\delta^{15}$N-NO$_3^-$ between Lochnagar and the River Etherow (t=-2.89, p=0.030) while the difference between Scoat Tarn and the River Etherow was not significant (t=-2.39, p=0.093). While there was a scatter around 0.0‰ at the Afon Gwy and River Etherow, most samples of bulk deposition NO$_3^-$ from Scoat Tarn and Lochnagar were depleted in $^{15}$N and only the River Etherow had a (slightly) positive mean value of $\delta^{15}$N.

At three sites the range of $\delta^{15}$N-NO$_3^-$ values in surface waters largely overlapped with that in atmospheric deposition. The exception was the River Etherow, where streamwater $\delta^{15}$N-NO$_3^-$ values were significantly higher than those in bulk deposition, especially for the two tributary streams sampled (Tukey’s HSD: Rose Clough, t=6.64, p<0.001; Swan Clough t=7.67, p<0.001; River Etherow t=3.92, p=0.002). Mean values of $\delta^{15}$N-NO$_3^-$ were +4.6‰ (n=12, SD= 2.0‰) for Rose Clough and +5.2‰ (n=12, SD= 1.1‰) for Swan Clough, in both cases exceeding the maximum values recorded in bulk deposition for the River Etherow catchment (+3.1‰ in December 2005). Mean $\delta^{15}$N values for surface waters in the Etherow catchment were 2.4 to 4.7‰ higher than those in bulk deposition. Despite the greater overlaps in $\delta^{15}$N values at other sites, surface water mean $\delta^{15}$N values were also higher (by 0.9 to 1.5‰) than those in bulk deposition.
Highly positive values of $\delta^{18}$O-NO$_3^-$ in bulk deposition were found at all four sites, with a minimum value of +48.3‰ and a maximum of +82.1‰, both at Lochnagar (Fig. 4). Much smaller ranges were found at the Afon Gwy and River Etherow sites (+60.4 to +71.6‰). Mean values from all sites were very similar, from +66.5 (River Etherow, n=12, SD=3.6‰) to +69.6‰ at Lochnagar (n=11, SD= 9.5‰). Analysis of variance showed no significant differences in bulk deposition $\delta^{18}$O-NO$_3^-$ between sites ($F(3,37)=1.02, p=0.394$). However, there were large and significant differences in $\delta^{18}$O-NO$_3^-$ between surface waters and bulk deposition at each site (Tukey HSD, p<0.001 for all sampled water bodies; see Fig.5). The maximum surface water $\delta^{18}$O value of +22.4‰, observed at Lochnagar in April 2006, was lower than the minimum value observed in deposition. This allowed the use of a simple two end-member mixing model to estimate the proportion of untransformed atmospheric NO$_3^-$ in surface waters (see below). In the few soilwater samples which yielded sufficient NO$_3^-$ for isotopic analysis, the $\delta^{18}$O-NO$_3^-$ values were comparable to surface waters at Scoat Tarn (+9.1 to +19.8‰), but intermediate between surface waters and bulk deposition at both the River Etherow ($\delta^{18}$O = +17.2 to +28.7) and Lochnagar ($\delta^{18}$O = +35.5 to +53.6‰ in bulked samples; see Fig. 5, Table S6).

While the differences in $\delta^{18}$O-NO$_3^-$ between bulk deposition and surface waters were much greater than for $\delta^{15}$N-NO$_3^-$, there was some evidence of inverse relationships between the $\delta^{18}$O and $\delta^{15}$N values of surface water NO$_3^-$ (i.e. decreasing $\delta^{15}$N with increasing $\delta^{18}$O) in the Etherow tributaries Rose Clough and Swan Clough, inflow 2 at Scoat Tarn, and both inflow and lake at Lochnagar (all p<0.05; see Fig. 5).
Theoretical $\delta^{18}O$-NO$_3$ of microbially produced NO$_3$:

Measurement of $\delta^{18}O$-H$_2$O at the four sites showed very similar mean values of -5.9 (Afon Gwy), -6.0 (River Etherow), -6.4 (Scoat Tarn) and -7.4‰ (Lochnagar). The only significant difference between sites was between Lochnagar and the River Etherow ($t$=-2.77, $p=0.035$). Mean theoretical $\delta^{18}O$-NO$_3$ values for microbially produced NO$_3$ were +3.7‰ (Afon Gwy, River Etherow), +3.4‰ (Scoat Tarn) and +2.8‰ (Lochnagar). The annual range of theoretical microbial $\delta^{18}O$-NO$_3$ for each site is shown in Fig. 5; monthly data are provided in Supplementary Information.

Derived contribution of untransformed atmospheric NO$_3$:

The simple two end-member mixing model employs an interpolation between the theoretical microbial value and the measured bulk deposition $\delta^{18}O$-NO$_3$ (in each case referring to $\delta^{18}O$-NO$_3$):

\[
\text{% atmospheric} = \frac{\delta^{18}O_{\text{surface water}} - \delta^{18}O_{\text{microbial}}}{\delta^{18}O_{\text{deposition}} - \delta^{18}O_{\text{microbial}}} \times 100\% \quad (2)
\]

Calculated values for each monthly sample are presented in supplementary Tables S2-S5 but here we present annual means based on % values calculated monthly from measured $\delta^{18}O_{\text{surface water}}$, $\delta^{18}O_{\text{microbial}}$ and $\delta^{18}O_{\text{deposition}}$.

Afon Gwy:

At the Afon Gwy, most measurements of $\delta^{18}O$-NO$_3$ in the stream lay within the theoretical range for microbially produced NO$_3$ (Fig. 5), suggesting that almost all the
NO$_3^-$ observed in the stream had been microbially produced, and that direct leaching of untransformed atmospheric NO$_3^-$ was negligible (Fig. 6). Very low NO$_3^-$ concentrations in the main channel at the Afon Gwy regularly prevented the collection of sufficient NO$_3^-$ on the anion resins for isotopic analysis. The highest proportion of atmospheric NO$_3^-$ was recorded from a tributary of the Afon Gwy (stream LB7a) with slightly higher NO$_3^-$ concentrations, indicating 7% untransformed NO$_3^-$ in June 2005; the annual mean value was only 3% (Table S2; SE = 1.4%). Due to bad weather, this site could not be accessed for sampling during February-March 2005. In order to investigate the possibility that peaks in both NO$_3^-$ concentration and atmospheric contribution had been missed as a result, the site was resurveyed as part of a subsequent study in February-April 2009. In this later study, bulk deposition $\delta^{18}$O-NO$_3^-$ fell within the range reported here while the proportion of atmospheric NO$_3^-$ was 3% in February 2009 and <1% in March 2009. Insufficient NO$_3^-$ was collected in April 2009 for dual isotope analysis.

River Etherow

At the River Etherow and its two sampled tributaries, all samples analysed had low $\delta^{18}$O-NO$_3^-$ values, close to or within the range for microbially produced NO$_3^-$ (Fig. 5). Less than 10% of streamwater NO$_3^-$ was untransformed atmospheric NO$_3^-$, with peak and mean values of only 9% and 6% (SE = 0.7%) respectively in the main channel (Fig. 6). In the Rose Clough and Swan Clough tributaries the $\delta^{18}$O-NO$_3^-$ signal was barely distinguishable from the theoretical range for microbially produced NO$_3^-$ (Fig. 5), with mean values for both streams indicating <3% (SE = 0.5 and 0.7% respectively) atmospheric NO$_3^-$ contribution (Table S3). Bulked samples from shallow soilwater lysimeters did show a larger proportion of atmospheric NO$_3^-$ in the upper
peat horizons, up to 40% for the period November 2005 to February 2006 (Fig. 5), but there was no evidence that this atmospheric NO$_3^-$ reached surface waters.

Scoat Tarn

Most outflow samples from Scoat Tarn showed $\delta^{18}$O-NO$_3^-$ values much greater than the range for microbially produced NO$_3^-$ at the site. The range of values was greater in the inflow streams than in the lake outflow, but the annual mean value was greater in the outflow (Fig. 5; Table S4). The proportion of untransformed atmospheric NO$_3^-$ indicated by $\delta^{18}$O-NO$_3^-$ values varied from 9-21% (mean 15%, SE= 1.0%) in the lake outflow, with a greater range of 5-23% (mean 11%, SE= 1.6%) in Inflow 1 and 1-24% (mean 9%, SE= 1.8%) in Inflow 2 (Figs. 5-6). The tray lysimeters at Scoat Tarn produced the greatest number of samples of sufficient size for isotopic analysis and indicated a similar range in $\delta^{18}$O values as surface waters, with inferred proportions of atmospheric NO$_3^-$ from 8-26% (Fig. 5, Table S6).

Lochnagar

Values of $\delta^{18}$O-NO$_3^-$ in the loch outflow at Lochnagar were all much higher than the range for microbial NO$_3^-$ (Table S5) although some of the inflow spring values overlapped the microbial range (Fig. 5). The calculated proportions of atmospheric NO$_3^-$ showed a greater range and higher mean values than other sites; 14-32% (mean 21%, SE= 1.7%) in the loch outflow and 0-30% (mean 13%, SE= 3.3%) in the inflow spring (Fig. 6). Lysimeter samples from this site were all very small and the only data obtained were for samples bulked over several months. However these samples had very high $\delta^{18}$O values of +35.5‰ and +53.6‰ in the two lysimeters, indicating 43 and 69% atmospheric NO$_3^-$ in the shallow tray lysimeters over this period.
Temporal patterns

Temporal variations in the direct contribution of atmospheric NO$_3^-$ to surface water concentrations are illustrated in Fig. 6. While there were too few samples to identify temporal patterns at the Afon Gwy, the other sites showed distinct seasonal patterns in the contribution of atmospheric NO$_3^-$ which were much more pronounced in streams and lake inflows than in lake outflows (Fig. 6). The contribution of atmospheric NO$_3^-$ was lowest in the autumn or early winter and increased to a maximum in the late winter or spring, when seasonal peaks in NO$_3^-$ concentration are normally observed. At Scoat Tarn both minimum and maximum atmospheric contributions occurred later in the lake outflow relative to inflow streams. Comparisons with rainfall data from the bulk deposition collectors (supplementary Fig. S3) show that while fewer high rainfall events occurred in summer there was no real pattern in rainfall seasonality corresponding with seasonal atmospheric contributions to surface waters.

Discussion

Consideration of deposition input fluxes and leaching fluxes alone suggests that the four study catchments leached varying proportions of bulk deposited N; from 57% at the Afon Gwy to 156% at the River Etherow as a proportion of bulk deposited NO$_3^-$, or from 26% at the Afon Gwy to 71% at the River Etherow when expressed as a proportion of total inorganic N (NH$_4^+$ + NO$_3^-$) in bulk deposition.
While surface water NO$_3^-$ concentrations were comparable to those in bulk deposition at three of the four study sites, the dual isotope data indicated that most of the NO$_3^-$ reaching surface waters had been microbially cycled and was not simply atmospheric NO$_3^-$ transported hydrologically through catchments into surface waters. These results correspond with previous studies using the same technique in North America (summarized in Curtis et al. 2011). This study also confirms the findings of previous authors that the $\delta^{18}O$ signature of NO$_3^-$ is much more useful than $\delta^{15}N$ for separating atmospheric and microbial sources of surface water NO$_3^-$ in upland catchments where there are no other important inputs of inorganic N (Kendall 1998; Hales et al. 2004; Piatek et al. 2005). A major implication of this study is that where microbially produced NO$_3^-$ is found, it may originate from both atmospheric NO$_3^-$ and NH$_4^+$ deposition which contribute to the overall biological N pool (cf. Durka et al. 1994; Campbell et al. 2002), and which were of very similar magnitude and significantly related in the sites studied here (Fig. 2). Nitrification is thus a key process controlling NO$_3^-$ concentrations in upland lakes and streams. Excess NH$_4^+$ availability not only promotes nitrification (for which it is the substrate) but may inhibit NO$_3^-$ immobilisation, either indirectly through preferential utilization of NH$_4^+$ by plants and microbes, or directly (Bradley 2001; Rennenberg and Gessler 1999). Nevertheless, the $\delta^{18}O$-NO$_3^-$ data showed that even if NO$_3^-$ immobilisation was reduced by NH$_4^+$ availability, cycling of NO$_3^-$ must have occurred at one or more locations within the soil-water continuum to drastically alter the $\delta^{18}O$ values of deposited NO$_3^-$.

$\delta^{15}N$-NO$_3^-$ and nitrogen biogeochemistry
The δ15N values of surface water NO3⁻ tended to be slightly higher than those of bulk deposition NO3⁻. While the δ15N value for bulk deposition NH4⁺ was not measured in this study, δ15N values of NH4⁺ in atmospheric deposition tend to be lower than those of NO3⁻ (Heaton et al. 1997) so a possible NH4⁺ deposition source for N in NO3⁻ produced by nitrification is unlikely to account for the elevated δ15N-NO3⁻ in surface waters. Instead, the predominant control on the δ15N value of the microbial NO3⁻ is more likely to be the large pool of soil organic N. Although the primary inputs of N to soils in uncultivated areas (atmospheric deposition and N fixation) both have δ15N values close to 0‰, soil δ15N values are commonly higher than this: up to +10‰ or more in some environments (Kendall 1998). The reasons for this are the subject of debate, but largely focus on the fact that isotope fractionation associated with processes which remove N from the soil (assimilation by plants, leaching losses of organic and inorganic N, denitrification and volatilization) tends to favour loss of 14N, leaving residual soil N enriched in 15N (Handley et al. 1999; Amundson et al. 2003; Kramer et al. 2003; Inglett et al. 2007; Conen et al. 2008). As a result, soils saturated with N, and more ‘open’ or ‘leaky’ with respect to N loss, may in time develop high soil total N δ15N values (Handley et al. 1999; Amundson et al. 2003; Inglett et al. 2007). This may explain why the largest difference between the δ15N values of NO3⁻ in surface water and atmospheric deposition are found in the River Etherow catchment, where the high concentrations of NO3⁻ in surface water may reflect a greater degree of soil N saturation. The abundance of peat in the Etherow catchment could also be relevant: 15N preferentially accumulates during humification of soil organic matter (Kramer et al. 2003; Conen et al. 2008), so that mineralisation of degraded peat might produce NO3⁻ with elevated δ15N.
\( \delta^{18}O-\text{NO}_3^- \) and nitrogen biogeochemistry

Mean bulk deposition \( \delta^{18}O-\text{NO}_3^- \) values from all sites were very similar (+66.5 to +69.6‰) and fell within the ranges published from studies elsewhere (e.g. Kendall et al. 2007; Granger et al. 2008). The two end-member mixing model (Equation 2) showed that at all sites in this study a large proportion of leached \( \text{NO}_3^- \), which had much smaller \( \delta^{18}O-\text{NO}_3^- \) values than deposition, was therefore microbially produced by nitrification (monthly range 68-100%, annual mean 79-98%).

At the River Etherow site, this result appears to contradict a previous study on nitrification potentials in catchment soils, which were very small in the highly acidic upper horizons of the degraded peat soils at this site (Curtis et al. 2004). \( \text{NO}_3^- \) production at the Etherow must therefore be occurring elsewhere within the catchment, either deeper in the soil profile, in microbial hotspots (e.g. riparian zones) or perhaps in-stream (Curtis et al. 2011). At the two other sites common to the previous study (Afon Gwy, Scoat Tarn) much higher nitrification potentials were found in catchment soils, which is consistent with the isotope results reported here showing the dominance of microbially produced \( \text{NO}_3^- \).

The relatively high proportion of atmospheric \( \text{NO}_3^- \) found in soilwater lysimeters (up to 41% at the Etherow and 69% at Lochnagar) supports the idea that progressive transformation may occur deeper in the soil profile or in-stream, particularly at the River Etherow where there is little evidence of atmospheric \( \text{NO}_3^- \) in surface waters. Other studies have also found a high proportion of atmospheric \( \text{NO}_3^- \) in lysimeters.
(e.g. Sebestyen et al. 2008) while increasingly “microbial” $\delta^{18}O$ values with soil depth were reported by Ohte et al. (2004).

The results presented here are consistent with the NO$_3^-$ flushing hypothesis (e.g. Williard et al. 2001) whereby a large proportion of the NO$_3^-$ observed in surface waters during rainfall events has been displaced from soil waters where it was produced by microbial nitrification. The relative importance of different hydrological flowpaths is key to determining the proportion of untransformed atmospheric NO$_3^-$ reaching surface waters, e.g. by overland flow or through preferential flowpaths (cf. Curtis et al. 2005b, 2011). A tracer $^{15}$NO$_3^-$ addition study at the Afon Gwy (Evans et al. 2008) showed however that NO$_3^-$ immobilisation could occur within hours along preferential flowpaths, implying that there is potential for microbial cycling (and thus isotopic transformation) of atmospheric NO$_3^-$ even in water passing quite quickly through the catchment. This could be considered analogous to the ‘nutrient spiralling’ concept described for streams (Curtis et al. 2011).

Microbial nitrification model

The inverse relationship between $\delta^{15}N$ and $\delta^{18}O$ values of surface waters at a number of sites provides additional evidence that greater leaching of untransformed atmospheric NO$_3^-$ is related to both higher $\delta^{18}O$ and lower $\delta^{15}N$ values, i.e. is consistent with the simple two end-member mixing model. As noted in the Methods section, our mixing model assumed a theoretical $\delta^{18}O$ value for the microbial NO$_3^-$ end-member, calculated assuming stoichiometry in which NO$_3^-$ derives one O atom from atmospheric O$_2$, and two O atoms (i.e. 67%) from water (H$_2$O). Experiments on soils incubated with $^{18}$O-labeled H$_2$O have sometimes suggested a very different
relationship, with the proportion of O derived from H₂O ranging from extremes of 32% for possible heterotrophic nitrification (Mayer et al. 2001), up to 96% in systems subject to significant O exchange with nitrite (Snider et al. 2010). However, the general applicability of laboratory experiments, and prevalence of high nitrite concentrations in natural systems may be questioned (Snider et al. 2010). For our waters, moreover, calculations assuming 32% O or 96% O derived from H₂O would yield theoretical δ¹⁸O values for the microbial NO₃⁻ of +14 to +16‰ or -7 to -3‰, respectively. If the former range was applicable we would not be able to explain the measured δ¹⁸O-NO₃⁻ of the great majority of our surface waters, whose values are much lower than this. If the latter range was applicable it would imply that all of our waters had a minimum of 10% atmospheric nitrate (i.e. none were purely microbial nitrate), a feature we consider highly unlikely. In contrast, the assumption that microbial NO₃⁻ derives 67% (two-thirds) of its O from water would yield theoretical δ¹⁸O values of +2 to +5‰, which exactly correspond to the lowest end of the measured range of δ¹⁸O-NO₃⁻ values in our surface waters. Being mindful of the uncertainties involved, we therefore calculated the δ¹⁸O value of our microbial NO₃⁻ from Equation 1.

Between site differences in streamwater δ¹⁸O-NO₃⁻

While comparison of data from lake and stream sites must consider residence times in lakes and direct atmospheric deposition to lake surfaces (see below), differences in the transport of atmospheric NO₃⁻ to streams may be assessed using inflow data for the lake sites to compare with the stream catchments, i.e. comparing streams in each catchment (Fig. 7). Values of δ¹⁸O-NO₃⁻ were significantly lower in Rose Clough and Swan Clough than all other streams including the River Etherow (p<0.05), into which
they flow. Scoat Inflow 1 had a significantly greater δ¹⁸O-NO₃⁻ than the River Etherow. No other significant differences were found. The greatest proportions of atmospheric NO₃⁻ were found in streams at Lochnagar (13%), then Scoat Tarn (Inflow 1 = 11%, Inflow 2 = 9%) and the main channel of the Etherow (6%). The mean proportion was only 2-3% in the Afon Gwy and the Etherow tributaries, Rose Clough and Swan Clough.

While the differences between sites are not significant, the higher proportions of atmospheric NO₃⁻ leaching are associated with the highest altitude, steepest catchments of Lochnagar and Scoat Tarn (Table 1). Lochnagar also has a particularly high proportion of bare rock compared with the other catchments. The streamwater data are therefore consistent with previous studies showing a greater amount of hydrological bypass transportation and/or lower microbial immobilisation of atmospheric NO₃⁻ associated with these physical catchment attributes (e.g. Evans et al. 2004, 2006; Helliwell et al. 2007).

**Importance of streamflow and hydrological flowpaths**

Very detailed isotopic studies in gauged catchments in North America have demonstrated the importance of stream flow conditions and the timing of sampling relative to baseflow and extreme flow events in affecting the proportion of atmospheric NO₃⁻ in surface waters (e.g. Sebestyen et al. 2008, 2009). In particular, several studies in snowmelt dominated systems have shown that a relatively large proportion of annual NO₃⁻ fluxes are transported during very high flow events e.g. during snowmelt (Campbell et al. 2002; Schiff et al. 2002; Ohte et al. 2004; Pardo et al. 2004; Sebestyen et al. 2008, 2009; Goodale et al. 2009), and at these times the
proportion of atmospheric NO$_3^-$ may be elevated. Sampling regimes which do not include these high flow events may therefore underestimate both total NO$_3^-$ fluxes and the proportion of untransformed atmospheric NO$_3^-$ exported from catchments.

In our study, only one of the four catchments (Afon Gwy) was instrumented for flow measurement throughout the study period. At the Afon Gwy, flow was measured every 15 minutes and streamwater sampled weekly. The flows sampled during the monthly isotope sampling programme cover a wide range of the mean flows observed on a daily basis but do not represent the most extreme conditions (see supplementary Fig. S1). However, a sample from 11$^{th}$ November 2004 was taken during very high flow (only 3% of daily mean flows were higher over the sampling year) and yet yielded insufficient NO$_3^-$ for dual isotope analysis. Furthermore, high NO$_3^-$ concentrations were seen under both high and low flow conditions (Fig. S2). Rainfall data for the study catchments show that high rainfall may occur throughout the year and does not appear to account for seasonal variations in the proportion of atmospheric NO$_3^-$ (Fig. S3). At Scoat Tarn, the highest rainfall recorded for a two-week period during the study (20.5mm per day for sample dated 15$^{th}$ November 2005) corresponded with the lowest proportion of atmospheric NO$_3^-$ recorded for the site (Table S4).

Therefore, while our monthly sampling regime could not capture extreme flow events and may therefore underestimate annual mean NO$_3^-$ fluxes, there is no evidence from the highest resolution data at the Afon Gwy that any underestimate of either NO$_3^-$ fluxes or the contribution of atmospheric NO$_3^-$ is likely to be large. Indeed, the high-resolution event-based study of Sebestyen (2009) found that although atmospheric
contributions to streamwater NO$_3^-$ could increase greatly during high flow events, the
effect of this increase on total annual contributions was small. Hence while our study
cannot provide detailed information about atmospheric contributions to NO$_3^-$ leaching
fluxes on an event basis or during extremes of flow, it does provide robust estimates
of the importance of direct atmospheric NO$_3^-$ leaching on an average annual basis.

Finally, the issue of short-lived, very high flow events is less important for lakes with
long residence times compared to streams. The average residence time of water in
Lochnagar is 242 days (Jenkins et al. 2007) while at Scoat Tarn it is 49 days. Hence
lakes are ideal integrators of both varying bulk deposition inputs and flow conditions
in inflow streams and show a damped temporal pattern in both NO$_3^-$ concentrations
and atmospheric contributions relative to streams (Fig. 6).

Lochnagar is the most snow-dominated site in our study at the highest altitude, with
approximately 20% of precipitation falling as snow in an average year (Jenkins et al.
2007). Periods of significant snowmelt usually occur in late April/early May but short
periods of extremely high flow (1-2 days) occur throughout the year (Jenkins et al.
2007). This study does indeed show that both NO$_3^-$ concentrations in the lake and the
greatest proportion of atmospheric NO$_3^-$ occur at this time of year, i.e. the monthly
sampling regime has successfully captured this seasonal pattern. It must however be
recognized that our data could underestimate the total contribution of directly leached
atmospheric NO$_3^-$ and especially the maximum contributions which may be attained
during extreme hydrological events.
Lakes versus streams

It might be expected that a higher proportion of untransformed atmospheric NO$_3^-$ would be found in lakes relative to streams because of direct deposition to lake surfaces, bypassing the terrestrial processing of deposition inputs. At the two lake sites in this study, Scoat Tarn and Lochnagar, both lake outflows and major inflow streams were sampled at the same time. Mean values of $\delta^{15}$N were slightly lower in lake outflows relative to inflow streams (Fig. 5). Values of $\delta^{18}$O-NO$_3^-$ were significantly higher in Scoat Tarn (mean= +12.6‰) than in Inflow 2 (mean= +9.0‰, Fig. 8: t=3.31, p=0.009) and while also higher than in Inflow 1 (+10.3‰) on average, this difference was not significant (t=2.35, p=0.095). At Lochnagar $\delta^{18}$O-NO$_3^-$ was significantly higher in the loch outflow (mean= +16.7‰) than in the inflow spring (mean = +10.9‰, t=3.36, p=0.005).

These data provide isotopic evidence that there was indeed a greater proportion of untransformed atmospheric NO$_3^-$ in the two lakes compared with their inflow streams, presumably due to direct deposition to the lake surface. For Scoat Tarn the annual mean proportion of atmospheric NO$_3^-$ in the lake was 15% compared with 11% in inflow 1 and 9% in inflow 2, i.e. was greater by 4-6% in the lake outflow. The surface area of Scoat Tarn is 4.3 ha and represents 5% of the total catchment area. Likewise, at Lochnagar the mean proportion of atmospheric NO$_3^-$ in the loch was 21% compared with 13% in the inflow spring i.e. 8% greater in the outflow, while the loch has a surface area of 9.9 ha representing 9% of total catchment area. These figures are remarkably consistent with the idea that direct deposition to lake surfaces contributes to catchment scale leaching of untransformed atmospheric NO$_3^-$ in direct proportion to
lake:catchment area ratios. There appears to be relatively little cycling of directly
deposited NO$_3^-$ within the lakes themselves since all of the directly deposited NO$_3^-$ is
recovered in the lake outflows. An alternative hypothesis is that the greater proportion
of untransformed NO$_3^-$ in lake outflows relative to their inflow streams simply reflects
elevated, event-based inputs of atmospheric NO$_3^-$ in streams which are not captured in
the monthly streamwater sampling but which increase the overall proportion of
atmospheric NO$_3^-$ in the receiving lakes. Further work on a greater number of lakes
and associated inflows would be required to test these hypotheses.

While catchment hydrology is a key factor determining the delivery of untransformed
atmospheric NO$_3^-$ to upland streams, the surface area of upland lakes relative to their
catchments is also an important factor controlling their exposure to direct inputs of
atmospheric NO$_3^-$.

Lake:catchment ratio must therefore also be an important
determinant of exposure to other pollutants subject to terrestrial retention and
processing, e.g. NH$_4^+$ deposition.

Although microbially produced NO$_3^-$ dominates in the four sites studied here, the
persistent contribution of atmospheric NO$_3^-$ all year round in lakes and especially
inflow streams indicates a chronic atmospheric NO$_3^-$ leaching problem which has
seldom been seen in other isotopic studies, where streamwater $\delta^{18}$O-NO$_3^-$ falls within
the microbial range for much of the year (e.g. Piatek et al. 2005; Campbell et al. 2006;
Hales et al. 2007; Barnes et al. 2008; Sebestyen et al. 2008; Burns et al. 2009;
Goodale et al. 2009). Notable exceptions showing persistent contributions of
atmospheric NO$_3^-$ (though still predominantly microbial) through most of the year
include the studies of Spoelstra et al. (2001) in forested catchments in the Turkey
Lakes Watershed, Canada (10-15% atmospheric) and Campbell et al. (2002) in alpine watersheds in the Rocky Mountains (up to 50% atmospheric during snowmelt, but much less at other times - and these catchments had >80% bare rock). Hence the results of the present study show a greater (and more persistent) atmospheric contribution to \(\text{NO}_3^-\) leaching in terms of annual fluxes than most other isotopic studies.

Conclusions

The major proportion of \(\text{NO}_3^-\) observed in surface waters in upland catchments, for which atmospheric deposition is the only source of anthropogenic N inputs, is derived from microbial production. While mindful of the uncertainty in knowing the precise \(\delta^{18}O\) value for microbial \(\text{NO}_3^-\), our best estimates for late winter / early spring peak contributions of untransformed atmospheric \(\text{NO}_3^-\) at the four study sites ranged from 5% to around 30%, but annual means varied from just 2-13% for streams and 15-21% for lakes. Crucially, this means that deposition of reduced N compounds cannot be ignored as a possible source of the N leached as \(\text{NO}_3^-\), since only a small proportion of surface water \(\text{NO}_3^-\) derives directly from \(\text{NO}_3^-\) in bulk deposition. For upland streams, other studies have shown that catchment hydrology and linked physical attributes such as slope, bare rock and amount of organic matter in soils, are key determinants of the delivery of atmospheric \(\text{NO}_3^-\) to surface waters, while we show here that for lakes an important additional source is direct deposition to lake surfaces. In the two lakes studied here, all the directly deposited \(\text{NO}_3^-\) appeared to be recovered from the outflows. Microbial \(\text{NO}_3^-\) production is however the major overall determinant of surface water \(\text{NO}_3^-\) concentrations in upland catchments, and hence future trends in
NO$_3^-$ leaching will be intimately linked with global change impacts on microbial macronutrient cycling.

While the greatest proportion of untransformed atmospheric NO$_3^-$ was found in the headwater lakes, the greatest leaching flux of NO$_3^-$ in both absolute terms and as a proportion of deposition inputs was found at the River Etherow, which has the lowest altitude, gentlest slopes and greatest proportion of organic soils. Hence, there is a clear distinction to be made between the physical attributes of a site which may allow direct leaching of atmospheric inputs to surface waters, and the nitrogen saturation status of a site. In the case of the River Etherow, the catchment is a net source of NO$_3^-$ despite very high NO$_3^-$ concentrations in deposition and has negligible scope for direct leaching of deposition inputs without biological cycling.

Acknowledgements

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Figure 1: Location, catchment outlines and sampled subcatchments streams at a) Scoat Tarn, b) Lochnagar, c) Afon Gwy and d) River Etherow. Subcatchments of sampled streams are shaded. Contour intervals in metres above sea level.
Figure 2: Inorganic nitrogen concentrations in bulk deposition (NH$_4^+$, NO$_3^-$) and surface waters (NO$_3^-$)
Figure 3: Box and whisker plot of $\delta^{15}$N-NO$_3^-$ in bulk deposition at the four study sites. Boxes represent median and inter-quartile range, whiskers extend to $1.5 \times$ inter-quartile range and individual points represent data outside this range.
Figure 4: Box and whisker plot of $\delta^{18}$O-NO$_3^-$ in bulk deposition at the four study sites (see Fig. 3 for explanation)
Figure 5: Results of dual isotope analysis ($\delta^{15}$N vs SMOW and $\delta^{18}$O vs Air, ‰) of NO$_3^-$ in surface water, soilwater and bulk deposition samples
Figure 6: Seasonal variation in contribution of atmospheric NO$_3^-$ to surface waters
Figure 7: Box and whisker plot of δ¹⁸O-NO₃⁻ in streamwaters at the four study catchments (see Fig. 3 for explanation)
Figure 8: Box and whisker plot of $\delta^{18}$O-NO$_3^-$ in bulk deposition, inflow streams and lake outflows at a) Scoat Tarn and b) Lochnagar (see Fig. 3 for explanation)
Table 1: Description of study sites with selected long-term mean surface water chemistry, April 1998 – March 2006 (from Monteith and Shilland 2007) and bulk deposition chemistry (1999-2006; source – Lawrence et al. 2008)

<table>
<thead>
<tr>
<th>Site:</th>
<th>Afon Gwy</th>
<th>River Etherow</th>
<th>Scoat Tarn</th>
<th>Lochnagar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling location</td>
<td>Flume</td>
<td>Main channel</td>
<td>Outflow</td>
<td>Outflow</td>
</tr>
<tr>
<td>OS grid Ref</td>
<td>SN82400, 85350</td>
<td>SK11557, 99691</td>
<td>NY15819, 10344</td>
<td>NO25317, 86268</td>
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<td>Altitude (m)</td>
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<td>280</td>
<td>595</td>
<td>788</td>
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<tr>
<td>Max. altitude</td>
<td>741</td>
<td>633</td>
<td>841</td>
<td>1155</td>
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<td>Catchment area (ha)</td>
<td>389</td>
<td>1295</td>
<td>87</td>
<td>109</td>
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<tr>
<td>Bare ground</td>
<td>&lt;2%</td>
<td>4%</td>
<td>&lt;2%</td>
<td>24%</td>
</tr>
<tr>
<td>Sampled inflows / tributaries</td>
<td>LB7a: SN81450, 86500</td>
<td>Rose Clough: SK12181, 99532</td>
<td>1 (Scoat Fell): NY15866, 10423</td>
<td>Spring: NO25279, 85770</td>
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<tr>
<td>Rainfall (mm)</td>
<td>2021</td>
<td>1004</td>
<td>2313</td>
<td>1279</td>
</tr>
<tr>
<td>Concentration (µeq l⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₃⁻ (water body)</td>
<td>6.9</td>
<td>42.0</td>
<td>15.5</td>
<td>18.5</td>
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<tr>
<td>NO₃⁻ (bulk depn.)</td>
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<td>28.3</td>
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<td>NH₄⁺ (bulk depn.)</td>
<td>11.8</td>
<td>31.8</td>
<td>18.3</td>
<td>17.0</td>
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<tr>
<td>Deposition flux (kgN ha⁻¹ yr⁻¹)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>NO₃⁻</td>
<td>2.7</td>
<td>3.9</td>
<td>4.8</td>
<td>3.8</td>
</tr>
<tr>
<td>DIN (NO₃⁻ + NH₄⁺)</td>
<td>6.0</td>
<td>8.4</td>
<td>10.6</td>
<td>6.7</td>
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</table>
Table 2: Mean concentrations (µeq l⁻¹) and fluxes (kgN ha⁻¹ yr⁻¹) of N species in surface waters and bulk deposition. Figures in parenthese indicate flow-weighting (surface waters) or volume weighting (bulk deposition). No flow data were available at the River Etherow or Scoat Tarn. NH₄⁺ is negligible in surface waters. See text for further details.

<table>
<thead>
<tr>
<th>Site</th>
<th>Sampling Stream / Rainfall ET NO₃⁻ -</th>
<th>Bulk deposition concentration</th>
<th>Deposition flux</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Period</td>
<td>(mm)</td>
<td>(%)</td>
</tr>
<tr>
<td>Afon Gwy</td>
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<td>15</td>
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<td>19</td>
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<td>Lochnagar</td>
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<td>1099</td>
<td>20</td>
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