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 (NO_3^-) 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, $^{15}N/^{14}N$ and $^{18}O/^{16}O$, (the "dual isotope" technique) have previously been used in biogeochemical studies of alpine and forested ecosystems to demonstrate that most of the NO_3^- 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 NO_3^- concentrations of bulk deposition and surface waters at three sites are very similar. While noting the constraints imposed by uncertainty in the precise $\delta^{18}O$ value for microbial NO_3^-, however, we estimate that 79-98% of the annual mean NO_3^- has been microbially produced. Direct leaching of atmospheric NO_3^- is a minor component of catchment NO_3^- export, although greater than in many similar studies in forested watersheds. A greater proportion of atmospheric NO_3^- is seen in the two lake sites relative to their inflow streams, demonstrating the importance of direct NO_3^- 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 NO_3^- 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 NO_3^- leaching at the Afon Gwy, high NO_3^- leaching at Scoat Tarn and Lochnagar and extremely high NO_3^- (for a semi-natural upland site) in the River Etherow (Table 1). These patterns reflect NO_3^- concentrations in bulk deposition, with comparable concentrations between surface waters and bulk deposition at all sites except the River Etherow, where mean NO_3^- 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 $\delta^{15}N$ values versus air (atmospheric N_2) and $\delta^{18}O$ 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 $\delta^{18}O$ 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 qualitical 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 $\delta^{18}O-H_2O$. 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_2O, which is measured directly (Amberger and Schmidt 1987; Kendall 1998):

$$\delta^{18}O\text{-}NO_3^- = (2/3\ \delta^{18}O\text{-}H_2O\ \text{soilwater}) + (1/3\ \delta^{18}O\text{-}O_2\ \text{atmosphere}) \qquad (1)$$

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_2O 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

(Table 2). 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 $\delta^{15}N\text{-}NO_3^-$ 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 $\delta^{15}N\text{-}NO_3^-$ 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 $\delta^{15}N\text{-}NO_3^-$ 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 $\delta^{15}N\text{-}NO_3^-$ values in surface waters largely overlapped with that in atmospheric deposition. The exception was the River Etherow, where streamwater $\delta^{15}N\text{-}NO_3^-$ 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 $\delta^{15}N\text{-}NO_3^-$ 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 δ^{18}O-H_2O 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 δ^{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 δ^{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 δ^{18}O-NO_3^- (in each case referring to δ^{18}O-NO_3^-):

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

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 δ^{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 $\delta^{15}N$ values of surface water NO_3^- tended to be slightly higher than those of bulk deposition NO_3^-. While the $\delta^{15}N$ value for bulk deposition NH_4^+ was not measured in this study, $\delta^{15}N$ values of NH_4^+ in atmospheric deposition tend to be lower than those of NO_3^- (Heaton et al. 1997) so a possible NH_4^+ deposition source for N in NO_3^- produced by nitrification is unlikely to account for the elevated $\delta^{15}N$-NO_3^- in surface waters. Instead, the predominant control on the $\delta^{15}N$ value of the microbial NO_3^- 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 $\delta^{15}N$ values close to 0‰, soil $\delta^{15}N$ 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 ^{14}N, leaving residual soil N enriched in ^{15}N (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 $\delta^{15}N$ values (Handley et al. 1999; Amundson et al. 2003; Inglett et al. 2007). This may explain why the largest difference between the $\delta^{15}N$ values of NO_3^- in surface water and atmospheric deposition are found in the River Etherow catchment, where the high concentrations of NO_3^- in surface water may reflect a greater degree of soil N saturation. The abundance of peat in the Etherow catchment could also be relevant: ^{15}N preferentially accumulates during humification of soil organic matter (Kramer et al. 2003; Conen et al. 2008), so that mineralisation of degraded peat might produce NO_3^- with elevated $\delta^{15}N$.

δ^{18}O-NO_3^- and nitrogen biogeochemistry

Mean bulk deposition δ^{18}O-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 NO_3^-, which had much smaller δ^{18}O-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). 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 NO_3^-.

The relatively high proportion of atmospheric 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 NO_3^- in surface waters. Other studies have also found a high proportion of atmospheric 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_2O). Experiments on soils incubated with ^{18}O-labeled H_2O have sometimes suggested a very different relationship, with the proportion of O derived from H_2O 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 H_2O would yield theoretical $\delta^{18}O$ values for the microbial NO_3^- of +14 to +16‰ or -7 to -3‰, respectively. If the former range was applicable we would not be able to explain the measured $\delta^{18}O-NO_3^-$ 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 NO_3^- derives 67% (two-thirds) of its O from water would yield theoretical $\delta^{18}O$ values of +2 to +5‰, which exactly correspond to the lowest end of the measured range of $\delta^{18}O-NO_3^-$ values in our surface waters. Being mindful of the uncertainties involved, we therefore calculated the $\delta^{18}O$ value of our microbial NO_3^- from Equation 1.

Between site differences in streamwater $\delta^{18}O-NO_3^-$

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 NO_3^- 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 $\delta^{18}O-NO_3^-$ 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 $\delta^{18}O-NO_3^-$ than the River Etherow. No other significant differences were found. The greatest proportions of atmospheric NO_3^- 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% 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 NO_3^- 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 NO_3^- 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 NO_3^- 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 NO_3^- 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 11th 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 15th 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 NO_3^- leaching in terms of annual fluxes than most other isotopic studies.

Conclusions

The major proportion of 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 NO_3^-, our best estimates for late winter / early spring peak contributions of untransformed atmospheric 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 NO_3^-, since only a small proportion of surface water NO_3^- derives directly from 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 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 NO_3^- appeared to be recovered from the outflows. Microbial NO_3^- production is however the major overall determinant of surface water 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.

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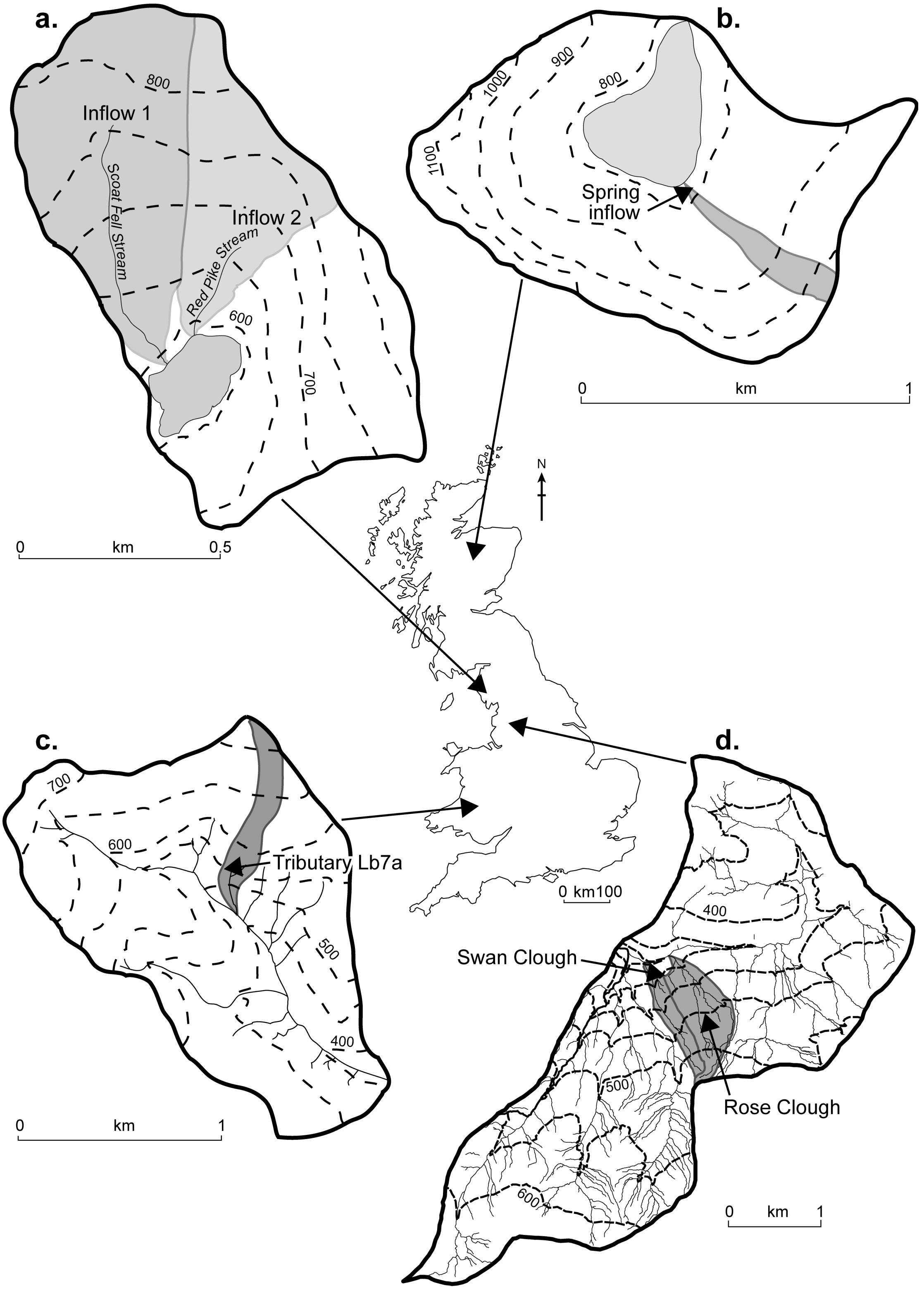


Figure 1: Location, catchment outlines and sampled subcatchments streams at a) Scoat Tarn, b) Lochnagar, c) Afon Gwy and d) River Etherow. Subcatchments of sampled streams are shaded. Contour intervals in metres above sea level.

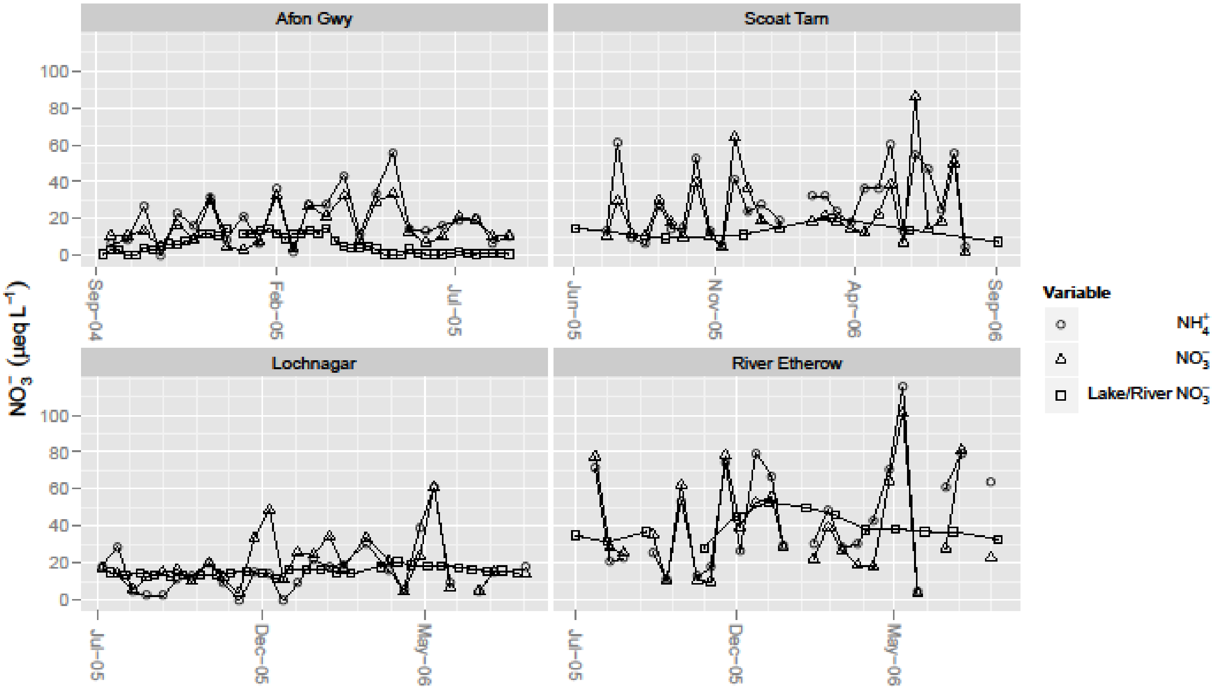


Figure 2: Inorganic nitrogen concentrations in bulk deposition (NH_4^+, NO_3^-) and surface waters (NO_3^-)

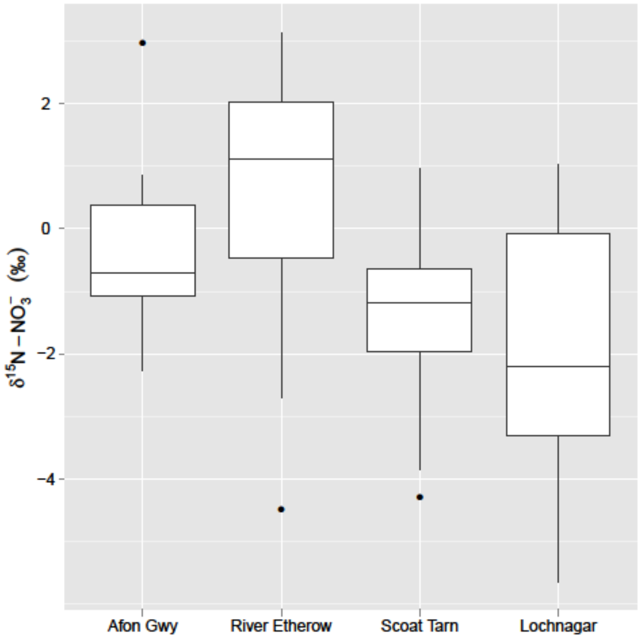


Figure 3: Box and whisker plot of δ^{15}N-NO$_3^-$ in bulk deposition at the four study sites. Boxes represent median and inter-quartile range, whiskers extend to 1.5 × inter-quartile range and individual points represent data outside this range

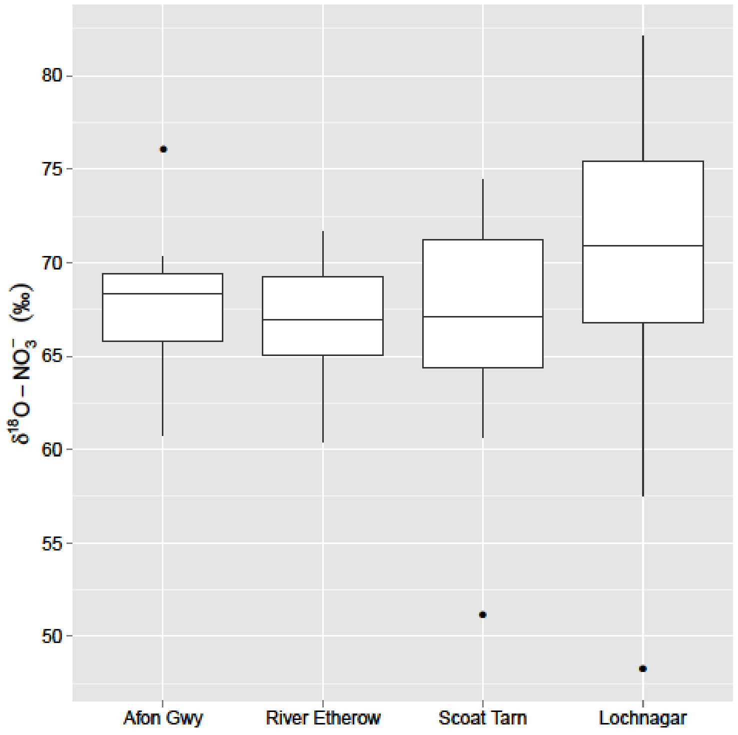
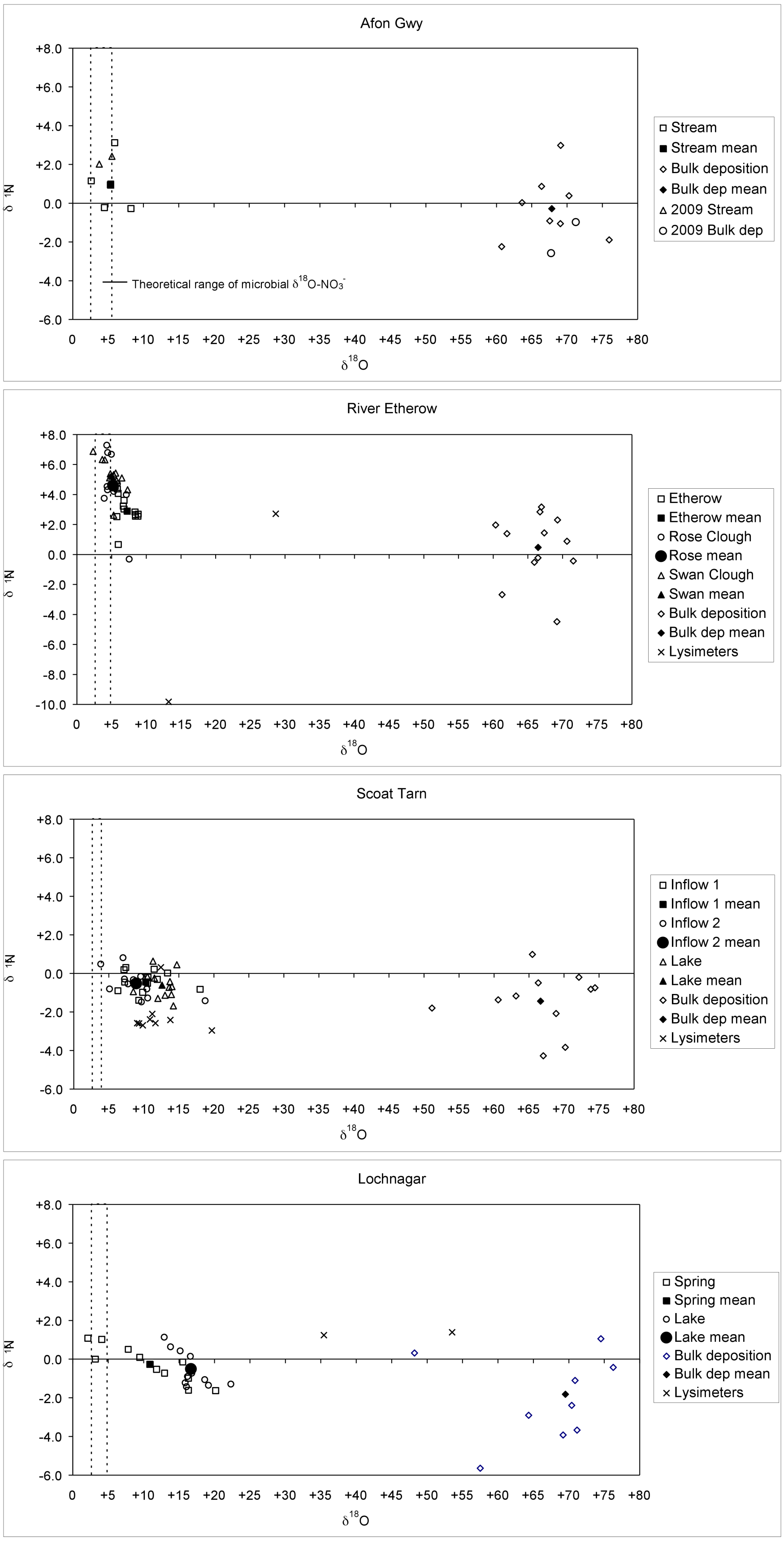


Figure 4: Box and whisker plot of $\delta^{18}O$-NO_3^- in bulk deposition at the four study sites (see Fig. 3 for explanation)

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



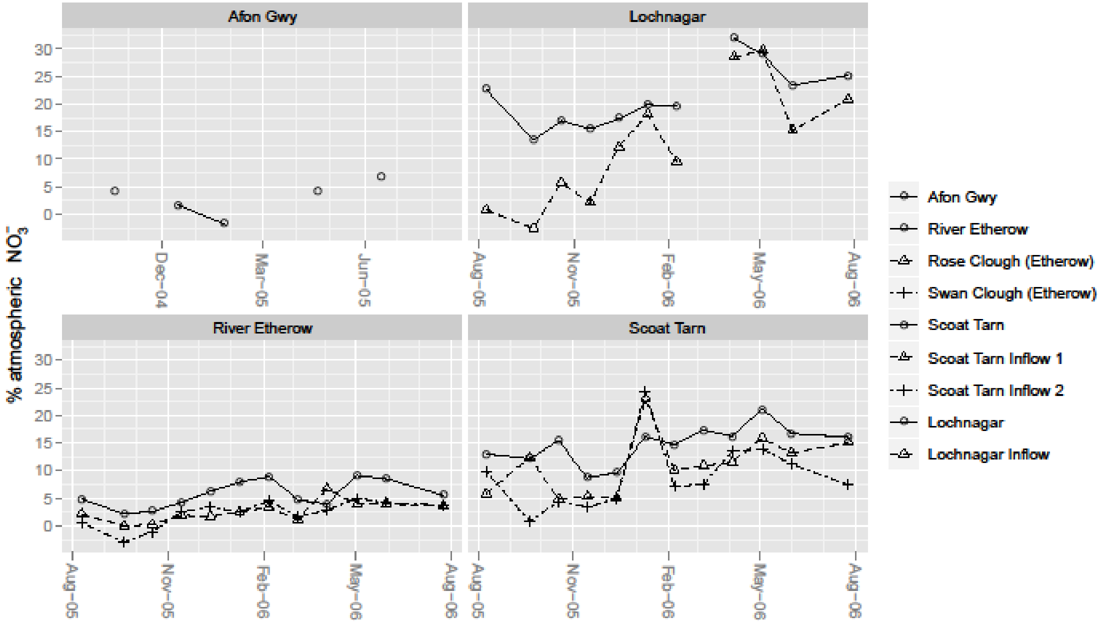


Figure 6: Seasonal variation in contribution of atmospheric NO_3^- to surface waters

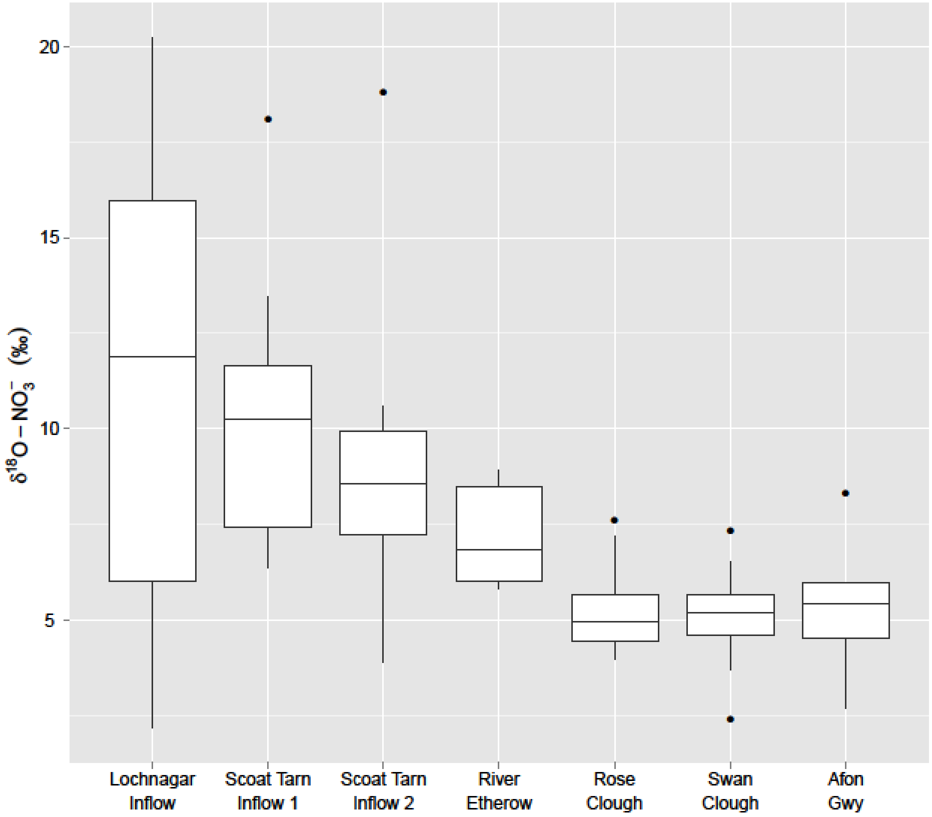


Figure 7: Box and whisker plot of δ^{18}O-NO_3^- in streamwaters at the four study catchments (see Fig. 3 for explanation)

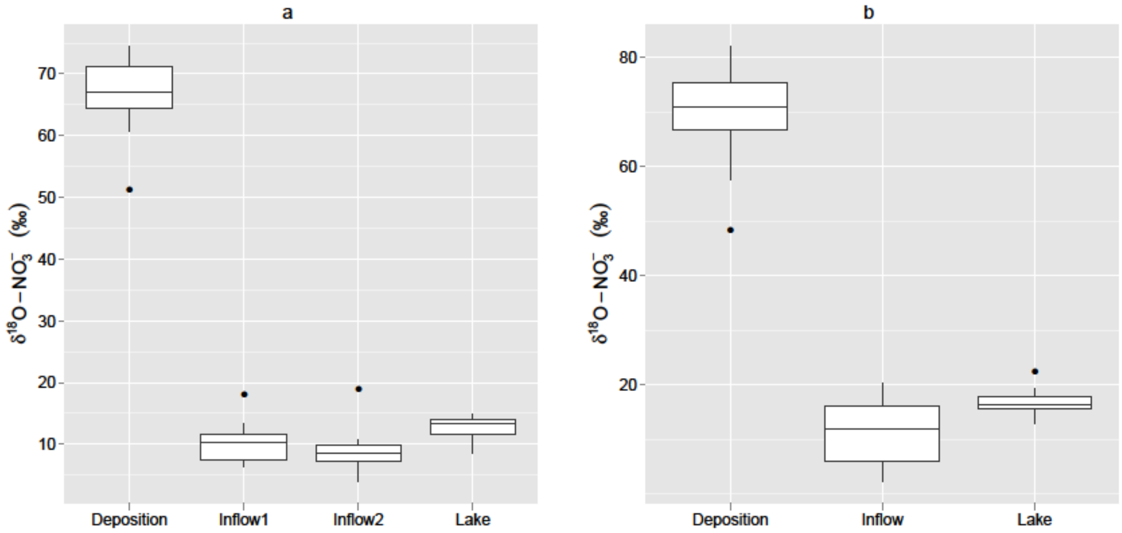


Figure 8: Box and whisker plot of $\delta^{18}O-NO_3^-$ in bulk deposition, inflow streams and lake outflows at a) Scoat Tarn and b) Lochnagar (see Fig. 3 for explanation)

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)

Site:	**Afon Gwy**	**River Etherow**	**Scoat Tarn**	**Lochnagar**
Sampling location	Flume	Main channel	Outflow	Outflow
OS grid Ref	SN82400, 85350	SK11557, 99691	NY15819, 10344	NO25317, 86268
Altitude (m)	385	280	595	788
Max. altitude	741	633	841	1155
Catchment area (ha)	389	1295	87	109
Bare ground	<2%	4%	<2%	24%
Sampled inflows / tributaries	LB7a: SN81450, 86500	Rose Clough: SK12181, 99532 Swan Clough: SK11909, 99453	1 (Scoat Fell): NY15866, 10423 2 (Red Pike): NY15935, 10503	Spring: NO25279, 85770
Rainfall (mm)	2021	1004	2313	1279
Concentration (μeq l^{-1})				
NO_3^- (water body)	6.9	42.0	15.5	18.5
NO_3^- (bulk depn.)	9.5	28.3	14.7	20.8
NH_4^+ (bulk depn.)	11.8	31.8	18.3	17.0
Deposition flux (kgN ha^{-1} yr^{-1})				
NO_3^-	2.7	3.9	4.8	3.8
DIN (NO_3^- + NH_4^+)	6.0	8.4	10.6	6.7

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. NH_4^+ is negligible in surface waters. See text for further details.

						Bulk deposition concentration			Deposition flux		
Site	**Sampling Period**	**Stream / lake NO_3^-**	**Rainfall (mm)**	**ET (%)**	**NO_3^- flux**	**NH_4^+**	**NO_3^-**	**DIN**	**NH_4^+**	**NO_3^-**	**DIN**
Afon Gwy	07/9/04- 06/9/05	6.0 (6.8)	2050	15	1.4	19.4 (12.7)	15.5 (10.1)	22.8	3.6	2.9	6.5
River Etherow	10/8/05- 08/8/06	39.6 (n/a)	984	29	3.9	45.2 (30.7)	37.8 (25.4)	56.1	4.2	3.5	7.7
Scoat Tarn	9/8/05- 04/8/06	13.8 (n/a)	2316	19	3.6	28.4 (21.1)	24.1 (15.4)	36.5	6.9	5.0	11.9
Lochnagar	10/8/05- 10/8/06	15.8 (15.2)	1099	20	2.0	15.3 (14.4)	20.4 (17.7)	32.1	2.2	2.8	5.0

Figure S1: Cumulative distribution functions for stream flow according to daily mean data and corresponding weekly or monthly sampling dates for water chemistry at the Afon Gwy

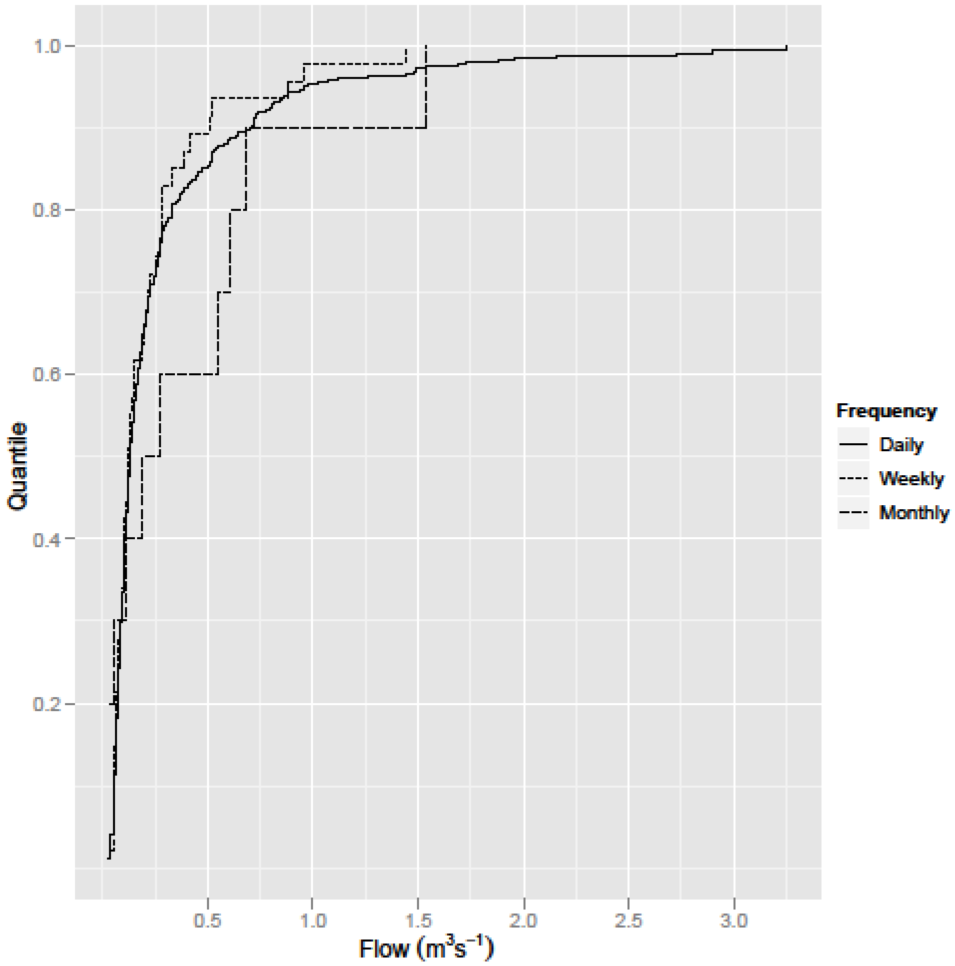


Figure S2: Nitrate concentration versus daily mean flow for weekly water chemistry samples at the Afon Gwy

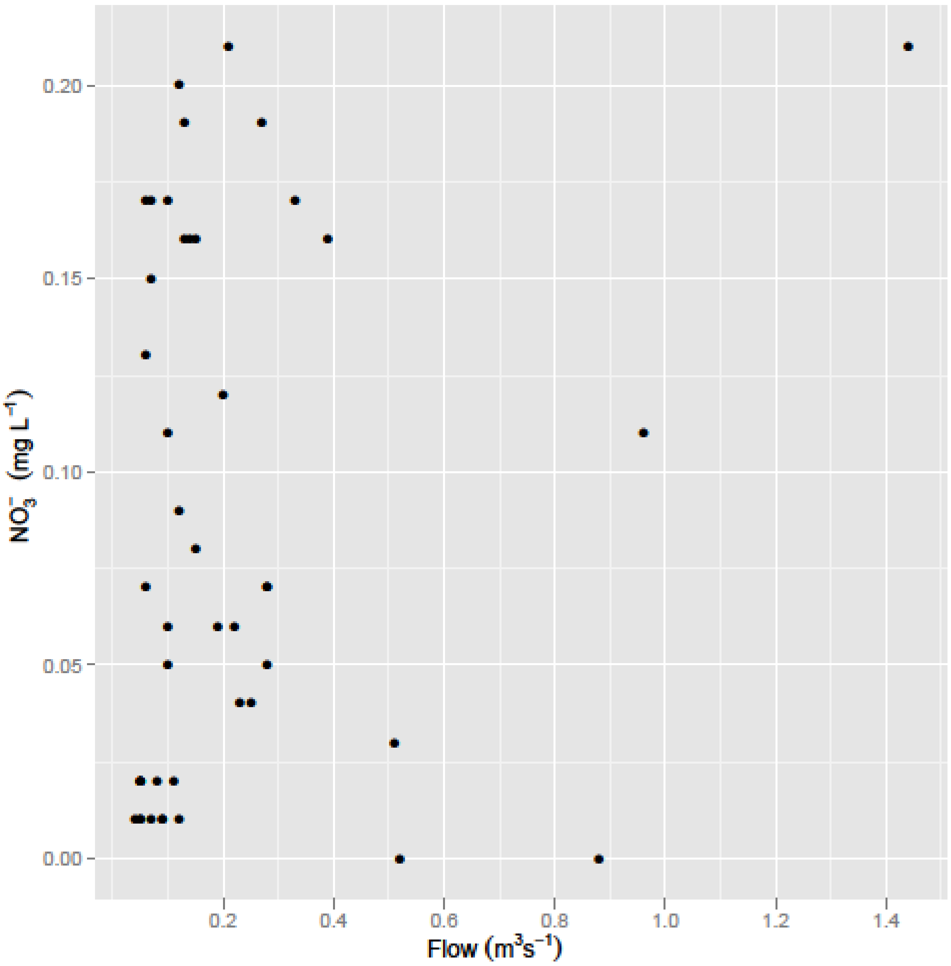


Figure S3: Rainfall patterns (expressed as mm per day for each 2-weekly sample) over the monitoring period at the four study catchments

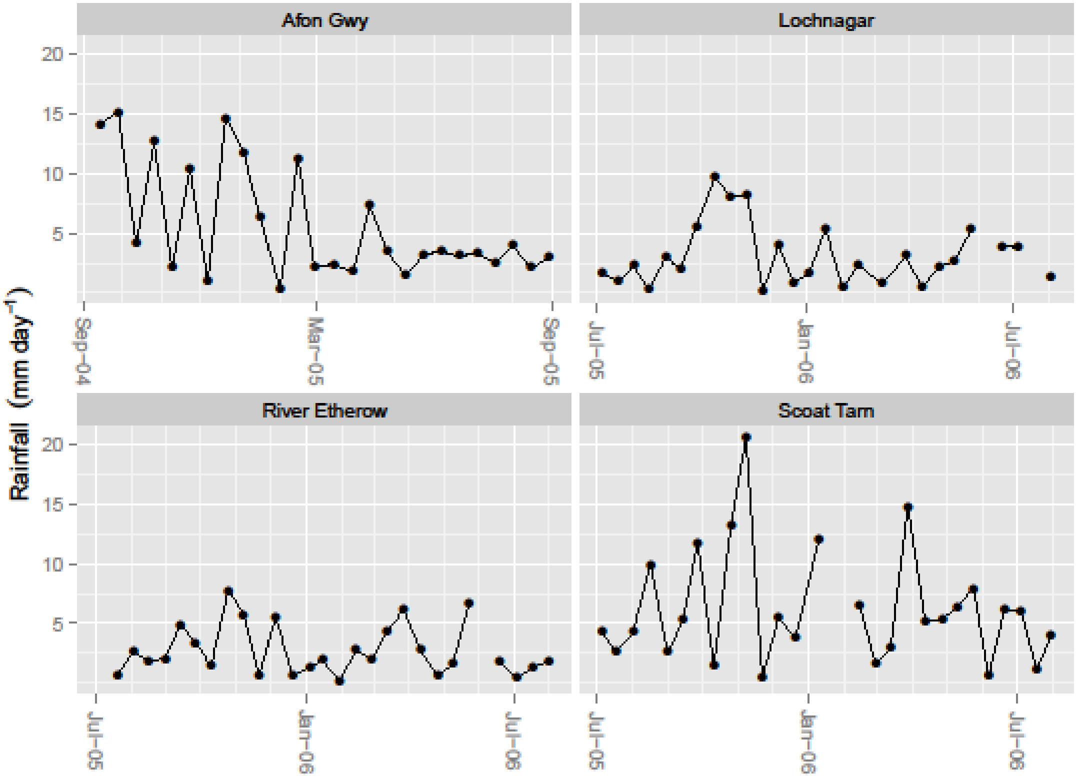


Table S1: Location of additional sampling points in each study catchment. Lysimeters and new bulk deposition collectors for isotope samples were installed as close as possible to ADMN collectors used for precipitation chemistry.

Site:	Afon Gwy	River Etherow	Scoat Tarn	Lochnagar
Bulk deposition (ADMN)	SN82403, 85418	SK12408, 98885	NY15813, 10268	NO25409, 86121
Bulk deposition (isotopes)	As above	As above	NY15797, 10061	As above
Soilwater lysimeters	SN81967, 86100	As above	As above	NO25380, 86096
Inflows / tributaries	LB7a: SN81450, 86500	Rose Clough: SK12181, 99532 Swan Clough: SK11909, 99453	1 (Scoat Fell): NY15866, 10423 2 (Red Pike): NY15935, 10503	Spring: NO25279, 85770

Table S2: Results of isotopic analysis of soilwater H_2O and NO_3^- in bulk deposition and surface waters at the Afon Gwy

Sample		**Soilwater δ^{18}O-H_2O**		**Bulk deposition**		**Streamwater**		**%**
Date	**n**	**Mean**	**Theor. Microbial**	**δ^{15}N**	**δ^{18}O**	**δ^{15}N**	**δ^{18}O**	**atmospheric**
20-Sep-04	3	-4.8	+4.5	-	-	-	-	-
20-Oct-04	3	-7.7	+2.6	+0.4	+70.3	+1.0	+5.4	4
18-Nov-04	3	-5.8	+3.8	+3.0	+69.2			
16-Dec-04	3	-6.6	+3.3	-1.9	+76.0	-0.3	+4.5	2
26-Jan-05	3	-5.8	+3.8	-0.9	+67.6	+1.1	+2.7	-2
28-Feb-05	-	-	-	-	-	-	-	-
19-Mar-05	-	-	-	-	-	-	-	-
20-Apr-05*	3	-6.4	+3.4	-1.1	+69.1	*+3.1*	*+6.0*	*4*
18-May-05	1	-7.0	+3.0	+0.9	+66.4	-	-	-
16-Jun-05*	1	-5.1	+4.3	+0.0	+63.7	*-0.3*	*+8.3*	*7*
15-Jul-05	2	-3.1	+5.6	-2.3	+60.8	-	-	-
11-Aug-05	1	-6.7	+3.2	-0.7	-	-	-	-
n:		**10**	**10**	**9**	**8**	**5**	**5**	**5**
Mean:		**-5.9**	**+3.7**	**-0.3**	**+67.9**	**+0.9**	**+5.4**	**3**
SD:		**1.3**	**0.9**	**1.6**	**4.6**	**1.4**	**2.1**	**3.2**

*Data from subcatchment LB7a as insufficient NO_3^- in main channel for isotopic analysis

Table S3: Results of isotopic analysis of soilwater H_2O and NO_3^- in bulk deposition and surface waters at the River Etherow and its tributary streams Rose Clough and Swan Clough

Sample		**Soilwater (H_2O)**		**Bulk deposition**		**River Etherow**		**Rose Clough**		**Swan Clough**		**Etherow**	**Rose**	**Swan**
Date	**n**	**$\delta^{18}O$**	**Theor. Microbial**	**$\delta^{15}N$**	**$\delta^{18}O$**	**$\delta^{15}N$**	**$\delta^{18}O$**	**$\delta^{15}N$**	**$\delta^{18}O$**	**$\delta^{15}N$**	**$\delta^{18}O$**	**% atmospheric**		
10-Aug-05	1	-6.0	+3.7	-0.5	+66.0	+3.2	+6.7	+6.7	+5.0	+6.3	+4.1	5	2	1
20-Sep-05	2	-4.9	+4.4	-0.5	+71.6	+4.3	+5.9	+7.3	+4.4	+6.9	+2.4	2	0	-3
17-Oct-05	2	-4.9	+4.4	+2.8	+66.8	+4.1	+6.0	+6.8	+4.5	+6.3	+3.7	3	0	-1
14-Nov-05	2	-6.5	+3.3	-2.7	+61.3	+2.5	+5.8	+4.5	+4.4	+5.1	+4.8	4	2	3
12-Dec-05	2	-7.2	+2.9	+3.1	+67.0	+3.6	+6.9	+3.7	+4.0	+4.8	+5.1	6	2	3
09-Jan-06	-	-	+3.7	+2.3	+69.3	+2.7	+8.9	+4.2	+5.4	+5.3	+5.3	8	3	3
06-Feb-06	2	-7.4	+2.7	+1.4	+67.4	+2.5	+8.5	+4.6	+4.9	+5.4	+5.6	9	3	4
06-Mar-06	-	-	+3.7	+0.9	+70.7	+3.0	+6.9	+4.3	+4.5	+5.4	+4.9	5	1	2
03-Apr-06	-	-	+3.7	+1.4	+62.0	+0.6	+6.0	-0.3	+7.6	+2.6	+5.3	4	7	3
02-May-06	-	-	+3.7	+2.0	+60.4	+2.5	+8.9	+4.7	+5.9	+5.1	+6.5	9	4	5
30-May-06	2	-6.9	+3.1	-0.2	+66.5	+2.6	+8.5	+4.4	+5.6	+4.9	+5.8	9	4	4
24-Jul-06	1	-4.1	+4.9	-4.5	+69.2	+2.8	+8.4	+4.0	+7.2	+4.3	+7.3	5	4	4
n:		**8**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**
Mean:		**-6.0**	**+3.7**	**+0.5**	**+66.5**	**+2.9**	**+7.3**	**+4.6**	**+5.3**	**+5.2**	**+5.1**	**6**	**3**	**2**
SD:		**1.2**	**0.6**	**2.3**	**3.6**	**0.9**	**1.3**	**2.0**	**1.1**	**1.1**	**1.3**	**2.4**	**1.9**	**2.4**

Table S4: Results of isotopic analysis of soilwater H_2O and NO_3^- in bulk deposition and surface waters at Scoat Tarn and inflow streams

Sample		**Soilwater (H_2O)**		**Bulk deposition**		**Inflow 1**		**Inflow 2**		**Lake outflow**		**Inflow 1**	**Inflow 2**	**Outflow**
Date	**n**	**$\delta^{18}O$**	**Theor. Microbial**	**$\delta^{15}N$**	**$\delta^{18}O$**	**$\delta^{15}N$**	**$\delta^{18}O$**	**$\delta^{15}N$**	**$\delta^{18}O$**	**$\delta^{15}N$**	**$\delta^{18}O$**		**% atmospheric**	
9-Aug-05	2	-5.8	+3.8	-1.2	+63.2	+0.2	+7.2	-1.5	+9.7	-0.3	+11.6	6	10	13
20-Sep-05	-	-	+3.4	-2.1	+68.9	+0.2	+11.5	+0.5	+3.9	+0.6	+11.4	12	1	12
18-Oct-05	2	-5.4	+4.0	-0.8	+73.9	+0.3	+7.5	+0.8	+7.1	+0.4	+14.8	5	4	15
15-Nov-05	2	-6.9	+3.0	+1.0	+65.5	-0.9	+6.4	-0.8	+5.2	-1.0	+8.6	5	3	9
13-Dec-05	2	-5.8	+3.8	-0.8	+74.5	-0.5	+7.3	-0.3	+7.3	-0.2	+10.6	5	5	10
10-Jan-06	-	-	+3.4	-	-	-0.8	+18.1	-1.4	+18.8	-0.7	+13.7	23	24	16
07-Feb-06	2	-5.9	+3.7	-0.2	+72.2	-0.2	+10.6	-0.5	+8.6	-0.4	+13.8	10	7	15
07-Mar-06	1	-7.0	+3.0	-0.5	+66.4	-1.0	+9.9	-0.6	+7.8	-1.1	+14.0	11	8	17
04-Apr-06	2	-7.5	+2.7	-1.4	+60.6	-1.4	+9.3	-0.8	+10.5	-1.3	+12.1	11	14	16
03-May-06	2	-7.1	+2.9	-1.8	+51.2	-0.5	+10.6	-0.2	+9.6	-1.2	+13.1	16	14	21
31-May-06	2	-6.9	+3.1	-3.9	+70.2	-0.3	+12.0	-1.3	+10.6	-1.7	+14.3	13	11	17
25-Jul-06	2	-5.6	+3.9	-4.3	+67.1	-	+13.5	-0.4	+8.6	-0.7	+14.1	15	7	16
n:		**10**	**12**	**11**	**11**	**11**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**
Mean:		**-6.4**	**+3.4**	**-1.5**	**+66.7**	**-0.5**	**+10.3**	**-0.5**	**+9.0**	**-0.6**	**+12.6**	**11**	**9**	**15**
SD:		**0.8**	**0.5**	**1.5**	**6.7**	**0.6**	**3.3**	**0.7**	**3.7**	**0.7**	**1.9**	**5.5**	**6.2**	**3.4**

Table S5: Results of isotopic analysis of soilwater H_2O and NO_3^- in bulk deposition and surface waters at Lochnagar and inflow spring

Sample	**Soilwater (H_2O)**			**Bulk deposition**		**Spring inflow**		**Loch outflow**		**Inflow**	**Outflow**
Date	**n**	**$\delta^{18}O$**	**Theor. Microbial**	**$\delta^{15}N$**	**$\delta^{18}O$**	**$\delta^{15}N$**	**$\delta^{18}O$**	**$\delta^{15}N$**	**$\delta^{18}O$**	**% atmospheric**	
08-Aug-05	-	-	-	-5.7	+57.5	-0.0	+3.2	+0.4	+15.2	1	23
23-Sep-05	1	-5.6	+3.9	-2.4	+70.5	+1.1	+2.2	+1.1	+13.0	-3	14
20-Oct-05	2	-6.6	+3.3	-2.2	+82.1	+0.5	+7.9	+0.1	+16.7	6	17
17-Nov-05	1	-7.5	+2.7	+1.0	+74.6	+1.0	+4.2	+0.6	+13.9	2	16
15-Dec-05	2	-8.0	+2.3	+0.9	+80.2	-0.6	+11.9	-1.3	+15.9	12	17
12-Jan-06	1	-8.3	+2.1	-0.4	+76.3	-0.2	+15.6	-0.8	+16.9	18	20
09-Feb-06	1	-7.1	+3.0	-1.1	+70.9	+0.1	+9.5	-0.9	+16.3	10	20
Mar-06	-	-	-	-	-	-	-	-	-	-	-
06-Apr-06	2	-7.5	+2.7	-2.9	+64.4	-1.7	+20.2	-1.3	+22.4	28	32
04-May-06	1	-7.2	+2.9	+0.3	+48.3	-1.6	+16.4	-1.5	+16.1	30	29
02-Jun-06	2	-7.6	+2.6	-3.7	+71.2	-0.8	+13.0	-1.1	+18.7	15	23
26-Jul-06	2	-7.9	+2.4	-3.9	+69.2	-1.0	+16.4	-1.4	+19.2	21	25
n:		**10**	**10**	**11**	**11**	**11**	**11**	**11**	**11**	**11**	**11**
Mean:		**-7.3**	**+2.8**	**-1.8**	**+69.6**	**-0.3**	**+10.9**	**-0.5**	**+16.7**	**13**	**21**
SD:		**0.8**	**0.5**	**1.8**	**9.5**	**1.0**	**5.8**	**0.9**	**2.7**	**10.7**	**6.0**

Table S6: Results of isotopic analysis of soilwater NO_3^- from tray lysimeters. Where insufficient sample volume was collected, soilwater samples from consecutive months were bulked together and mean values for theoretical microbial and bulk deposition $\delta^{18}O\text{-}NO_3^-$ were calculated for the corresponding period from monthly data.

Site / Tray Lysimeter (TL)	Sampling date / period	$\delta^{15}N$	$\delta^{18}O$	Theor. Micr. $\delta^{18}O\text{-}NO_3^-$	Bulk dep. $\delta^{18}O\text{-}NO_3^-$	% atmospheric
Etherow TL1	12-Dec-05		+17.2	+2.9	+67.0	22
Etherow TL1	Bulked Nov05-Feb06	+2.7	+28.7	+3.2	+66.3	41
Etherow TL2	Bulked Oct05-Feb06	-9.9	+13.3	+3.4	+66.4	16
Scoat Tarn TL1	13-Dec-05	-2.6	+9.1	+3.8	+74.5	8
Scoat Tarn TL1	Bulked Oct05-Feb06	-2.4	+10.9	+3.6	+71.5	11
Scoat Tarn TL2	Bulked Oct05-Feb06	-2.1	+11.3	+3.6	+71.5	11
Scoat Tarn TL1	07-Mar-06	-3.0	+19.8	+3.0	+66.4	26
Scoat Tarn TL1	04-Apr-06	-2.6	+11.7	+2.7	+60.6	16
Scoat Tarn TL2	04-Apr-06	-2.4	+13.8	+2.7	+60.6	19
Scoat Tarn TL1	03-May-06	-2.7	+9.9	+2.9	+51.2	15
Scoat Tarn TL2	03-May-06	-2.6	+9.4	+2.9	+51.2	13
Scoat Tarn TL1+2	25-Jul-06	+0.3	+12.5	+3.9	+67.1	14
Lochnagar TL1	Bulked Oct05-Dec05	+1.2	+35.5	+2.8	+79.0	43
Lochnagar TL2	Bulked Oct05-Feb06	+1.4	+53.6	+2.7	+76.8	69