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Research Report # XXX

Defra Freshwater Umbrella Programme - Final Report

The effect of nitrogen deposition on freshwaters in the UK

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FRESHWATER UMBRELLA - THE EFFECTS OF NITROGEN DEPOSITION ON FRESHWATERS IN THE UK

FINAL REPORT

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Report to DEFRA under Contract AQ0803

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

In upland areas of the UK located away from direct human disturbance through agriculture, industrial activities and urban pollution, atmospheric pollution poses one of the major threats to the chemical and biological quality of lakes and streams. One of the most important groups of pollutants is nitrogen (N) compounds, including oxidised forms of N called NO_x, generated mainly by fossil fuel combustion especially in motor vehicles, and reduced forms of N (ammonia gas or dissolved ammonium compounds) generated mainly from agricultural activities and livestock. These nitrogen compounds may dissolve in rain or soilwater to form acids, or may be taken up as nutrients by plants and soil microbes in upland catchments, and then subsequently released in acid form associated with nitrate leaching at a later date. It is well established that nitrate leaching contributes to acidification of upland waters, with damage to aquatic ecosystems including plants, invertebrates and fish. However it has recently been suggested that nitrate leaching may also be associated with nutrient enrichment of upland waters that contain biological communities adapted to very low nutrient levels. Furthermore, important interactions have been found elsewhere between acid deposition and leaching of dissolved organic carbon (DOC) which has been found to be increasing in many upland waters of the northern hemisphere.

Issues of acidification, nutrient enrichment and biological controls on nitrate and DOC leaching are therefore intrinsically linked and need to be understood in order to determine current and future impacts of N deposition on water quality and ecological status of upland waters. This second phase of the Freshwater Umbrella programme was designed to build on the novel findings of the first phase, with key objectives and methods to:

1. update and review the FAB critical load model which shows that a significant proportion of sampled water bodies in the UK continue to exceed critical loads beyond 2020 after all currently planned emissions reductions have been implemented;

2. continue and expand method development for monitoring and assessing the ongoing and potential impacts of deposited nitrogen acting as a nutrient in upland lakes and streams;

3. explore the potential for studies of lake sediment cores (palaeolimnology) to determine historical changes in the nutrient status of upland lakes, in particular assessing the evidence for increased phytoplankton (primary) productivity;

4. quantify the relative importance of NO_3^- leaching sources between atmospherically deposited nitrate that is leached unchanged from catchments (linked to NOx deposition) and nitrate produced from ammonium by the microbial process of nitrification in soils (originating from an excess reactive nitrogen pool

to which both NOx and reduced N deposition contribute) using both isotopic and empirical modelling approaches;

5. experimentally test the interactions between acid deposition inputs and the release of both NO_3^- and DOC from soils in moorland catchments.

The national freshwater critical loads dataset was updated in 2008 with data from an under-represented, acid-sensitive region of the North York Moors; modelling using FRAME deposition projections for 2020 show that 25% of all sampled sites in the UK continue to exceed critical loads for acidity across the UK. Since the achievement of critical loads becomes ever more difficult in policy and financial terms as available emission abatement measures are implemented, it is important to test our confidence of modelled critical load values. A thorough sensitivity and uncertainty analysis of the terms in the FAB (and inherent SSWC) model demonstrated that there appears to be no single term in the model which might systematically influence calculated critical loads in a particular direction, and uncertainty in the deposition data is more important than uncertainty in the FAB model. However, we found evidence that for some sites there are underlying trends in calculated critical loads which are decreasing as recovery from acidification progresses. Future modelling efforts should prioritise the identification of such sites from catchment and soil characteristics and employ alternative dynamic models to explain the underlying trends and how to compensate for them in the critical load model. A random resurvey of one third of water bodies exceeding critical loads beyond 2020 found no systematic change in calculated critical load values, demonstrating the difficulty in identifying underlying changes from spot sampled data but also suggesting that the initial assessment of exceedance for 2020 was not unduly pessimistic.

We also explored the implications of revised empirical critical loads for nutrient nitrogen in upland lakes and found that under existing recommendations from the WGE, all 959 alpine lakes in the UK (assumed to be those at >600m altitude) exceeded a mid-range nutrient N critical load of 5 kgN ha^{-1} yr^{-1} and 79% including all in England and Wales exceed the uppermost recommended critical load of 10 kgN ha^{-1} yr^{-1} using deposition for 2004-06. Furthermore, all lakes in SACs designated for aquatic habitats in England and Wales exceed this upper critical load for nutrient N, although in Scotland the figure is 18% and many designated lake sites lie in a region where deposition falls with the recommended range for nutrient N critical loads. Assessing the ecological impacts of nutrient N deposition on these lakes should therefore be an urgent priority.

The role of N deposition in causing changes to lake primary productivity has been demonstrated directly through measurement of phytoplankton (microscopic, free-floating aquatic algae) responses to nutrient additions in laboratory bioassay studies. Growth and productivity are limited almost as frequently by N availability as by P in upland lakes but joint- or co-limitation of growth by both N and P together is the most common status. A pilot study of a periphytometer method to test for nutrient

limitation of primary production in streams was only partially successful due to loss of samples (requiring the design of more robust equipment for field deployment in streams) but the data that were obtained supported the pattern of N limitation in low NO_3^- streams and P limitation in NO_3^- rich streams. The technique clearly has potential for wider application but requires methodological development. A study of the stable isotope ^{15}N in aquatic macrophytes as a potential indicator of N limitation status showed a key problem to be the identification of a sufficiently common indicator species and the need for a large number of sample sites to better explore the method.

Upscaling of pilot studies of N stable isotopes in lake sediments to 19 lakes around the UK has demonstrated that most lakes show a 20th Century depletion in the stable isotope ^{15}N attributed by other studies to increased inputs of isotopically light N deposition. We assessed the relationships between the isotopic signatures of NO_3^- and NH_4^+ in monthly samples of deposition and lakewater and their relationship with ^{15}N in sedimenting organic material collected in sediment traps or filtered from the water column, and found no clear relationship on a seasonal basis. Comparison of material from annual sediment traps in 12 lakes around the UK also showed no clear relationship with isotopic signals in deposition, suggesting that a direct reflection of the isotopic signature of anthropogenic N deposition in lake sediments is unlikely. This does not, however, rule out the possibility that ^{15}N trends in lake sediments reflect biogeochemical changes in lake processing of N in response to elevated N deposition, the timing of which will depend upon the cumulative N deposition load required to saturate the terrestrial ecosystems and lead to enhanced NO_3^- leaching. Compelling evidence for a link between the ^{15}N signal and productivity increases in lakes came from a pilot study using fossil pigments in Small Water, which clearly showed an increase in indicators of productivity and shifts in algal assemblages coincident with the isotopic signal. This method shows great potential for direct assessment of changing productivity in lakes, while future work should focus on demonstrating the mechanistic link between enhanced NO_3^- leaching and the ^{15}N signal.

A monthly limnological study of nutrients and phytoplankton in three contrasting lakes showed that a strong seasonal pattern in NO_3^- leaching was inversely related to chlorophyll-a. However, phytoplankton haul samples showed that biovolume was unrelated to NO_3^- and it appears that the primary control on seasonal NO_3^- patterns is top-down (i.e. linked to supply from outside the lake). However, strong seasonal patterns in phytoplankton species assemblages suggest that further studies of the links between NO_3^- availability and the species best able to utilise it could be fruitfully linked to fossil pigment studies to determine possible impacts of N deposition on biodiversity. This study also demonstrated that lake outflow sampling is not a reliable indicator of in-lake chlorophyll-a as a proxy for phytoplankton, although NO_3^- in the lake centre was very closely related to that in the outflow.

The “dual isotope” approach was successfully upscaled to 19 catchments around the UK and provided further evidence that most NO_3^- in upland waters has been microbially produced. An alternative isotopic method was also used for >150 resurvey sites around the UK; both studies showed the dominance of microbial NO_3^- but also that a few sites were susceptible to high levels of atmospheric NO_3^- leaching, presumably linked to site-specific physical catchment attributes.

Compilation of new catchment attribute datasets was carried out for modelling NO_3^- concentrations and direct atmospheric contributions of NO_3^- calculated from stable isotope signatures. As in a previous study, N deposition and the biomass of bryophytes and lichens were found to be the most significant predictors of NO_3^- concentrations, together explaining 66% of the variance in the NO_3^- data. Lake:catchment surface area ratio was the most important predictor of the proportion of atmospheric NO_3^- in lakes, highlighting the importance of direct deposition to lake surfaces as a pathway for atmospheric NO_3^-, but only explained 25% of the variance in the data.

Three years of experimental pH manipulation on four sets of moorland plots in England and Wales show that recovery from acidification is the primary driver of UK-wide increases in dissolved organic carbon (DOC), observed in the UK Acid Waters Monitoring Network and elsewhere. Importantly, the study also shows that this DOC increase has been accompanied by an increase in dissolved organic nitrogen (DON), providing a relatively benign pathway for loss of deposited N from upland organic-rich soils (although the ultimate fate of this DON is not yet fully known). The effects of recovery from acidification on inorganic N loss, on the other hand, remain equivocal. Further work is merited to determine the potential magnitude of enhanced DON leaching as a loss pathway for deposited N which could have a major bearing on critical load and dynamic modelling of N deposition impacts.

EXECUTIVE SUMMARY

Work package 1: Critical loads for acidity – updates, uncertainty analysis and links to dynamic modelling

The aims of this task were:

a) to provide updates on modifications to national critical loads datasets and methodological changes or new approaches to critical loads modelling (Task 1.1);
b) carry out comprehensive sensitivity and uncertainty analysis to assess our confidence in FAB model applications showing continued critical load exceedance in a large number of surface waters beyond 2020 (Task 1.2);
c) provide a methodology for the determination of lake chemical analogues to link unsampled lakes to those with water chemistry data using catchment attributes, allowing upscaling of models to the whole UK lake population (Task 1.3);
d) Compare policy approaches of the Gothenburg Protocol and the EU Water Framework Directive in terms of chemical and biological targets (Task 1.4); and
e) Design and carry out a targeted resurvey of a random subset of the most impacted surface waters across the UK in terms of continued critical load exceedance and provide a regional assessment of the key contributory factors, with additional assessment of nutrient N impacts (Task 1.5).

Task 1.1: Watching brief on critical loads methodology and new datasets

1) In 2008 the national critical loads dataset for freshwaters submitted to the National Focal Centre was updated to include a new regional dataset from a previously under-represented region, the North York Moors, showing a cluster of heavily acidified sites among the most impacted in the UK.
2) FRAME 2020 deposition datasets indicate that critical loads continue to be exceeded in a quarter of sampled sites even after planned emissions reductions under the Gothenburg protocol and the EU NECD.
3) The application of empirical critical loads for nutrient nitrogen to freshwater habitats was assessed following a downward revision of the recommended values at the ICP Mapping and Modelling Workshop in 2010.
4) On the basis of empirical critical loads for nutrient N, 959 alpine lakes (>600m altitude) in the UK are an extremely threatened habitat with 79% receiving deposition loads >10 kgN ha^{-1} yr^{-1} (including all in England and Wales) and 100% receiving more than 5 kgN ha^{-1} yr^{-1}.
5) There are 4627 lakes of EUNIS Types C1.1 and C1.4 in 69 SACs designated for lake habitat types H3130, H3110 or H3160 in Great Britain. 833 (18%) of these lakes experience N deposition > 10 kgN ha^{-1} yr^{-1} including all 79 designated lakes in England and 85 in Wales. All sites experience deposition > 3 kgN ha^{-1} yr^{-1}. In Scotland, 73% receive > 5kgN ha^{-1} yr^{-1} and 15% > 10

kgN ha^{-1} yr^{-1} hence the choice of critical load in this range has a major effect on calculated exceedance.

6) 193 designated lakes lie above 600m altitude and may therefore be considered alpine sub-types of H3130 lakes. 75% receive deposition >10 kgN ha^{-1} yr^{-1} and 25% receive 5-10 kgN ha^{-1} yr^{-1}; all above the recommended range for alpine lakes. For 4434 non-alpine designated lakes, 73% would exceed a critical load of 5 kgN ha^{-1} yr^{-1} while only 16% would exceed the upper limit of 10 kgN ha^{-1} yr^{-1}.

Task 1.2: Uncertainty and sensitivity analysis of critical loads and exceedances

1. Sensitivity analysis of the FAB and SSWC model terms using broad ranges in each parameter and 10,000 Monte-carlo model runs indicate that most sites fall into "definitely not exceeded" or "definitely exceeded" categories. An appreciable minority of sites fall into the low probability of exceedance class (0-0.2; 180/1752 sites).Very few sites fall into the moderate probability of exceedance classes, but 299 sites (17%) in the national dataset have <60% probability of exceedance, compared with 534 sites (30%) that are definitely exceeded.
2. Sensitivity to uncertainty in model parameters is very site specific and the majority of sites are either definitely not exceeded, or definitely exceeded, even employing the wide parameter ranges allowed here.
3. The use of 5^{th} and 95^{th} centile HARM deposition datasets taken from a previous study (Page *et al.*, 2008) and comparison with equivalent centiles for the FAB model Monte Carlo runs indicates that uncertainty in deposition contributes more to uncertainty in exceedance than does uncertainty due to model parameters.
4. Analysis of temporal variations in calculated critical loads (and hence exceedances) using AWMN time series data shows that some sites vary between exceedance and non-exceedance according to when sampling was carried out. The use of annual mean data reduces but does not eliminate this problem, suggesting that e.g. climatic effects may influence year-to-year variations in critical load.
5. Some sites show apparent declining trends in critical load which may be linked, counter-intuitively, to recovery from acidification which allows measured base cations to decline below the pre-industrial rate. Exceedances at these sites show apparent increasing trends with a constant deposition load. It is therefore possible that for a proportion of sites, exceedances calculated beyond 2020 are exaggerated and future work should aim to identify which sites are affected by this phenomenon.
6. Overall, there is little evidence that the FAB model is particularly sensitive to any parameters which might lead to systematic under- or over- estimates of critical load or exceedance, but there is some evidence that longer-term trends in chemical recovery from acidification are impacting calculated critical loads. It is therefore possible that the exceedances beyond 2020 at a quarter of FAB modelled sites is an over-estimate of the true extent of exceedance.

Task 1.3: Classification of lake types / analogues according to catchment attributes for upscaling critical load and dynamic modelling outputs to unsampled lakes from the national database

1. National scale datasets were successfully used to test analogue models to identify the nearest analogue from a database of sampled lakes for any lake in the UK. The final R code was provided to the CLDM for use in MAGIC Library work (see CLDM Final Report).

Task 1.4: Comparison of policy approaches under CLRTAP, WFD and Habitats Directive

1. Current freshwater critical loads provided to the UK NFC employ fixed ANC_{crit} values of either 20 µeq l^{-1}, or 0 µeq l^{-1} where other modelling (from site specific MAGIC or palaeolimnological assessments) indicates a pre-industrial ANC which was less then 20 µeq l^{-1}.
2. Using the broad-brush SSWC ANC hindcasting approach, a wide range of Reference ANC values is modelled within each region of the UK where FAB model exceedance persists beyond 2020.
3. A few regions have Reference ANC values of less than 0 µeq l^{-1} suggesting that current critical loads may be over-protective (too low) – the largest proportions in the North York Moors (22%) and Pennines (12%).
4. In most regions, the majority of sites have Reference ANC values of >20 µeq l^{-1} indicating that current critical loads allow a decline in ANC, which can range up to a decrease of >80 µeq l^{-1} in the least sensitive sites.
5. In north-west Scotland, Reference ANC values are very low, and while the SSWC hindcasts are highly uncertain, it is likely that some sites may have had pre-industrial ANC values of less than 20 µeq l^{-1} which means that current critical load targets could never be achieved.
6. In regions with very low critical loads under the current approach, more sophisticated modelling may help to determine whether sites can ever achieve the critical ANC values of 20 µeq l^{-1} and improve confidence in the exceedances calculated for these regions using FAB beyond 2020.
7. While the approaches used under the WFD and Gothenburg Protocol are fundamentally different, it would be possible to develop a compromise approach using some index of change from Reference, but better modelling of Reference ANC and ecological response to chemical deviations from Reference would be required.

Task 1.5 Regional re-sampling and analysis of factors preventing achievement of critical loads

1. Application of the FAB model to the UK critical loads dataset for freshwaters using FRAME 2020 deposition shows that 445 sites continue to exceed critical loads beyond planned emissions reductions to 2020.
2. Regional analysis of the factors preventing achievement of critical loads reveals that the most acid sensitive sites with the lowest critical loads for sulphur are located in the North Yorkshire Moors and North West Scotland, while the least sensitive (of this acidified subset of UK sites) regions include the Lake District and Galloway. The pattern is similar for the regions most sensitive to N deposition (lowest values of $CL_{max}N$ - the critical load for N deposition alone) but the least sensitive regions are the Pennines, Galloway and mid & South Wales.
3. By 2020, the lowest median values of S deposition occur in NW Scotland, Northern Ireland and the Cairngorms while the highest occur in the Pennines

and SW England. For N deposition the lowest values occur in the Cairngorms and especially NW Scotland, while the highest occur in the Lake District and the Pennines.

4. Regional patterns of exceedance are dictated by the combination of deposition and critical loads. North West Scotland shows by far the lowest range of exceedance values despite high sensitivity. The highest exceedances are found (in descending order) in the North Yorkshire Moors, South West England, the Lake District and the Pennines, i.e. the five most exceeded regions are all in England.
5. The FAB model suggests that NO_3^- makes up the larger part of the acidity flux indicated by exceedance, from a minimum of around 50% in some sites in NW Scotland, to a median of around 80% in the Lake District and Northern Ireland. The nitrogen mass balance in the FAB model therefore continues to account for a large proportion of exceedances in most regions. The implication is that a more conservative (rather than the worst-case indicated by FAB) estimate of NO_3^- leaching would result in fewer exceeded sites in most regions, and a reduced magnitude of exceedance in all sites.
6. Resurveys of around one third of exceeded sites in each region (and all in N. Ireland) showed little evidence that critical loads had been systematically under-estimated (and exceedance over-estimated) by the original datasets sampled early in the recovery trajectory in the 1990s, as hypothesized by Rapp & Bishop (2009).
7. Analysis of chlorophyll-a (an index of phytoplankton productivity) and total phosphorus in 106 resurveyed standing waters showed no significant relationship between the chlorophyll:TP ratio and N deposition, in contrast to the study of Bergström and Jansson (2006) in Swedish and North American lakes. This may however be due to the different sampling seasons in the present study and the sampling bias towards the most acidified lakes in the UK resurveys. The highest chlorophyll-a values were generally recorded in the Pennines and southern regions of England and the lowest in Scotland. The highest TP values were similarly found in the Pennines and southern England with a few high values in Snowdonia.

Work Package 2: Nitrogen as a nutrient – contemporary monitoring and assessment

The primary aims of this Work Package were to further develop methods to test for present-day effects of deposited nitrogen as a nutrient in oligotrophic lakes and streams, through the following series of tasks:

a) Previous phytoplankton bioassay work undertaken only during the summer growing season was extended throughout the whole year at three lakes with contrasting nutrient-limitation characteristics in the Lake District to look for evidence of seasonal responses to nutrient availability (Task 2.1);
b) A new method for assessing periphyton nutrient responses in upland streams was tested (Task 2.2);
c) Empirical modelling approaches for the prediction of atmospheric NO_3^- leaching were expanded using new catchment predictors for core sites where isotopic methods provided estimates of direct NO_3^- inputs into surface waters (Task 2.3);

d) A pilot study into the use of aquatic macrophyte $\delta^{15}N$ as a proxy for N deposition impacts on nutrient limitation in upland lakes was carried out (Task 2.4); and
e) Limnological monitoring methods were tested at three Lake District sites to determine their utility for identifying seasonal impacts of N deposition on phytoplankton production and community composition, with a comparison of in-lake and outflow sampling methods to assist in the design of nutrient monitoring programmes.

Task 2.1: Seasonal variations in N limitation at three contrasting sites

1. Previous bioassay work under the Freshwater Umbrella demonstrated that during the growing season, N-limitation of phytoplankton growth in upland lakes was as commonly observed as P limitation, while co-limitation by both nutrients was the most frequent response.
2. Here, monthly phytoplankton nutrient bioassays over a whole year at three contrasting Lake District Tarns has demonstrated that there are seasonal changes in N and P limitation but they do not follow a coherent seasonal pattern and can be event-based, suggesting possible links to catchment inputs, flushing and phytoplankton blooms. Scoat Tarn was primarily P-limited, while Burnmoor Tarn and Moss Eccles Tarn were mainly co-limited with periods of N limitation, although occurrences of P limitation were found in Burnmoor Tarn in this study, in contrast with an earlier study in 2000. Seasonal changes were mainly from co-limitation to N- or P- limitation and back, rather than a switch from N- to P- limitation between seasons.
3. Observations of N-limitation suggest that elevated N deposition is likely to increase phytoplankton production in some lakes on a seasonal basis. P-limited lakes generally have high NO_3^- concentrations and may have been N-limited under pre-industrial reference conditions prior to the onset of enhanced NO_3^- leaching.

Task 2.2: Development of new methods for bioassay in streams at AWMN sites

1. A new method was developed for nutrient bioassay of periphyton growth in streams to provide a comparable method for the phytoplankton bioassay successfully used in lakes.
2. The stream periphytometer produced results that appeared to be ecologically reasonable: Allt a'Mharcaidh had low nitrate concentrations and was N-limited, Dargall Lane had intermediate concentrations of nitrate and was co-limited and the River Etherow had high concentrations of nitrate and was P-limited. However, the loss of sample filters during spates was a problem and a more robust design would be required for routine use of the method to assess streamwater nutrient limitation.

Task 2.3: Development of catchment-based empirical models for prediction of N leaching and N limitation for upscaling and "stock at risk" assessment of N deposition impacts across the UK

1. A compilation of potential catchment predictors of NO_3^- concentrations or proportion of atmospheric NO_3^- was completed to produce two largely overlapping datasets; i) the FUMBLE1 subset of 16 unafforested AWMN sites for which soil O horizon depth, C:N ratio and moss biomass were available, and ii) the FUMBLE2 dataset (Task 4.1) of 19 AWMN and other sites with isotope-based estimates of atmospheric NO_3^- inputs.
2. The best model for the FUMBLE1 sites used only N deposition (2004-06) and moss biomass to explain 66% of the variance in NO_3^- concentrations, confirming earlier results from FUMBLE1 using slightly different predictors.
3. No significant relationships between concentrations of N species in deposition or surface waters and atmospheric NO_3^- contributions were found for the FUMBLE2 dataset of 19 sites.

Task 2.4: Assessing macrophyte $\delta^{15}N$ as an indicator of N limitation status

1. The purpose of this work was to test whether the ^{15}N ratio of attached (rootless) aquatic plants, such as bryophytes or filamentous algae, could be used to diagnose nitrogen limitation as recently suggested in other studies.
2. At 18 sites around the UK, water samples were collected for water chemistry and phytoplankton nutrient bioassays, while non-rooted macrophytes, attached filamentous algae and bryophytes were collected for stable isotope analysis.
3. The nutrient bioassays confirmed the widespread nature of nitrogen-limitation or co-limitation, rather than phosphorus-limitation as found in previous studies under the Freshwater Umbrella and elsewhere.
4. The stable isotope analyses also confirmed the large amount of ^{15}N variability within aquatic plants, both for a given species in different lakes and the community of plants within a given lake.
5. The sample size was too small to establish robust correlations between water chemistry or nitrogen deposition on one hand and ^{15}N values of macrophytes or phytoplankton nutrient-limitation on the other due to the lack of very widely distributed indicator species in all waters sampled. Some interesting results e.g. for the leafy-liverwort *Nardia compressa* suggest that if the effects of confounding factors, such as the source value of nitrate and ammonium, can be minimised, the ^{15}N values of bryophytes may be useable as a means of assessing nutrient limitation without the costly and artificial use of bioassays, but more research is needed to firmly establish the method.

Task 2.5 Seasonal variations in nutrient status at three sites

1. Strong seasonal patterns are observed at all sites in NO_3^- concentrations which peak in mid- to late winter and tend to be inversely correlated to chlorophyll-a concentrations.
2. There are very marked seasonal variations in the dominant phytoplankton groups at each site, with major differences between summer and winter and also between sites.
3. Although summer minima in NO_3^- concentrations coincide with high chl-a concentrations, phytoplankton blooms in terms of biovolume are not closely

related to either chl-a or low NO_3^- concentrations and occur in spring and/or autumn at Scoat Tarn and Small Water. Concentrations of NO_3^- are driven by seasonal patterns of supply rather than bottom-up primary production controls.

4. While most samples show a close correspondence between outflow and lake centre samples for NO_3^-, the relationship between outflow and lake centre chl-a appears to be dictated by the relative locations of inflows and outflows and the presence of other sources of chl-a from macrophytes and filamentous green algae in the outflows. Hence the use of throw-bottles or boats is recommended for the monitoring of chl-a as an indicator of phytoplankton production, rather than outflow sampling.

Work package 3: Nitrogen as a nutrient – historical reconstruction of deposition and effects using palaeolimnological methods

The primary aim of this task was to test the use of lake sediment $\delta^{15}N$ as an historical indicator of both N deposition inputs and impacts through the following tasks:

a) Sampling bulk deposition, lakewater and sedimenting organic material in two lakes to determine whether deposition is a driver of isotopic signatures in organic material on a seasonal basis (Task 3.1);
b) The use of annual sediment traps to upscale the assessment of deposition and lake sediment $\delta^{15}N$ to 12 lakes around the UK with palaeolimnological $\delta^{15}N$ data (Task 3.2);
c) A detailed palaeolimnological study of lake sediment $\delta^{15}N$ records from 19 upland lakes to look for coherent patterns which might support the use of sediment $\delta^{15}N$ as an indicator of N deposition inputs and impacts (Task 3.3); and
d) To look for direct evidence of historical changes in phytoplankton productivity and composition through the use of fossil pigment signatures in conjunction with sediment $\delta^{15}N$ records (Task 3.4).

Task 3.1: Seasonal variation in drivers of sediment $\delta^{15}N$

1. Sedimenting organic material in the two study lakes does not closely track the isotopic signature of lakewater NO_3^- or of bulk deposition NO_3^- or NH_4^+ on a seasonal basis. Hence it cannot be assumed that sediment core records of bulk organic matter $\delta^{15}N$ are a simple proxy for N deposition inputs through the incorporation of isotopically depleted deposition NO_3^-.

Task 3.2: Drivers of Sediment $\delta^{15}N$ – upscaling

1. A statistically significant relationship was found between annual sediment trap material $\delta^{15}N$ and deposition $\delta^{15}N$-NO_3^- but this was driven largely by high values in an outlying site. Exclusion of the site resulted in a lack of significant relationships.

2. Hence results from this study do not support the use of sediment core $\delta^{15}N$ as a proxy for N deposition inputs although other methods could be employed to more thoroughly test the relationships under future work.

Tasks 3.3-3.4: Palaeolimnological assessment of changes in lake nutrient status ($\delta^{15}N$), including additional fossil pigment analysis (Task 3.4)

1. Analysis of $\delta^{15}N$ in 13 of 19 lake sediment cores taken from upland lakes indicate changes to N biogeochemistry which in most cases coincide with changes in sediment C:N ratios suggesting a stimulation of in-lake production.
2. The timing of change varies between sites and may be linked to differences in the onset of significant nitrate leaching due to differing catchment soils and vegetation.
3. Analysis of fossil pigments in one site which does not suffer from the confounding effects of acidification indicates wholesale increases in algal production comcomitant with changes in sediment $\delta^{15}N$.
4. These studies provide compelling evidence for a widespread effect of N deposition on the biogeochemistry and biology of oligotrophic lakes in the UK as found in other studies of remote alpine and Arctic lakes.

Work package 4: Improved understanding of nitrate leaching pathways for model development

It has been demonstrated using stable isotope methods that a major proportion of the NO_3^- recovered in upland waters has been microbially produced, implying that the source of the N in the NO_3^- may be either NO_3^- or NH_4^+ deposition which contributes to the labile N pool in upland ecosystems. The main aim of this Work Package was to upscale and test the isotopic methods used under the previous Freshwater Umbrella programme which allow the quantification of direct atmospheric inputs of NO_3^- into lakes:

a) Upscaling of the dual isotope approach from four previously studies catchments to a total of 19 lakes including new sites not sensitive to the confounding effects of acidification (Task 4.1);
b) Directly test one of the assumptions of the $\delta^{18}O\text{-}NO_3^-$ method about the isotopic signature of microbial NO_3^- through soil core incubation experiments to isolate microbially produced NO_3^- (Task 4.2);
c) Further upscale the dual isotope approach, using a new bacterial denitrifier method which allows the isotopic analysis of NO_3^- in much smaller samples than previous techniques, to the 163 sites in the critical loads resurvey under Task 1.5, with additional empirical modelling of catchment predictors of atmospheric NO_3^- (Task 4.3).

Task 4.1: Dual isotope ($\delta^{15}N$- and $\delta^{18}O\text{-}NO_3^-$) and $\delta^{15}N\text{-}NH_4^+$ studies at 19 mid-level study catchments including one in Northern Ireland (quarterly)

1. The dual isotope work piloted in the first Freshwater Umbrella programme was successfully upscaled to 19 sites around the UK confirming that the major

proportion of surface water NO_3^- has been microbially produced, with 0-24% deriving from direct leaching of atmospheric NO_3^- on a mean annual basis.

2. A greater proportion of atmospheric NO_3^- was found in lake sites relative to their inflow streams, indicating the importance of direct deposition to lake surfaces.
3. Regional and seasonal patterns in the $\delta^{15}N$ of bulk deposition NO_3^- and NH_4^+ suggest there may be potential to use isotopes to link back to dominant emission sources.

Task 4.2: Dual isotope analysis of incubated soil microbial NO_3^- to validate "theoretical" $\delta^{18}O$ values used at present

1. There is no evidence from the soil incubation study that the $\delta^{18}O$-NO_3^- method has under-estimated the proportion of untransformed atmospheric NO_3^- in surface waters; in fact it is possible the atmospheric component has been over-estimated.

Task 4.3: Dual isotope ($\delta^{15}N$- and $\delta^{18}O$-NO_3^-) analysis at 150 regional study sites in Great Britain plus 15 Northern Irish sites (regional assessment and upscaling of controls on N leaching)

1. There are significant differences between regions in the isotopic signature of NO_3^- in surface waters, with the Cairngorms being most distinctive in having a large median $\delta^{18}O$-NO_3^- indicative of major atmospheric contributions.
2. Very high proportions of atmospheric NO_3^- were found in a small number of sites, predominantly in the Cairngorms and north Pennines in autumn and also Snowdonia and one site in central Scotland in the spring.
3. Lake:catchment area ratio is the most important predictor of atmospheric NO_3^- in surface waters, highlighting the importance of direct deposition to lake surfaces, but explains only around a quarter of the variance in the data. Other site-specific catchment attributes are also clearly very important in determining the transport of untransformed atmospheric NO_3^- into surface waters.

Work Package 5: Interactions between acidity, DOC and nitrate leaching

This Work Package aims to test through field manipulation of acid inputs and isotope tracer experiments the relationships between acid deposition, leaching of dissolved organic carbon and interactions with NO_3^- leaching. Three years of experimental pH manipulation on four sets of moorland plots in England and Wales show that:

1. recovery from acidification due to declining S deposition is having profound effects on C and N cycling in these ecosystems. The results strongly support the hypothesis that recovery from acidification is the primary driver of UK-wide increases in dissolved organic carbon (DOC), observed in the UK Acid Waters Monitoring Network and elsewhere.
2. They further indicate that this DOC increase has been accompanied by an increase in dissolved organic nitrogen (DON), providing a relatively benign

pathway for loss of deposited N from upland organic-rich soils (although the ultimate fate of this DON is not yet fully known).

3. The effects of recovery from acidification on inorganic N loss, on the other hand, remain equivocal. Some experimental data suggest that acidic soils have reduced rates of NO_3 turnover, which may lead to increased NO_3 leaching following acidification, and reduced NO_3 leaching following recovery. However, clear effects of pH change on NO_3 leaching were not observed in the main plots. Data from a related study of European forest plots exposed to large ambient changes in S deposition, on the other hand, do demonstrate clear and dramatic reductions in NO_3 leaching following recovery from acidification (Oulehle *et al.*, 2011).
4. Data from our ^{15}N addition study also confirm the importance of bryophytes in the initial uptake and assimilation of atmospheric N, suggesting that the loss of bryophyte cover due to acid deposition in the past contributed to increased NO_3 leaching in areas such as the Peak District, and that the recovery of bryophyte cover in recent years has thus led to improved N retention by terrestrial ecosystems in this area. The long-term impact of reduced levels of sulphur deposition may therefore be greater terrestrial N retention, possibly leading to accelerated eutrophication, whereas N impacts on surface waters may decline.

Key findings and policy implications

The work completed under this contract has further strengthened the evidence base for the adverse effects of N deposition as an agent of both acidification and especially nutrient N enrichment in sensitive water bodies of the UK.

Acidification continues to be a problem in 25% of sampled sites beyond 2020 and while detailed uncertainty analysis has found that in some sites recovering from acidification, critical loads may be underestimated due to structural problems with the underlying model, there is no doubt that extensive acidification will persist for some decades yet in many sites. The UK national critical loads dataset is therefore a valuable contribution to the evidence base being used in the currently ongoing revisions to the UNECE Gothenburg Protocol. Substantial further reductions in emissions targets for total N would be required to prevent critical load exceedance in the majority of acid-sensitive freshwaters, and the isotope work done under this programme demonstrates convincingly that leached NO_3^- is mostly microbially produced from a reactive pool which is increased by both oxidised and reduced forms of N deposition.

The role of N deposition in causing changes to lake nutrient cycles and increasing productivity has been demonstrated through present-day bioassay studies as well as lake sediment studies of isotopes and fossil pigments. The potential impacts of N

deposition in increasing lake productivity are highly relevant to N emissions policy with respect to several international directives:

1. Oligotrophic lakes in the UK uplands designated under the EU Habitats Directive are particularly sensitive to the effects of N deposition, with probable changes to phytoplankton species and productivity and possibly to macrophyte flora. All alpine lakes as well as lakes in SACs designated for aquatic habitat features exceed nutrient N critical loads in England and Wales, as well as in large proportions in Scotland. The key unknown is the ecological significance of these exceedances.
2. Changes in the productivity and natural nutrient limitation status of upland lakes may be considered a deviation from the good ecological status required under the EU Water Framework Directive. This is an additional pressure to the widely demonstrated acidification problem and hindcasting of Reference chemical conditions suggests that current modelling approaches using fixed ANC values in acidification models are allowing major chemical changes to occur without exceedance of acidity critical loads in some sites.

Confirmation that N deposition may lead to both acidification and nutrient enrichment highlights the need to understand the processes that determine whether N deposition causes enhanced nitrate leaching over the short- or longer term. Results from this project have greatly expanded the evidence that only a small proportion of leached nitrate is rapidly transported from NO_x deposition; the remainder is generated within soils by microbial processes. Hence a fraction of leached nitrate will respond rapidly to changes in NO_x deposition. However, a larger proportion of leached nitrate is generated in soils from N pools that consist of accumulated N deposition (both NO_x and reduced N) as well as recently deposited N. Therefore a large proportion of nitrate leaching may respond slowly to changes in N deposition while current levels of nitrate must indicate advanced terrestrial N saturation. Lakes have been shown to be particularly vulnerable to atmospheric NO_3^- inputs via direct deposition to their surfaces.

Evidence of altered N cycling due to altered S deposition has implications for the combined assessment of these two key air pollutants. Our results suggest that declining S emissions have increased ecosystem export of N as DON, but may have reduced the amount of inorganic N leaching. In areas of high NO_3 leaching and less organic soils, the overall impact of these processes may have been greater terrestrial N retention, with beneficial impacts for freshwaters but possibly detrimental impacts for terrestrial ecosystems. The clear evidence of increased DOC loss following reduced S deposition has implications for the wider ecosystem service impacts of atmospheric pollutants; DOC is often viewed as a negative aspect of water quality due to the costs and health risks associated with its removal from water supplies. Our results suggest that it is, nevertheless, a natural property of waters draining upland organic soils, and that rising trends represent a return to these natural conditions.

Work package 1: Critical loads for acidity – updates, uncertainty analysis and links to dynamic modelling

Contributors:
Chris Curtis, Gavin Simpson, Chris Evans

Task 1.1: Watching brief on critical loads methodology and new datasets

1) Status of UK National Dataset for Critical Loads of Acidity for Freshwaters

In response to the CCE voluntary call for data for 2008, the UK freshwaters critical loads data submission was updated in March 2008 with the inclusion of a new regional dataset for the North Yorkshire Moors (NYM), generated by CEH Bangor under a previous contract to DEFRA (CPEA19: Evans *et al.*, 2005). The update was carried out using the same procedures and screening criteria applied under the previous freshwaters data submission in March 2004. A total of 35 new sites and 3 water chemistry updates were added to the existing FAB mapping dataset, raising the total number of mapping sites from 1722 to 1757. Of the 35 new sites added to the mapping dataset, 34 are stream sites and one is a lake. Five of the new stream sites would have zero critical loads with an ANC_{crit} of 20 µeq l^{-1} so the lower ANC_{crit} of 0 µeq l^{-1} was selected. For the data submission to CCE, five sites were excluded as being too small (CEHL24/27/44/54 and CZSX27) hence the actual officially submitted dataset contains 1752 sites.

CEH North Yorkshire Moors sites

Of the 38 NYM sites/samples used to update the FAB mapping dataset, 36 exceeded their critical loads for 2003-2005 deposition, i.e. 95% of the total. 33 of the 38 NYM sites have both S deposition > $CL_{max}S$ and N deposition >$CL_{max}N$ i.e. are "double-exceeded". The sites in this region are clearly highly impacted by both S and N deposition which is a function of both their great acid-sensitivity and the high deposition in the region due to the proximity of several major coal-fired power stations. Their inclusion in the national FAB mapping dataset has resulted in the presence of an additional regional cluster of acidified sites and a net deterioration in the national critical load exceedance statistics for freshwater acidity. A detailed assessment of acidification in this region is included in a paper submitted to the AWMN/Upland Waters Special Issue of Ecological Indicators (Evans, CD et al., in revision. Persistent surface water acidification in a peat-dominated upland region subject to high atmospheric deposition: The North York Moors, UK. Submitted to *Ecological Indicators*).

Critical Load Exceedance and RoTAP Datasets

Following the submission of the revised freshwater critical loads dataset to the CCE Voluntary Call for Data in April 2008 (see First Annual Report and Curtis *et al.*, in prep), new national critical load exceedance maps were produced by Jane Hall at CEH Bangor which feature in the RoTAP Freshwaters Chapter (Figs 1.1.1a-d below). Summary exceedance data are provided in Table 1.1.1.

The exceedance data show major improvements in terms of reducing critical load exceedance over the last 20 years, especially for Scotland and Wales. While the improving picture in these regions is due in part to a large number of marginally exceeded sites in the past, the opposite is true for England and Northern Ireland where

clusters of sites showing very high exceedances in the 1980s and 1990s continue to show exceedance to 2020. Almost half of sampled sites in England still exceed critical loads in 2020 and this is due largely to nitrogen deposition and the high rates of nitrate leaching predicted by the FAB model. Note that these proportions are not representative for regions as a whole because of the non-random sampling strategies and composite nature of the current mapping dataset including several regionally focused surveys in the most sensitive upland areas (see Curtis *et al.*, in prep).

Table 1.1.1: Breakdown of critical load exceedance for freshwaters in RoTAP assessment periods

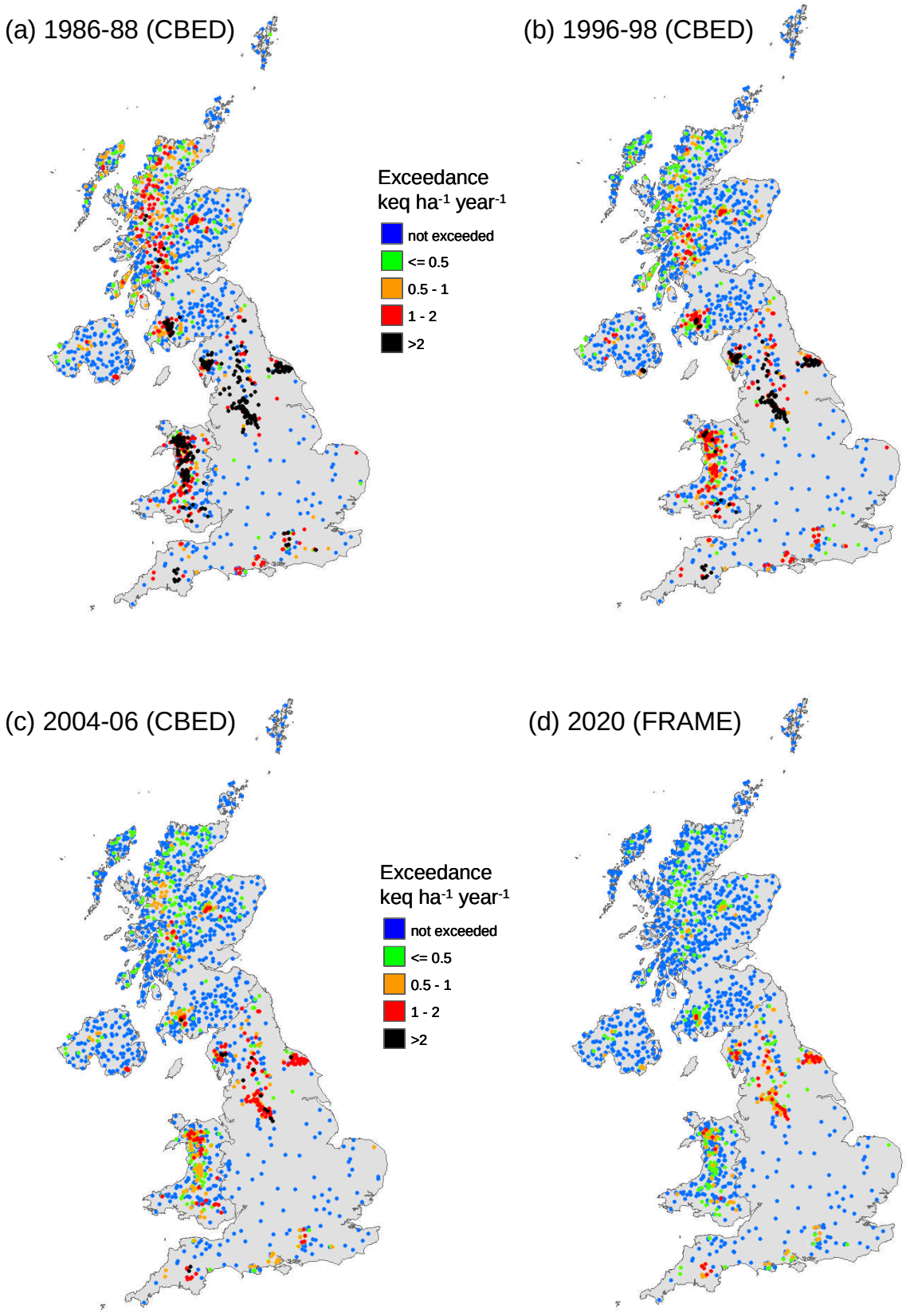
Country	Number FAB sites	Number & percentage of sites exceeded by acid deposition for:			
		1986-88	1996-98	2004-06	2020
England	**425**	**284 (67%)**	**265 (62%)**	**230 (54%)**	**198 (47%)**
Wales	**344**	**273 (79%)**	**222 (65%)**	**168 (49%)**	**103 (30%)**
Scotland	**856**	**400 (47%)**	**302 (35%)**	**232 (27%)**	**128 (15%)**
NI	**127**	**24 (19%)**	**27 (21%)**	**22 (17%)**	**16 (13%)**
UK	**1752**	**981 (56%)**	**816 (47%)**	**652 (37%)**	**445 (25%)**

Publication of FAB model and datasets

A manuscript is in preparation for submission to Environmental Pollution which describes all modifications to the FAB model in the UK application in the last 10 years and the implications of continued critical load exceedance in certain regions of the UK beyond 2020. This will take into account recent methodological publications on the steady-state critical load models.

Chris Curtis is also leading a book chapter on freshwater critical load applications in Europe as a contribution to a book on Critical Loads Modelling and Applications due for submission in December 2011: Curtis *et al.* (in prep) Assessment of critical loads for acidity and their exceedances for European lakes. In: W. de Vries & J.-P. Hettelingh (Eds.), *Critical loads for nitrogen, acidity and metals for terrestrial and aquatic ecosystems.* Environmental Pollution Series, Springer, The Netherlands.

Figure 1.1.1: FAB model critical load exceedance maps for freshwaters for RoTAP standard assessment periods



2) *Empirical critical loads for nutrient N*

The case for nutrient N critical loads was revisited by Gavin Simpson and Chris Curtis who gave both oral and poster presentations of DEFRA funded work at the INI Workshop on Nitrogen Deposition, Critical Loads and Biodiversity at the George Hotel, Edinburgh on 16-18th November 2009. A chapter in the published Proceedings of this workshop is in press (Curtis et al., *in press*).

An ICP Mapping and Modelling workshop to revise empirical critical loads for nutrient N was held in Noordwijkerhout, The Netherlands on 23-25th June 2010 and was attended by Jane Hall on behalf of the UK. Evidence for revisions to empirical critical loads for surface waters was presented and new critical loads recommended for EUNIS ecosystem types C1.1 (soft-water lakes) and C1.4 (permanent dystrophic lakes and ponds) (Table 1.1.2). These revisions were formally presented at the 29th Session of the Working Group on Effects in Geneva on 22-24th September 2010 and are to be incorporated into the next revision of the Mapping Manual*.

*http://www.unece.org/env/documents/2010/eb/wge/ece.eb.air.wg.1.2010.14.e.pdf

Table 1.1.2: Revisions to empirical critical loads for surface waters, WGE September 2010
= reliable, (#) = expert judgement

Ecosystem type	*EUNIS code*	*2003 kg N ha^{-1} year^{-1} and reliability*	*2010 kg N ha^{-1} year^{-1}*	*2010 reliability*	*Indication of exceedance*
Inland surface water habitats (C)					
Soft-water lakes (permanent oligotrophic waters)	C1.1[c]	5–10 ##	3–10	##	Change in the species composition of macrophyte communities, increased algal productivity and a shift in nutrient limitation of phytoplankton from N to phosphorous (P)
Dune slack pools (permanent oligotrophic waters)	C1.16	10–20 (#)	10–20	(#)	Increased biomass and rate of succession
Permanent dystrophic lakes, ponds and pools	C1.4[d]		3–10	(#)	Increased algal productivity and a shift in nutrient limitation of phytoplankton from N to P

[c] This critical load should only be applied to oligotrophic waters with low alkalinity with no significant agricultural or other human inputs. Use the lower end of the range for boreal and alpine lakes, use the higher end of the range for Atlantic softwaters.

[d] This critical load should only be applied top waters with low alkalinity with no significant agricultural or other direct human inputs. Use the lower end of the range for boreal and alpine dystrophic lakes.

Identification of lakes in the UK for application of empirical critical loads

There are large populations of lakes in the UK of EUNIS types C1.1 (permanent oligotrophic waters) and C1.4 (permanent dystrophic lakes, ponds and pools) which are located across much of Scotland as well as (primarily) upland areas of England, Wales and Northern Ireland. Some additional populations of C1.4 dystrophic lakes and ponds are located in lowland areas of heathland, ombrotrophic mires or blanket bog. However, while the total population of lakes in the UK is described in the UKLakes inventory, there is (to our knowledge) no such UK-wide inventory of lakes falling into EUNIS types C1.1 or C1.4. However, it is possible to identify lakes of these types which are located in conservation areas, specifically SACs, via GIS techniques using SAC polygons and lakes >0.5ha in size included in UKLakes.

Note: Use of empirical critical loads in the UK – JNCC context (from Clare Whitfield at JNCC)

JNCC now provide "site relevant critical loads" through an on-line tool (via APIS) for SACs, SPAs and SSSIs in the UK. Relevant critical loads are assigned to each of the interest features, with EUNIS-based critical loads assigned for Annex 1 features or BAP priority habitats for SSSIs, based on JNCC correspondence tables. Nutrient N critical loads have been assigned for C1.1 to Annex 1 types H3130, H3110 and for C1.4 to H3160. In both cases the empirical critical load range is given as 3-10 kgN ha^{-1} yr^{-1} with the statement that the lower end of the range is intended only for boreal and alpine lakes, while the upper range of 5-10 kgN ha^{-1} yr^{-1} applies to the Atlantic softwaters found in the UK. It was noted by JNCC that the Noordwijkerhout report provided no guidelines on the definition of alpine lakes which required the lower end of the empirical CL range.

These lake populations in freshwater habitat designated SACs are the subject of a manuscript being prepared by Kernan et al. (in prep) which identifies the lakes most impacted by acidification and exceeding critical loads. The same population of lakes may be considered appropriate for the application of empirical nutrient N critical loads.

In the UK, alpine lakes are a subset of the total population of lake types C1.1 but are not subject to the restriction of being identifiable only within designated conservation areas, since their definition is based only on altitudinal criteria, potentially with regional variations. Hence it is possible to identify both the total UK population of alpine lakes as well as the sub-population located with conservation areas designated for freshwater habitat types C1.1 and C1.4.

Here we identify the three relevant groupings of lakes in Great Britain, although the same exercise could be carried out for Northern Ireland given the availability of appropriate GIS data:

a) all alpine lakes in Great Britain based on altitude criteria;
b) lakes of EUNIS Types C1.1 and C1.4 lying within SACs designated for freshwater habitat types H3130, H3110 and H3160; and

c) the sub-population of alpine lakes lying within the above designated SACs, i.e. the subset of EUNIS Type C1.1 lakes which qualify as alpine lakes.

a) Definition of alpine lakes in the UK

Use of the upper empirical nutrient N critical loads range by JNCC was based on the fact that published literature in support of the lower range comes from Scandinavia (boreal lakes) and North America (alpine lakes), i.e. may not be appropriate in a UK context. Indeed, a new paper by Jill Baron *et al.* published in August 2011 (Empirical critical loads of atmospheric nitrogen deposition for nutrient enrichment and acidification of sensitive US lakes. *BioScience* **61**: 602-13) proposes a nutrient enrichment critical load of just 1-3 kgN ha^{-1} yr^{-1} for western US lakes and 3.5-6 kgN ha^{-1} yr^{-1} for northeastern US lakes.

Previous work done under EU funded projects in the UK has however defined populations of "alpine" lakes for Scotland, e.g. the work of Kernan *et al.* (2009) in the EMERGE project. The definition of "alpine" in this context means above the theoretical, natural tree-line and four separate regions were defined for Scotland; Western Grampians (716m), eastern Grampians (700m), Cairngorms (793m) and north-west Highlands (500m) (references in Kernan *et al.*, 2009). Using a modified scheme with a combined tree-line for the Grampians of 700m and for the Cairngorms of 750m, 399 alpine lochs with a surface area >0.5ha were identified in Scotland. Furthermore, a number of these alpine lochs are located in SACs (Kernan *et al.*, in prep).

However, other possible values for a theoretical tree-line have been proposed in other sources. The alpine zone is described in a key SNH Advisory Note (**http://www.snh.org.uk/publications/on-line/advisorynotes/26/26.htm)** as lying above the upper limit of tree growth. According to the JNCC entry for the Cairngorms SAC: "In common with the rest of Scotland, the upper limits of the pine woodland are mostly artificially depressed by grazing, but a more natural tree-line occurs at 640 m on Creag Fhiachlach. This is the highest altitudinal limit of woodland in the UK." (http://jncc.defra.gov.uk/protectedsites/sacselection/sac.asp?EUCode=UK0016412)

Other sources of data for altitudinal tree-line limits include one for Wales (CCW Habitats Series: The Mountains of Wales) which suggests a natural treeline for Wales of 500-700m, and two for England suggesting a 600m natural tree-line (Natural England Report "The Heather and Grass Burning Code 2007"; http://www.naturalengland.org.uk/Images/senareasweb2_tcm6-7797.pdf; 600m in Northern England according to "New Native Woodlands for Nidderdale AONB" - http://www.ydmt.org/assets/x/50248). Hence there are various values proposed for defining the tree-line across Great Britain, ranging from 500m to >750m. The populations of lakes lying within selected altitude ranges are shown in Table 1.1.3 and mapped in Fig. 1.1.2a.

Given the range of values proposed to define the alpine tree-line cut-off, it may be argued that as a first approximation for Great Britain, the value of 600m could be used as a cut-off for defining national populations of alpine lakes (bold in Table 1.1.3; Figure 1.1.2b). There are almost one thousand lakes in Great Britain above 600m altitude with the vast majority in Scotland but small localised populations in England and Wales. Future work could include the identification of more regionally appropriate tree-lines through review of the available literature.

The recommended nutrient N critical loads for alpine lakes are at the lower end of the range 3-10 kgN ha^{-1} yr^{-1}, assumed to be 3-5 kgN ha^{-1} yr^{-1}. Total CBED 2004-06 N deposition across the UK is mapped in Figure 1.1.3a, and split into categories of 0-3, 3-5, 5-10 and >10 kgN ha^{-1} yr^{-1} in Figure 1.1.3b. Nowhere in the UK experiences N deposition below the minimum critical load value of 3 kgN ha^{-1} yr^{-1} (Fig. 1.1.3b) and most of the country experiences deposition >10 kgN ha^{-1} yr^{-1}. Hence the selection of suitable empirical critical load ranges for alpine lakes is most critical in northern and western Scotland where lower N deposition coincides with the location of alpine lakes (Fig. 1.1.4).

Table 1.1.3: Alpine lakes in Great Britain according to various altitude categories

Altitude > than	England	Scotland	Wales	Great Britain
500m	68	1594	103	1765
600m	**30**	**907**	**22**	**959**
650m	17	658	6	681
700m	4	445	5	454
750m	2	267	2	271

All alpine lakes in the UK lie in areas experiencing N deposition loads greater than 5 kgN ha^{-1} yr^{-1} (Figure 1.1.4). There is one lake (WBID=11586) at altitude 585m which has a total N deposition load of 4.8 kgN ha^{-1} yr^{-1}, i.e. the closest we have to a non-exceeded alpine lake in the UK, if the upper value of the range 3-5 kgN ha^{-1} yr^{-1} is used. The only lakes in the UK with altitude >500m and deposition below or equal to 10 kgN kgN ha^{-1} yr^{-1} occur in Scotland. The breakdown of sites versus deposition classes up to and including 10 kgN ha^{-1} yr^{-1} for 500m and 600m altitude cut-offs is shown in Table 1.1.4. Using the chosen value of 600m to define alpine lakes, only one does not exceed a critical load of 6 kgN ha^{-1} yr^{-1} while 203 alpine lochs experience deposition in the range 6-10 kgN ha^{-1} yr^{-1}. With 959 alpine lakes in the UK, 756 therefore exceed the uppermost value for nutrient N critical loads of 10 kgN ha^{-1} yr^{-1}.

Figure 1.1.2: Candidates for "alpine" lakes by altitude class. Each class is exclusive of lower altitude classes – see Table 1.1.3. a) All classes, b) Qualifying lakes >600m altitude.

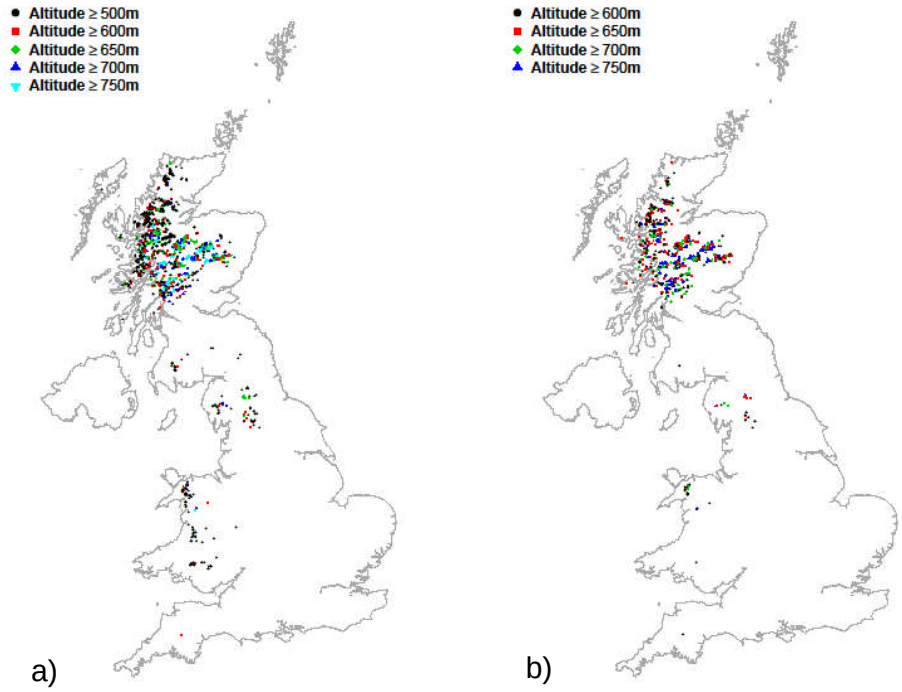


Figure 1.1.3: CBED Total N Deposition for 2004-06 showing a) hotspots of N deposition and b) regions experiencing deposition in ranges linked to nutrient N critical loads (NB needs legend correcting to kgN ha^{-1} yr^{-1})

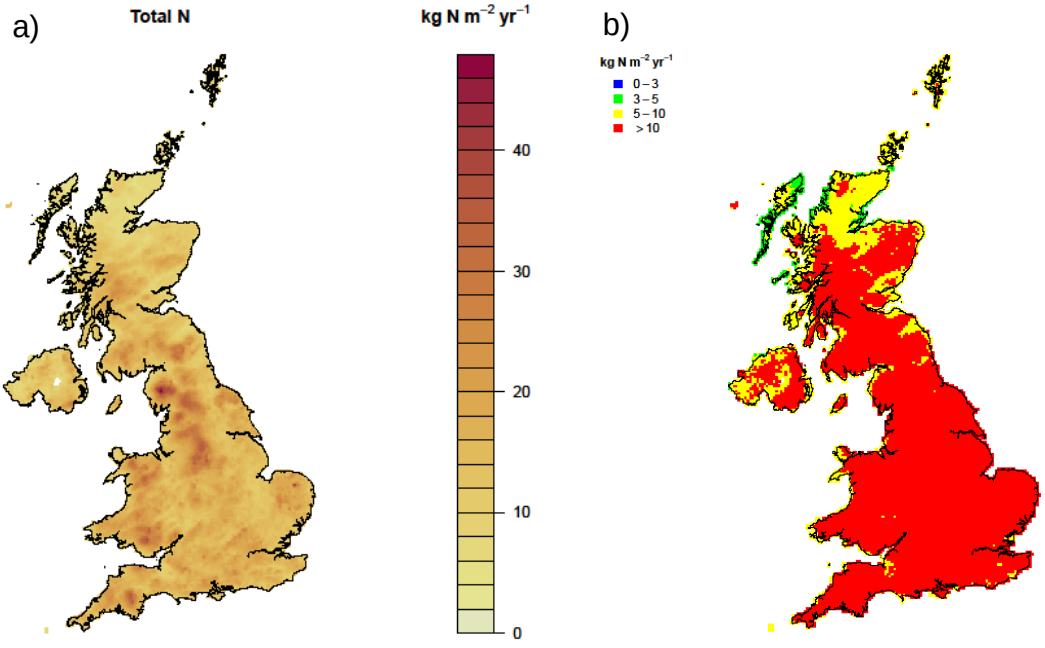
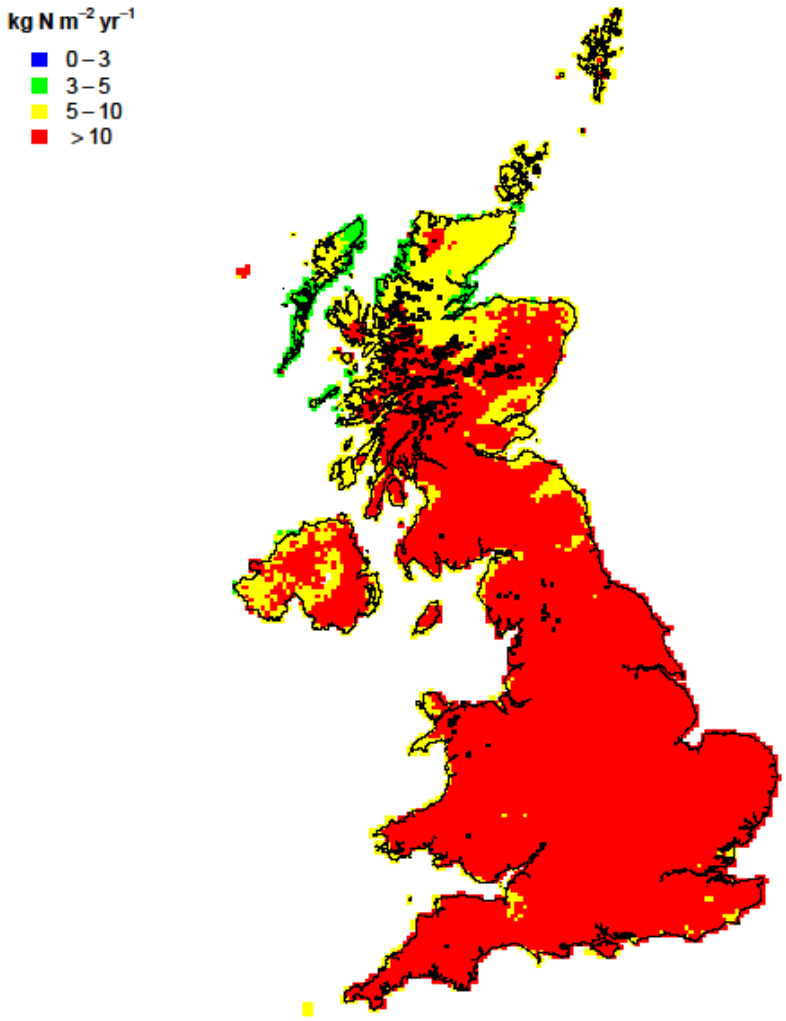


Table 1.1.4: Number of Scottish lochs <u>below</u> critical deposition loads in the range 5-10 kgN ha^{-1} yr^{-1}

	CBED 2004-06 Total N Deposition less than or equal to (kgN ha^{-1} yr^{-1})					
Min. altitude	**5**	**6**	**7**	**8**	**9**	**10**
500m	1	8	43	141	360	545
600m	**0**	**1**	**12**	**48**	**139**	**203**

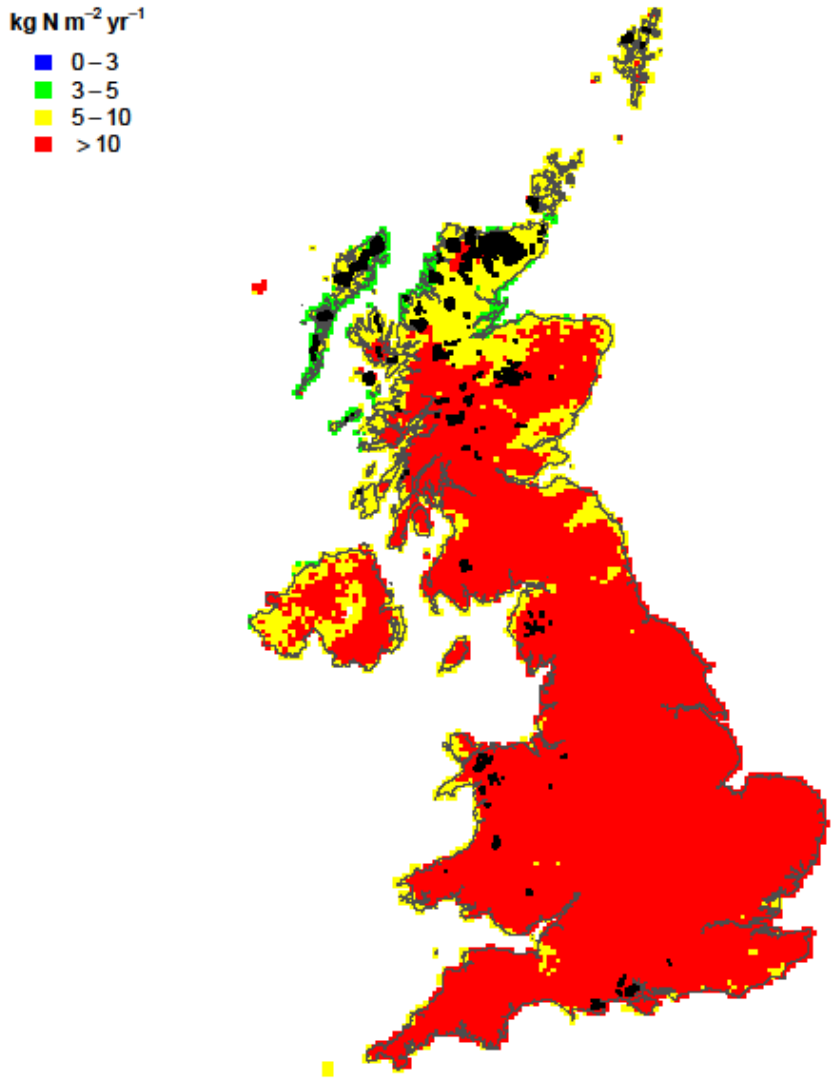
Figure 1.1.4: Location of alpine lakes (>600m altitude) in empirical nutrient N critical load categories



b) Lakes of EUNIS Types C1.1 and C1.4 in designated conservation areas

While the British population of alpine lakes may be estimated from the GBLakes database using only altitude criteria, the population of sensitive oligotrophic lakes is more difficult to identify without site-specific information. However, it is possible to identify lakes in designated areas of conservation importance through a GIS overlay of SAC boundaries with lakes >0.5ha included in the GBLakes database. Only 76 SACs in Great Britain are designated for at least one of the three acid- or nutrient-N sensitive lake habitat types H3130, H3110 or H3160. Of these, 69 SACs include lakes large enough to be included in the GB Lakes inventory, with a total of 4627 lakes qualifying for empirical critical loads for N in designated conservation areas (Figure 1.1.5). 285 of these lakes lie at more than 500m altitude, 193 at >600m, 134 at >700m, and 104 at >750m.

Figure 1.1.5: EUNIS Type 1.1 and 1.4 lakes in SACs designated for freshwater habitat types H3130, H3110 and H3160 (n=4627 lakes) with 2004-06 deposition classes



Of the 4627 lakes in freshwater designated SACs, 833 (18%) exceed even the highest nutrient N deposition load of 10 kgN ha^{-1} yr^{-1} including all 79 designated lakes in England, all 85 in Wales and 669 (15%) in Scotland. 74% of all sites exceed a critical load of 5 kgN ha^{-1} yr^{-1}, the lower limit prior to the 2010 revision, while all sites exceed the lowest recommended critical load of 3 kgN ha^{-1} yr^{-1} (Table 1.1.5).

In Scotland there are many sites with deposition in the range 3-10 kgN ha^{-1} yr^{-1} hence the proportion exceeding critical loads varies widely depending on the selected value in this range, from 73% at 5 kgN ha^{-1} yr^{-1} to 15% at 10 kgN ha^{-1} yr^{-1}. Choice of an appropriate value within the recommended range is therefore critical for Scottish sites only, since all sites in England and Wales exceed even the highest value of 10 kgN ha^{-1} yr^{-1}.

Table 1.1.5: Number of designated lakes in GB and separately for Scotland exceeding critical deposition loads in 2004-06

Designated	**Empirical nutrient N critical load (kgN ha^{-1} yr^{-1})**						
Lakes	**3**	**5**	**6**	**7**	**8**	**9**	**10**
Not exceeded	0	1197	1975	2711	3153	3426	3794
Exceeded	**4627**	**3430**	**2652**	**1916**	**1474**	**1201**	**833**
Exceeded (SCO)	4463	3266	2488	1752	1310	1037	669
%	100	73.2	55.7	39.3	29.4	23.2	15.0

c) Alpine lakes as a sensitive sub-type of H3130 designated SACs

For all alpine lakes (sub-type of H3130) the total GB population can be identified (n=959 using 600m cut-off) as well as the population in designated SACs (n=193). A further 4434 lakes are non-alpine but are located in SACs designated for at least one of the three sensitive habitat types.

For empirical nutrient N critical loads, it may be argued that the upper range (5-10 kgN ha^{-1} yr^{-1}) should be used for the 4434 non-alpine lakes in relevant SACs, while 3-5 kgN ha^{-1} yr^{-1} should be used for the 959 more sensitive alpine lakes of which 193 lie in these freshwater SACs.

The numbers of alpine and other designated lakes falling within the threshold deposition values of 0, 3, 5 and 10 kgN ha^{-1} yr^{-1} are shown in Table 1.1.6. If the "lower range" recommended for alpine lakes is assumed to be 3-5 kgN ha^{-1} yr^{-1} then all alpine lakes in SACs exceed their empirical critical loads for nutrient N. If the upper range of 5-10 kgN ha^{-1} yr^{-1} is used for all non-alpine lakes then 1197 do not exceed critical loads while a further 2549 would exceed the lower end of this range. 688 non-alpine lakes exceed the highest empirical critical load for nutrient N of 10

kgN ha^{-1} yr^{-1}, including all designated sites in England and Wales as described previously.

Table 1.1.6: Number of alpine and other designated lakes in deposition categories for 2004-06. Shading indicates exceedance even for highest CL values in range

Lake subset	Deposition category (kgN ha^{-1} yr^{-1})				
	0-3	**>3-5**	**>5-10**	**>10**	**All**
Alpine	0	0	48	145	**193**
Other designated	0	1197	2549	688	**4434**

Summary

1. On the basis of empirical critical loads for nutrient N, the 959 alpine lakes (>600m altitude) in the UK are an extremely threatened habitat with 79% receiving deposition loads >10 kgN ha^{-1} yr^{-1} (including all in England and Wales) and 100% receiving more than 5 kgN ha^{-1} yr^{-1}.

2. There are 4627 lakes of EUNIS Types C1.1 and C1.4 in 69 SACs designated for lake habitat types H3130, H3110 or H3160 in Great Britain. 833 (18%) of these lakes experience N deposition > 10 kgN ha^{-1} yr^{-1} including all 79 designated lakes in England and 85 in Wales. All sites experience deposition > 3 kgN ha^{-1} yr^{-1}. In Scotland, 73% receive > 5kgN ha^{-1} yr^{-1} and 15% > 10 kgN ha^{-1} yr^{-1} hence the choice of critical load in this range has a major effect on calculated exceedance.

3. 193 designated lakes lie above 600m altitude and may therefore be considered alpine sub-types of H3130 lakes. 75% receive deposition >10 kgN ha^{-1} yr^{-1} and 25% receive 5-10 kgN ha^{-1} yr^{-1}; all above the recommended range for alpine lakes. For 4434 non-alpine designated lakes, 73% would exceed a critical load of 5 kgN ha^{-1} yr^{-1} while only 16% would exceed the upper limit of 10 kgN ha^{-1} yr^{-1}.

Task 1.2: Uncertainty and sensitivity analysis of critical loads and exceedances

Aim of the Task

At the request of DEFRA a comprehensive sensitivity and uncertainty analysis of acidity critical load models for freshwaters was undertaken with the latest available datasets for water chemistry, catchment parameters and deposition, including:

1. Sensitivity analyses of empirically derived model parameters in the Steady State Water Chemistry (SSWC) and First-order Acidity Balance (FAB) models.
2. Analysis of uncertainty in critical loads exceedances associated with uncertainty in deposition data input, derived from many runs of the Hull Acid Rain Model (HARM) to provide a range of possible deposition values for individual sites for which exceedances may be calculated.
3. Uncertainty in critical loads based on temporal variability in measured water chemistry - how representative is a critical load based on a single spot sample?

Uncertainty and sensitivity to input parameters in critical loads models were assessed using computer-intensive Monte Carlo re-sampling techniques to produce credible confidence intervals for critical loads based on perturbation of model input parameters.

Methods

Sensitivity analysis is a means by which we can understand how the outputs from deterministic models, such as critical loads models, varies as a function of the model parameters. Furthermore, by varying the model parameters in a stochastic manner over reasonable ranges of uncertainty in the actual parameters, we can derive an idea of the uncertainty in the model output that arises from uncertainty in the model inputs.

Here we investigate the sensitivity of the FAB critical load model to a number of parameters specific to FAB and to the combination of SSWC and FAB parameters (Table 1.2.1). These parameters control the calculation of the critical leaching threshold above which critical load exceedance will take place. In addition, we consider the uncertainty in critical load exceedance due to uncertainties in deposition scenarios using a representative 95% higher posterior distribution of deposition from the Hull Acid Rain Model.

Sensitivity or uncertainty analysis proceeds by sampling parameter sets from assumed distributions for each of the model parameters. The assumed distribution and associated parameters for each of the model parameters (Table 1.2.1) reflects current understanding of the possible magnitudes or ranges of values that may be encountered for UK lakes and rivers, and reflect expert judgement based on knowledge of the types of system in the UK critical loads data set. For example, for the *S* parameter, we assumed that 400 was the most likely value but that there was a considerable degree

of uncertainty both above and below this value and hence represented this parameter using a suitably parameterised Gaussian or Normal distribution.

Several of the terms listed in Table 1.2.1 appear directly in FAB; the two woodland terms and the Sn and Ss terms. The N immobilisation and N denitrification terms do not appear in standard FAB formulations as model parameters. Instead these are best thought of as implied parameters taking a default value of 1. Terms related to N immobilisation and denitrification are included in FAB but are dependent upon literature values and the precise values that are used for a given site will depend upon the relative proportions of various soil types in the catchment. As such, the N immobilisation and denitrification terms in FAB represent input data that are based themselves on a set of soil "meta" parameters. It is not our intention to investigate the uncertainty of and sensitivity to these soil "meta" parameters here.

Instead, our interest is in the effect of uncertainty in N immobilisation and denitrification on critical load and critical load exceedance. As such, we take as given the soil "meta" parameters and introduce a pair of new parameters into FAB to control the *fraction* of N indicated by the combination of soil "meta" parameters and proportions of soils within individual catchments that is immobilised and denitrified respectively. To maintain back compatibility with previous FAB runs, these parameters are set at 1 meaning that the amount of immobilised and denitrified N indicated by the combination of soil "meta" parameters and soil proportions is respected. We allow these parameters to vary from 20% to 200% representing the high degree of uncertainty we attach to the soil "meta" parameters. This approach is justified because we are interested in the uncertainty in input data for the soils as a whole rather than the uncertainties on the individual soil types. Our approach allows us to isolate the uncertainty into two key terms rather than many tens of soil "meta" parameters.

10,000 random draws from the chosen distributions were made, each one representing a parameter set with which to parameterise the FAB model and thence calculate a critical load. Initial runs varied a single FAB term (indicated by "FAB" in the Model Part column of Table 1.2.1) at a time with a final global run in which all of the FAB terms were varied. For all of these runs, the SSWC parameters were held fixed at their default values. A final run was then performed, where all of the parameters in Table 1.2.1 were varied. We report only the result for the full FAB and SSWC runs here as these provide direct evidence as to the uncertainty in critical load calculations and the sensitivity of the model to the various input parameters.

Results are presented at the national scale via critical loads maps. Each set of maps shows i) the critical load exceedance using the default parameterisation of the FAB model, ii) the lower 5^{th} quantile, iii) the median ($=50^{th}$ quantile), and iv) the upper 95^{th} quantile of the distribution of 10,000 critical load exceedances for each site. We also

calculate the probability of exceedance for each site as the number of runs for which the sites had exceedance > 0, divided by 10,000 (the number of runs).

To investigate to which input parameters FAB is most sensitive, we computed the rank correlation coefficient between the 10,000 parameter values and the critical load exceedance for each site, and aggregated the results in the form of boxplots. A parameter that is closely correlated with the critical load exceedance over the 10,000 runs is one for which, at a given site, the FAB model output is sensitive to.

Results

Figure 1.2.1 shows the observed critical load exceedance map for the UK FAB dataset plus critical load exceedance maps for the median (50^{th}) and 5^{th} and 95^{th} percentiles of the distribution of 10,000 critical loads simulated by varying the model terms described in Table 1.2.1. The exceedances shown are based on the 2004-2006 CBED deposition data set. As we would expect, the observed critical load exceedance map and the median map are very similar, because many of the parameters varied as part of the Monte Carlo simulation have their stated FAB values near to or at the centres of the distributions assumed for these parameters (see Table 1.2.1 for details).

Critically, at the most optimistic end of the assumed uncertainty range, there remains a population of sites where current deposition is sufficient to exceed the 5^{th} percentile critical load. These sites are found in the North Yorkmoors, the Pennines, the Lake District, SW England, Galloway and inland areas of central & northern Scotland.

Given that we have varied parameters in the FAB model over realistic ranges, the results of this analysis suggest a modest population of 180 sites in the FAB mapping data set that are on the border-line of exceedance and would move into the not exceeded class if certain model parameter sets were used. A substantial number of sites (534) are found in the 0.8-1 probability of exceedance class. These sites represent a population within the FAB mapping data set for which, regardless of the parameter set used, are likely to be exceeding their critical load. That these sites exceed their critical load seems outwith the uncertainty in the FAB model itself.

A probability of exceedance can be computed from the 10,000 Monte Carlo runs by computing the number of runs where a site was not exceeded under 2004-2006 deposition as a proportion of the total number of runs. A map of the probability of exceedance is shown in Figure 1.2.2

The probability of exceedance maps (Figure 1.2.2) show the degree of uncertainty in exceedance of the critical load associated with uncertainty in the FAB model parameters, based on whether the SSWC parameters remained fixed at their default values or were allowed to vary as per Table 1.2.1. Table 1.2.2 shows the number of sites in each probability of exceedance class for the FAB model based on whether or not the SSWC-specific parameters were allowed to vary.

Table 3.2.1: Parameters used in the FAB model, their definition and defaults, plus the assumed distribution for the Monte Carlo analysis. The Model Part column refers to the part of FAB, which includes the SSWC model, where the parameters are used. μ = mean and σ = standard deviation of the normal or Gaussian distribution, α and β are shape parameters for the beta distribution. For the deposition data we use the indicated percentiles of the distribution of HARM runs produced as part of an uncertainty analysis of the HARM model itself.

Parameter	Model part	Definition	Default	Assumed Distribution
S	SSWC	The concentration of base cations representing a weathering rate that can effectively neutralise all acid inputs	400	Normal (μ=400, σ=100)
SO_4 Weathering	SSWC	Fraction of geological SO_4 in the sample, estimated as a fraction of total base cations	0.16	Beta (α=2, β=5)
SO_4 Zero	SSWC	Assumed background concentration of SO_4 from natural sources (e.g. volcanoes)	5	Uniform (min=0, max=20)
NO_3 Zero	SSWC	Assumed background concentration of NO_3 from natural sources	0	Exponential (rate=0.75, truncated at 5)
Coniferous Woodland[1]	FAB	Net flux of N taken up by coniferous forest	0.21	Uniform (min=0.1, max=0.4)
Broadleaved Woodland[1]	FAB	Net flux of N taken up by broadleaf forest	0.42	Uniform (min=0.2, max=0.8)
N immobilisation	FAB	Fraction of the amount of N immobilised by catchment soils. The amount of N immobilised is given by literature values for specific soil types. We include a parameter to control the fraction of this N that is immobilised thus allowing an assessment of uncertainty in the group of parameters associated with soil N immobilisation	1	Uniform (min=0.2, max=2)
N Denitrification	FAB	Fraction of the amount of N denitrified by catchment soils. The amount of N denitrified is given by literature values for specific soil types. We include a parameter to control the fraction of this N denitrified thus allowing an assessment of uncertainty in the group of parameters associated with soil N denitrification	1	Uniform (min=0.2, max=2)
Sn[2]	FAB	Mass-transfer coefficient for in-lake retention or removal of N, based on literature defaults with a range of 2-8 m yr^{-1}	5	Triangle (min=2, max=8, mode=5)
Ss[2]	FAB	Mass-transfer coefficient for in-lake retention or removal of S, based on literature defaults with a range of 0.2-0.8 m yr^{-1}	0.5	Triangle (min=0.2, max=0.8, mode=0.5)
N deposition	HARM	HARM-modelled total N deposition	N/A	Used 5th, 50th and 95th percentiles of HARM runs
S deposition	HARM	HARM-modelled total S deposition	N/A	Used 5th, 50th and 95th percentiles of HARM runs

[1] The coniferous and broadleaf woodland parameter sets were constructed to be highly correlated with each other, reflecting that a low value for one parameter should not be offset by a high value on the other. By enforcing a strong correlation, both forest terms are high together and vice versa.

[2] The Sn and Ss terms were simulated to be highly correlated in the parameter sets for the reasons outlined above.

Figure 1.2.1: Maps showing the observed, median, 5th quantile (Q05), and 95th quantile (Q95), FAB critical load exceedance for the UK critical loads data set for 10,000 random draws manipulating FAB model parameters.

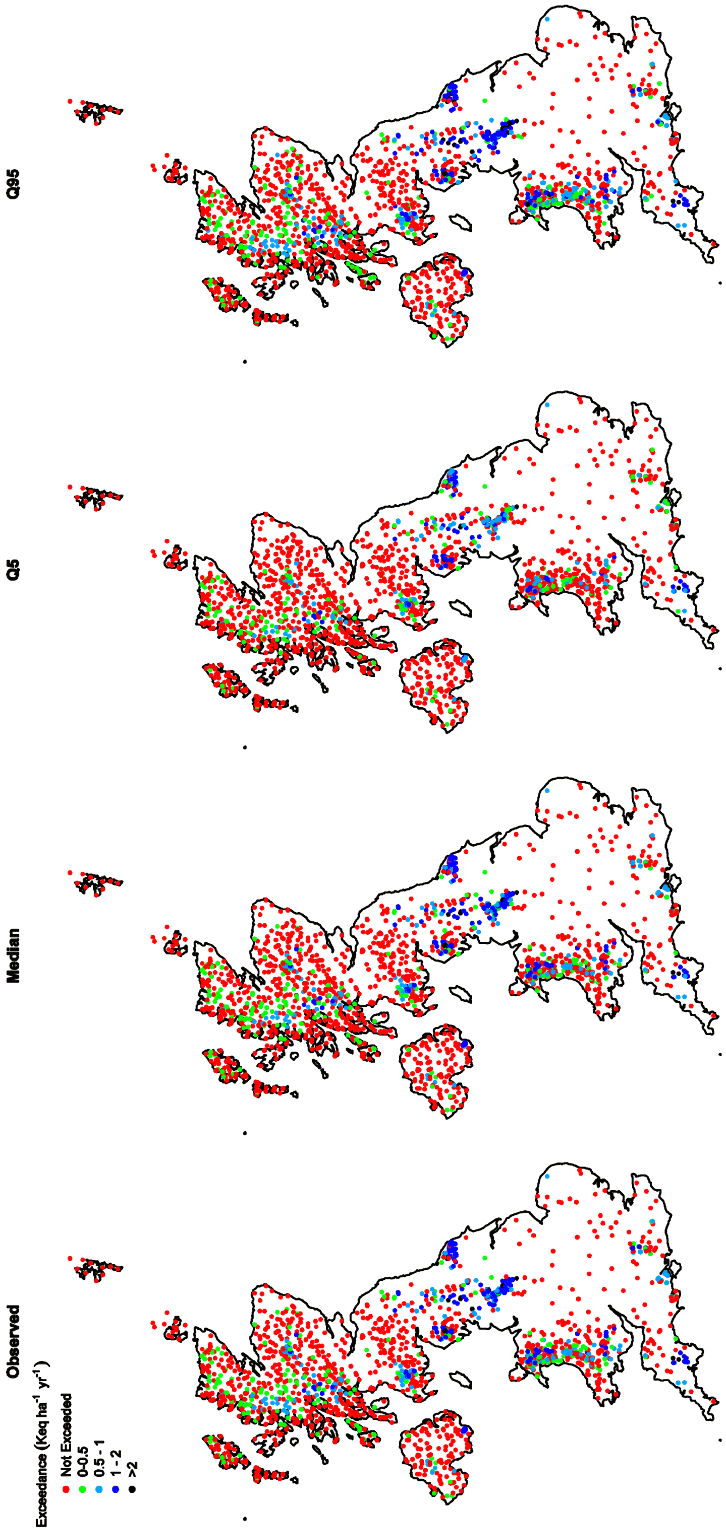


Figure 1.2.2: Probability of exceedance map for the UK FAB mapping dataset sites based on simulated parameter sets reflecting uncertainty in the FAB model terms.

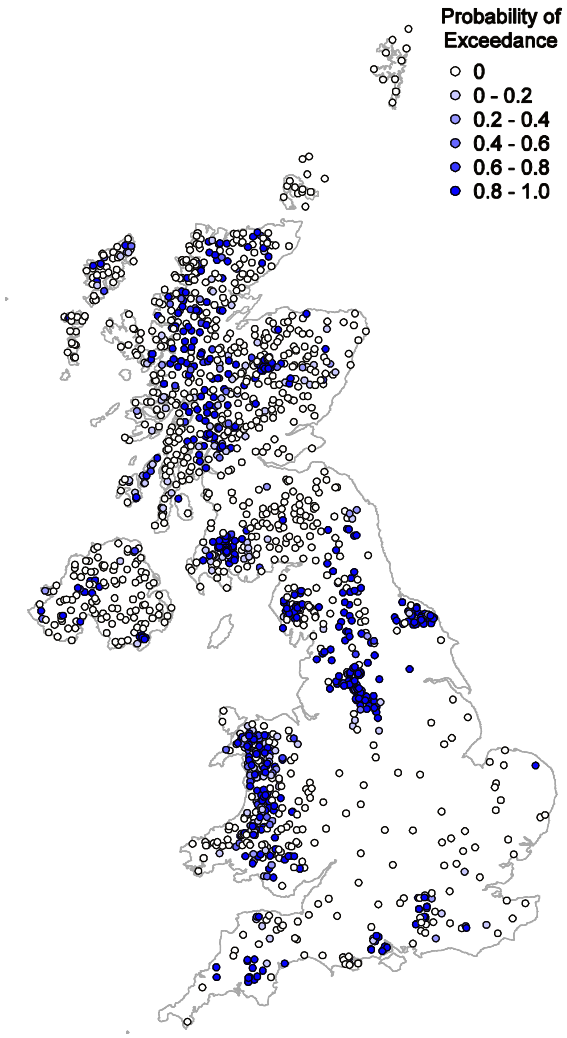


Table 1.2.4: The number of sites within probability of exceedance classes. Not exceeded indicates that sites did not exceed the critical load for any of the 10,000 parameter runs. These numbers are based on the model runs in which all FAB parameters were manipulated concurrently.

Not exceeded	0-0.2	0.2-0.4	0.4-0.6	0.6-0.8	0.8-1
919	180	36	44	39	534

Table 1.2.3 collates information from Figure 1.2.1 giving the actual number of sites in the 1752-site UK critical loads data set that fall into each of the usual exceedance classes. As mentioned earlier, we again note that the observed exceedances using the standard FAB terms and the median of the Monte Carlo uncertainty analysis runs are very similar to one another. Whilst this was largely by design given our choices for assumed distributions on the parameters, it does give some level of comfort that we can interpret the other quantiles of the distribution as appropriate confidence limits on the number of exceedances.

Table 1.2.3: Number of sites that exceed their critical load under 2004-2006 deposition. Observed is the currently reported number of exceedances using the standard FAB model terms. The remaining data are the number of exceeded sites in each exceedance class for the 5th, 50th and 95th percentiles of the 10,000 Monte Carlo simulation runs. The 5th and 95th percentiles reflect the expected uncertainty in critical load calculation, arising from uncertainty in FAB terms, with 90% coverage. The values in the first column are critical load exceedance in Keq ha^{-1} yr^{-1}.

	Observed	5th Percentile	50th Percentile	95th Percentile
Not Exceeded	1104	1271	1154	1042
0 – 0.5	251	211	246	246
0.5 – 1	137	161	173	214
1 – 2	184	103	168	217
2 +	16	6	11	33

Figure 1.2.3 shows the rank correlation coefficient between parameter values and critical load exceedance at the FAB mapping sites for the 10,000 randomly drawn parameter sets. A term that the model is sensitive to is one that would have a large rank correlation with the model output (critical load or critical load exceedance). In other words, the critical load exceedances at an individual site will be highly correlated with any terms in the model that the model is sensitive to for the assumed parameter values. Figure 1.2.3 summarises this sensitivity for the entire FAB mapping data set of 1752 sites.

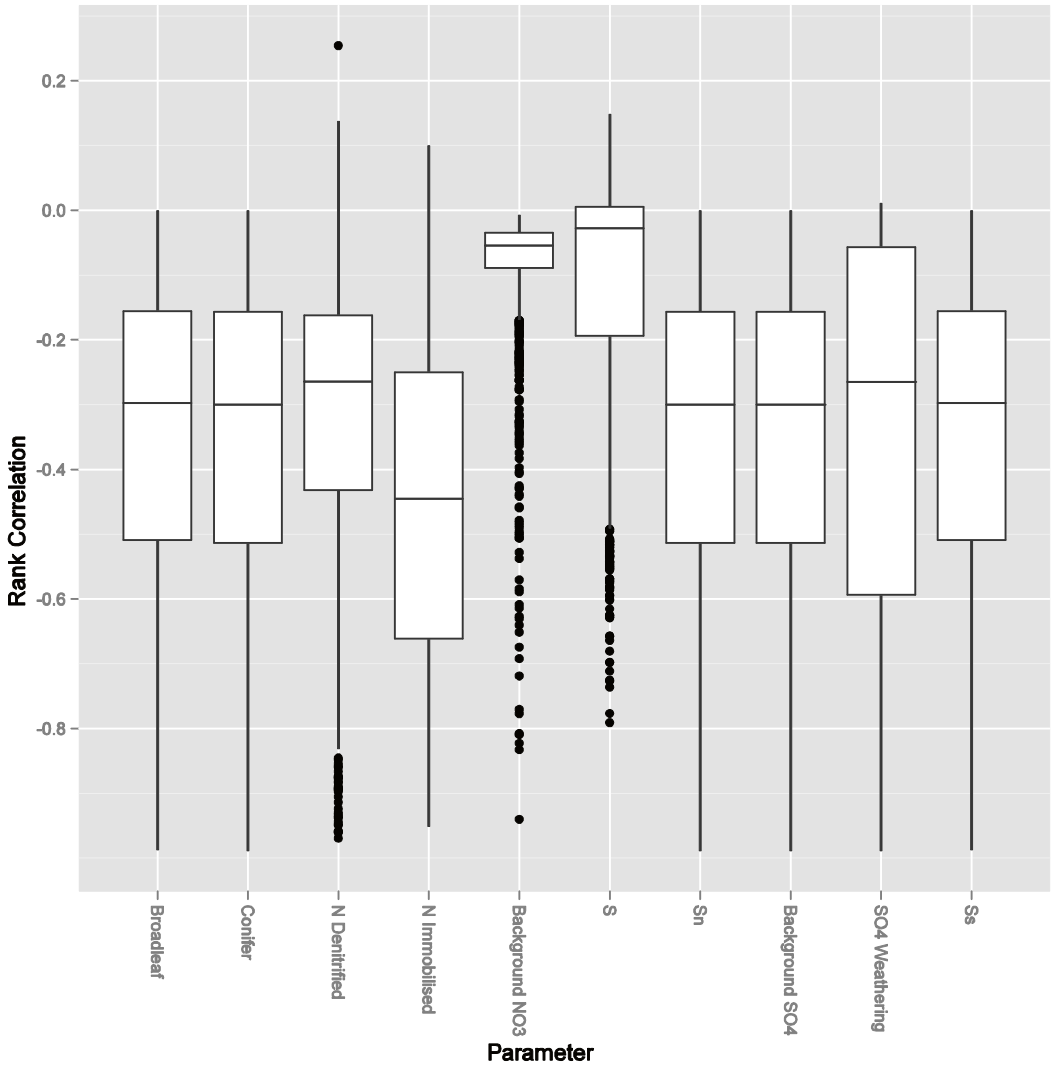
The main observation to be drawn from Figure 1.2.3 is that the sensitivity to a given model term is site specific. Very high rank correlations of greater than -0.8 are observed for some sites for all terms except the S term, whilst at other sites in the national data set, the model appears not particularly sensitive to the model parameter values used.

The second observation to make in regard to the sensitivity of the FAB model to specific terms is that, with a few minor exceptions, the rank correlations are all negative, such that as the value for a parameter is increased the critical load exceedance is reduced. That on average, all terms are negatively correlated with critical load exceedance is a reflection of the way the model is parameterised; the terms in the model represent processes that buffer the lake or stream from the effects of acid inputs.

With the exception of background nitrate concentration and the S model term, on average across the entire UK critical loads data set the FAB terms are of relatively similar sensitivity (compare the median bars in each boxplot). The term associated with the immobilisation of nitrate in soils is, on the whole the term that the FAB model is most sensitive two, with a median rank correlation of -0.45 across the national data set.

The results presented above suggest that the uncertainty in critical load exceedance is a site specific phenomenon. There are a body of sites that are sufficiently well buffered that they have effectively zero probability of being exceeded under reasonable limits of uncertainty in the FAB parameters. This body of sites numbers 919 out of a total of 1752 sites in the national data set. A number (260 with probability of exceedance <= 0.6) of other sites are only moderately exceeded under 2004-2006 deposition. For these sites, uncertainty in the parameter values used within FAB could move these sites from critical load exceedance into the not exceeded category. For a number of other sites in the national data set (534 with probability of exceedance > 0.8), these remain exceeded at all but the most extremely optimistic limits of the uncertainty in the currently used parameterisation of FAB.

Figure 1.2.3: Boxplots showing the rank correlation coefficient between the key parameter values and critical load exceedance for all FAB terms runs, for each of 1752 sites in the critical load mapping data set.



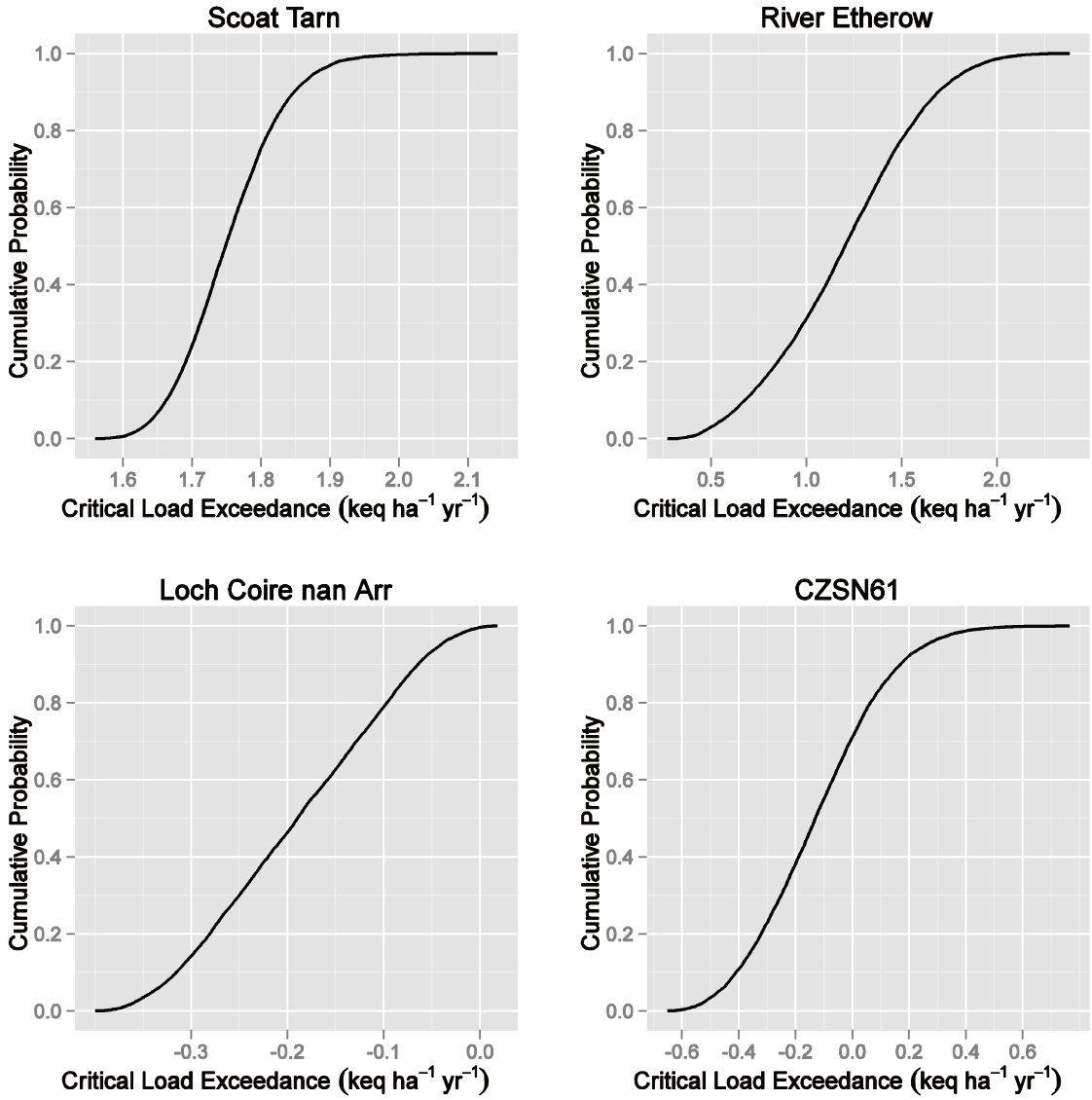
To illustrate the effects of uncertainty in FAB model terms on these various types of sites, we now present site-specific critical load exceedances for the 10,000 Monte Carlo runs of the FAB model. Figure 1.2.4 shows empirical cumulative distribution functions (ECDF) for four sites in the national FAB data set; Scoat Tarn, River Etherow, Loch Coire nan Arr and CZSN61 (Nant Gwythwch). The ECDF shows the cumulative probability, or the number of observations as a proportion of the total (10,000), on the y-axis where the critical load exceedance is less than or equal to a particular value of critical load exceedance as shown on the x-axis. For Scoat Tarn and River Etherow, two highly acidified sites in the Lake District and the Pennines respectively, none of the 10,000 Monte carlo-based critical load exceedances is less than or equal to zero, indicating no uncertainty in the statement that these two sites are exceeded under 2004-2006 deposition. The two sites differ in the range of uncertainty in critical load exceedance. The critical load exceedance for Scoat Tarn is quite tightly constrained, and is little affected by the uncertainty in the FAB terms. For the River Etherow, despite there being little or no uncertainty in whether the site is exceeded, the actual degree of exceedance is very uncertain, with values lower than 0.5 and greater than 2.25 observed over the 10,000 Monte Carlo runs. In terms of the critical loads exceedance maps, the River Etherow could be represented by any of the exceeded classes (green through to black on the maps). For the River Etherow therefore, despite the high degree of uncertainty in the actual critical load for the site, current deposition is sufficiently large at this site that this uncertainty does not change our assessment that the site is exceeded.

Two counter examples are presented in the lower panels of Figure 1.2.4. Loch Coire nan Arr in NW Scotland, an area of low acid deposition, is not exceeded up to all but the most pessimistic parameter sets encountered in the Monte Carlo runs. This is reflected in the low, but not zero, probability of exceedance for this particular site. In contrast to the other three sites, for CZSN61 the uncertainty in the FAB parameters has a marked effect on the degree of confidence in the current assessment that the site is not exceeded. This particular site is receiving deposition that is very close to but not exceeding the critical leaching threshold. A small decrease in the critical leaching threshold due to the use of slightly *lower* parameter values in FAB would push the site into the lowest critical load exceedance class. Conversely, slightly higher parameter values in FAB would afford a *higher* critical leaching threshold and consequently less chance of exceedance. CZSN61 is one of the few sites that have low to moderate probabilities of exceedance.

In terms of the national data set and reported exceedances, only 299 sites fall into intermediate probability of exceedance classes, representing ~17% of the national data set. 534 (~30%) sites are highly likely to remain exceeded regardless of the current uncertainty in FAB model parameters. Over half the sites in the national data set (919 sites) can be confidently deemed non-exceeded despite the uncertainty in the FAB model terms. These results indicate that approximately a third of the sites in the

national data set are exceeded despite the uncertainty in the FAB model used, while 20% of sites may not be exceeded under 2004-2006 deposition.

Figure 1.2.4: Empirical cumulative distribution functions (ECDF) for four sites in the national FAB mapping data set. The x-axis is critical load exceedance and the y-axis is the cumulative probability of the distribution of critical load exceedances up to and including a particular value of critical load exceedance. The ECDFs represent the 10,000 critical load exceedances produced from the Monte Carlo runs of the FAB model with all parameters varied at the same time.



Uncertainty in critical load exceedance due to uncertainty in deposition

To address the degree to which uncertainty in deposition data affects the uncertainty in critical load exceedance we use data from an assessment of uncertainty in the output from the Hull Acid Rain Model (HARM; Page *et al.*, 2008). The deposition data generally used for national assessments and critical load estimates (FRAME and

CBED) have not yet been subject to the same type of uncertainty analysis as that to which HARM has been subjected. As a result, and instead of assuming a distribution for the uncertainties in the outputs from FRAME and CBED, we use the 5th, 50th and 95th percentiles of the outputs from the HARM uncertainty runs as input into our assessment of uncertainties in critical load exceedance. HARM runs for 2005 and 2020 deposition were used here.

To allow for a real assessment of the effects of deposition uncertainty on critical loads computation and comparison with the uncertainty due to the FAB model, we conducted two sets of analyses here. The first analysis simply computed critical load exceedance under HARM 2005 and 2020 deposition for critical loads calculated using the standard FAB parameterisation. However, as HARM tends to produce lower deposition fields compared to FRAME and CBED, to provide a valid comparison of the deposition uncertainty with FAB model uncertainty we re-ran a Monte Carlo simulation for FAB parameters using the same parameter set described in Table 1.2.1, but this time calculated critical load exceedance using HARM deposition for each of the 10,000 Monte Carlo runs.

Table 1.2.4: Number of sites that exceed their critical load under median HARM 2005 deposition. The numbers are of exceeded sites in each exceedance class for the 5th, 50th and 95th percentiles of the HARM uncertainty analysis. The values in the first column are critical load exceedance in Keq ha^{-1} yr^{-1}.

	5th Percentile	50th Percentile	95th Percentile
Not Exceeded	1522	1408	1239
0 – 0.5	213	232	237
0.5 – 1	16	105	197
1 – 2	1	9	77
2 +	0	0	2

Table 1.2.5: Number of sites that exceed their critical load under median HARM 2020 deposition. The numbers are of exceeded sites in each exceedance class for the 5th, 50th and 95th percentiles of the HARM uncertainty analysis. The values in the first column are critical load exceedance in Keq ha^{-1} yr^{-1}.

	5th Percentile	50th Percentile	95th Percentile
Not Exceeded	1596	1512	1399
0 – 0.5	151	219	228
0.5 – 1	5	19	228
1 – 2	0	2	11
2 +	0	0	0

Table 1.2.6: Number of sites that exceed their critical load under median HARM 2005 deposition. The numbers are of exceeded sites in each exceedance class for the 5th, 50th and 95th percentiles of the 10,000 Monte Carlo simulation runs. The 5th and 95th percentiles reflect the expected uncertainty in critical load calculation, arising from uncertainty in FAB terms, with 90% coverage. The values in the first column are critical load exceedance in Keq ha^{-1} yr^{-1}.

	5th Percentile	50th Percentile	95th Percentile
Not Exceeded	1525	1455	1338
0 – 0.5	195	232	252
0.5 – 1	31	63	147
1 – 2	1	2	15
2 +	0	0	0

Table 1.2.7: Number of sites that exceed their critical load under median HARM 2020 deposition. The numbers are of exceeded sites in each exceedance class for the 5th, 50th and 95th percentiles of the 10000 Monte Carlo simulation runs. The 5th and 95th percentiles reflect the expected uncertainty in critical load calculation, arising from uncertainty in FAB terms, with 90% coverage. The values in the first column are critical load exceedance in Keq ha^{-1} yr^{-1}.

	5th Percentile	50th Percentile	95th Percentile
Not Exceeded	1618	1550	1451
0 – 0.5	131	192	262
0.5 – 1	3	10	38
1 – 2	0	0	1
2 +	0	0	0

The results of these two analyses are summarised in Tables 1.2.4-1.2.7. Tables 1.2.4 and 1.2.5 show the number of exceeded sites under HARM 2005 and 2020 deposition respectively in each of the critical load exceedance classes. It is clear that HARM deposition is lower than the CBED deposition used to produce the median values (50th percentile) in Table 1.2.3. As a result we cannot directly compare the degrees of uncertainty imparted via deposition or the FAB model from these results alone. We can however use the relative change in numbers between the 5th and 90th percentiles in Table 1.2.3 and Table 1.2.4 as a guide to the relative magnitudes of uncertainty due to deposition and that due to FAB. It is immediately clear that there are larger relative shifts between deposition classes for the 5th and 95th percentiles in Table 1.2.4 than in 1.2.3, despite the much lower number of exceeded sites in the latter. This result suggests that uncertainty in deposition is larger in magnitude than the uncertainty due to FAB.

To fully address this issue, we reran the Monte Carlo uncertainty analysis of the FAB model but using the median HARM deposition for 2005 and 2020. These results are shown in Tables 1.2.6 and 1.2.7. The difference in number of sites within each exceedance class between the 5th and 95th percentiles is far greater for the HARM uncertainty data than for the FAB uncertainty data for all classes bar the 0-0.5 Keq ha^{-1} yr^{-1} class. This result indicates that, using the best current available knowledge as to the uncertainties in deposition and in FAB, it is the uncertainty in deposition that leads to the greater uncertainty in critical loads exceedance calculations.

Uncertainty in critical loads due to temporal variability in measured water chemistry

Critical loads models assume that a lake or stream is in steady-state and use the measured water chemistry as an estimate of this steady-state chemistry. However, no lake or stream is actually in a steady state owing to the effects of reduced acid deposition across the UK, the result of emission reduction policies, and other factors including local variations in weather and climate. In this section we investigate the degree of uncertainty in the computed critical load that is due to variation in the specific water chemistry sample used. For this we use the UK Acid Waters Monitoring Network (UKAWMN) water chemistry time series and compute FAB-based critical loads and exceedances under 2004-2006 and 2020 predicted deposition. Here we use the standard parameterisation of the FAB model and do not vary any of the FAB terms as we are specifically interested in the effect of timing of water chemistry sampling on the critical load calculation.

Figure 1.2.5 shows the FAB critical loads (critical leaching threshold L_{crit}) for the UKAWMN sites. Each of the panels is drawn on a different scale to allow the within site variability to be ascertained, at the expense of between site comparisons. A large degree of variability in critical load is observed, dependent upon the particular chemistry sample input to the FAB model. Consequently, when a constant deposition is applied to the critical load to generate an exceedance, substantial variability is propagated through to the exceedance computation (Figure 1.2.6). Clearly this has implications for i) the representativity from spot samples of prevailing water chemistry conditions, and ii) the use of spot samples for critical loads computations against a changing background of water chemistry, such as the general chemical recovery from acidification observed in the UK AWMN sites (Monteith *et al.*, 2010).

The type of site also has a bearing on the degree of variability in water chemistry observed and thence on the calculated critical load. In Figure 1.2.5, the river sites have the largest variation in critical load. The lake sites tend to exhibit a lower degree of variance in water chemistry parameters owing to the time-averaging effects and mixing of water within the lake due to the water body residence time, the average time that water incoming from the catchment remains in the lake before exiting via the outflow.

A number of UKAWMN sites appear to have trends in L_{crit} with the direction of the trend, upwards or downwards, varying among these sites. Old Lodge (LODG) for example saw an increase in L_{crit} from the start of monitoring to the middle of the 1990s whereupon the trend has levelled out. The change in the critical load is modest however, on the order of 0.2 Keq ha^{-1} yr^{-1}. In contrast, lake sites such as Lochnagar (NAGA) and Scoat Tarn (SCOATT) have seen a trend throughout the period of monitoring to progressively lower critical loads. Again, the degree of change is small, on the order of 0.2-0.3 Keq ha^{-1} yr^{-1}. Whilst a full trend analysis of the critical loads data shown in Figure 1.2.5 is outwith the remit of this report, it is interesting to note the wide variation in trends in critical loads at UKAWMN sites despite the largely consistently improving conditions across the network in terms of acidity. As the critical load is a combination of acid inputs and ionic composition of the waters, it acts as an integrator of a wider range of acidity-related parameters than the determinand-specific trend analyses performed by Monteith *et al.* (2010). Further work is required to understand the drivers of the patterns in critical loads by examination of the multivariate inputs to the computation.

Figure 1.2.6 shows the exceedances for the critical loads shown in Figure 1.2.5 using 2004-2006 CBED deposition. Here we have used the same scale on each panel to emphasise the between site variability. The increased variance of the stream sites is still clearly visible. The critical load exceedances for a number of sites traverse the 0 line indicating that whether a site would or would not exceed its critical load is very much dependent upon which water sample is used to compute the critical load.

A number of sites are always indicated to have exceeded critical loads, where the time series does not fall below the zero line of exceedance. For these sites, currently any of the spot samples over the 20 years of monitoring would have led us to conclude that they were exceeded under current deposition and as such the temporal variability does not add to the uncertainty in critical load exceedance. For the remaining sites that regularly traverse the critical load exceedance threshold (the 0 line), whether or not the site is exceeded is highly dependent upon which sample is used to perform the critical load calculations.

For the majority of sites in the national critical loads data set we do not have time series of observations with which to quantify the effects of temporal variability on the national critical load exceedance statistics. In many cases however the degree of exceedance that occurs periodically due to variation in input chemistry at many sites is small. For selected regions, Task 1.5 considers the change in critical load exceedance between the original survey and winter 2010 and spring 2011 resampling campaigns.

Figure 1.2.5: FAB critical load (critical leaching threshold L_{crit}) for the UKAWMN sites.

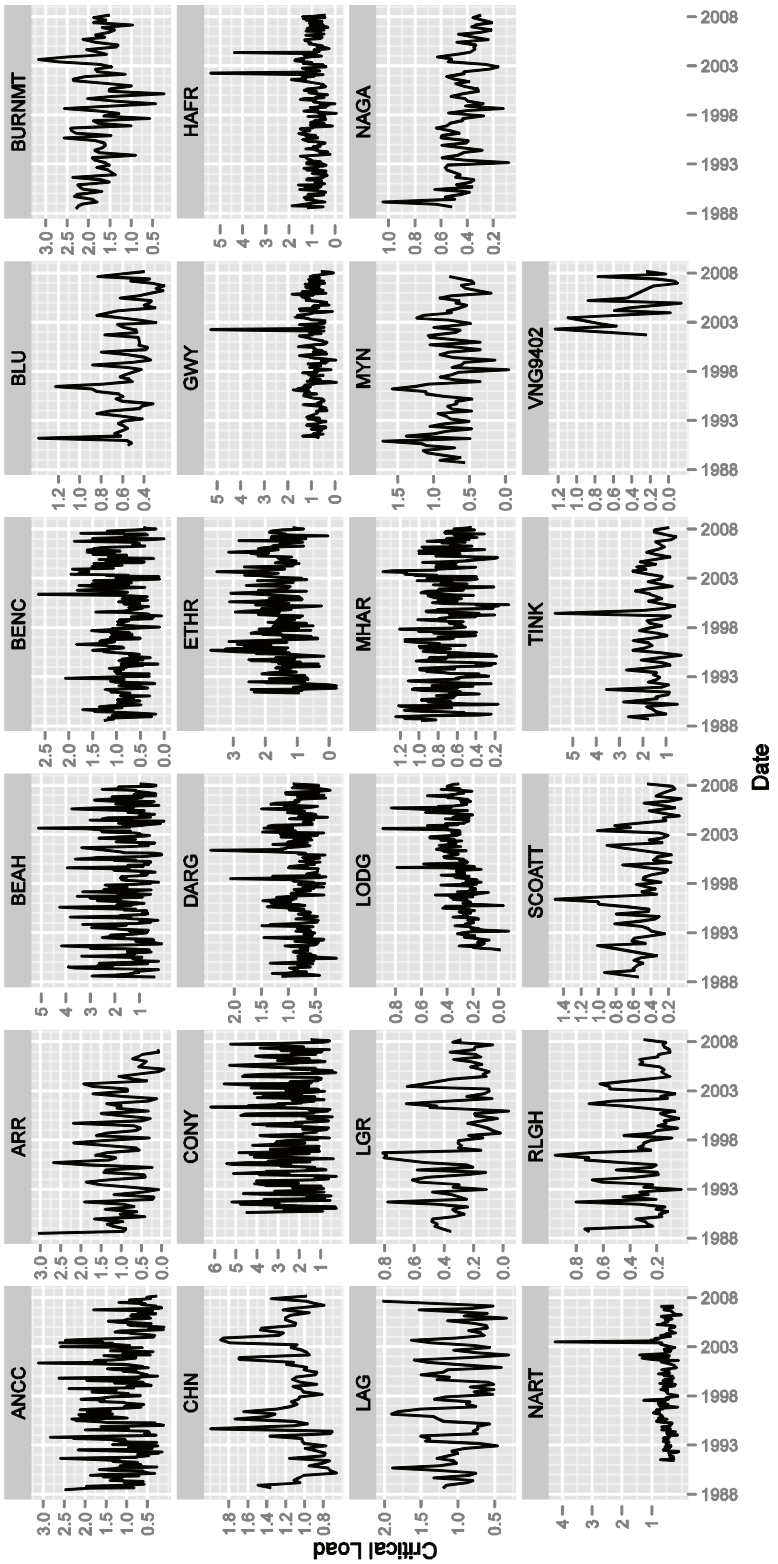


Figure 1.2.6: FAB critical load exceedance (Keq ha^{-1} yr^{-1}) for the UKAWMN sites, based on critical loads shown in Fig. 1.2.5 and 2004-2006 CBED deposition. Positive values indicate critical load exceedance at the fixed deposition level used.

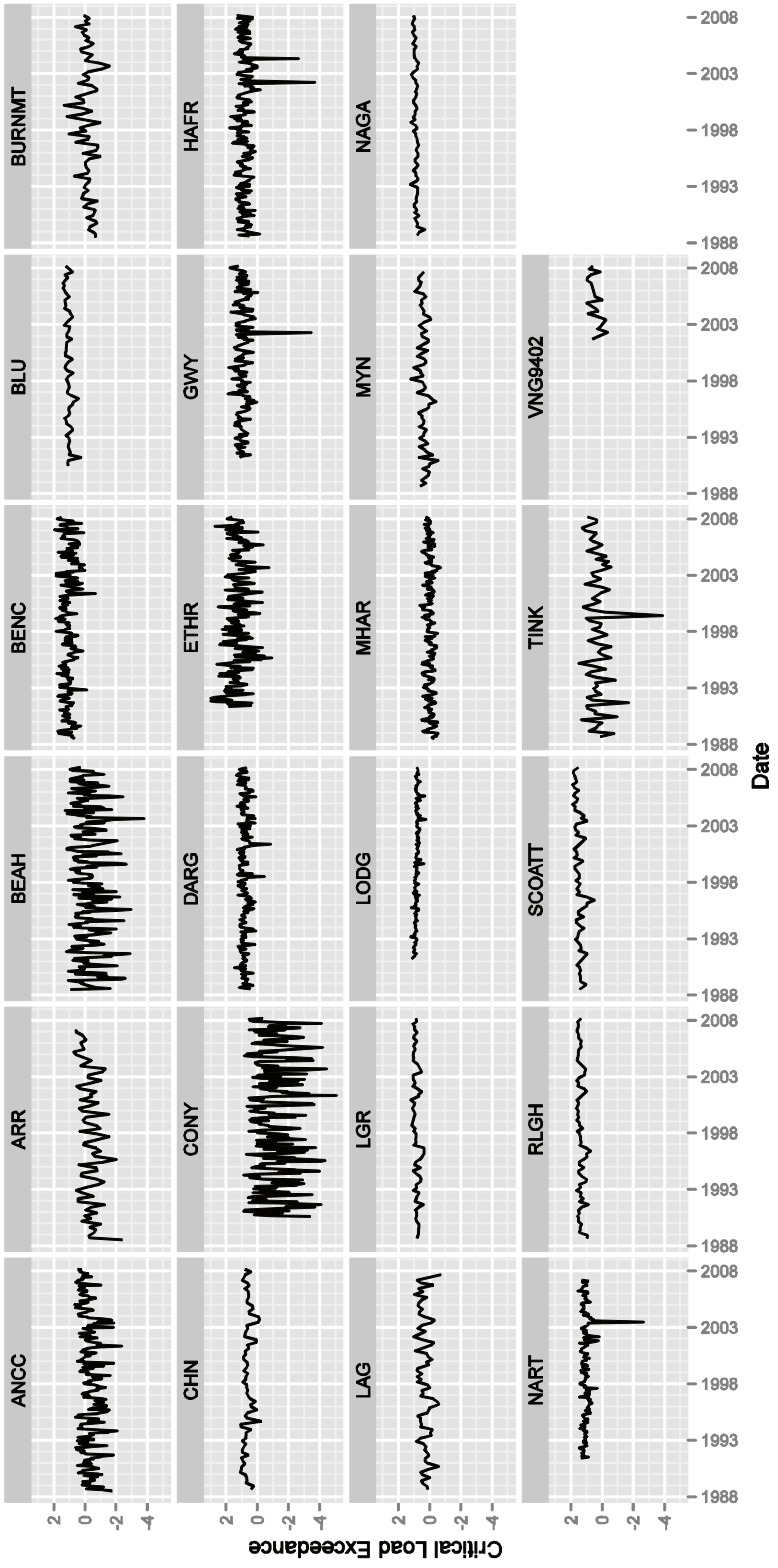
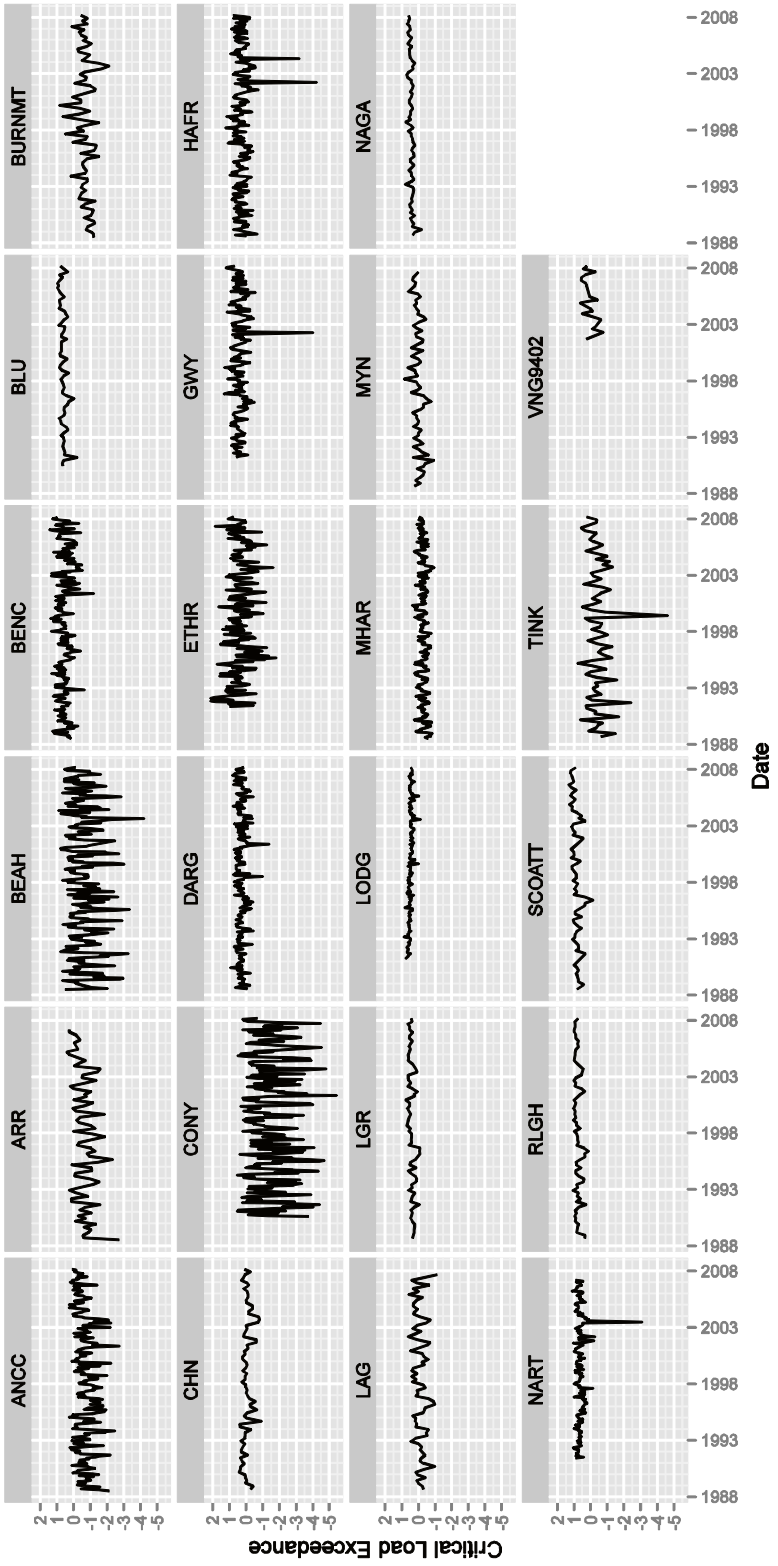


Figure 1.2.7: FAB critical load exceedance (Keq ha^{-1} yr^{-1}) for the UKAWMN sites, based on critical loads shown in Figure 1.2.5 and predicted 2020 deposition. Positive values indicate critical load exceedance at the fixed deposition level used.



Critical loads outputs are widely used to inform on the efficacy of emission reduction policies. As such, we might wish to consider the effect of temporal variation on the exceedance or otherwise of the UKAWMN sites using the FRAME 2020 deposition scenario considered by RoTAP (in press). Figure 1.2.7 shows exceedance time series for the UKAWMN sites based on the critical loads in Figure 1.2.5 but using the FRAME 2020 deposition. In general, this has the effect of lowering the mean exceedance value relative to Figure 1.2.6 because the 2020 scenario includes the effects of emission control measures to that date. Overall, for the majority of sites, the number of exceedances is reduced, sites continue to traverse the 0 lines moving from exceedance to non-exceedance depending on which sample is considered.

The effect of temporal variation on critical load exceedance uncertainty may be reduced by using annual average chemistry instead of individual spot samples. Figures 1.2.8 and 1.2.9 show the critical load exceedances for UKAWMN sites using current (2004-2006) and future (2020) deposition. The trends in critical load exceedance are now far more apparent in a number of sites (e.g. BEAH, LODG, NAGA, SCOATT).

Of the 23 UKAWMN sites (including the now discontinued Loch Coire nan Arr, ARR), eight show movement from non-exceedance to exceedance at one or more points during the 20 years of monitoring (ANCC, ARR, BEAH, BURNMT, CONY, LAG, MHAR, TINK). This change in status is *not* the result of changes in deposition as this has been held constant here at 2004-2006 CBED deposition. For CONY (Coneyglen Burn in Northern Ireland) only one point shows exceedance in the final year, which may be an artefact of incomplete sampling throughout the year at the end of the time series. At LAG (Llyn Llagi) all years show exceedance except for 1996. At the other sites in this group there is greater variation between exceedance and non-exceedance, although at most sites the range is quite small and applies to sites showing only borderline exceedance on occasion.

Under 2020 deposition, ten sites now move between exceedance and non-exceedance (ARR, BURNMT, CHN, DARG, ETHR, HAFR, LAG, MYN, TINK, VNG9402). Three sites that previously moved between exceedance and non-exceedance (ANCC, CONY and MHAR) are consistently non-exceeded under 2020 deposition. Again, whilst the degree of exceedance is small, temporal variation in water chemistry in the UKAWMN has considerable effect on the number of sites exceeded under the future deposition scenario.

The apparent positive trends in exceedance at some sites using fixed deposition values indicate that calculated critical loads, in particular the pre-industrial leaching flux of base cations, appear to decrease as sites recover from acidification. This phenomenon is consistent with the findings of Rapp & Bishop (2009) that the F-factor component of the SSWC and hence FAB model does not perform well during the recovery phase.

Figure 1.2.8: FAB critical load exceedance (Keq ha^{-1} yr^{-1}) under 2004-2006 CBED deposition for the UKAWMN sites based on annual average chemistry. Positive values indicate critical load exceedance at the fixed deposition level used.

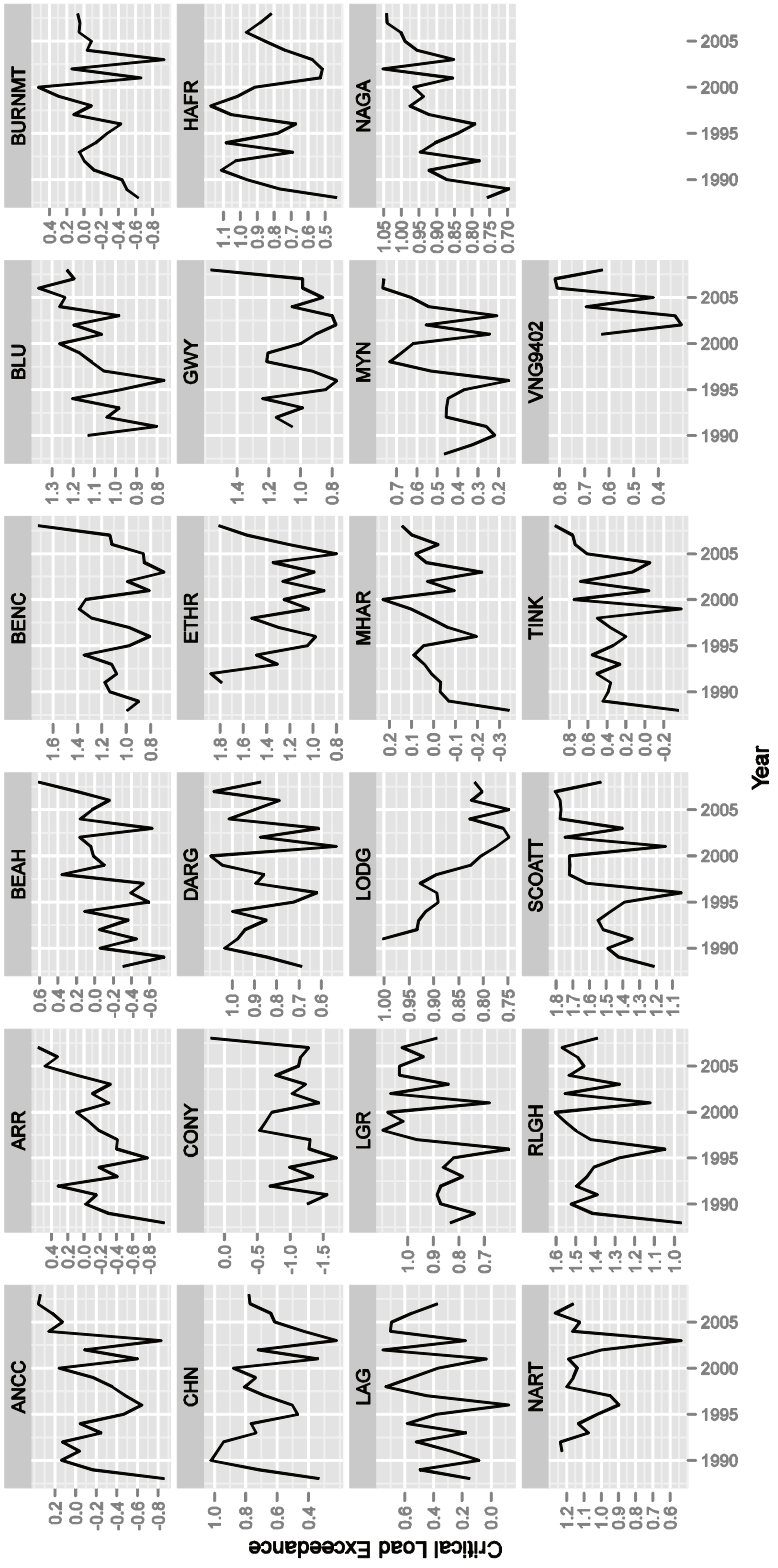
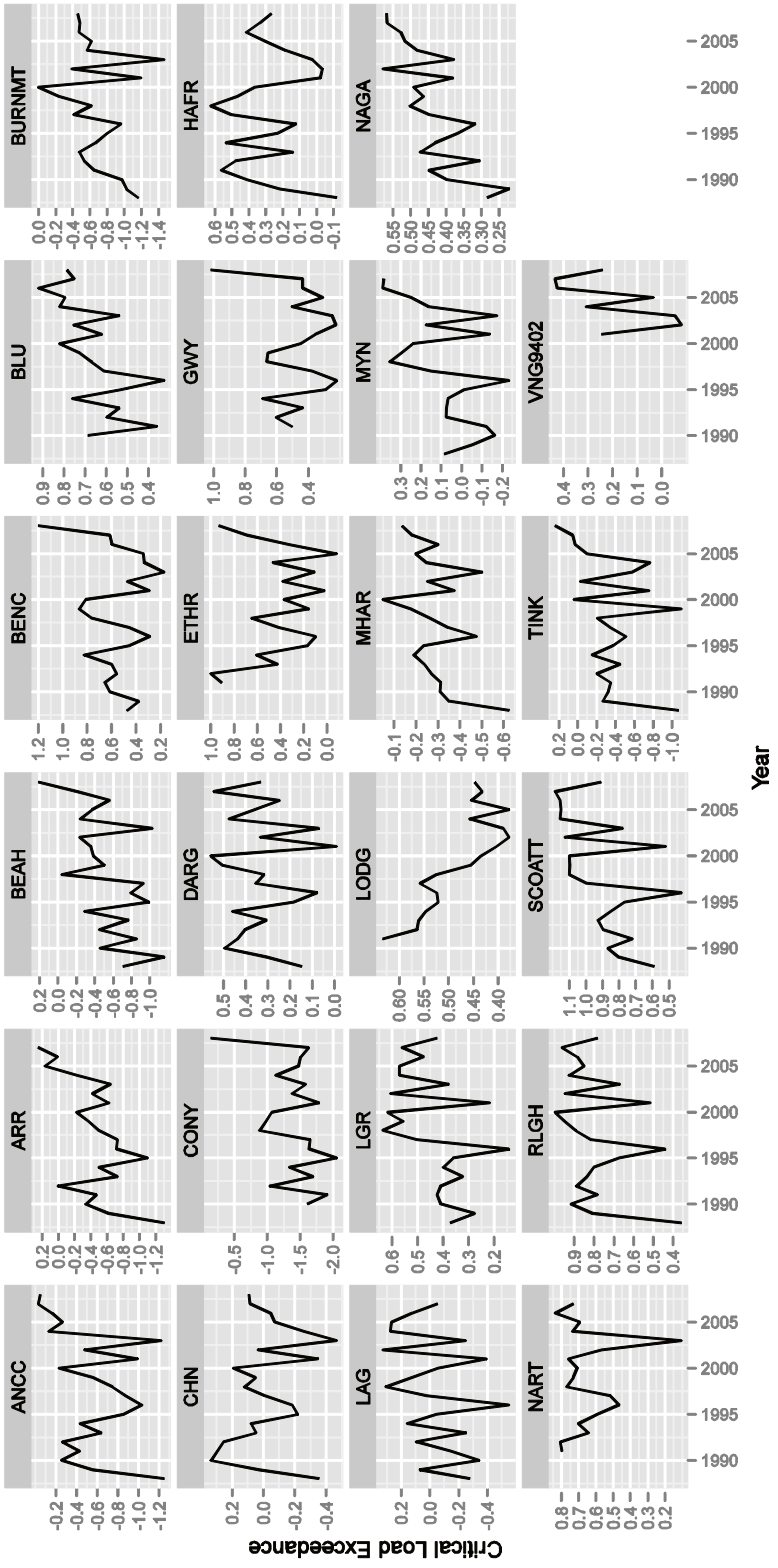


Figure 1.2.9: FAB critical load exceedance (Keq ha^{-1} yr^{-1}) for 2020 deposition for the UKAWMN sites based on annual average chemistry. Positive values indicate critical load exceedance at the fixed deposition level used.



The issue is linked to the depletion of soil base cations during the acidification phase, so that when the acid deposition load is reduced, the leaching flux of base cations may decline to below the pre-industrial rate which sets the critical load. There does appear to be evidence from the current analysis that for some sites at least, modelled critical loads are declining, leading to over-estimation of exceedance. The issue certainly merits more thorough statistical analysis of the noisy time series data and the identification of site characteristics which lead to this situation.

Summary

1. Sensitivity analysis of the FAB and SSWC model terms using broad ranges in each parameter and 10,000 Monte-carlo model runs indicate that most sites fall into "definitely not exceeded" or "definitely exceeded" categories. An appreciable minority of sites fall into the low probability of exceedance class (0-0.2; 180/1752 sites).Very few sites fall into the moderate probability of exceedance classes, but 299 sites (17%) in the national dataset have <60% probability of exceedance, compared with 534 sites (30%) that are definitely exceeded.
2. Sensitivity to uncertainty in model parameters is very site specific and the majority of sites are either definitely not exceeded, or definitely exceeded, even employing the wide parameter ranges allowed here.
3. The use of 5^{th} and 95^{th} centile HARM deposition datasets taken from a previous study (Page *et al.*, 2008) and comparison with equivalent centiles for the FAB model Monte Carlo runs indicates that uncertainty in deposition contributes more to uncertainty in exceedance than does uncertainty due to model parameters.
4. Analysis of temporal variations in calculated critical loads (and hence exceedances) using AWMN time series data shows that some sites vary between exceedance and non-exceedance according to when sampling was carried out. The use of annual mean data reduces but does not eliminate this problem, suggesting that e.g. climatic effects may influence year-to-year variations in critical load.
5. Some sites show apparent declining trends in critical load which may be linked, counter-intuitively, to recovery from acidification which allows measured base cations to decline below the pre-industrial rate. Exceedances at these sites show apparent increasing trends with a constant deposition load. It is therefore possible that for a proportion of sites, exceedances calculated beyond 2020 are exaggerated and future work should aim to identify which sites are affected by this phenomenon.

Task 1.3: Classification of lake types / analogues according to catchment attributes for upscaling critical load and dynamic modelling outputs to unsampled lakes from the national database

This task provides the necessary data on lake modern analogues for use with the MAGIC Library under the CLDM contract (MAGIC is a dynamic acidification model used to determine timescales of acidification and recovery and to set Target Loads). It entails a logical extension of the catchment modelling approach adopted for predicting nutrient N effects under Task 2.3 of this contract. Catchment attribute data (geology, soils, land cover, area, slope etc.) are used to link unsampled lakes to the most similar sites for which water chemistry, critical loads data and MAGIC model runs are available. The sites from the MAGIC Library that are identified as being most similar to the unsampled site of interest for which a model run is required are used to produce a best estimate of MAGIC hindcast and forecasts under future deposition scenarios. Suitable confidence intervals are derived where there are more than two close modern analogues in the MAGIC Library for the site of interest.

CLDM have done the MAGIC runs for the FAB dataset sites, and constructed the MAGIC library which provides a 'front end' for assigning the appropriate MAGIC run from a modelled site to an unmodelled site. The Freshwater Umbrella undertook the statistical analysis which underpins this link. Results of the MAGIC Library work are presented in the CLDM report; here we describe the contributed work done under the Freshwater Umbrella.

Phase 1: Analogue method trials
Following initial discussions with Chris Evans and members of the CLDM umbrella project, the first attempt at building an analogue-based tool for upscaling FAB and MAGIC to the national level was undertaken in 2008. A methodology was determined, based on the use of Gower's mixed dissimilarity coefficient, which although requiring future tweaking and enhancement, could be implemented in a first version of a UK MAGIC library or FAB upscaling tool. Here we briefly describe the approach taken, some preliminary results for upscaling FAB and discuss ways in which the method could be improved in the future.

Analogue-based methods are grounded on the use of dissimilarity coefficients and a set of predictor variables that allow discrimination between like and unlike sites. In building a MAGIC library or upscaling tool for FAB, we are interested in applying these models to sites for which the data required to run the actual model are lacking. Instead we wish to use national-scale mapping data, such as soils or land cover measurements, to attempt to find sites in the FAB data set that are most similar to sites at which we wish to apply FAB or MAGIC but have insufficient primary data.

The data collation work undertaken as part of Task 2.3 has provided a data set of 1752 sites with soils and land cover data. Further variables from the FAB mapping data set on site type (river, lake, artificial), forest land cover, run-off, N and S deposition, and catchment area, plus lake area and lake area to catchment area ratio where applicable,

were also available for this analysis. These data have been used here in this first attempt at an analogue-based upscaling tool.

To allow for comparison between sites on the basis of soils data, where some soil sub-types have been recorded only for subsets of the full 1752-site data set, all soil sub-types were merged into the general type. For example, 2 soil sub-types were recorded for some sites for peaty soils. For the analogue-based approach, we aggregated the values for these two sub-types into the main category for peaty soils, soil-type 10, by summing across the main soil-type and the two sub-types.

To measure the dissimilarity between sites using mixed-mode data, where different types of variables and variables measured in different units are used in the comparison, a suitable dissimilarity coefficient must be chosen. The most common dissimilarity coefficient of this type is Gower's general coefficient of dissimilarity. In this coefficient, each variable is considered separately, and for binary or categorical variables a similarity between two sites is recorded if the variable is present in both (binary variables) or of the same class (semi quantitative class variables) in the two sites. For quantitative variables, the standardised absolute difference between the value of the variable in the two sites is returned as a measure of similarity. The total dissimilarity between two sites is just the average of the dissimilarities recorded for each variable. This process if repeated for all pairs of sites. Weightings for variables can be used to give greater or lesser importance to one or more variables. In this case, the final dissimilarity is a weighted average of the individual variable-based dissimilarities for a pair of sites.

In this first attempt, all variables were given the same weighting.

Analogue-based upscaling proceeds by computing the pair-wise dissimilarity between each site and every other site in the FAB data set. The distribution of these dissimilarities can be used as a guide to determine whether a site is a close match (close analogue) or not. Low percentiles (say the 1%) of the distribution of these pair-wise dissimilarities are used for this purpose, recognising that within a given data set, few sites will be very similar or dissimilar.

An alternative is to use Monte Carlo resampling from the pair-wise dissimilarities to produce a new data set of dissimilarities that better reflects the distribution we might have obtained if a different sample of sites had been collected. Monte Carlo resampling was performed, by randomly selecting two sites from the FAB data set and recording the dissimilarity between the two sites. This was repeated a very large number of times (n = 100,000) to build up a Monte Carlo sample of pair-wise dissimilarities. Low percentiles of this distribution are then used to determine if any two sites are close matches or not.

Figure 1.3.1 shows the distribution of the 100,000 Monte Carlo samples of pair-wise dissimilarity for the FAB mapping data set. The shaded area on the right-hand plot shows the area under the cumulative distribution function that corresponds to a 95% significance level. The dissimilarity cut-off that corresponds to this significance level is 0.049, with the equivalent value from the observed data being somewhat higher at 0.055. It is worth noting that the total range of dissimilarity values observed is quite low, suggesting that discrimination between similar and dissimilar sites on the basis of the chosen set of variables and weightings is difficult.

To apply this methodology to upscale FAB or MAGIC, measurements on the set of predictor variables mentioned above are required for each site at which we want a FAB or MAGIC run. The dissimilarities between the test site and the FAB mapping sites are computed. Any sites from the FAB mapping data set that are as close as or closer to the test site than a dissimilarity of 0.049 are considered close matches and the FAB critical loads or MAGIC runs for the close matches are then taken to be the inferred or upscaled output from the tool.

In an attempt to determine the level of uncertainty that would be associated with upscaling the FAB model for critical loads of S and N, we undertook a modern analogue technique (MAT) analysis of the FAB data set described above and looked at the ability of the MAT model to predict critical loads of S and N for sites in the FAB data set where these values are known. MAT is a *k*-nearest neighbours method of smooth regression and works by selecting the *k*-closest analogues to each site and taking the average of these *k*-closest sites as the fitted value for that site. In this regard it is somewhat different to the critical values of dissimilarity we derived above as the use of a fixed *k* in MAT results in a variable cut-off being applied for each site. However, MAT provides an indication of the ability to discriminate between sites on the basis of the chosen set of predictors. It is worth noting that MAT can be fitted using a variable *k*, and part of the work under this task was to update the code in the analogue package, written by Gavin Simpson for the R statistical software, to allow this.

MAT models were fitted to the FAB data set to predict S and N critical loads using Gower's general dissimilarity coefficient. The maximum bias statistic was used to guide the choice of *k*, where models with low maximum bias were selected in preference to models with overall lower prediction error. This resulted in models that both used $k = 2$ analogues for predicting N and S critical loads. The root mean squared error of prediction (RMSEP) was determined by leave-one-out cross-validation. The RMSEPs of the two models were 5.77392 and 3.607252 K eq ha^{-1} $year^{-1}$ for N and S critical loads respectively. Whilst these uncertainty values are reasonably low given the range of critical loads in the FAB data set, they are quite high when we consider upland sites at which the model is most likely to be applied.

Part of the problem with these models is that the dissimilarity between any two sites is being biased low due to the sparse nature of the soils and land cover data sets. As an illustration, consider two sites dominated by single but different soil types and having zero proportions for all other soil types. Contributions from all the zero valued soil types would be considered as similarities under the coefficient described here and the only source of dissimilarity would come from the two soil types upon which the sites differed. However, the zero-valued soil types do not in reality reflect a similarity between these two sites, but they carry undue weight as such in the analysis.

Two solutions to this problem could be tried:

a) Discretize each soil-type and land cover-type into a few classes and treat each soil-type as a semi-quantitative variable, or
b) Convert the soils and land cover data into two single categorical variables by clustering the sites in some way to produce soil and land cover "types".

The first option above does not circumvent the zero-valued issue but may act to lessen it. The second option provides perhaps the best way to proceed with the method. Soil type and land cover type will contribute only once each to the dissimilarity and a similarity between sites will only be recorded if the two sites in question belong to the same soil or land cover type respectively.

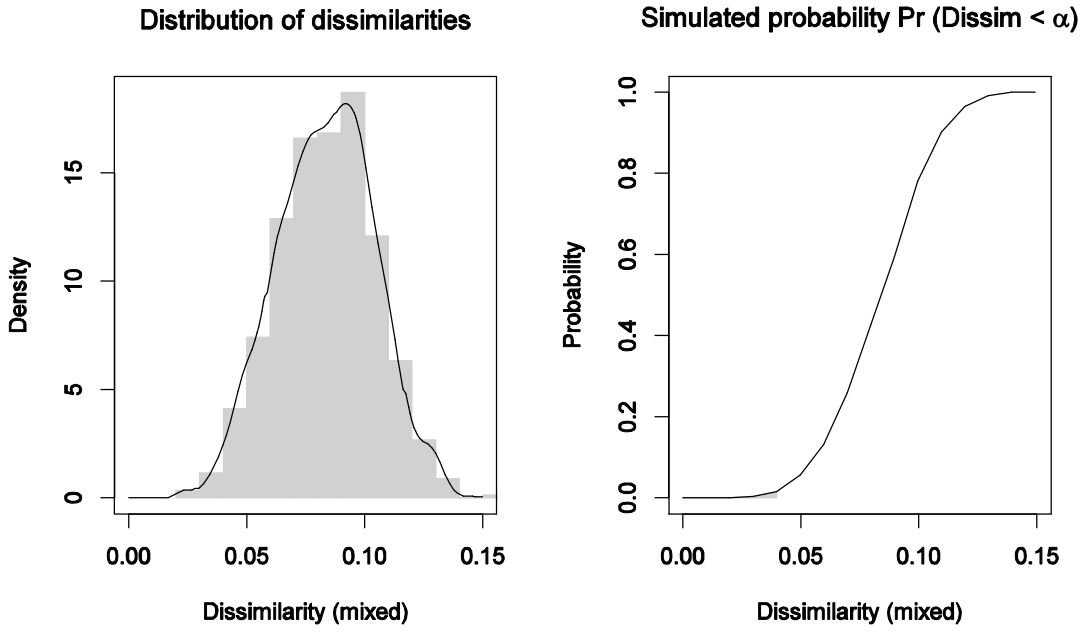
Alternatively, we could compute the dissimilarity for the soils and the land cover variables in two separate analyses, and combine these with equal weight with a dissimilarity computed using the remaining deposition, forestry, area etc. variables described above. The dissimilarities for the soils and land cover variables could be computed using an asymmetric dissimilarity coefficient, where double-zeros are not considered a similarity.

Work under this task continued by providing the methodology and critical values to the CLDM umbrella project, whilst improvements in the methodology were attempted.

Second phase

Subsequent developments to the methods followed on from meetings between CLDM and Freshwater Umbrella researchers during 2009, and all necessary R code was provided to the CLDM for use in development of the MAGIC library approach. The UKlakes GIS coverage of all lake catchments within the UK was also provided to the CLDM to allow upscaling to all lakes in the UK. This work is reported in detail in the CLDM Final Report.

Figure 1.3.1: Distribution of the 100,000 Monte Carlo samples of pair-wise dissimilarity for the FAB mapping data set (left panel); empirical cumulative distribution function showing 95% significance region (right panel)



Task 1.4: Comparison of policy approaches under CLRTAP, WFD and Habitats Directive

Aims

- Regional modelling of appropriate ANC limits based on reference conditions from MAGIC/SSWC and palaeo hindcasting methods
- Assessment of where ANC20 (CLRTAP/NECD) or $ANC_{reference}$ (WFD) provides more stringent policy targets for emissions
- Assess extent of probable impacts on Natura 2000 sites in terms of both acidification and nutrient N impacts including all designated aquatic features (3 main habitat types).

The aim of this task is to compare policy approaches under the primary driver of critical loads modelling work, the CLRTAP, with other policy areas making use of critical loads datasets for freshwaters. FAB datasets provided by the UK NFC to the integrated assessment modelling carried out under the Gothenburg Protocol employ critical values of ANC of either 20 μeq l^{-1} (the majority of cases) or 0 μeq l^{-1} where palaeolimnological or dynamic modelling data indicate a pre-industrial ANC of <20 μeq l^{-1} making a target value of 20 μeq l^{-1} impossible to achieve through emissions reductions. The EU Water Framework Directive employs a rather different approach to protecting aquatic ecosystems, requiring the achievement of good ecological status assessed according to deviation from a Reference condition which is assumed to be unimpacted by human disturbance, including air pollution.

The use of critical loads to assess damage to Natura 2000 sites of relevance under the Habitats Directive is described under Task 1.1 above.

Here we employed a rather simplistic approach using the steady-state water chemistry (SSWC) model of Henriksen et al. (1992) to provide pre-industrial (i.e. Reference) ANC. This is calculated from the pre-industrial base cation concentration $[BC]_0^*$ and pre-industrial SO_4^{2-} concentration $[SO_4^{2-}]_0^*$ which are both outputs of the SSWC model. Pre-industrial NO_3^- is assumed to be negligible, which is supported by present concentrations close to detection limits under low but anthropogenically elevated N deposition in regions of NW Scotland and northern Scandinavia. "Reference" ANC is calculated as the difference between $[BC_0^*]$ and $[SO_4^{2-}]_0^*$. It should be noted from the outset that this approach is very approximate since the SSWC model makes several assumptions which may not hold true for all sites. However, this exercise should provide a broad picture of which sites were likely to have Reference ANC values which differ from the fixed values used under the Gothenburg Protocol at present.

Using the SSWC modelled Reference ANC we categorised sites from the dataset used in Task 1.5 (see below) comprising 445 sites exceeding FAB critical loads according to FRAME 2020 deposition data. Data were subdivided by region, and split into SSWC Reference ANC classes (Table 1.4.1). The number of sites employing ANC_{crit} values of 0 or 20 μeq l^{-1} in each region is provided for comparison, while the same

figures expressed as a percentage of the total number of exceeded sites in each region is provided in Table 1.4.2.

Results

In several regions, a small proportion of sites has a Reference ANC of <0 μeq l^{-1} which suggests that the target of 0 or even 20 μeq l^{-1} under the Gothenburg Protocol is not achievable. The greatest proportion occurs in the North York Moors (22%), while 12% of Pennine sites and 9% of Lake District sites also have negative values of modelled Reference ANC. In these sites, the hypothetical use of Reference ANC as a target under the WFD would indicate that present critical loads are under-estimates and some of these sites should have greater critical loads.

For all regions except S&E England, a much greater proportion of sites have modelled Reference ANC values of 0-20 μeq l^{-1}, ranging from 4-72%. In the case of NW Scotland, 72% of sites have Reference ANC values of <20 μeq l^{-1} whereas 88% have a target ANC_{crit} of 20 μeq l^{-1}. If the modelled Reference ANC values are accurate, the suggestion here is that the majority of exceeded sites in NW Scotland actually have critical loads set too low under the current FAB methodology, and a lower value of ANC_{crit} should be used. Again, the WFD Reference approach would allow higher deposition loads than the current approach for these sites.

Conversely, most regions have >50% of sites with Reference ANC values of >20 μeq l^{-1}, the largest proportions being 92% in S&E England, 82% in mid & South Wales and 79% in the Trossachs & Central region. For these sites, the WFD approach would require lower critical loads to prevent deviation from the Reference ANC. In the most extreme cases where Reference ANC is modelled to be >100 μeq l^{-1} (e.g. in one third of sites in S&E England), the current approach using 0 or 20 μeq l^{-1} ANC_{crit} allows a very major change in ANC from reference conditions before the FAB critical load is exceeded. The distribution of sites within each region into each modelled Reference ANC class (Fig. 1.4.1) gives some indication of the relative differences in the two approaches; sites with the lowest Reference ANC values are being set unachievable targets while those in the higher ANC classes are being allowed to undergo major chemical changes and loss of ANC before critical loads are exceeded, suggesting a major deviation from a chemical Reference condition.

One advantage of the Reference condition approach which could be appropriate under the WFD is that critical loads are set specifically according to a modelled baseline condition, without a uniform value (or one of only two very low ANC values) being assumed to be appropriate for all sites. A major disadvantage though is that if no deviation from Reference is allowed, zero critical loads would have to be set for all acid-sensitive sites where any acid deposition would lead to an ANC decline, effectively invalidating the whole critical loads approach. Hence some compromise would have to be employed, for example to allow a proportional (percentage) or fixed

ANC decline (e.g. a 20% decline in ANC from Reference, or a fixed decline of 20 μeq l^{-1}).

A second problem with the Reference conditions approach is that the WFD assumes that Reference conditions are set according to biological rather than chemical targets, requiring knowledge of the pre-industrial biota. While it is known that some groups, such as diatoms, would respond very rapidly to even small changes in acid inputs causing a decline in pH, the deviation from Reference would have to be quantified.

Hence there is still some way to go to reconcile the critical loads approach currently employed under the Gothenburg Protocol and the WFD approach. However, the wide range of baseline or Reference ANC values illustrated in this exercise does suggest that further work employing other approaches would be valuable in assessing the degree of impact suggested for the 445 sites exceeding critical loads beyond 2020. The use of more sophisticated models, such as the dynamic models MAGIC or VSD, may allow more confident estimates of Reference ANC to be modelled. In may also be possible to develop a combined approach whereby a proportional or fixed ANC decline from Reference is allowed for WFD, but with a cutoff ANC_{crit} value of say 0 or 20 μeq l^{-1} as per the current method used under the Gothenburg Protocol.

Summary

1. Current freshwater critical loads provided to the UK NFC employ fixed ANC_{crit} values of either 20 μeq l^{-1}, or 0 μeq l^{-1} where other modelling (from site specific MAGIC or palaeolimnological assessments) indicates a pre-industrial ANC which was less then 20 μeq l^{-1}.
2. Using the broad-brush SSWC ANC hindcasting approach, a wide range of Reference ANC values is modelled within each region of the UK where FAB model exceedance persists beyond 2020.
3. A few regions have Reference ANC values of less than 0 μeq l^{-1} suggesting that current critical loads may be over-protective (too low) – the largest proportions in the North York Moors (22%) and Pennines (12%).
4. In most regions, the majority of sites have Reference ANC values of >20 μeq l^{-1} indicating that current critical loads allow a decline in ANC, which can range up to a decrease of 80 μeq l^{-1} in the least sensitive sites.
5. In north-west Scotland, Reference ANC values are very low, and while the SSWC hindcasts are highly uncertain, it is likely that some sites may have had pre-industrial ANC values of less than 20 μeq l^{-1} which means that current critical load targets could never be achieved.
6. In regions with very low critical loads under the current approach, more sophisticated modelling may help to determine whether sites can ever achieve the critical ANC values of 20 μeq l^{-1} and improve confidence in the exceedances calculated using FAB beyond 2020.
7. While the approaches used under the WFD and Gothenburg Protocol are fundamentally different, it would be possible to develop a compromise approach using some index of change from Reference, but better modelling of Reference ANC and ecological response to chemical deviations from Reference would be required.

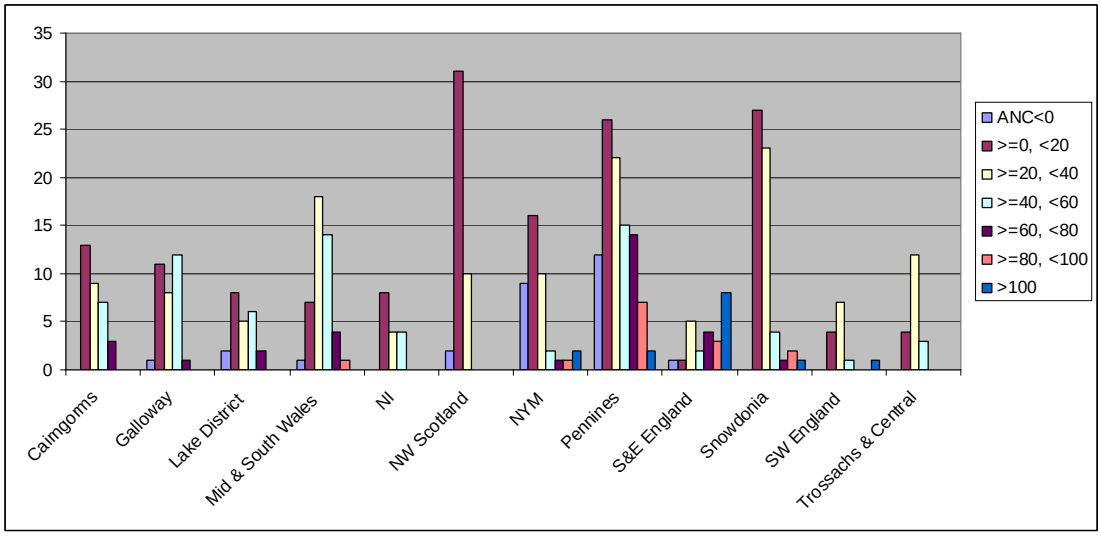
Table 1.4.1: Pre-industrial Reference ANC ranges in 445 sites exceeding FAB critical loads beyond 2020, with selected ANC_{crit} (µeq l^{-1})

		SSWC Hindcast Reference ANC							ANCcrit	
Region	n	<0	>=0, <20	>=20, <40	>=40, <60	>=60, <80	>=80, <100	>100	0	20
Cairngorms	32	0	13	9	7	3	0	0	3	29
Galloway	33	1	11	8	12	1	0	0	3	30
Lake District	23	2	8	5	6	2	0	0	4	19
Mid & South Wales	45	1	7	18	14	4	1	0	1	44
NI	16	0	8	4	4	0	0	0	5	11
NW Scotland	43	2	31	10	0	0	0	0	5	38
NYM	41	9	16	10	2	1	1	2	8	33
Pennines	98	12	26	22	15	14	7	2	12	86
S&E England	24	1	1	5	2	4	3	8	1	23
Snowdonia	58	0	27	23	4	1	2	1	1	57
SW England	13	0	4	7	1	0	0	1	0	13
Trossachs & Central	19	0	4	12	3	0	0	0	0	19

Table 1.4.2: Proportion of sites in Pre-industrial Reference ANC ranges (%)

		SSWC Hindcast Reference ANC							ANCcrit	
Region	n	<0	>=0, <20	>=20, <40	>=40, <60	>=60, <80	>=80, <100	>100	0	20
Cairngorms	32	0	41	28	22	9	0	0	9	91
Galloway	33	3	33	24	36	3	0	0	9	91
Lake District	23	9	35	22	26	9	0	0	17	83
Mid & South Wales	45	2	16	40	31	9	2	0	2	98
NI	16	0	50	25	25	0	0	0	31	69
NW Scotland	43	5	72	23	0	0	0	0	12	88
NYM	41	22	39	24	5	2	2	5	20	80
Pennines	98	12	27	22	15	14	7	2	12	88
S&E England	24	4	4	21	8	17	13	33	4	96
Snowdonia	58	0	47	40	7	2	3	2	2	98
SW England	13	0	31	54	8	0	0	8	0	100
Trossachs & Central	19	0	21	63	16	0	0	0	0	100

Figure 1.4.1: Reference ANC classes of exceeded sites within each region



Task 1.5 Regional re-sampling and analysis of factors preventing achievement of critical loads

Many of the 445 freshwater sites showing FAB critical load exceedance to 2020 occur in the most acid sensitive regions of high N deposition. These sites are the most difficult for achievement of critical loads required under the Gothenburg Protocol and the NECD. In some regions of high exceedance (e.g. Pennines, North Yorkshire Moors) S deposition continues to play a major role and there is the additional possibility of net export of S stored in peat soils. In other regions (e.g. the Lake District), the worst-case nitrate leaching scenario of the FAB model may be largely responsible for continued exceedance. The primary aim of this Task is to analyse current regional critical load model outputs for each region and then to carry out a targeted random survey of a subset of sites exceeded in 2020 (approx. 150 sites stratified by region) to

1) provide a regional assessment of the main drivers preventing achievement of critical loads,
2) provide a second point in time for chemical calibration of MAGIC model runs which for many sites are based on single spot samples or sampling years;
3) provide a broad comparison of calculated critical loads in the most impacted areas to assess the likely importance of major seasalt inputs during much of the initial survey work (1990-92) in producing spurious critical load values and to assess the possible importance of changing relationships between base cations and acid anions leaching during the recovery trajectory (Rapp & Bishop, 2009) which affects critical load calculations and assumptions of fixed critical load values.

These aims are to be achieved through three staged sub-tasks:

- 1.5.1) identification of resurvey sites for testing steady-state model developments/temporal changes and linking the MAGIC upscaling approach to sites with repeat chemistry data
- 1.5.2) regional chemistry surveys (autumn 2010 / spring 2011) including additional nutrient measurements (Chlorophyll-a = Chl-a) and total phosphorus (TP) measurements to repeat studies of Bergström & Jansson (2006, *Global Change Biology* 12, 635-43) and Elser *et al.* (2009, *Science* 326, 835-7) where in regions of high N deposition in Scandinavia and North America, they recorded greater Chl-a per unit P concentration relative to low N deposition regions, indicating stimulation of algal productivity that is of poorer quality for zooplankton with effects up the food chain.
- 1.5.3) Northern Ireland Resurveys (originally given as Option). There is a small number of sites exceeding critical loads in Northern Ireland including the AWMN sites Blue Lough and Bencrom River in the Mourne Mountains and a resurvey of the 16 most sensitive sites in the region would be carried out twice, in parallel with the main sampling programme under Task 1.5.2.

Task 1.5.1: Identification of resurvey sites

According to FRAME modelled deposition for 2020, 445 FAB sites across the UK still exceed critical loads beyond 2020. This group of sites forms the basis for this Task. To allow a regional analysis of factors causing continued critical load exceedance across the UK, sites were grouped into sub-regions which in some cases

are very distinct geographically and in others are somewhat arbitrary (Figure 1.5.1). A random sampling strategy was employed, stratified by geographical region. Each of the 445 sites was assigned a random number and then the data were sorted by increasing random number for each region. The first *n* sites were selected in ascending order where *n* is the smallest number to give a proportion of at least one third (33%) of sites for subsampling. An exception is the region Northern Ireland, where all 16 exceeded sites (100%) were included.

The total number of exceeded sites and the proportion subsampled are provided in Table 1.5.1. Numbers per region vary from five in SW England to 33 in the Pennines, with 163 sites selected overall. These sites form the basis of the sampling campaigns planned for October/November 2010 and April/May 2011. A breakdown of site types and altitude ranges is provided for all 445 sites in Table 1.5.2.

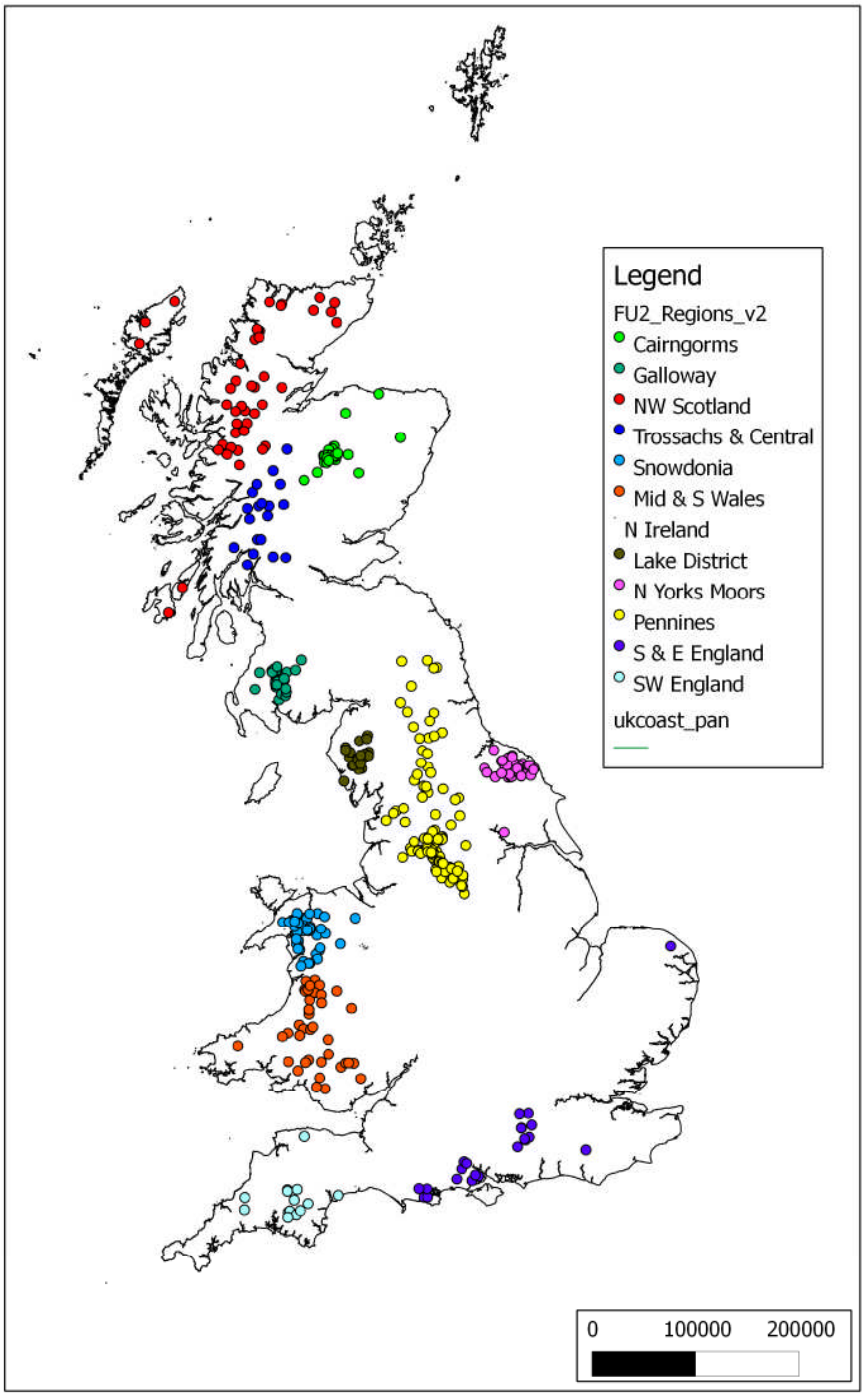
Table 1.5.1: Stratified random subsampling by region

Region	Subregion	Sites	Subsample	%
Scotland	Cairngorms	32	11	34
Scotland	Galloway	33	11	33
Scotland	NW Scotland	43	15	35
Scotland	Trossachs & Central	19	7	37
Scotland	*Subtotal*	*127*	*44*	*35*
Wales	Snowdonia	58	20	34
Wales	Mid & South Wales	45	15	33
Wales	*Subtotal*	*103*	*35*	*34*
N Ireland	*NI*	*16*	*16*	*100*
England	Lake District	23	8	35
England	NYM	41	14	34
England	Pennines	98	33	34
England	S&E England	23	8	35
England	SW England	14	5	36
England	*Subtotal*	*199*	*68*	*34*
UK	**Total**	**445**	**163**	**37**

Table 1.5.2: Site types (L=lake; A=Artificial; S=Stream) and altitude range by region

Region	L	A	S	Total	%L	%A	%S	Min Alt	Max Alt
Cairngorms	30	0	2	32	93.8	0.0	6.3	120	1123
Galloway	28	0	5	33	84.8	0.0	15.2	120	536
Lake District	21	1	1	23	91.3	4.3	4.3	18	718
Mid & South Wales	12	8	25	45	26.7	17.8	55.6	160	597
NI (Northern Ireland)	12	3	1	16	75.0	18.8	6.3	140	470
NW Scotland	42	0	1	43	97.7	0.0	2.3	27	927
NYM	4	1	36	41	9.8	2.4	87.8	8	400
Pennines	18	66	14	98	18.4	67.3	14.3	114	646
S&E England	16	5	3	24	66.7	20.8	12.5	8	225
Snowdonia	45	4	9	58	77.6	6.9	15.5	45	765
SW England	1	8	4	13	7.7	61.5	30.8	38	480
Trossachs & Central	17	1	1	19	89.5	5.3	5.3	92	907

Figure 1.5.1: Regional subdivision of sites exceeding FAB critical loads in 2020

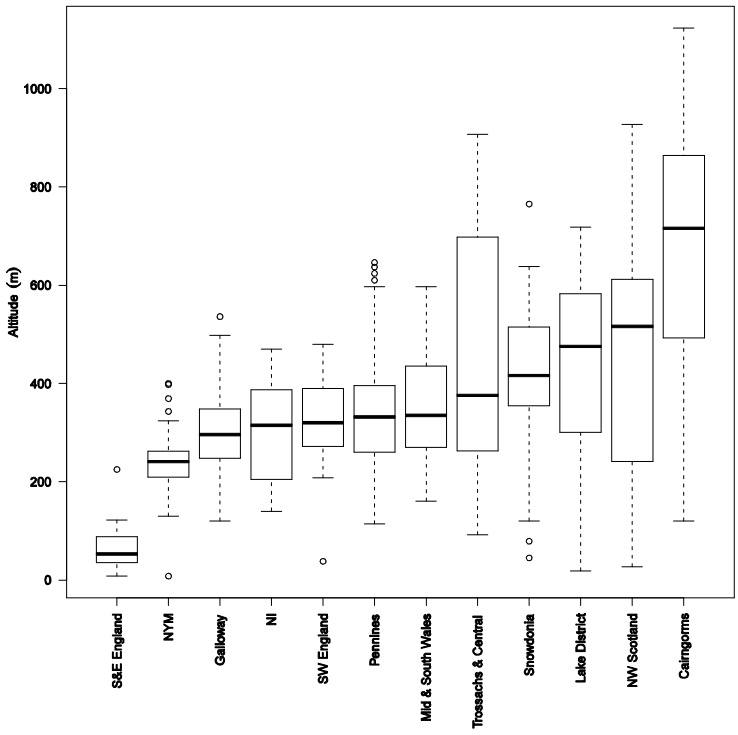


A summary of site characteristics (physical, critical loads, deposition and exceedance) is provided for all regions below, followed by individual descriptions for each region.

Regional Summaries

The majority of sites exceeding critical loads in 2020 are upland sites at an altitude >300m above sea level, but there are large regional differences in median altitude from <100m in S&E England to >700m in the Cairngorms (Fig. 1.5.2).

Figure 1.5.2: Boxplot of site altitude by region



Sensitivity to acidification by sulphur deposition alone (i.e. assuming N deposition is zero) is represented by the term $CL_{max}S$ in FAB, i.e. the maximum theoretical deposition load of sulphur the system can receive without exceeding critical loads. There is a fairly smooth gradient in median values of $CL_{max}S$ with the most sensitive sites (lowest critical loads) in the North Yorkshire Moors and NW Scotland and the least sensitive exceeded sites according to median values in Galloway, Mid & South Wales and the Lake District (Fig. 1.5.3).

While sensitivity to sulphur deposition is dictated largely by the weathering rate (estimated using the F-factor in the SSWC and FAB models), nitrogen deposition is subject to several potential retention mechanisms in soils and vegetation and hence the critical load for N alone ($CL_{max}N$) shows a slightly different pattern to $CL_{max}S$ through regional differences in soil and vegetation types. All median values of $CL_{max}N$ are > 0.5 keq ha^{-1} yr^{-1} while for S most medians are below this value. The lowest regional medians are found in the North Yorkshire Moors, NW Scotland and Northern Ireland while the largest occur in the Pennines, Galloway and Mid & South Wales.

Deposition of sulphur and nitrogen shows strong regional gradients. The lowest median values of S deposition occur in NW Scotland, Northern Ireland and the Cairngorms while the highest occur in the Pennines and SW England (Fig. 1.5.5). For N deposition the lowest values occur in the Cairngorms and especially NW Scotland, while the highest occur in the Lake District and the Pennines (Fig. 1.5.6).

Figure 1.5.3: Boxplot of critical load for sulphur alone ($CL_{max}S$) by region

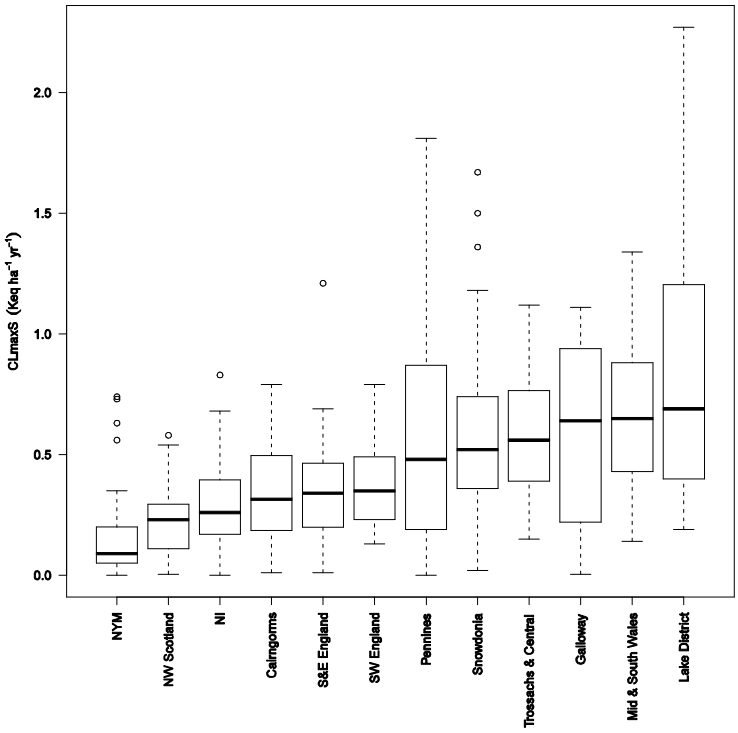


Figure 1.5.4: Boxplot of critical load for nitrogen alone ($CL_{max}N$) by region

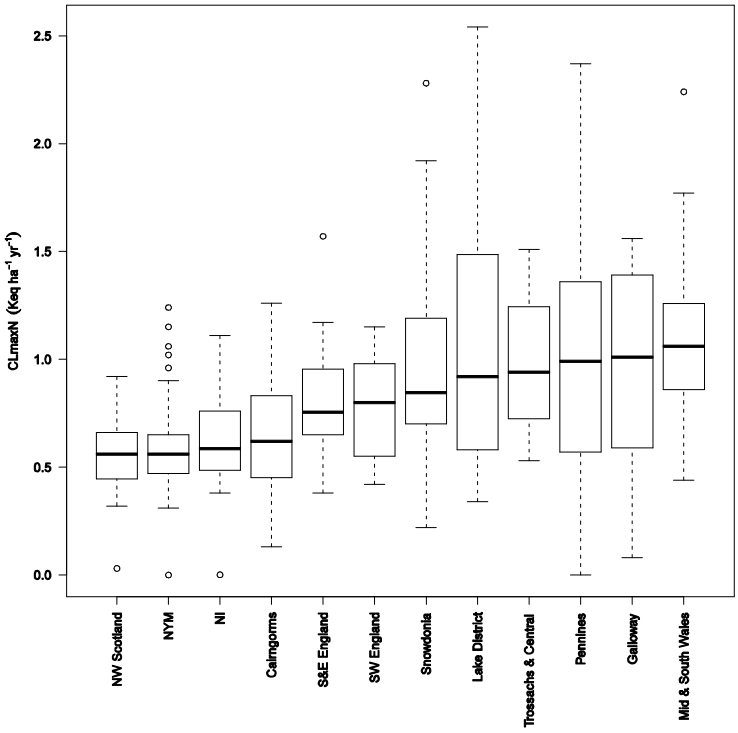


Figure 1.5.5: Boxplot of catchment-weighted Sulphur deposition by region

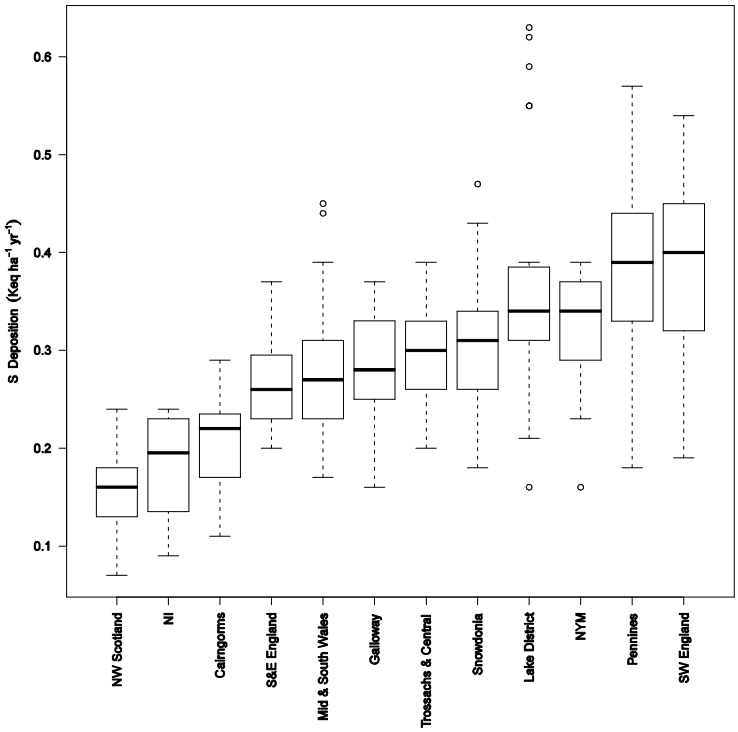
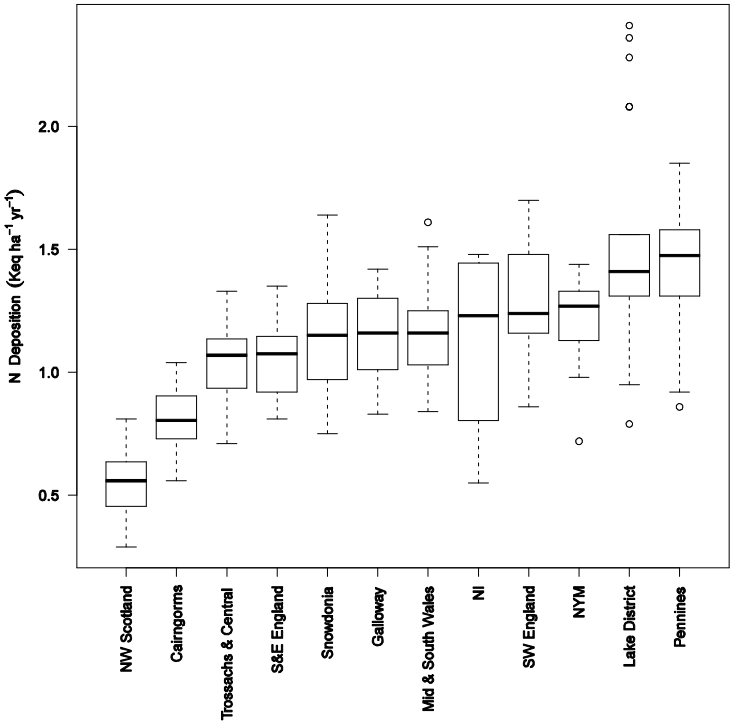


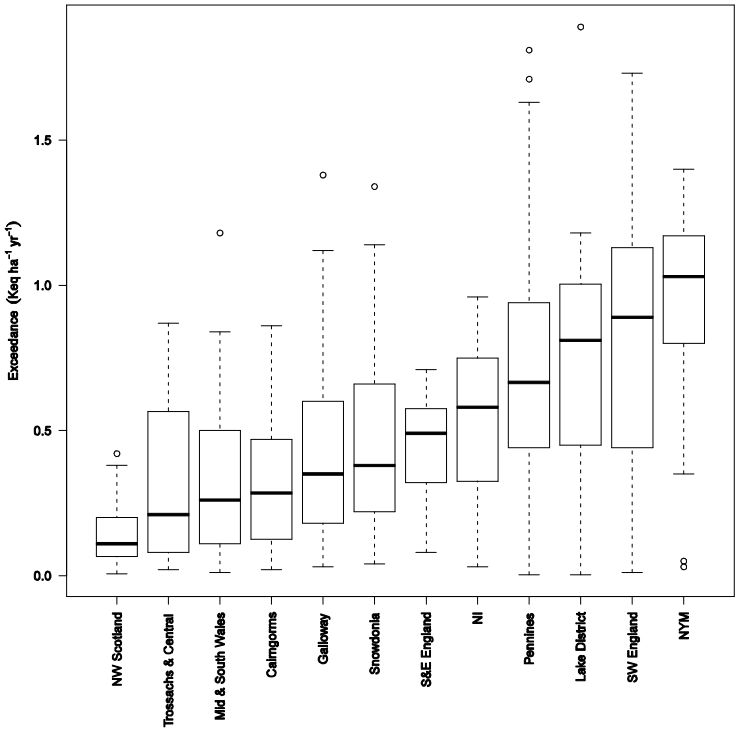
Figure 1.5.6: Boxplot of catchment-weighted Total Nitrogen deposition by region



The combined effect of sensitivity (as critical load) and deposition is shown by critical load exceedance, calculated as a flux of acidity. While this figure quantifies the combined effect of S and N deposition, it does not indicate by how much deposition must be reduced to protect the site because reductions in S or N do not have the same effect on exceedance. It does though provide an index of where acidification impacts are likely to be greatest. By far the lowest and smallest range of exceedances are found in NW Scotland, reflecting the low deposition but very high sensitivity of surface waters in the region (Fig. 1.5.7). The highest median exceedance values are found in the North Yorkshire Moors, followed by SW England, the Lake District and the Pennines, i.e. the most exceeded sites are found largely in England.

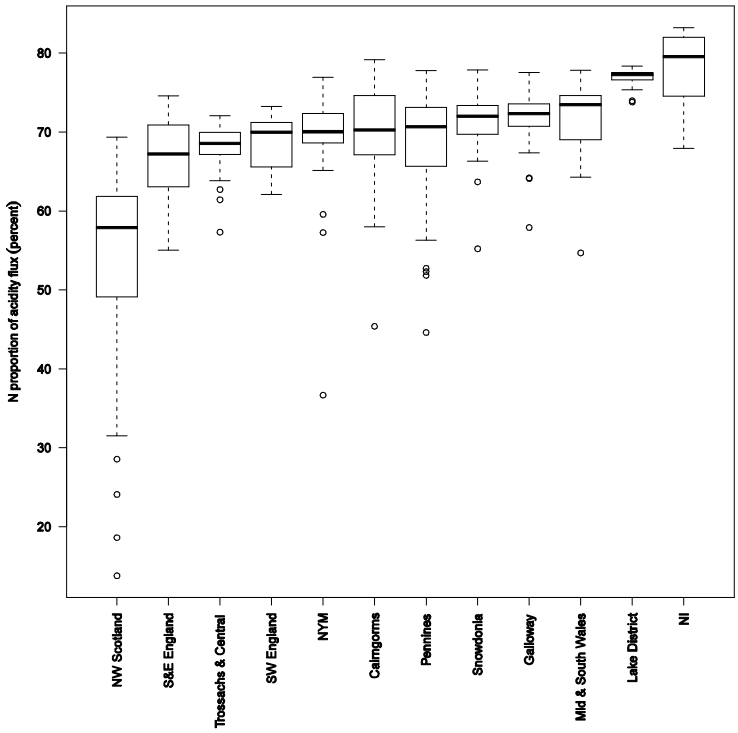
The modelled (i.e. long-term steady-state predicted) contribution of nitrate leaching to exceedance flux of acidity can be expressed as a proportion of the acidity flux (Fig. 1.5.8). At the great majority of exceeded sites, nitrate makes up 65-75% of predicted acidity fluxes, with only NW Scotland having a median value below 60%, although even here the lower quartile is around 50% nitrate. The relative contribution of nitrate is predicted to be particularly high in the Lake District and Northern Ireland, with median values approaching 80% of acidity fluxes in both. These plots show the major importance of nitrate in causing continued critical load exceedance at the majority of sites, with sulphate only a minor component at most of them.

Figure 1.5.7: Boxplot of FAB critical load exceedance by region



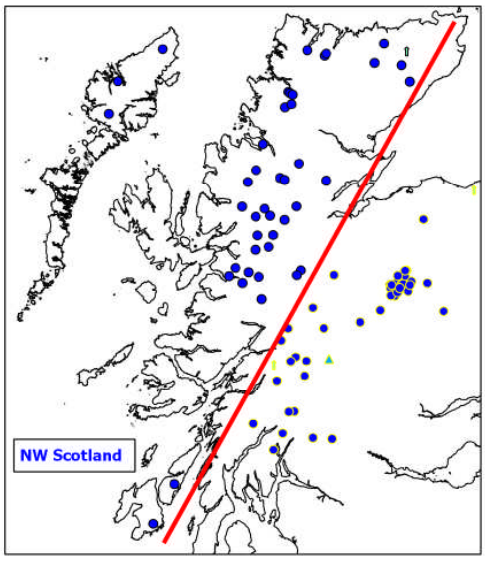
An individual summary for each region is provided in the following section, starting with NW Scotland and working roughly clockwise around the UK. Site types are represented in the regional maps with the following symbols: lake = ●, artificial (reservoir) = ▲, stream = ⇑.

Figure 1.5.8: Boxplot of proportional N contribution to exceedance by region



North-west Scotland

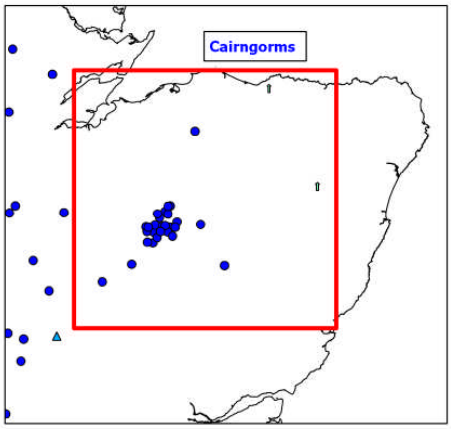
Figure 1.5.9a: 43 sites in NW Scotland exceeding critical loads beyond 2020



This region is dominated by lakes (42 of 43 sites) with only one stream site. Exceeded sites range from 27m to 927m in altitude with a median of >500m, second only to the Cairngorms. This region has the lowest deposition in the UK and the lowest median critical load exceedance, reflecting the extreme acid-sensitivity of surface waters here. 40% of sites have Sdep >$CL_{max}S$ while 42% have Ndep >$CL_{max}N$.

Cairngorms

Figure 1.5.9b: 32 sites in the Cairngorms / NE Scotland area exceeding critical loads beyond 2020

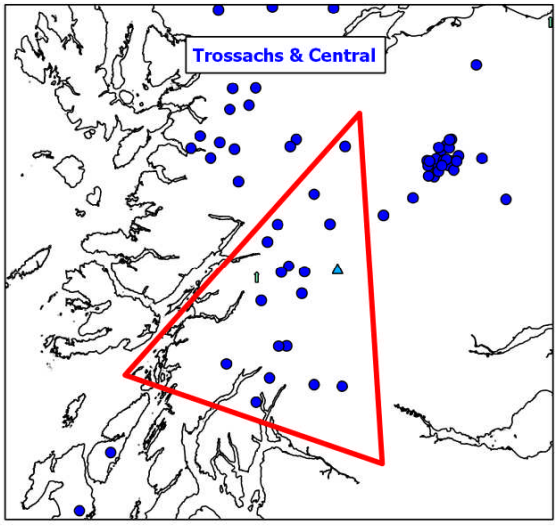


Thirty of the 32 exceeded sites in the Cairngorms are lakes, with only two streams, which are not located in the Cairngorms *per se* but are included in this region for convenience. Site altitude ranges from 120 to 1123m with the highest median altitude of all regions, at >700m. This region has the lowest N deposition after NW Scotland, while only NW Scotland and Northern Ireland have lower S deposition. Exceedances here therefore reflect low critical loads rather than high deposition, with 28% of sites having Sdep >$CL_{max}S$ and 63% having Ndep >$CL_{max}N$.

Trossachs & Central Scotland

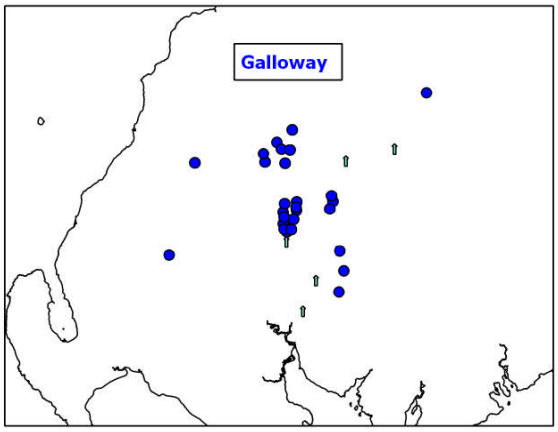
This rather arbitrarily defined region contains 17 lakes, one stream and one artificial water body. Site altitudes range from 92 to 907m with a median of >300m. Sulphur and nitrogen deposition levels are both moderate, as are critical loads, so this region has low exceedances, with only NW Scotland having a lower median value. Only 15% of sites exceed $CL_{max}S$ while 47% exceed $CL_{max}N$ which are among the lowest proportions of all regions. Even small further deposition reductions should therefore have beneficial effects in this region.

Figure 1.5.9c: 19 sites in the Trossachs & Central Scotland exceeding critical loads beyond 2020



Galloway

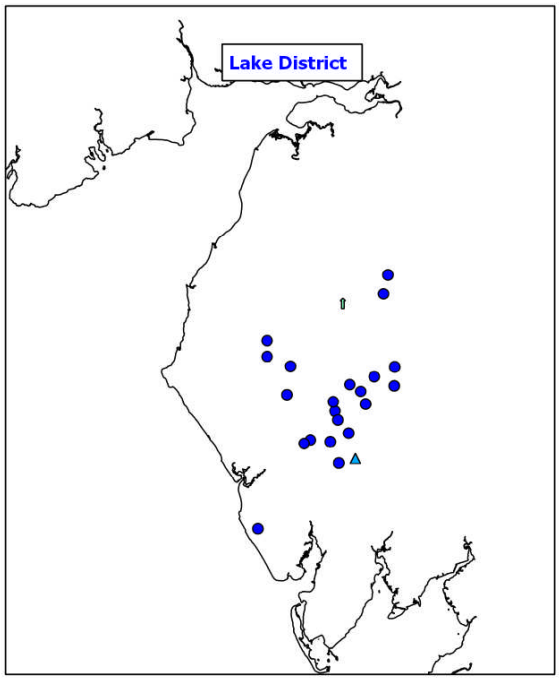
Figure 1.5.9d: 33 sites in Galloway exceeding critical loads beyond 2020



This region is spatially well defined, occupying an upland area in SW Scotland. Exceeded sites in Galloway include 28 lakes and 5 streams, ranging in altitude from 120 to 536m, with a moderate median altitude of around 300m. Deposition of both sulphur and nitrogen is close to the mid-range for all exceeded regions. Median values of both $CL_{max}S$ and $CL_{max}N$ are high, second only to Mid & South Wales for N, resulting in fairly low exceedances, mostly <0.5 keq ha^{-1} yr^{-1}. 27% of sites have Sdep >$CL_{max}S$ and 52% have Ndep>$CL_{max}N$.

Lake District

Figure 1.5.9e: 23 sites in the Lake District exceeding critical loads beyond 2020



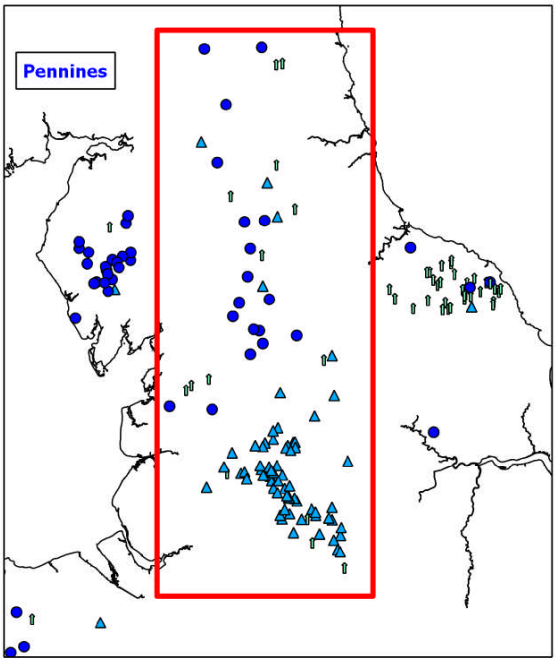
This is a spatially distinct region dominated by lakes, with only one stream and one artificial water body out of 23 exceeded sites. It contains one of the lowest lying exceeded sites at 18m altitude, with the regional maximum at 718m and a median of around 500m, third highest after NW Scotland and the Cairngorms. Sulphur and especially nitrogen deposition are high, with N deposition second only to the adjacent Pennines region. However, the region has the highest median value of $CL_{max}S$ indicating the least acid-sensitive mineralogy of all exceeded regions, while $CL_{max}N$ is also moderately high. Despite these high critical loads, the Lake District still has the third highest median exceedance of all regions, due to the very high deposition loads experienced. Only 17% of sites exceed $CL_{max}S$ but 74% exceed $CL_{max}N$, and nitrate makes up almost 80% of predicted acidity leaching at most sites, second only to Northern Ireland.

Pennines

This region is one of the largest and most diverse in the UK. It contains the largest number of exceeded sites at 98, and with SW England is one of only two regions dominated by artificial water bodies, mostly drinking water reservoirs in the Pennines (66, compared with 18 natural lakes and 14 streams). Altitude ranges from 114 to 646m with a median of around 300m, in the mid-range for all regions. Nitrogen deposition is the highest in the country and sulphur deposition is second only to SW

England, making this one of the most impacted regions in the country. Median $CL_{max}S$ is moderate but $CL_{max}N$ is high indicating a relatively low sensitivity to acid deposition. 44% of sites exceed $CL_{max}S$ and 83% exceed $CL_{max}N$.

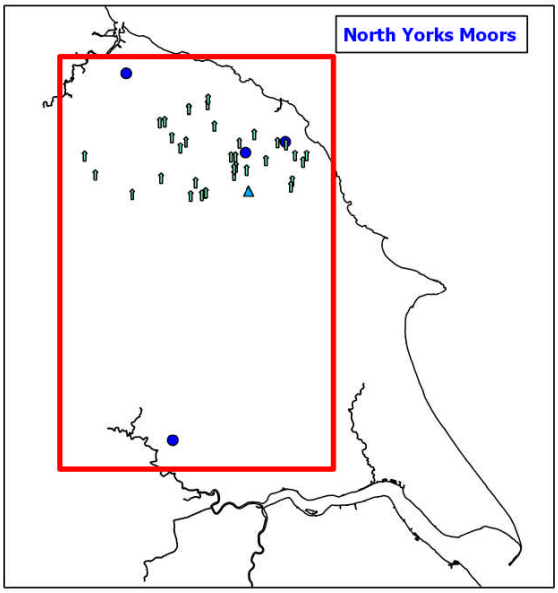
Figure 1.5.9f: 98 sites in the Pennines exceeding critical loads beyond 2020



North Yorkshire Moors

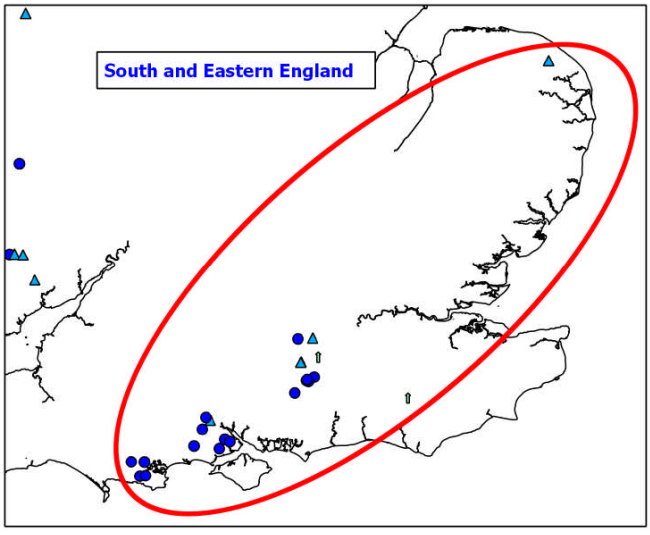
This is the only region heavily dominated by stream sites which make up 36 of the 41 exceeded sites, with only four lakes and one artificial site. The main region is very well defined in the uplands of the North Yorkshire Moors but also includes an outlying, low-lying site to the south, near Selby. Altitude ranges from just 8m above sea level to 400m, with a median around 250m. The region ranks third in the country for both sulphur and nitrogen deposition. Values of $CL_{max}S$ are the lowest in the country indicating extremely high sensitivity to acid deposition due to very low weathering rates, while critical loads for N deposition, $CL_{max}N$, are also extremely low, being very similar to the most sensitive region of NW Scotland. These extremely low critical loads combined with very high deposition result in the highest median value of exceedance for the whole country (>1 keq ha^{-1} yr^{-1}), with 35% of sites exceeding $CL_{max}S$ and 39% exceeding $CL_{max}N$. The streams of the North Yorkshire Moors are collectively the most impacted sites in the country.

Figure 1.5.9g: 41 sites in the North Yorkshire Moors region exceeding critical loads beyond 2020



Southern and Eastern England

Figure 1.5.9h: 23 sites in Southern and Eastern England exceeding critical loads beyond 2020



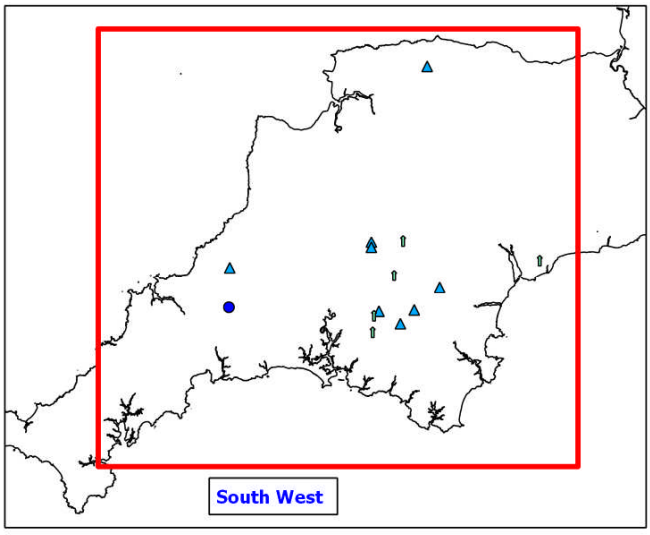
This region is really a combination of several “islands” of acid-sensitivity on lowland heathlands in southern England (the Surrey heaths, the New Forest and the Dorset heaths) plus outlying sites in the Ashdown Forest of Sussex and one site in Norfolk. Most of the sites are heathland ponds, 16 in total compared with five artificial water bodies and three streams. Although altitude ranges from 8m to 225m above sea level,

the median altitude is by far the lowest of all regions at around 50m. Levels of S and N deposition are moderate while critical loads for S ($CL_{max}S$) and N ($CL_{max}N$) are rather low. Exceedances are in the mid-range for all regions with a median value of around 0.5 keq ha^{-1} yr^{-1}. 33% of sites have $Sdep>CL_{max}S$ while 83% have $Ndep>CL_{max}N$.

South west England

This region was distinguished from Southern and Eastern England because it's acid sensitive areas are upland in nature, being located on Dartmoor, Bodmin Moor and Exmoor, with altitude ranging from 38m to 480m and a median value > 300m. It is one of only two regions (with the Pennines) dominated by artificial water bodies (8), with only one lake and four stream sites. Sulphur deposition is the highest of all exceeded regions with a median value greater even than the Pennines, while N deposition is also moderately high. Critical loads for both S and N are in the mid-range for all regions, but the very high deposition results in the second highest median exceedance after the North Yorkshire Moors. 54% of sites have $Sdep>CL_{max}S$ and 92% have $Ndep>CL_{max}N$, indicating that further reductions in both S and N deposition are required to prevent critical load exceedance in the majority of sites.

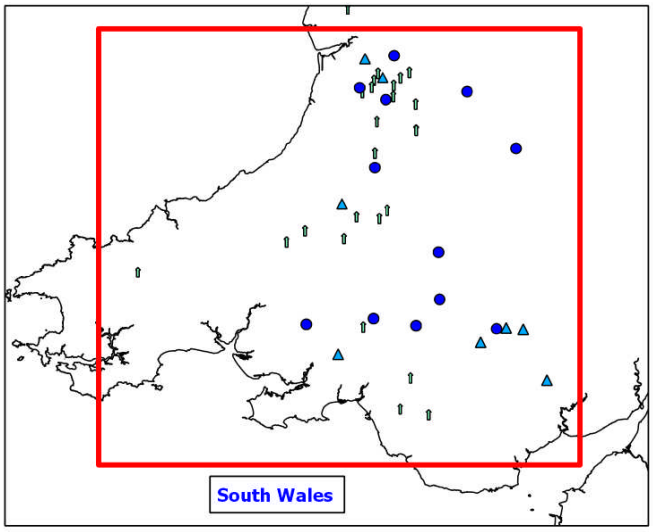
Figure 1.5.9i: 14 sites in South-West England exceeding critical loads beyond 2020



South and mid Wales

The sites in South and Mid-Wales are roughly evenly split between streams (25) and standing waters (12 lakes, 8 artificial). The exceeded sites are predominantly upland, with a minimum altitude of 160m (the highest of all regions), a maximum of 597m and a median of >300m. Both S and N deposition are moderate, while critical loads for S ($CL_{max}S$) and N ($CL_{max}N$) are high, for N the highest of all regions. Hence exceedances are low, with the third lowest median of all regions. S deposition exceeds $CL_{max}S$ in only 11% of sites, the lowest proportion of any region, while N deposition exceeds $CL_{max}N$ in 49% of sites which is also relatively low. Hence most sites in this region would be responsive to further reductions in deposition of either S or N.

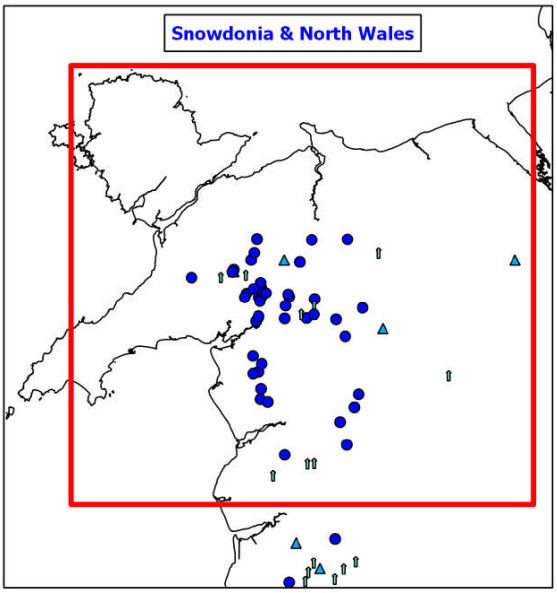
Figure 1.5.9j: 45 sites in South and Mid-Wales exceeding critical loads beyond 2020



Snowdonia and North Wales

The second largest exceeded region after the Pennines, Snowdonia and North Wales is dominated by lakes (45), with four artificial water bodies and nine streams. The sites range from 45m to 765m in altitude, with a median of >400m making this one of the higher elevation regions. Sulphur deposition levels are moderately high while N deposition is moderate. Median critical loads for both S ($CL_{max}S$) and N ($CL_{max}N$) are in the mid-range for all regions, resulting in fairly low exceedances. Only 14% of sites experience S deposition > $CL_{max}S$, while 69% have N deposition > $CL_{max}N$, a somewhat larger proportion than in South and Mid-Wales.

Figure 1.5.9k: 58 sites in Snowdonia and North Wales exceeding critical loads beyond 2020



Northern Ireland

All 16 sites exceeding critical loads in Northern Ireland were included in the resurveys of autumn 2010 and spring 2011. The region is dominated by lakes (12), with three artificial water bodies and one stream, with the lowest altitude site at 140m (second only to South and Mid-Wales in minimum altitude), the highest at 470m and a median value of just over 300m. Sulphur deposition is very low, with only NW Scotland experiencing lower values, but N deposition is high, making this region extreme in the dominance of N deposition over S. Northern Ireland has the third lowest median critical loads for both S and N making its water bodies the most sensitive after NW Scotland and the North Yorkshire Moors. The median value of exceedance is therefore rather high and is due mainly to N deposition, with only 13% exceeding $CL_{max}S$ but 94% exceeding $CL_{max}N$. This region shows the greatest proportion of nitrate in the predicted leaching flux of acidity, with a median value of almost 80%.

Tasks 1.5.2 & 1.5.3 Chemical resurveys of exceeded sites inc. all Northern Ireland: Part I – FAB Critical Load Exceedances in 2020

There are two aspects to these Tasks;

I) Calculation of FAB critical load exceedances for 2020 to compare with the original data described above (10-20 year old chemistry data)

II) Exploratory analysis of nutrient data and N deposition to compare with Scandinavian studies.

Resurveys of the 163 shortlisted sites took place in autumn 2010 (October-December) and spring 2011 (April-May). 161 sites were sampled in Survey 1 with two sites missed due to access problems (high storm flow at WAWSS067, deep snow on roads at CEHNI02). On the second survey, WAWS067 was omitted and CZNN88 was also not sampled since it was found on the autumn survey to have dried up almost entirely.

Dip samples taken from lake outflows or streams were filtered either on-site on later the same day through 1.2 μm GF/C pre-filters (retained for chlorophyll-a analysis; see below) and then 0.45 μm membrane filters. Separate 100ml sub-samples were frozen for major ions and isotopic analysis (see Task 4.3).

Derivation of new critical loads and exceedances

FAB model critical loads and exceedances for 2020 were calculated using the new resurvey chemistry data, for both autumn and spring surveys. Major ion chemistry was determined by ion chromatography (IC) at Queen Mary, University of London. Where major ion chemistry data were below detection limit, a concentration of 0.5 × detection limit was assumed (Table 1.5.3).

Since the resurvey sites were selected from an existing dataset of sites still exceeding FAB model critical loads in 2020, all necessary data for application of FAB were already available and the only change with the recalculated critical loads was due to the new water chemistry data.

A comparison of critical load exceedance using the new data with existing data is presented below. Exceedances were only calculated for sites with non-zero critical loads ($CL_{max}S$ and $CL_{max}N$ >0); 285 out of 322 samples from 160 sites met this criterion. Of these 160 sites, 45 no longer showed critical load exceedance by 2020 using the new water chemistry data. The breakdown by region is shown in Table 1.5.4; in most regions, most of the sampled sites still show exceedance. Where the sum of exceeded and not exceeded sites does not tally with the total for a region, the missing sites were either not sampled or screened out on the basis of zero critical loads (which would imply exceedance).

Table 1.5.3: IC detection limits and assumed concentrations in μeq l^{-1}

Analyte	IC Detection Limit mg l^{-1}	Conversion factor	DL μeq l^{-1}	DL ×0.5 μeq l^{-1}
Na	0.037	43.5	1.6	0.8
NH4	0.096	55.55	5.3	2.7
K	0.061	25.58	1.6	0.8
Mg	0.054	82.29	4.4	2.2
Ca	0.144	49.9	7.2	3.6
Cl	0.015	28.21	0.4	0.2
NO3	0.02	16.13	0.3	0.2
SO4	0.02	20.82	0.4	0.2

The biggest changes are seen in Galloway and NW Scotland. In Galloway, almost half of resurveyed sites no longer exceed critical loads, with autumn samples largely responsible for increased critical loads. In NW Scotland only one third of resurveyed sites exceed critical loads, with spring samples responsible for increased critical loads. In other regions, the proportion of sites continuing to show critical load exceedance varies from around two thirds to 100%.

Table 1.5.4: Number of sites per region showing FAB Model Exceedance for 2020 on the basis of average, Autumn and Spring survey chemistry

	All	Average			Autumn			Spring		
Region	**160**	**Ex.**	**%**	**Not Ex.**	**Ex.**	**%**	**Not Ex.**	**Ex.**	**%**	**Not Ex.**
Cairngorms	**11**	10	90.9	1	8	72.7	2	8	72.7	1
Galloway	**11**	6	54.5	5	5	45.5	6	8	72.7	2
Lake District	**8**	7	87.5	1	7	87.5	1	7	87.5	0
Mid & South Wales	**14**	9	64.3	5	10	71.4	4	4	28.6	4
NI	**16**	11	68.8	5	11	68.8	4	10	62.5	4
NW Scotland	**15**	5	33.3	10	9	60.0	5	4	26.7	9
NYM	**14**	9	64.3	5	14	100.0	0	8	57.1	6
Pennines	**33**	26	78.8	7	23	69.7	7	23	69.7	7
S&E England	**8**	5	62.5	3	6	75.0	2	4	50.0	2
Snowdonia	**18**	16	88.9	2	16	88.9	2	11	61.1	2
SW England	**5**	5	100.0	0	5	100.0	0	3	60.0	0
Trossachs & Central	**7**	6	85.7	1	6	85.7	1	3	42.9	1

The new exceedance data are compared with the original FAB data for 2020 in Figs. 1.5.10-11. It can be seen that there are major differences at some sites which show large negative exceedance values indicating high (non-sensitive) critical load values according to the more recent resurvey data. However, for the majority of sites which continue to show critical load exceedance (i.e. >0 on x-axis), there is a reasonable one to one relationship in the exceedance values with little evidence of a systematic change in exceedance from the original samples taken during the 1990s and the recent resurveys.

The reasons for large non-exceedance values at sites previously showing critical load exceedance are not known and require further investigation. An initial hypothesis, based on the paper by Rapp & Bishop (2009) was that there may be systematic changes through time in the relationship between the acid anions associated with acidification and base cation leaching from acidified soils, especially when moving from the acidification phase to the recovery phase. There is little evidence from these resurvey data for a systematic change in exceedance. The large negative (non-) exceedance values at some sites could be linked to this process, but comparison of

recent samples taken in autumn and spring (Figure 1.5.12) shows that in most cases, sites with large negative exceedance values on one survey do not show similar values in the other survey. The lack of a 1:1 relationship in non-exceeded sites suggests that the reasons for non-exceedance may be analytical and this issue is being investigated.

Figure 1.5.10: Comparison of original FAB model exceedance for 2020 with new values calculated from the autumn 2010 resurvey

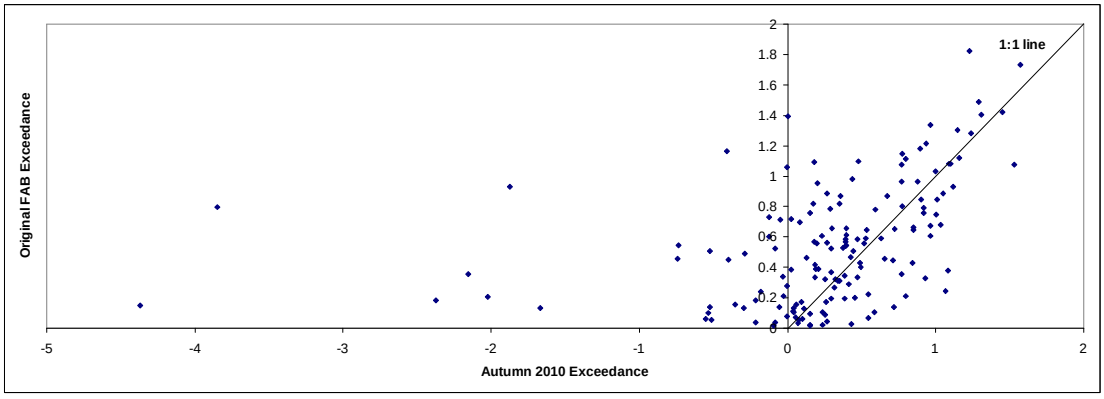
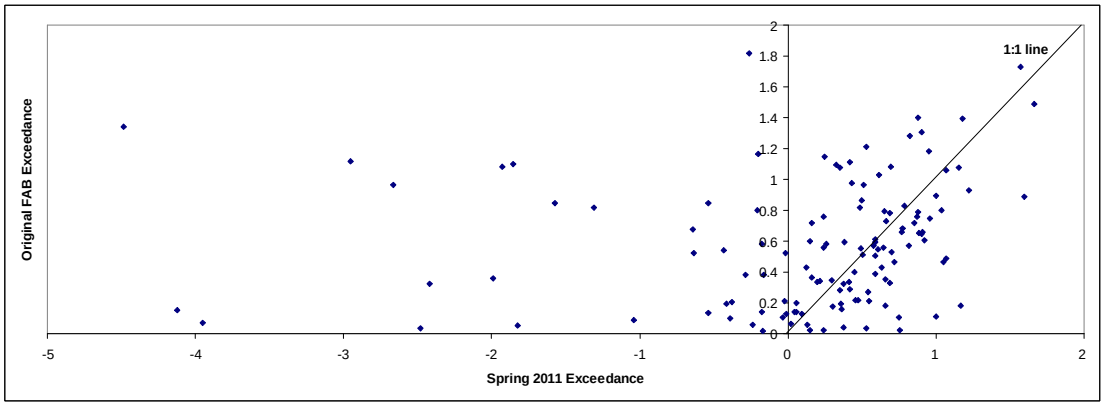


Figure 1.5.11: Comparison of original FAB model exceedance for 2020 with new values calculated from the spring 2011 resurvey



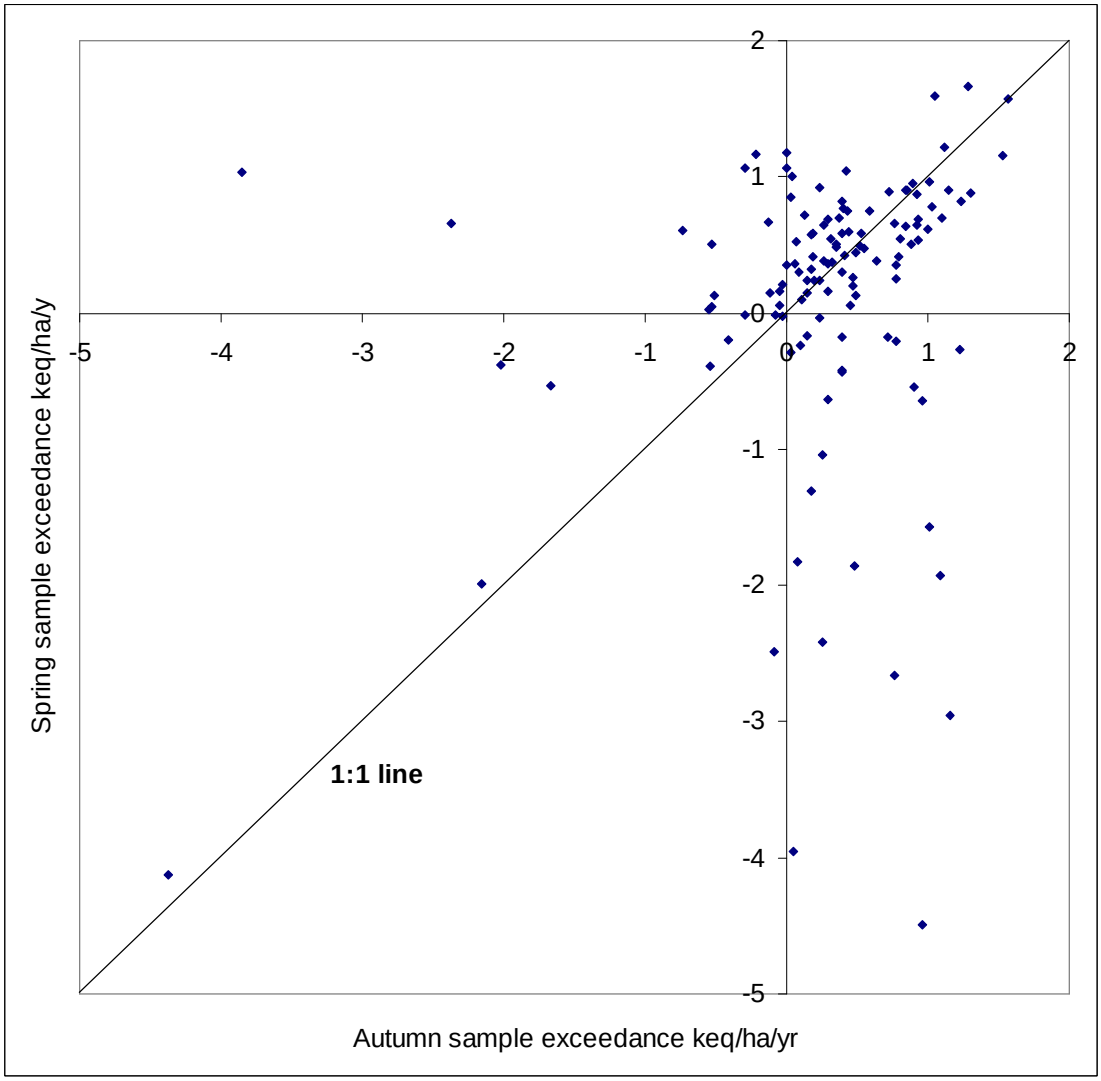
While there is some scatter in the relationship between exceedance values in recent samples and original samples from the 1990s (Figs. 1.5.10-11) the resurvey data do broadly corroborate the original exceedance data. It is therefore unlikely that the original calculated exceedances are due to some artefact of sampling period, such as the high seasalt inputs associated with the early 1990s phase of the North Atlantic Oscillation, and are in fact real. It is possible that some of the 28% of sites which do not show exceedance when averaged over the two resurveys do reflect a shift in critical loads in line with the hypothesis of Rapp and Bishop (2009) but this can only reflect a small proportion of sites overall, since a large number in this category seem

to reflect analytical problems or methodological issues. Furthermore it cannot be stated with any certainty that the new data indicate that critical load exceedance is not as widespread as shown by the older datasets; the lack of a systematic difference in exceedances suggests that more random factors may be responsible. It is possible that if non-exceeded sites from the original dataset had been included in the resurvey, some could have moved into the exceeded category on the basis of new data.

Conclusions

The random resurvey of approximately one third of the 445 sites showing exceedance in the official FAB dataset indicates that 28% of sites no longer show exceedance based on 2020 deposition, but the majority of sites continue to be exceeded. It is likely that the difference in exceedance figures is due to random analytical or methodological factors rather than real systematic change in critical loads in the great majority of sites.

Figure 1.5.12: Comparison of FAB model exceedance for 2020 using autumn 2010 and spring 2011 resurvey data



Tasks 1.5.2 & 1.5.3 Chemical resurveys of exceeded sites inc. all Northern Ireland: Part II – testing for increased phytoplankton productivity

For all resurvey sites described above, additional samples were taken for nutrients and chlorophyll-a analysis. Known sample volumes of at least 1L (where possible) were filtered through 1.2 μm GF/C filters which were immediately wrapped in aluminium foil, bagged in labelled whirlpak bags and frozen the same day for subsequent chlorophyll-a analysis using standard methods involving acetone extraction and spectrophotometry. Specific absorbance was measured at 663, 480, 430 and 410 nm.

Unfiltered 100ml samples were collected for TP analysis at the same time and also frozen the same day. TP was analysed following microwave digestion and measurement of the blue composite reagent complex at 885nm.

Results

Analysis for TP was problematic at the very low levels encountered in these acidified, oligotrophic systems with many values close to or below the detection limit of the method (5-10 μg l^{-1}). As a result, several batches had to be re-analysed to obtain acceptable calibrations using standard solutions. Even with these re-analyses, some batches were unreliable below ~5 or 10 μg l^{-1} and in these cases (depending on the calibrations obtained for each batch) a value of half the detection limit was used.

The spatial distribution of TP in resurvey sites is shown in Figures 1.5.13 - 14. Values are generally greater in the spring survey, and highest in the northern Pennines, Snowdonia and southern English sites.

Analysis for chlorophyll-a showed very low values, in the range 0-3 mg l^{-1}, for most sites in Wales and Scotland sampled during the autumn survey, with higher values clustered in the Pennine and southern English regions (Fig. 1.5.15). Only data for 106 standing waters are mapped because chl-a is not a standard measurement for productivity in streams which are dominated by benthic biomass. Higher values were found in the spring survey (Fig. 1.5.16) with far fewer sites falling into the <1 mg l^{-1} category. Boxplots of chl-a show that in the autumn, the highest values were found in S&E England (Fig. 1.5.17) with a median > 5 mg l^{-1}, while in the spring the highest values were found in SW England (Fig. 1.5.18), and median values for both SE and SW England exceeded 10 mg l^{-1}.

For standing water resurvey sites, the chl-a:TP ratio was compared with the most recent available deposition data (2004-06 CBED for total N) to test whether there was evidence for increased productivity in higher N deposition regions as found by Bergström and Jansson (2006) for Swedish and North American lakes. There was no relationship for either autumn or spring surveys in the present study (Figs. 1.5.19-20) despite a larger gradient in N deposition across the UK than in previous study regions.

There are at least two possible reasons that we did not find a productivity signal related to N deposition in terms of the chl:TP ratio. Our surveys were carried out in autumn and spring rather than during the summer peak in productivity as for the Swedish studies, because the primary purpose of the resurveys was to obtain appropriate data for calculating critical loads and calibrating MAGIC.

Figure 1.5.13: Total phosphorus concentrations (μg l^{-1}) for autumn 2010 survey

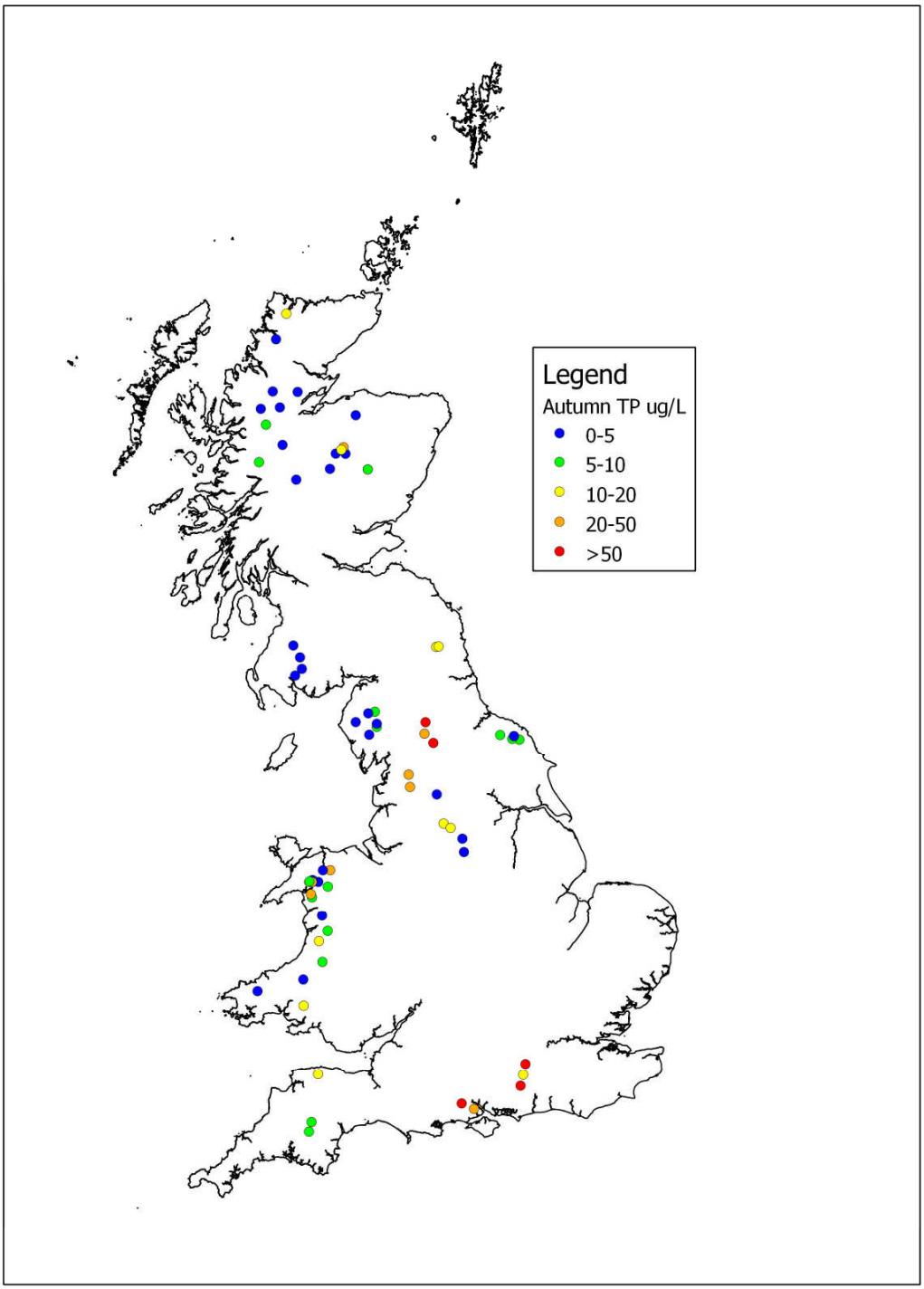


Figure 1.5.14: Total phosphorus concentrations (μg l^{-1}) for spring 2011 survey

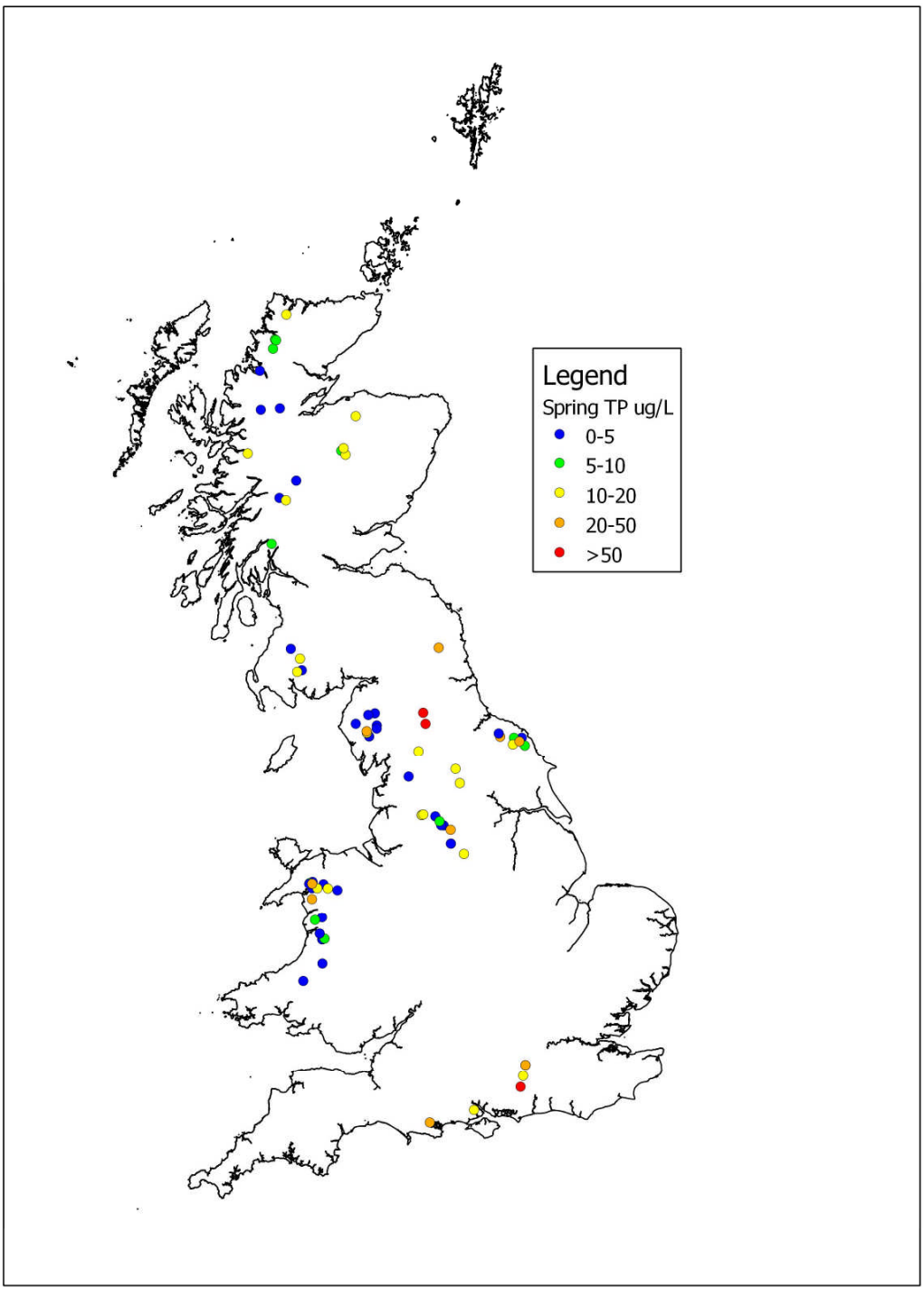


Figure 1.5.15: Chlorophyll-a concentrations (mg l^{-1}) in non-stream sites for autumn 2010 survey

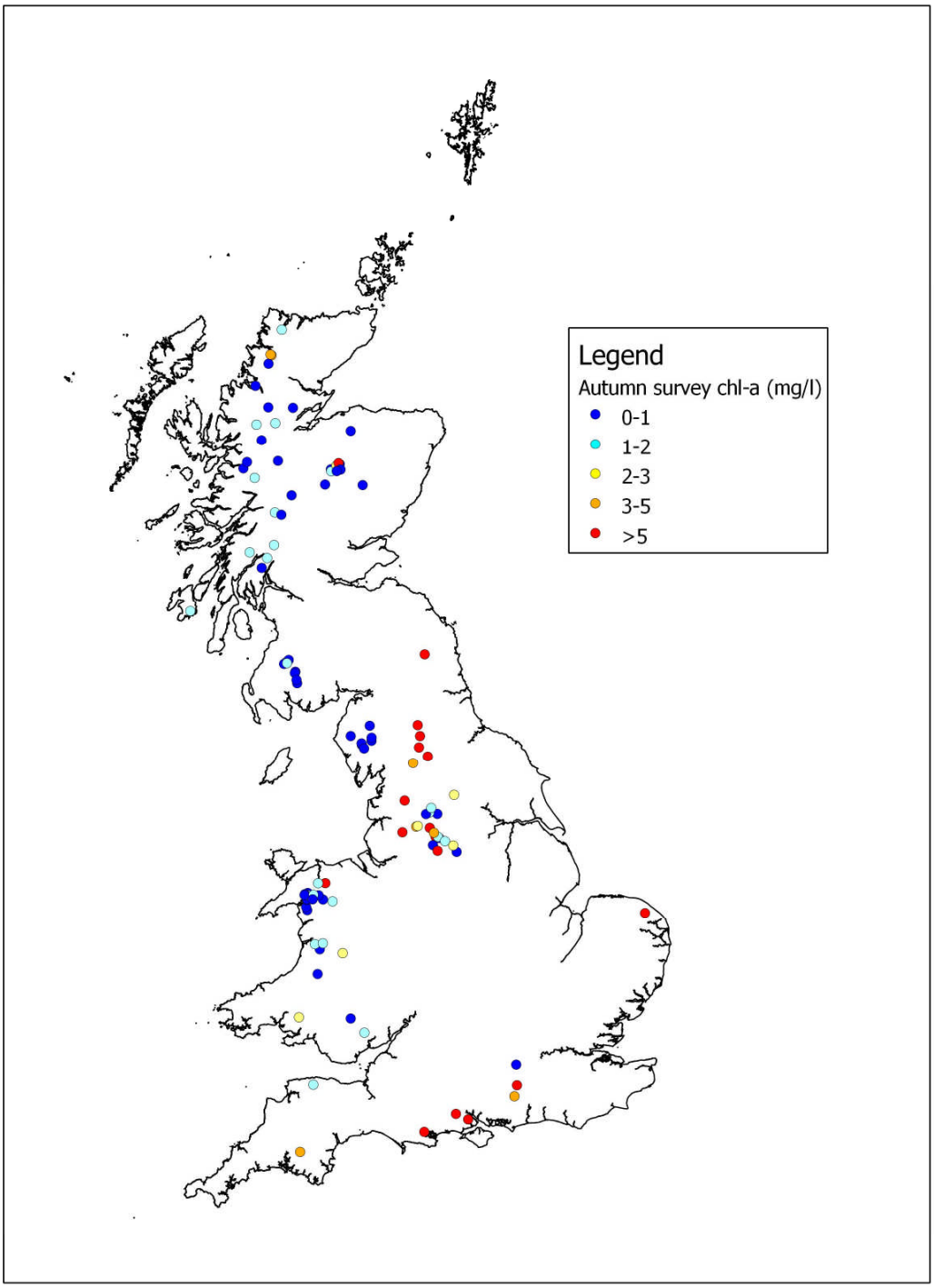


Figure 1.5.16: Chlorophyll-a concentrations (mg l^{-1}) in non-stream sites for spring 2011 survey

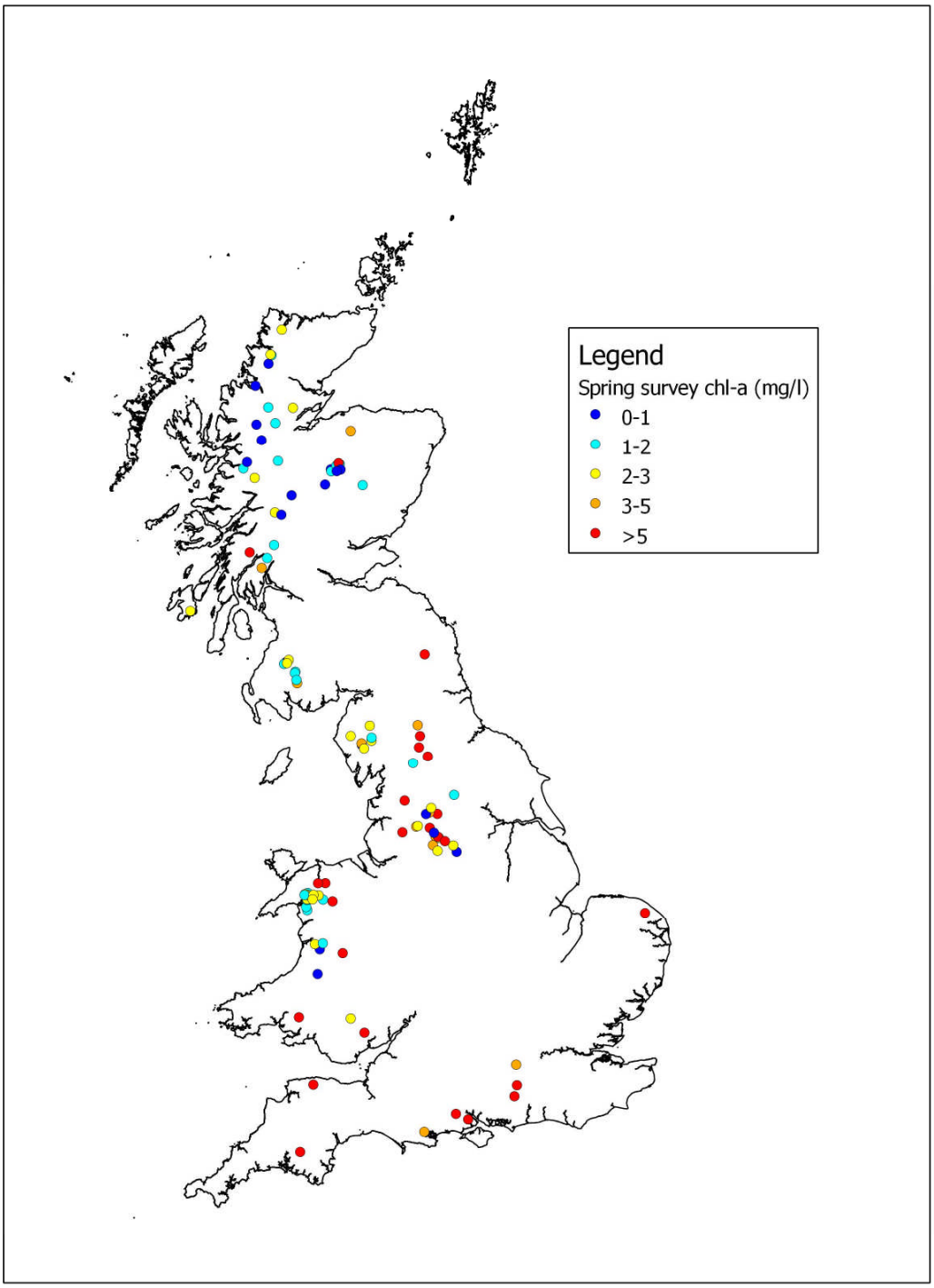


Figure 1.5.17: Boxplot of chl-a concentrations in autumn survey

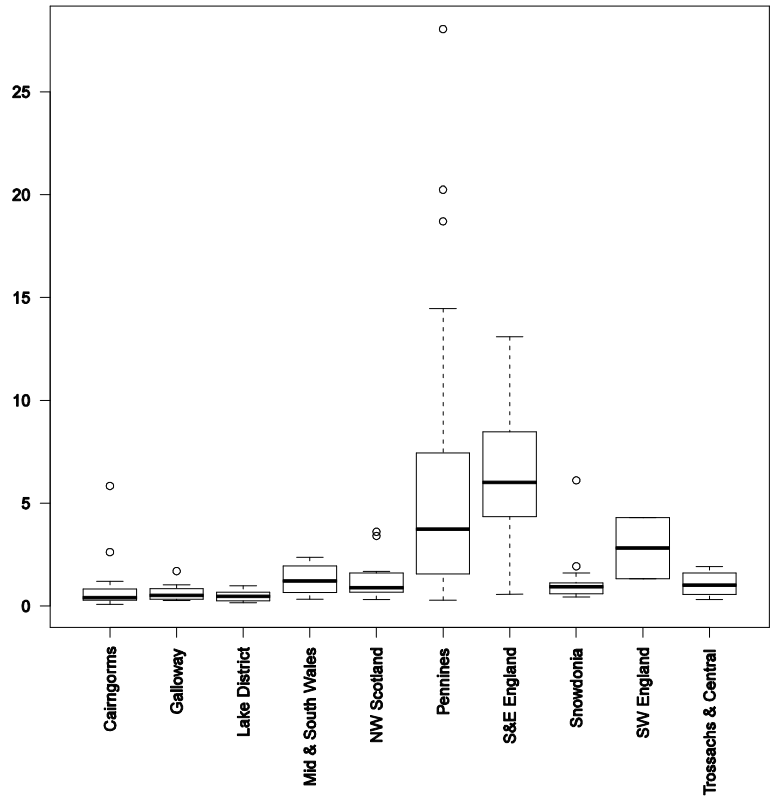


Figure 1.5.18: Boxplot of chl-a concentrations in spring survey

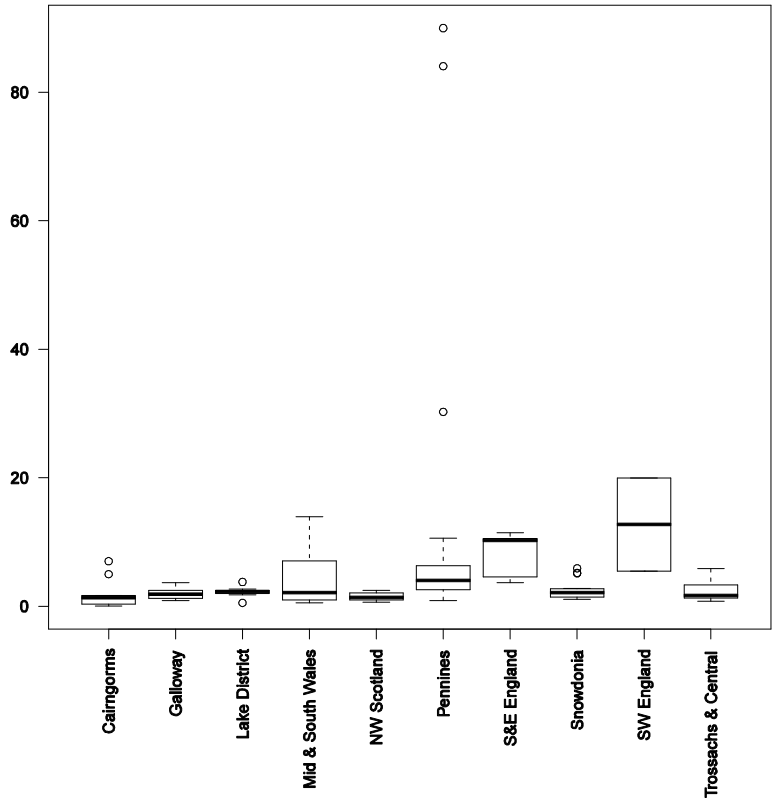


Figure 1.5.19: Plot of chlorophyll:TP ratio against total N deposition for autumn survey

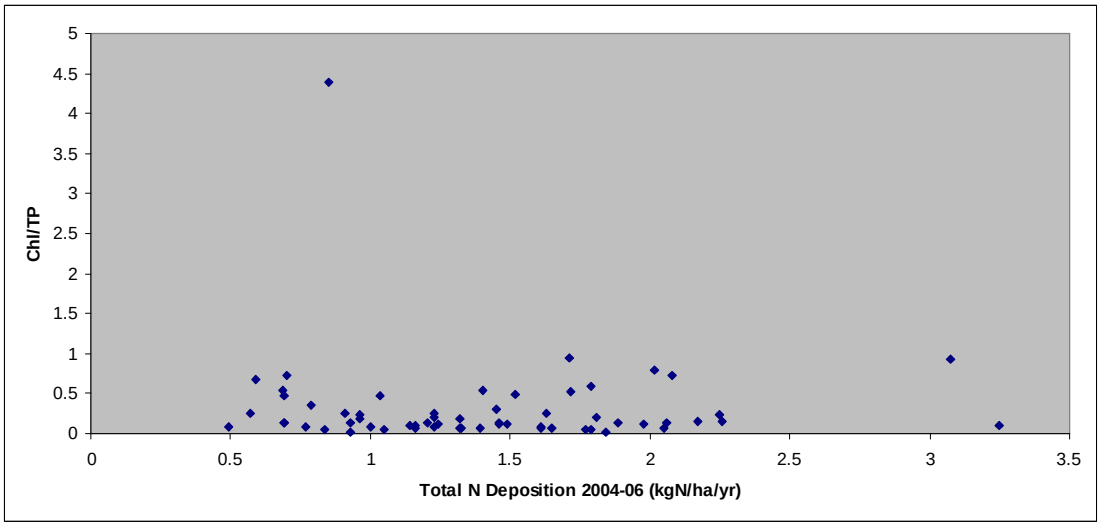
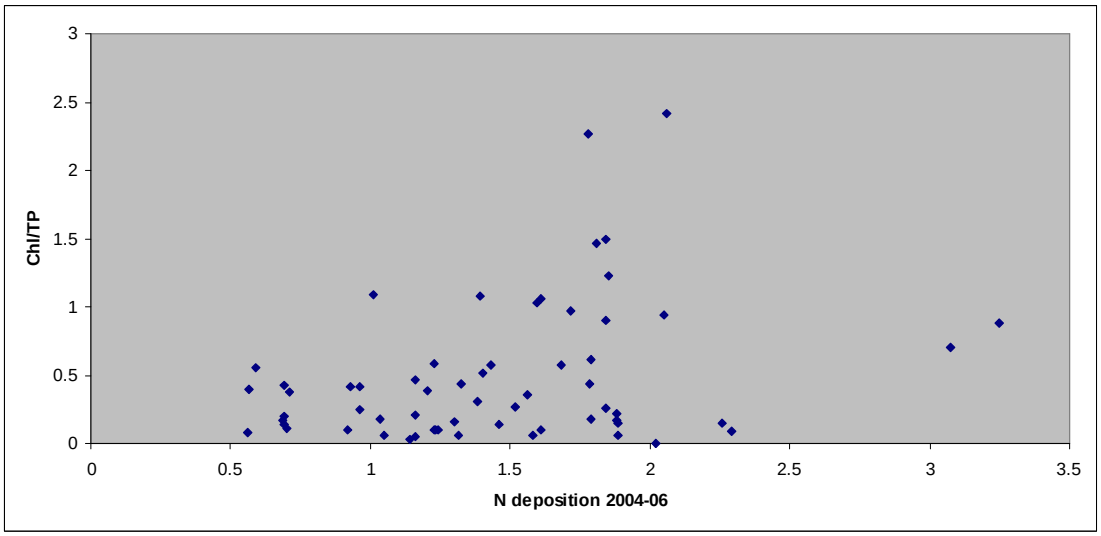


Figure 1.5.20: Plot of chlorophyll:TP ratio against total N deposition for spring survey



Both nutrient concentrations and productivity are known to vary on a seasonal basis so the two studies are not strictly comparable. Furthermore, our survey sites were a subset of sites exceeding critical loads of acidity and hence were heavily biased towards the most acidified sites in the UK, which provides a major confounding variable through potential influences on productivity. To properly address the question of whether N deposition increases phytoplankton productivity would require a survey design properly planned to cover potential N deposition gradients for sites where confounding factors of land-use and acidification could be minimised. Such a study is challenging in the UK because most upland waters remote from direct terrestrial sources of anthropogenic N are also acid-sensitive and exposed to relatively high levels of N deposition.

Summary

1. Application of the FAB model to the UK critical loads dataset for freshwaters using FRAME 2020 deposition shows that 445 sites continue to exceed critical loads beyond planned emissions reductions to 2020.
2. Regional analysis of the factors preventing achievement of critical loads reveals that the most acid sensitive sites with the lowest critical loads for sulphur are located in the North Yorkshire Moors and North West Scotland, while the least sensitive (of this acidified subset of UK sites) regions include the Lake District and Galloway. The pattern is similar for the regions most sensitive to N deposition (lowest values of CLmaxN - the critical load for N deposition alone) but the least sensitive regions are the Pennines, Galloway and mid & South Wales.
3. By 2020, the lowest median values of S deposition occur in NW Scotland, Northern Ireland and the Cairngorms while the highest occur in the Pennines and SW England. For N deposition the lowest values occur in the Cairngorms and especially NW Scotland, while the highest occur in the Lake District and the Pennines.
4. Regional patterns of exceedance are dictated by the combination of deposition and critical loads. North West Scotland shows by far the lowest range of exceedance values despite high sensitivity. The highest exceedances are found (in descending order) in the North Yorkshire Moors, South West England, the Lake District and the Pennines, i.e. the five most exceeded regions are all in England.
5. The FAB model suggests that NO_3^- makes up the larger part of the acidity flux indicated by exceedance, from a minimum of around 50% in some sites in NW Scotland, to a median of around 80% in the Lake District and Northern Ireland. The nitrogen mass balance in the FAB model therefore continues to account for a large proportion of exceedances in most regions. The implication is that a more conservative (rather than the worst-case indicated by FAB) estimate of NO_3^- leaching would result in fewer exceeded sites in most regions, and a reduced magnitude of exceedance in all sites.
6. Resurveys of around one third of exceeded sites in each region (and all in N. Ireland) showed little evidence that critical loads had been systematically under-estimated (and exceedance over-estimated) by the original datasets sampled early in the recovery trajectory in the 1990s, as hypothesized by Rapp & Bishop (2009).
7. Analysis of chlorophyll-a (an index of phytoplankton productivity) and total phosphorus in 106 resurveyed standing waters showed no significant relationship between the chlorophyll:TP ratio and N deposition, in contrast to the study of Bergström and Jansson (2006) in Swedish and North American lakes. This may however be due to the different sampling seasons in the present study and the sampling bias towards the most acidified lakes in the UK resurveys. The highest chlorophyll-a values were generally recorded in the Pennines and southern regions of England and the lowest in Scotland. The highest TP values were similarly found in the Pennines and southern England with a few high values in Snowdonia.

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Work Package 2: Nitrogen as a nutrient – contemporary monitoring and assessment

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Task 2.1: Seasonal variations in N limitation at three contrasting sites

Aims

This Task entails monthly sampling (water chemistry, phytoplankton bioassay) at three lakes which previous phytoplankton bioassay work suggests are contrasting in their nutrient limitation; Scoat Tarn is primarily P-limited, Burnmoor Tarn is primarily co-limited and Moss Eccles Tarn is co- or N-limited. The primary aim is to establish whether there are seasonal patterns in the limiting nutrients for phytoplankton production which may be affected by the availability of NO_3^- from anthropogenic deposition.

Sampling

Monthly sampling of lakewater, bulk deposition, phytoplankton (Task 2.1) and sediment traps (Task 3.1) at Scoat Tarn and Burnmoor Tarn was completed in May 2009. All monthly samples under Task 2.1 were successfully obtained except for one lost deposition sample in July 2008 at Scoat Tarn (Table 2.1.1).

All equipment for Tasks 2.1, 3.1 and 4.1 was decommissioned in May 2009 and samples processed and dispatched that month. Samples were analyzed at CEH Lancaster and the data are presented below.

Table 2.1.1: Details of sampling completed under Tasks 2.1 and 3.1

Month	Description	Samples	Task 2.1	Task 3.1
May-08	First priming trip	None	PRIMING	Deployment
Jun-08	First full sampling	All 19 sites	all	all
Jul-08	Scoat / Burnmoor monthly	SCOAT/BURNMT	SCOAT Dep. Missing	all
Aug-08	Second priming trip	SCOAT/BURNMT	all	all
Sep-08	Second full sampling	All 19 sites	all	all
Oct-08	Scoat / Burnmoor monthly	SCOAT/BURNMT	all	all
Nov-08	Third priming trip	SCOAT/BURNMT	all	all
Dec-08	Third full sampling	All 19 sites	all	all
Jan-09	Scoat / Burnmoor monthly	SCOAT/BURNMT	all	None - lakes frozen
Feb-09	Fourth priming trip	SCOAT/BURNMT	all	BURNMT trap on shore
Mar-09	Fourth full sampling	All 19 sites	all	BURNMT trap redeployed
Apr-09	Scoat / Burnmoor monthly	SCOAT/BURNMT	all	all
May-09	Decommissioning	SCOAT/BURNMT	all	all

Introduction

Although phosphorus is traditionally regarded as the primary limiting nutrient, especially in the sector of water management, it has been known for many years (e.g. Sakomoto, 1966) that nitrogen can also limit or co-limit phytoplankton biomass and productivity. Nitrogen is the primary or co–limiting nutrient for phytoplankton

production in lakes around the world (e.g. Elser, Marzolf & Goldman, 1990), South America (Diaz & Pedrozo, 1996), northern Sweden (Jansson et al., 1996), acidified lakes in central Sweden (Blomqvist et al., 1993) and some lowland German (Sommer, 1989; Sommer, 1993) and Dutch lakes (van der Molen et al., 1998). Nitrogen–limitation may be more general in tropical lakes (Vincent et al., 1984; Harding et al., 1997; Talling & Lemoalle, 1998). Within the UK, nitrogen–limitation has been found in some Cheshire meres (Moss et al., 1992, 1994; James et al., 2003), other shallow eutrophic and mesotrophic lowland lakes around the UK and upland UK lakes (Maberly et al., 2002). N–limitation does not appear to be confined to eutrophic lakes, and has been reported in mesotrophic lakes (Hough & Thompson, 1996) and in oligotrophic lakes for periods during the late summer (Chang & Petersen, 1995; Matthews et al., 2002) in North America.

A recent meta-analysis of nutrient enrichment experiments from over 500 freshwater studies (Elser et al. 2007) showed that on average freshwater phytoplankton are as commonly nitrogen-limited as phosphorus-limited and addition of both nutrients typically produced the strongest response. A similar conclusion was reached by Lewis & Wurtsbaugh (2008) based on a review of the available literature. An intriguing possibility was raised by Bergstrom & Jansen (2006), namely that in the northern hemisphere atmospheric nitrogen deposition has driven some lakes from their natural N-limited state towards P-limitation: in other words before Man's increase in N-supply via atmospheric deposition, most lakes in the northern hemisphere would have been nitrogen-limited. This has been supported by more recent work experimental work (Elser et al. 2009a,b).

Even where lakes are not predominately N–limited, the N–limitation of phytoplankton can occur even for short periods (Sommer, 1989). For example, the phytoplankton of Lough Neagh is P–limited for much of the year but becomes N–limited in the late summer (DOE–DARD, 2002). Periods of possibly limiting N concentrations have also been reported from Lough Erne and from a number of shallow lakes in Northern Ireland (DOE–DARD, 2002; Maberly et al., 2002; Fisher, 2003) and in some oligotrophic lakes in the summer (Chang & Petersen, 1995; Matthews et al., 2002). In upland lakes in the UK, Maberly et al. (2002) showed different seasonal patterns of nutrient limitation. For example, some sites changed from P limitation in spring to co-limitation in summer or autumn. Others changed from N limitation in spring to co-limitation in summer and autumn. One changed from P-limited in spring to N-limited in summer and co-limited in autumn.

Maberly et al. (2002) only assessed on three occasions during the main growing season. The aim of this task was to assess the extent of seasonal changes in nutrient limitation by making approximately monthly measurements at three sites with contrasting nutrient limitation (Burnmoor Tarn, Moss Eccles Tarn and Scoat Tarn). Maberly et al. (2002) found that Moss Eccles Tarn was co-limited in spring and N-

limited in autumn. Curtis et al. (2007) found that Scoat Tarn was P-limited on the three survey occasions and that Burnmoor Tarn was co- or N-limited.

Summary of methodology

The methods and approach follow that in Maberly et al. (2002). Water samples collected from each site were transported back to CEH Lancaster or posted back and were generally processed within 3 days of collection. Water was filtered through a net (120 μm) to remove larger zooplankton. Bioassays were carried out in glass boiling tubes of 50 cm^3 capacity stoppered with a foam bung and containing 35 cm^3 of mixed lake water. Three treatments in triplicate were established: control (no addition), P (NaH_2PO4 stock, final concentration 6 mmol P m^{-3}), N ($NaNO_3$ stock, final concentration 90 mmol N m^{-3}), P + N (additions as above). Tubes were incubated in racks in a constant room temperature at 20ºC and illuminated continuously from below by fluorescent tubes adjusted with neutral density filters to give a photon irradiance of 50 (range 42–62) μmol m^{-2} s^{-1} PAR (400–700 nm) measured with a cosine-corrected sensor and meter (Macam Q102, Macam Photometrics, Livingston, UK). After 14 days, chlorophyll *a* concentration was then measured by filtering the contents of the tube through 4.7 cm diameter Whatman GF/C filter paper and stored in a freezer prior to analysis. Filters were extracted in 7.1 cm^3 of industrial methylated spirits (96% ethanol, 4% methanol) and briefly brought to boiling point. Following cooling in the dark, they were centrifuged and optical density was read in a spectrophotometer at 665 and 750 nm. Concentrations of chlorophyll a were calculated using the equations of Talling (1974). Statistical differences among treatments were assessed using t-tests.

Results

Scoat Tarn always responded strongly to addition of both phosphorus and nitrogen with a mean concentration of 130 mg m^{-3} (Table 2.1.2, Fig. 2.1.1). Overall, phosphorus addition produced the second highest mean concentration of chlorophyll *a* while the average for nitrogen was very similar to the control (Table 1). The tarn was primarily phosphorus-limited (Fig. 2.1.2), however in June and July 2008, the tarn was co-limited by both phosphorus and nitrogen (i.e. neither phosphorus nor nitrogen alone caused a significant increase in chlorophyll a compared to the control). In September 2008, although the NP treatment produced a large increase in chlorophyll a over the control the variability was quite high in this and the control and the increase was not quite significant (P=0.05).

Burnmoor Tarn always responded strongly to addition of both phosphorus and nitrogen with a mean concentration of 133 mg m^{-3} (Table 2.1.2, Fig. 2.1.3). Overall, phosphorus addition produced the second highest mean concentration of chlorophyll *a* while the average for nitrogen was slightly higher than the control (Table 2.1.2). Burnmoor Tarn was phosphorus-limited on two occasions and co-limited on six

occasions and not statistically significant on August, September and October 2008, although in each of these months the response was probably co-limited but could not be resolved because of high variability (Fig. 2.1.4).

Figure 2.1.1: Mean concentration of chlorophyll a in Scoat Tarn with standard deviation in parenthesis for Control (no additions), plus-phosphorus, plus-nitrogen and addition of both phosphorus and nitrogen. Note the y-axis is on a logarithmic scale.

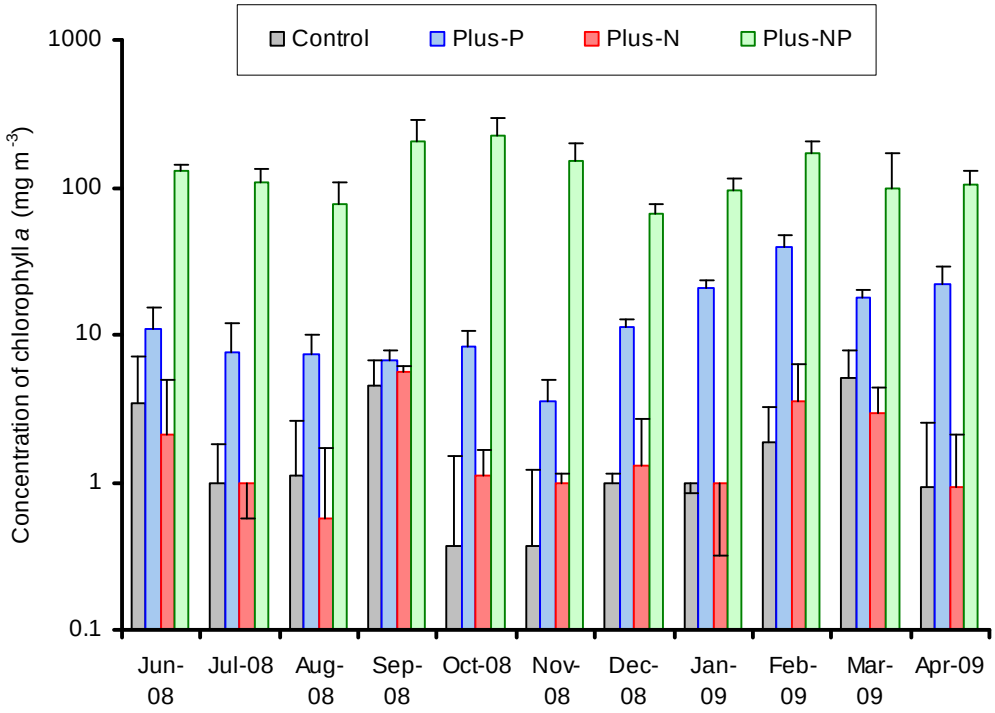


Table 2.1.2: Average concentration of chlorophyll a (mg m^{-3}) for the different sites and seasons. The values in parentheses are the ratio against the control concentration for each site.

Site	Control	Plus-P	Plus-N	Plus P + N
Scoat Tarn	1.6	14 (4.8)	1.7 (1.1)	130 (81)
Burnmoor Tarn	3.7	9.2 (2.5)	4.6 (1.2)	133 (36)
Moss Eccles Tarn	6.4	18 (2.8)	11 (1.7)	99 (15)

Figure 2.1.2: Probability in Scoat Tarn of the named nutrient being limiting- based on the probability of a difference against the control. The horizontal line shows the P<0.05 significance.

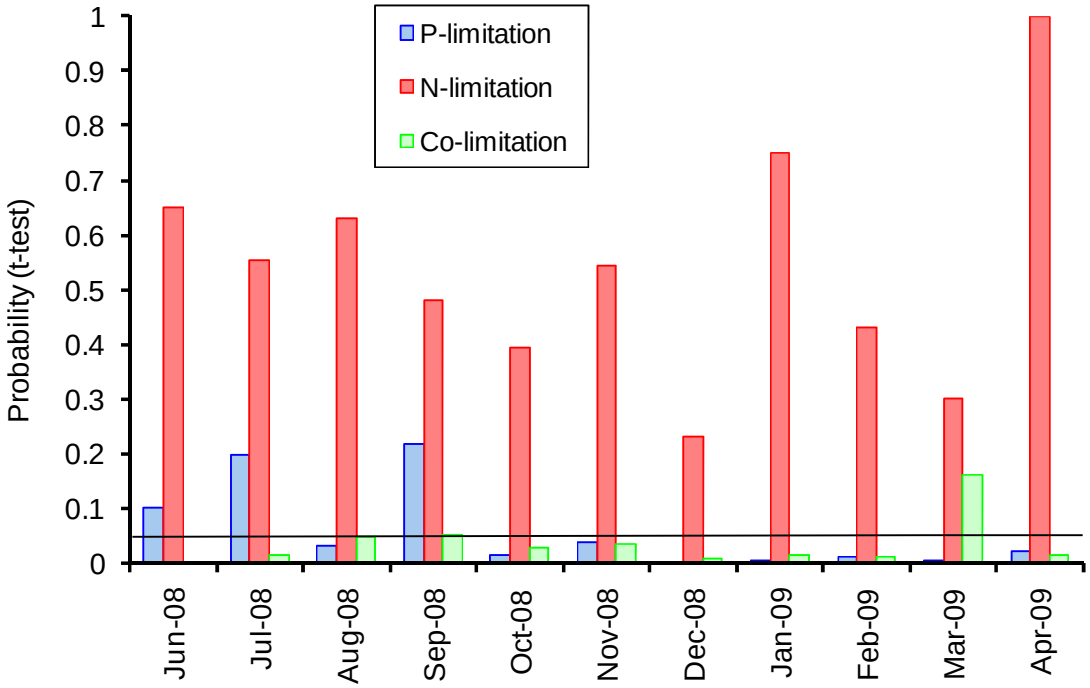


Figure 2.1.3: Mean concentration of chlorophyll a in Burnmoor Tarn with standard deviation in parenthesis for Control (no additions), plus-phosphorus, plus-nitrogen and addition of both phosphorus and nitrogen. Note the y-axis is on a logarithmic scale.

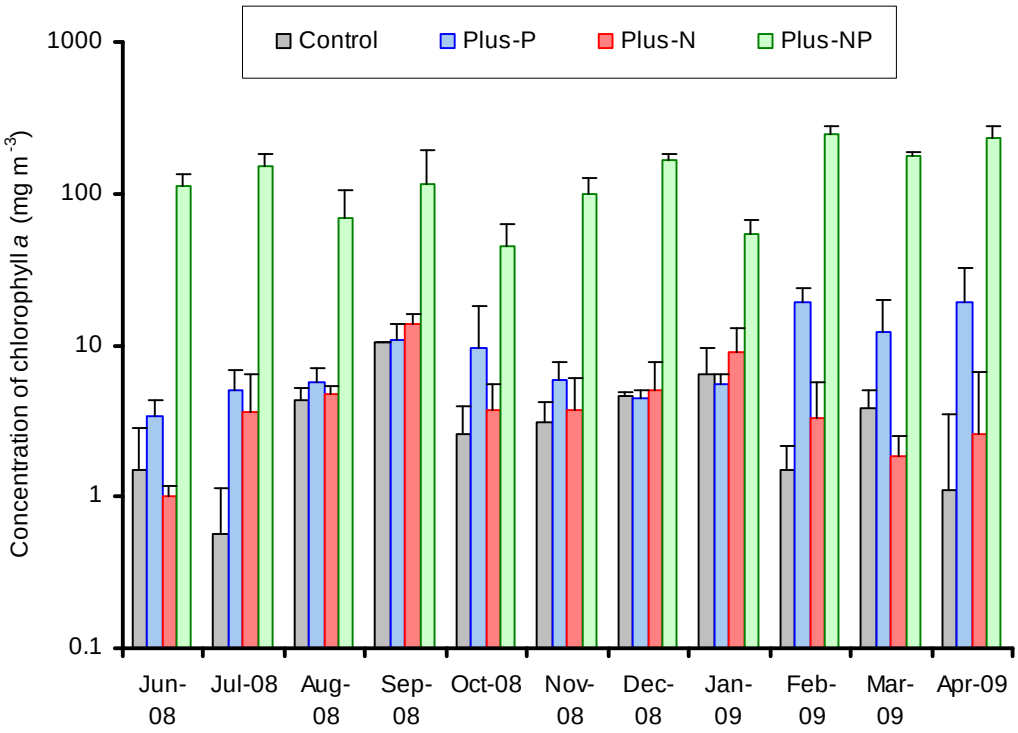
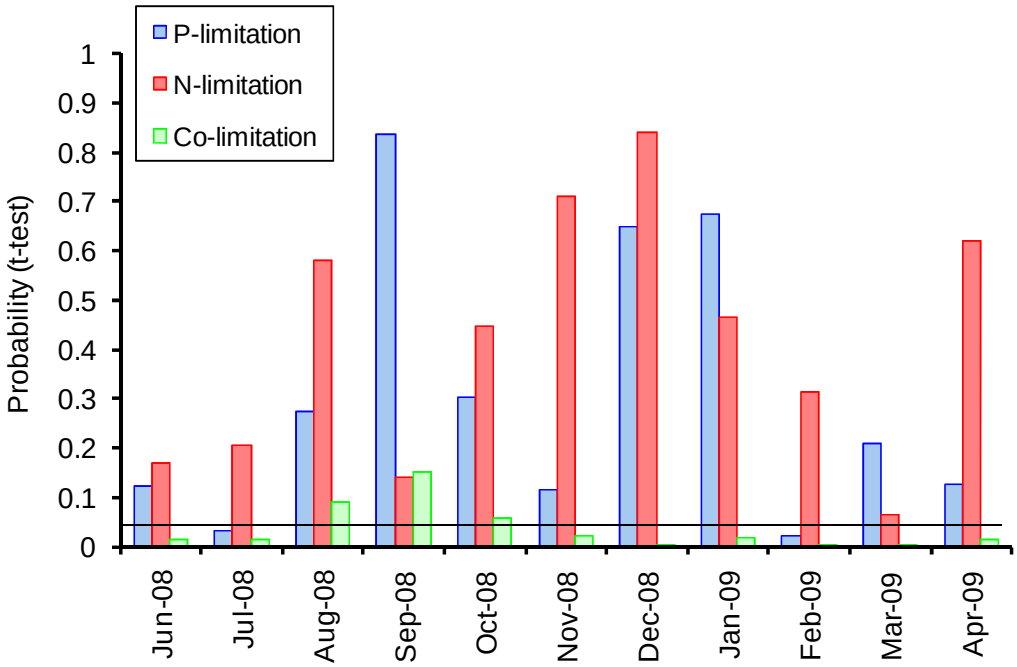


Figure 2.1.4: Probability in Burnmoor Tarn of the named nutrient being limiting- based on the probability of a difference against the control. The horizontal line shows the P<0.05 significance cut-off.



Moss Eccles Tarn always responded strongly to addition of both phosphorus and nitrogen although the mean concentration of 99 mg m^{-3} (Table 2.1.2, Fig. 2.1.5) was lower than at the two other sites for this treatment. Overall, phosphorus addition produced a slightly higher concentration of chlorophyll *a* than nitrogen (Table 2.1.2). Moss Eccles Tarn was co-limited on 7 occasions, nitrogen-limited on one occasion (May 2009) and there was no statistically significant effect in January or February 2009 (Fig. 2.1.6). In this latter month, phosphorus was close to being limiting (P = 0.06).

Discussion and conclusions

The frequency of nutrient limitation varied across the three sites (Table 2.1.3) and broadly followed what had been found before. Scoat Tarn was primarily phosphorus limited (Curtis et al. 2007), Burnmoor Tarn was mainly co-limited. In 2008-09 Burnmoor Tarn was P-limited on two occasions while in 2005 it was N-limited in July. Moss Eccles is also predominantly co-limited and was N-limited on occasion in 2008-09 which is in agreement with what was found in 2000 (Maberly 2002). Nutrient limitation did alter seasonally, although mainly e.g. between P and co-limited or N and co-limited in these three tarns. However, in contrast to the general agreement over the main nutrient that was limiting phytoplankton, the seasonal pattern of nutrient-limitation did not appear to vary coherently: episodes of, for example, P-limitation in Burnmoor Tarn occurred for a single month while phytoplankton in months either side were co-limited. This suggests that small events within a Tarn, possible linked to flushing and input of nutrients, since all these tarns are small and

probably relatively rapidly flushed, can alter nutrient-limitation, especially since the catchment is known to control water quality in these types of tarn (Maberly et al. 2003).

Figure 2.1.5: Mean concentration of chlorophyll a in Moss Eccles Tarn with standard deviation in parenthesis for Control (no additions), plus-phosphorus, plus-nitrogen and addition of both phosphorus and nitrogen. Note the y-axis is on a logarithmic scale.

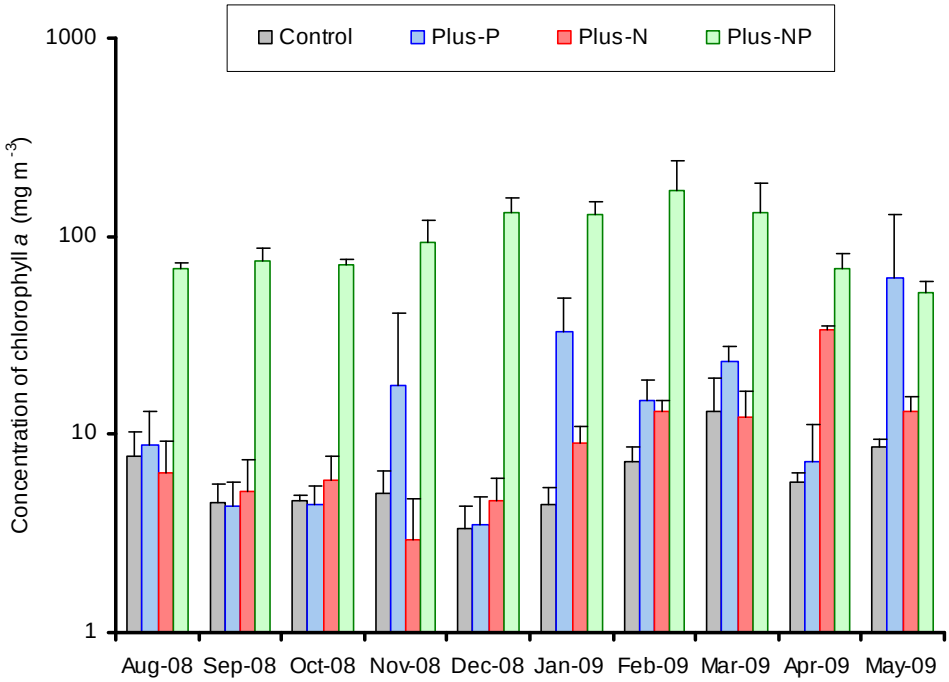
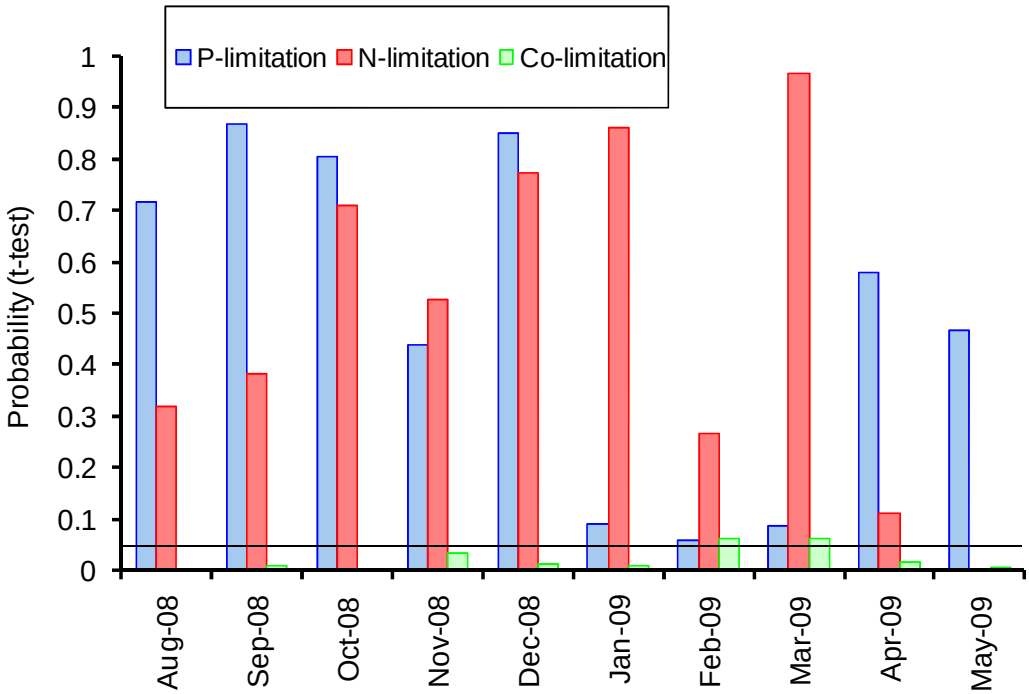


Table 2.1.3:. Frequency of nutrient-limitation as a percent of the occasions when a statistically significant result was obtained

Site	P- limited	Co-limited	N-limited
Scoat Tarn	80	20	0
Burnmoor Tarn	25	75	0
Moss Eccles Tarn	0	87.5	12.5

There has been a recent controversy about the role of N and P-limitation in lakes and estuaries and the effectiveness of reducing only one of these nutrients (Schindler et al. 2008; Howarth & Pearl 2008; Schindler & Heckey 2008). In a Policy Forum Review in Science, Conley et al. (2009) rehearsed the various arguments and concluded that effective control of the negative impacts of nutrient enrichment should be made by control and reduction of both nitrogen and phosphorus. If many lakes are co-limited or can switch seasonally between different types of nutrient limitation, this also suggests that both nitrogen and phosphorus will need to be controlled to manage the water quality of these upland tarns.

Figure 2.1.6: Probability in Moss Eccles Tarn of the named nutrient being limiting- based on the probability of a difference against the control. The horizontal line shows the P<0.05 significance cut-off.



Summary

Monthly phytoplankton nutrient bioassays over a whole year at three contrasting Lake District Tarns has demonstrated that there are seasonal changes in N and P limitation but they do not follow a coherent seasonal pattern and can be event-based, suggesting possible links to catchment inputs, flushing and phytoplankton blooms. Scoat Tarn was primarily P-limited, while Burnmoor Tarn and Moss Eccles Tarn were mainly co-limited with periods of N limitation, although occurrences of P limitation were found in Burnmoor Tarn in this study, in contrast with an earlier study in 2000. Seasonal changes were mainly from co-limitation to N- or P- limitation and back, rather than a switch from N- to P- limitation between seasons.

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Task 2.2: Development of new methods for bioassay in streams at AWMN sites

This Task aims to develop and test a new method for nutrient bioassay in streams to determine whether it is appropriate for wider monitoring of stream nutrient limitation status in the uplands. The streams selected at the start of 2008 from the AWMN were:

1. Allt a'Mharcaidh – possibly N limited due to inorganic N concentrations usually below detection limits;
2. River Etherow – probably P limited due to very high NO_3^- concentrations throughout the year ;
3. Dargall Lane – fairly high loads of both S and N deposition; intermediate between the Mharcaidh and Etherow sites and could be co-limited by N and P.

Introduction

Different approaches have been used to assess nutrient limitation in inland waters. These include: i) calculation of ratios of N:P concentration and comparison with empirical thresholds or more physiologically-based ratios, such as the Redfield ratio; ii) analysis of seasonal water-chemistry data to identify seasonal (usually summer) decline in concentration below a threshold; iii) use of models at a range of complexities that relate nutrient supply to biological demand; iv) changes in stable isotope (applicable to nitrogen) discrimination and v) physiological indices related to regulation of genes or enzymes involved in nutrient acquisition (Maberly *et al*., 2004). A final, direct and widely-used approach is the use of a bioassay. This measures the response of algae to addition of a potentially-limiting nutrient. The response measured is normally growth after a period from several days to several weeks. Variants on the method include: i) using natural phytoplankton populations *versus* a test, single species in culture; ii) measuring the response under controlled, standardised laboratory conditions *versus* measuring under semi-natural conditions in the field and iii) measuring a short-term photosynthesis response rather than long-term growth. This approach is usually regarded as the 'gold-standard' for identifying nutrient-limitation although it also has inherent problems associated with artificiality of conditions, 'snap-shot' responses and removal from natural supplies of nutrients that can occur in the field. Bioassays have been applied most frequently to phytoplankton in standing water. In flowing water, phytoplankton can be investigated by bioassay, but the ecological value of this is limited because, apart from long, slow-moving rivers in spring and early summer, phytoplankton tend to be sparse in flowing water because of rapid hydraulic loss and low light availability. Instead, attached benthic algae, the periphyton, are often important in flowing water and many of the approaches outlined above are also applicable to this group. However, periphyton offers the opportunity to undertake more ecologically relevant bioassay experiments that can be carried out without significant investment of money in mesocosms. Two general approaches are used: i) nutrient-diffusing substrates and ii) 'spiking' a stretch or small section of a stream or river with additional nutrient. The spiking experiments are technologically more complicated as they require a means of constant nutrient addition; see Bowes *et al*. (2007). The nutrient-diffusing substrate approach to

assessing nutrient limitation of periphyton was developed by Fairchild *et al.* (1985), initially for use in lakes. They used a terracotta pot, both as the substrate for periphyton attachment and growth and as the material through which potentially limiting nutrients could diffuse. The centre of the pot was filled with agar made up in distilled water (control) or in a solution containing phosphate or nitrate or combinations of both. The top was sealed and attached to the substratum and incubated in a lake for about 50 days before chlorophyll was measured. This approach has been used successfully in UK lakes where the response of the periphyton was similar to measurements made on phytoplankton at a similar time (Maberly *et al*., 2002). A variation on this theme was developed by Matlock et al. (1998) who used plastic bottles filled with nutrient solution (or distilled water for the control) and a glass-fibre filter paper attached over the mouth of the bottle as the substrate for algal growth and the medium through which nutrient could diffuse. The advantage of this approach is that the filter paper can be directly extracted for chlorophyll analyses without the need to scrape a surface to remove periphyton. This 'periphytometer' method was evaluated here to determine nutrient limitation in UK streams.

Summary of methodology

The method generally followed that in Rodriguez & Matlock (2008). Widely available standard plastic pipes and connectors were used to build the experimental frame to which wire-mesh was attached to the bottom and side (see Figure 1 b,c,d). The mesh on the side was for protection, preventing stones or debris carried by the river in high flow events from damaging the bottles and filters but still allowing water to flow through. The bottom mesh was used to attach the experimental 500 ml bottles containing different nutrient solutions. The phosphorus treatment bottles were filled with a solution of 6.2 mg P L^{-1} (0.2 mmol P L^{-1}) of sodium phosphate and the nitrogen treatment bottles were filled with a solution of 14 mg N L^{-1} (1.0 mmol N L^{-1}) of sodium nitrate, an approximate 100- to 10-fold increase to the concentrations occurring in the rivers respectively. Phosphorus and nitrogen treatment solutions contained both nutrient concentrations and the control treatment was filled with distilled water. The lids of the bottles were cut on the top, giving a diameter of 40 mm and an exposed area of 12.6 cm^2. A glass-fibre filter was placed on top of a dialysis membrane (porosity of 12-14 kDaltons, to prevent bacterial ingress to the solution) and held in place by the remains of the lid (see Figure 2.2.1 a) to allow passive diffusion. Each treatment was replicated three-fold and placed randomly on the mesh frame with the filter parallel to the direction of flow. Two deployments were made during 2008, in June and September, with incubation time of from two to four weeks depending on staff availability to visit the sites.

Results

Water chemistry

Background water chemistry for the three sites is shown in Table 2.2.1. All three have low alkalinity and concentrations of soluble reactive phosphorus and total phosphorus,

but there is a gradient in concentration of nitrate ranging from extremely low (Allt a'Mharcaidh) to high (River Etherow), with Dargall Lane being intermediate.

Loss of samples

The current design suffered from loss of samples (Table 2.2.2). On some occasions all the filters were recovered, but on one occasion only 3 (25%) were recovered. One cause of filter loss resulted from shear forces that tore the filter from the mouth of the bottle. This could perhaps be reduced by reducing the tightness of the lid and adding a washer to spread the force of the bottle closure on the filter when it is screwed tight. The second cause was wholesale loss of a bottle. On some occasions, for example at Dargall Lane in June, the force of the water was so great that the chicken wire to which the bottles were attached was broken. This cause of loss could be reduced by using stronger wire.

Bioassay results

Despite the problems of sample loss, the periphyton bioassay did seem to produce reliable and reasonable results. At Allt a'Mharcaidh, the periphyton were nitrogen limited on both occasions (Fig. 2.2.2a) and this agrees with the very low nitrate concentrations at this site (Table 2.2.1). At Dargall Lane, sample loss made nutrient-limitation more difficult to evaluate. In June no P-spiked samples survived and the single high chlorophyll value for the N+P treatment indicates either co-limitation or P-limitation at this site (Fig. 2.2.2b). In September the site appeared to be co-limited by both nitrogen and phosphorus. At the River Etherow the periphyton were P-limited in June but limitation could not be assessed in September because of loss of sample (Fig. 2.2.2c). This was the site with the highest nitrate concentration.

Conclusions

The stream periphytometer produced results that appeared to be ecologically reasonable: Allt a'Mharcaidh had low nitrate concentrations and was N-limited, Dargall Lane had intermediate concentrations of nitrate and was co-limited and the River Etherow had high concentrations of nitrate and was P-limited. However, in the 'flashy' systems the periphytometer was used in, the attachment of the filters in the lid of the bottle and the attachment of the bottle to the wire frame will need to be modified and made more robust to minimise loss of samples. Nevertheless, these preliminary results are encouraging and could form the basis of further work in the future.

Summary

The stream periphytometer produced results that appeared to be ecologically reasonable: Allt a'Mharcaidh had low nitrate concentrations and was N-limited, Dargall Lane had intermediate concentrations of nitrate and was co-limited and the River Etherow had high concentrations of nitrate and was P-limited. However, the

loss of sample filters during spates was a problem and a more robust design would be required for routine use of the method to assess streamwater nutrient limitation.

Figure 2.2.1: The periphytometer in the laboratory and at the River Etherow: a) single bottle showing cut-lid replaced with glass-fibre filter; b) installing the frame in the Etherow; c) the installed frame, note filters are orientated parallel to the flow; d) the frame at the end of the incubation.

Table 2.2.1: Background water chemistry for the three study sites between 1999 and 2000 (1999 and 2006 at Dargall Lane) based on data supplied by UCL.

Site	Alkalinity (μEquiv L^{-1})	Soluble reactive P ($\mu g\ L^{-1}$)	Nitrate-N ($\mu g\ L^{-1}$)	Total P ($\mu g\ L^{-1}$)	Total N ($\mu g\ L^{-1}$)
Allt a'Mharcaidh*	41	<detection	13	4.1	125
Dargall Lane	12	1.8	178	4.3	275
River Etherow	57	2.4	606	8.5	877

* Mean for three sites

Table 2.2.2: Number of filters recovered on the two sampling occasions at the three sites. Twelve filters were deployed on each occasion.

Site	June	September
Allt a'Mharcaidh	10	11
Dargall Lane	6	12
River Etherow	12	3

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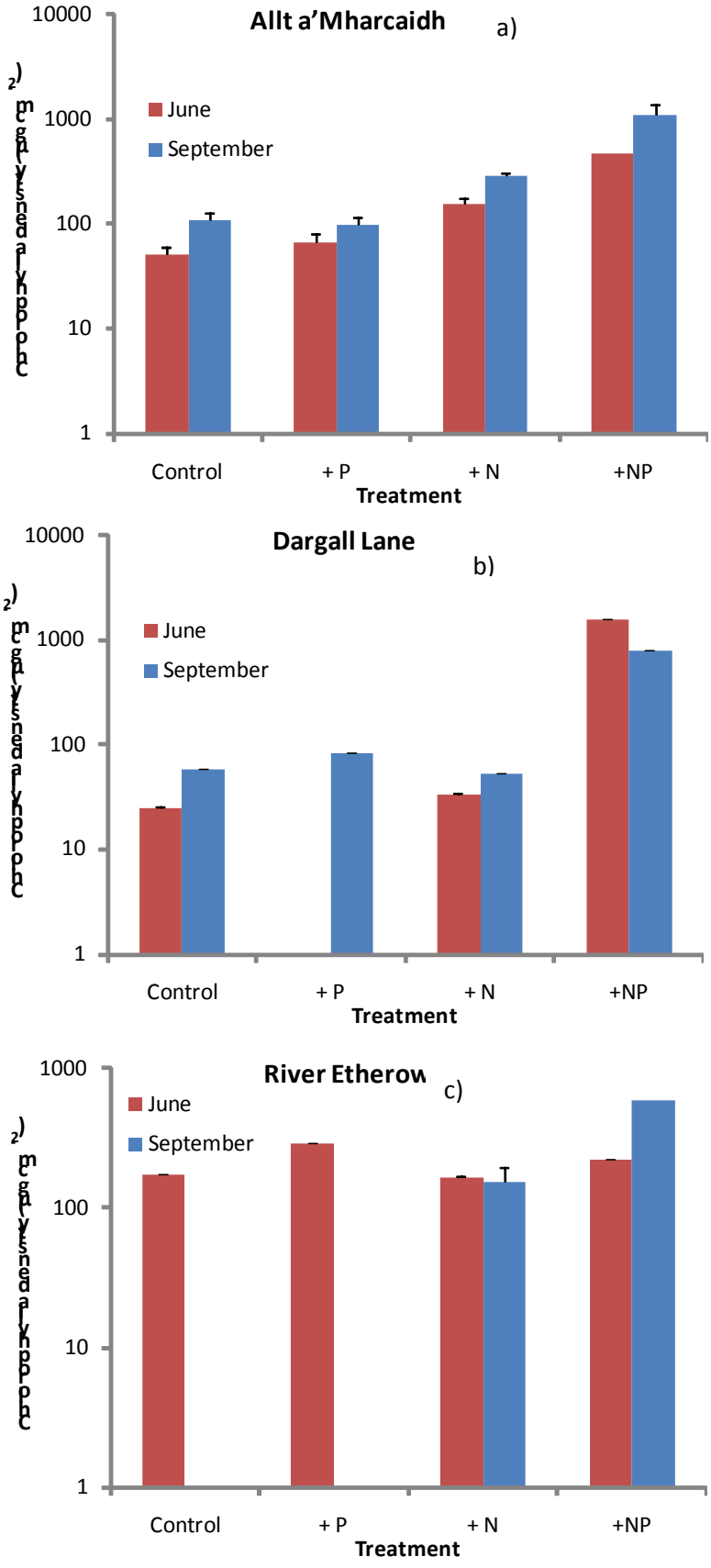
Figure 2.2.2: Stream periphyton bioassay results from three sites in June and September 2008.



Task 2.3: Development of catchment-based empirical models for prediction of N leaching and N limitation for upscaling and "stock at risk" assessment of N deposition impacts across the UK

The primary aims of this task were:

1. collation of catchment attribute data available at regional/subproject and national scales for prediction of NO_3^- leaching, including new published datasets not previously included in analyses – with tabulated metadata;
2. statistical analysis of available catchment-scale predictors of NO_3^- leaching including new variables not previously considered e.g. soil depth, C:N ratio.

2.3.1 Collation of catchment attribute data

National datasets

This task was discussed in parallel with Task 1.3 following the Annual Meeting on 11th February 2009. It was decided to combine efforts in the generation of catchment attribute datasets as these are equally useful for both dynamic modelling work (especially MAGIC library) under the Freshwater Umbrella and CLDM as well as the empirical catchment model development under this task. Hence a subsequent meeting was planned to determine which additional catchment attribute data may be generated at UCL and CEH Bangor.

Major progress has been made with the collation of new datasets through the compilation of raw soil cover data for all FAB mapping sites. Previously, only derived N sink data had been compiled for these sites and collated only at the final stage due to problems with the different soil type descriptions used for England+Wales, Scotland and Northern Ireland. In particular, the expansion of the UK mapping dataset in 2004 led to the amalgamation of datasets from many sources (eg GANE, CEH and MLURI regional surveys, Welsh Acid Waters Survey, EHS Northern Ireland Survey, FFL/NIVA Scottish Random Survey) with soils derived independently for each. The new master table includes original soil type and data source, proportional cover and generalised soil type used in the lookup table of literature default N sink terms compiled for the Terrestrial Umbrella in the 1990s. This exercise was a substantial undertaking (c. 8 days work) but provides valuable additional data for catchment based modelling under the Freshwater Umbrella and especially for parallel dynamic modelling activities under the CLDM contract.

In addition, new high resolution land-cover data have been derived for all sites in the updated (March 2008) FAB mapping dataset. Previously, only approximate LCM data based on the 1km resolution dataset was used but this has now been upgraded to the highest resolution catchment-weighted 25m raster LCM2000 data.

Modified catchment-weighted deposition datasets have also been derived under the CLDM at CEH Bangor, using for the first time the actual land-cover within each catchment to weight CBED deposition data for 2004-06 according to forest and

moorland/grassland proportions. Previously, only 5km average gridded data have been used.

AWMN and FUMBLE modelling datasets

In the first phase of the Freshwater Umbrella programme, modelling showed that N deposition in combination with moss/bryophyte biomass explained a large proportion of the variance in NO_3^- leaching data observed at unafforested AWMN sites (Task 3.4 in Curtis and Simpson, 2007). It was speculated in that report that a possible reason for a lack of additional explanatory power given by soil C:N ratios could be related to peat erosion in certain catchments, whereby the exposure of older peat deposits invalidated the assumption that surface soil C:N ratios reflect recent modification by cumulative N deposition loads.

To test whether catchment scale data on peat erosion improved models for predicting NO_3^- leaching, publicly available catchment images from Google Earth were used in combination with GIS derived catchment outline shapefiles. In Google Earth it was possible to overlay a grid on the catchment and draw polygons around areas of eroding peat, assessed by eye (Fig. 2.3.1). The proportion of eroding peat within all AWMN and additional FUMBLE catchments was estimated by eye in this way; in the Bencrom River example (Fig. 2.3.2.) the proportion of eroding peat within the catchment was estimated as 20% and Blue Lough at 5%.

Figure 2.3.1: Delineating areas of eroding peat (yellow outlines) within a zoomed-in area of the catchment of the AWMN site Bencrom River (i.e. right of the red line) using Google Earth images

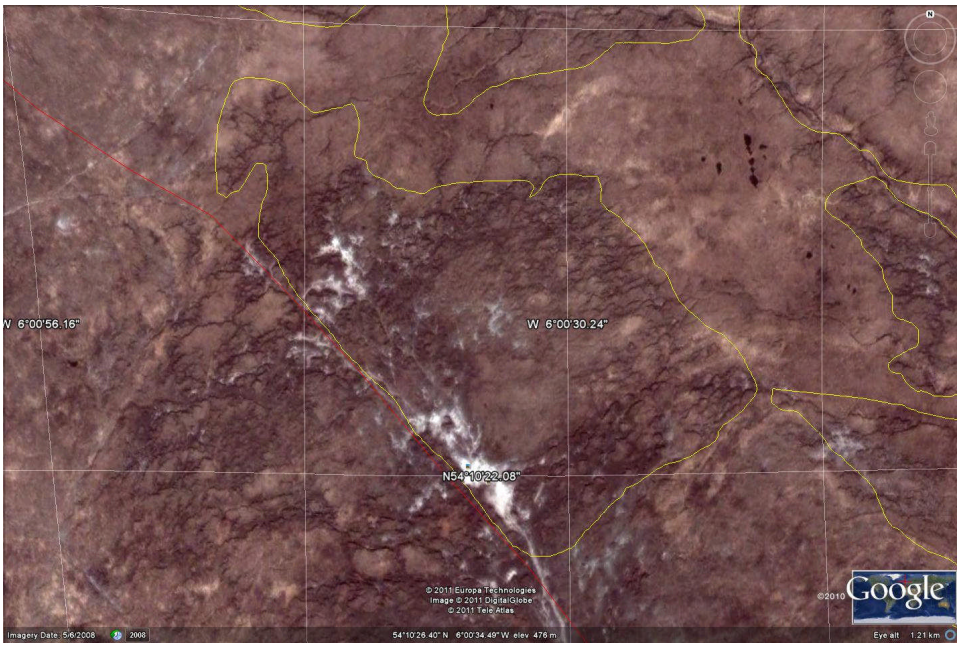
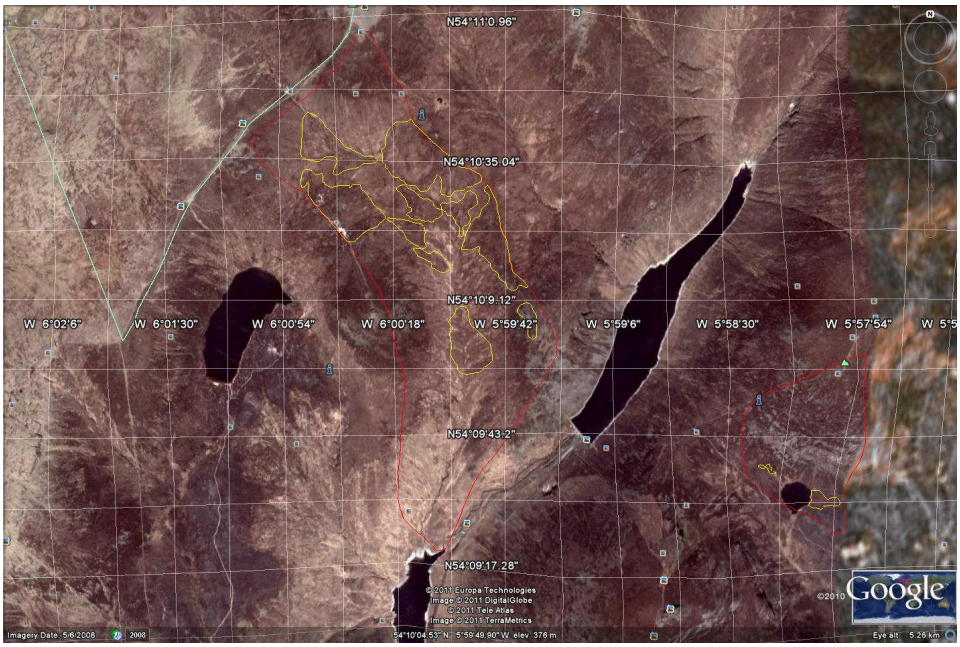


Table 2.3.1 shows the proportion of peat soil map units for all catchments studied under Phases 1 (FU1) and 2 (FU2) of the Freshwater Umbrella programme, plus other

AWMN sites not otherwise studied under these projects. For all cases except Borralie and Trout Beck, catchment weighted soil-cover data were available from the FAB Critical Loads database and derived originally from SSLRC, MLURI or DoE NI sources. The proportion of peat soils within these catchments varies from zero to 100% at Bencrom River and Llyn Conwy. The estimated proportion of eroding peat varies from zero to 35% at both the River Etherow and Trout Beck catchments. Note that due to coarse resolution soils data used within the FAB database, some catchments known to contain peat soils are shown as having zero (e.g. Lochnagar, Round Loch of Glenhead, Loch Tinker). Cases where the estimated proportion of eroding peat exceeds the proportion of mapped peat cover reflects the differences in data resolution.

Figure 2.3.2: Grid overlay with polygons denoting peat erosion (yellow) within the catchments (red outline) of Bencrom River (left; 20%) and the adjacent AWMN site Blue Lough (right; 5%)



The estimated eroding peat data were combined with other catchment predictor variables available for largely overlapping datasets from Phases 1 and 2 of the Freshwater Umbrella programme. In Phase 1, all non-forested AWMN sites were randomly surveyed for moss+bryophyte biomass and the depth and C:N ratio of the organic (O) horizon at 20 randomly selected points within the catchment (Curtis & Simpson, 2007). The unique predictor variables available for these 16 sites are presented in Table 2.3.2. These sites largely overlap with the 19 studied in Task 4.1 of the current programme, for which additional isotopic data on the proportion of atmospheric NO_3^- in surface waters are available (see Table 4.1.3 in WP4). Here, prediction of NO_3^- concentrations and % atmospheric NO_3^- was attempted using all available predictors for the FUMBLE1 and FUMBLE2 (Task 4.1) datasets respectively.

Table 2.3.1: Estimated proportion of peat soils (SSLRC / MLURI soils data) and eroding peat (from Google Earth). NB no digital soils data for Borralie; Trout Beck data from ECN website.

Site Code	Name	Project	Map Unit	Fraction	Eroding?
ANCC	Allt na Coire nan Con	AWMN	0	0.00	0.01
CHN	Loch Chon	AWMN	5.1.0	0.03	0.01
CONY	Coneyglen Burn	AWMN	5.1.0	0.60	0.01
MYN	Llyn Cwm Mynach	AWMN	1013b	0.24	0.01
ARR	Coire nan Arr	FU1	0	0.00	0.01
BEAH	Beaghs Burn	FU1	6	0.97	0.20
BENC	Bencrom River	FU1	5.1.0	1.00	0.20
BLU	Blue Lough	FU1/2	5.1.0	0.45	0.05
BURNMT	Burnmoor Tarn	FU1/2	0	0.00	0.02
DARG	Dargall Lane	FU1/2	0	0.00	0.01
ETHR	River Etherow	FU1/2	1011b	0.80	0.35
GWY	Afon Gwy	FU1/2	1013a	0.40	0.05
LAG	Llyn Llagi	FU1/2	1013a	0.44	0.00
LODG	Old Lodge	FU1/2	0	0.00	0
MHAR	Allt a'Mharcaidh	FU1/2	0	0.00	0.05
NAGA	Lochnagar	FU1/2	0	0.00	0.02
RLGH	Round Loch of Glenhead	FU1/2	0	0.00	0.01
SCOATT	Scoat Tarn	FU1/2	0	0.00	0
TINK	Loch Tinker	FU1/2	0	0.00	0.1
VNG9402	Loch Coire Fionnaraich	FU1/2	5.1.0	0.10	0.00
BORR	Borralie	FU2			0
CON	Llyn Conwy	FU2	1013b	1.00	0.04
HAFR	Afon Hafren	FU2	1013a	0.60	0.25
LGR	Loch Grannoch	FU2	5.1.0	0.19	0.03
TROUT	Trout Beck	FU2		0.90	0.35
VNY4101	Small Water	FU2	1011b	0.04	0.00

Table 2.3.2: Predictor variables for NO_3^- leaching available only for the Freshwater Umbrella Phase 1 dataset (all unafforested AWMN sites, n=16)

Sitecode	Count	C/N	%N	%C	O Depth cm	Moss g/m2
ARR	15	19.69	0.82	17	8	225.1
BEAH	20	26.08	1.69	43.64	50	325.6
BENC	18	18.99	1.77	32.87	39	53.4
BLU	17	21.51	1.38	27.78	15	162
BURNMT	19	15.74	2.09	31.88	10	80
DARG	19	12.15	1.56	18.4	18	179.6
ETHR	20	30.09	1.73	48.74	88	118.9
GWY	20	14.03	1.45	21.06	17	483.6
LAG	18	16.15	1.89	31.07	7	149.5
LODG	20	18.91	0.79	15.46	2	56.5
MHAR	17	25.33	1.14	28.14	16	568.3
NAGA	16	21.67	1.15	26.52	9	284.5
RLGH	20	17.98	2.11	37.77	41	106.4
SCOATT	18	14.37	1.56	21.61	7	124.4
TINK	19	24.78	1.55	36.82	23	377.1
VNG9402	20	22.32	1.16	25.87	23	313.7

2.3.2 Statistical Analysis

Several potential predictor variables were collated into data sets for use in a statistical modelling exercise to derive predictive models for lake water NO_3^-, NH_4^+ and the percentage of atmospheric NO_3^- derived from stable isotope methods (Task 4.1).

A number of statistical techniques can be used to develop a predictive model, including simple linear regression. Given the small number of observations available we decide to use simple statistical methods for our analysis, and as a result fitted models using least squares.

Step-wise model selection is problematic for several reasons, not least parameter bias in the selected model terms arising from the exclusion of insignificant terms from the model during fitting (Whittingham *et al.*, 2006). In the past couple of decades a number of methods have been developed that place model selection at the heart of the model building process which do not suffer the same problems as step-wise techniques (Hastie *et al.*, 2010). Loosely, we might term these techniques as shrinkage methods. One such shrinkage method is the lasso. This approach works by placing a penalty on the absolute size of the regression coefficients (Tibshirani, 1996).

A path of models exists between the full least squares estimates for a model containing all candidate predictor variables and a model containing an intercept term only. Each of the models intermediate between these two points is formed by shrinking the estimated regression coefficients away from the least squares solution by some amount, the penalty, λ. As the degree of shrinkage increases, predictor variables are selected out of the model if their estimated coefficient crosses the 0 line of no effect. As such, a predictor with a positive coefficient is removed as soon as shrinkage would move the coefficient to or past zero, and vice versa. The optimal model from the entire path of models is determined using a *k*-fold cross validation (CV) procedure and the model with i) the lowest error or ii) the simplest model within one standard error of the best model (lowest error) is chosen. The one standard error rule (1 SE) reflects an important model choice; we should prefer simpler models over more complex models unless the more complex models fit the data substantially better. A simpler model that is within one standard error of the best model is statistically equivalent to the best model in terms of model error and hence, following the maxim of preferring simpler models we should chose the simpler model in that case.

The final regression coefficients from the lasso procedure are biased estimates. As an alternative we can fit the least squares model using only those terms retained in the lasso model and use those regression coefficients as our predictive model (Hastie *et al.*, 2010). We adopt this approach here and refit least squares models using the predictors indicated by the results of the Lasso procedure.

Results

Figure 2.3.3 shows the lasso model path and CV error for the model to predict lake water NO_3^- concentrations using the FUMBLE1 group of 16 sites from Table 2.3.2. The simplest model within 1 SE of the best model contains two predictors, i) N deposition and ii) moss biomass, with standardised lasso coefficients of 0.76 and -0.0007 respectively, indicating a 3 orders of magnitude difference in the ability to predict NO_3^- concentration. The least squares model using only these two predictors is highly significant ($F_{2,13}$ = 12.86, p = 0.0008) and explains 66% of the variance in the measured lake water NO3$^-$ (adjusted R^2 0.61).

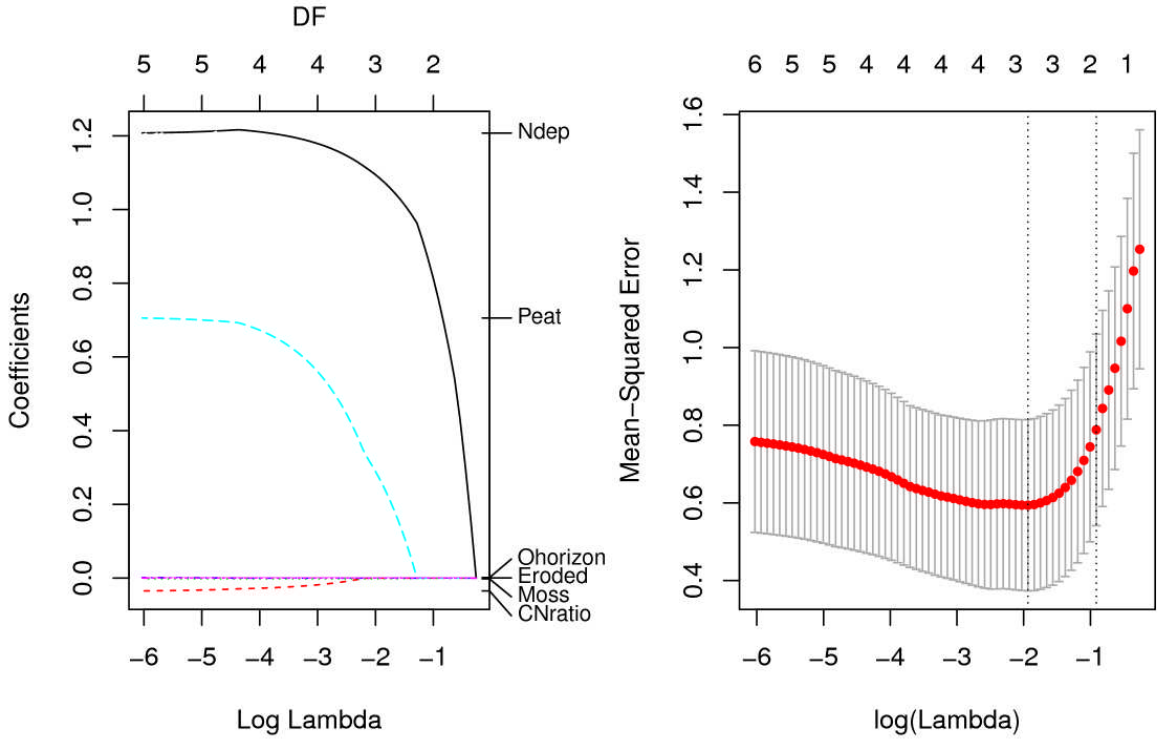
For the larger data set comprising 19 sites (Table 4.1.3), the lasso model chosen using the 1 SE rule included zero terms. The model with lowest CV mean squared error included NH_4^+ deposition as the single retained term. If we relax our parsimonious rule and take as our model the one with lowest CV error, then this suggests a weak relationship between lake NO_3^- and NH_4^+ deposition. The estimate for NH_4^+ deposition in the least squares solution is statistically significant ($F_{1,17}$ = 10.63, p = 0.005), explaining approximately 40% of the variation in the response (adjusted R^2 0.347). For the same data set, combining NH_4^+ and NO_3^- into a total N deposition variable further degraded model performance, owing to the lack of predictive power in the nitrate deposition variable weakening the relationships between lake water nitrate and ammonium in deposition.

The lasso fits for models with lake water NH_4^+ and % atmospheric nitrate indicated no significant relationships between the predictors and response in the 19 site data set.

In addition, we investigated the relationships between nitrate and ammonium in lake waters and deposition for the 19 site data set as well as the relationship between the % of atmospheric nitrate and nitrate and ammonium in lake waters and deposition. The observed data and fitted relationships are shown in Figure 2.3.4. In all comparisons except that between lake water and deposition NH_4^+ a positive correlation between response and predictor was observed. However, the relatively weak relationships coupled with the small, noisy data set is reflected in the lack of statistically significant relationships for all observations shown in Figure 2.3.4. The one exception to this is the relationship between % atmospheric nitrate in lake water and ammonium deposition (Figure 2.3.4 f), which is borderline significant at the 0.05 level ($F_{1,16}$, p = 0.065).

The proportion of eroding peat derived from GoogleEarth images for FUMBLE2 sites was not a significant predictor of NO_3^- leaching or atmospheric NO_3^-.

Figure 2.3.3: Summary of the Lasso model to predict lake NO_3^- concentration from the 16 site FUMBLE1 data set. Lasso model path showing the coefficient estimates (left panel) and CV mean squared error (right panel) as a function of the shrinkage penalty. In both panels, the full least squares solution is found to the far left. In the right hand panel, the two vertical dotted lines indicate the best model and the simplest model within 1 SE of the best model. The error bars represent the uncertainty in the CV mean squared error estimate. The numbers on the upper axis are an approximate indicator of the number of terms in the model



Summary

1. A compilation of potential catchment predictors of NO_3^- concentrations or proportion of atmospheric NO_3^- was completed to produce two largely overlapping datasets; i) the FUMBLE1 subset of 16 unafforested AWMN sites for which soil O horizon depth, C:N ratio and moss biomass were available, and ii) the FUMBLE2 dataset (Task 4.1) of 19 AWMN and other sites with isotope-based estimates of atmospheric NO_3^- inputs.
2. The best model for the FUMBLE1 sites used only N deposition (2004-06) and moss biomass to explain 66% of the variance in NO_3^- concentrations, confirming earlier results from FUMBLE1 using slightly different predictors.

No significant relationships between concentrations of N species and atmospheric NO_3^- contributions were found for the FUMBLE2 dataset of 19 sites.

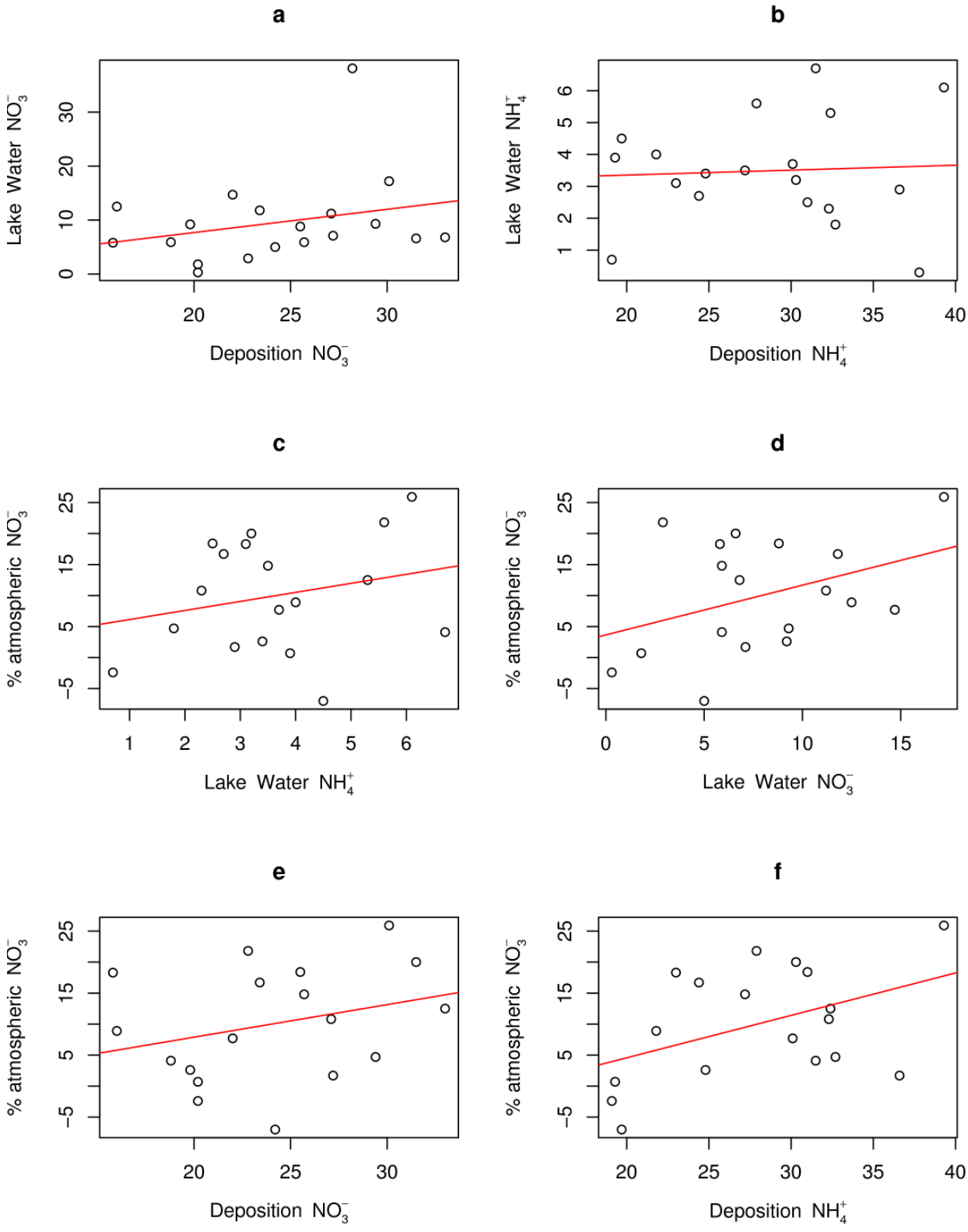
References

Hastie T, Tibshirani R, Friedman J (2011) The Elements of Statistical Learning (2nd edition). Springer, New York

Tibshirani R (1996) Regression shrinkage and selection via the lasso. J R Stat Soc Series B 58:267-288

Whittingham MJ, Stephens PA, Bradbury RB, Freckleton RP (2006) Why do we still use step-wise modelling in ecology and behaviour? J Animal Ecol 75:1182-1189

Figure 2.3.4: Observed data and fitted least squares relationship relating lake water and deposition N species and the % atmospheric nitrate with lake water and deposition N species in the 19 sites of the FUMBLE2 isotopic dataset. The red line indicates the least squares regression fit to the data shown in each panel.



Task 2.4: Assessing macrophyte $\delta^{15}N$ as an indicator of N limitation status

Background and Aims of the task

Earlier GANE work carried out by Stephen Maberly and co-workers showed a weak relationship between nitrogen limitation and the ^{15}N signature of lake sediment and macrophytes (Jones R.I., King L., Dent M.M., Maberly S.C. & Gibson C.E., 2004, Nitrogen stable isotope ratios in epilithon, macrophytes and surface sediments from upland lakes with differing nutrient status. *Freshwater Biology* **49**: 382-391). Subsequent work has shown that the relationship between macrophyte ^{15}N and nitrogen limitation is stronger if the analyses are restricted to species, such as mosses, that obtain their nutrients wholly from the water column (S.C. Maberly, King L. and Jones R.I. unpublished).

Macrophyte samples from up to 5 separate species (including mosses, liverworts, charophytes, filamentous algae) collected on one occasion in summer 2008 from 10-13 sites, where nutrient limitation has previously or is being assessed under phase 2 of the Freshwater Umbrella programme, were to be sent to CEH Lancaster where they would be prepared and analysed for natural abundance ^{15}N at the stable isotope facility there.

First sampling run

Sites for macrophyte sampling were agreed in Spring 2008 and were selected as far as possible from sites being studied elsewhere under the current Freshwater Umbrella programme (n=11; Table 2.4.1).

In addition, it was decided to sample the three stream sites selected for the nutrient diffusing substrate study in Task 2.2 (Allt a'Mharcaidh, Dargall Lane, River Etherow) since nutrient limitation data should also become available for these sites for comparison with $\delta^{15}N$ data.

Macrophyte sampling was undertaken in August 2008 at the majority of the shortlisted sites but in general only one or two of the requisite species were found at each site. Furthermore, some sites could not be sampled because of extremely high water levels (e.g. Blue Lough, Llyn Llagi) which prevented access to the littoral zone. Hence this sampling run was incomplete and a resampling during late spring 2009 was agreed with DEFRA.

During Spring 2009 a student (Rebecca Bromley) on the MSc Freshwater and Coastal Science (FACS) course run jointly by UCL and Queen Mary College, University of London opted to work on this task for her MSc Dissertation which allowed the scope of the work to be expanded beyond that originally contracted. A total of 18 lakes were

sampled for macrophytes (Table 2.4.2, Fig. 2.4.1), with 14 species being analysed for $\delta^{15}N$ (Table 2.4.3).

In addition, phytoplankton bioassays were repeated at all sites to verify previous bioassay studies carried out over several years prior to the present study. Four treatments, each with three replicates, were applied: control (no nutrients added), P (0.2 cm^3 NaH_2PO_4 stock solution added), N (0.3 cm^3 $NaNO_3$ stock solution added), and P+N (0.2 cm^3 NaH_2PO_4 and 0.3 cm^3 $NaNO_3$ stock solution added). Following incubation, chlorophyll *a* levels in each sample were analysed using a U-2800 spectrophotometer at wavelengths of 665 and 750 nm, according to the methods of Maberly et al (2002).

Table 2.4.1: Original lake sites selected for macrophyte sampling in 2008

Easting	Northing	Lake	Site Code	FU2 Sites	Bioassayed?	Country
337152	496838	Moss Eccles Tarn	-	Yes	GANE	ENG
264506	359587	Llyn Idwal	IDWA	EA Survey	GANE	WAL
264911	356269	Llyn Cwm-y-ffynon	CFYN	EA Survey	GANE	WAL
264902	348240	Llyn Llagi	LAG	Yes	GANE	WAL
244982	580392	Round Loch of Glenhead	RLGH	Yes	GANE	SCO
315900	510400	Scoat Tarn	SCOATT	Yes	FUMBLE1	ENG
318400	504300	Burnmoor Tarn	BURNMT	Yes	FUMBLE1	ENG
345500	510000	Small Water	VNY4101	Yes	FUMBLE1	ENG
194500	849800	Loch Coire Fionnaraich	VNG9402	Yes	FUMBLE1	SCO
325200	785900	Lochnagar	NAGA	Yes	FUMBLE1	SCO
J328252		Blue Lough	BLU	Yes	FUMBLE1	NI

Results

Chlorophyll *a* data were analysed using a series of one way ANOVAs and Tukey's post hoc test. The definitions of nutrient limitation status were those used by Bergström et al., (2008): N-limitation, which shows a significantly greater response in chl *a* compared to the control after N-addition, and P-limitation, which shows a significantly greater response in chl *a* compared to the control after P addition. Dual limitation by N and P was equated to a significantly higher response in chl *a* relative to controls when both N- and P-additions alone led to increased chl *a.* Co-limitation by N and P was equated with an increase in the N+P treatment relative to the control when there was no response to N added alone or to P added alone.

Table 2.4.2: Expanded list of sampling sites for macrophyte $\delta^{15}N$ study

Site No.	Site name	Lake area (ha)	Catchment area (ha)	L: C ratio	N deposition (keq ha^{-1} yr^{-1})	Grid Ref.
1	Scoat Tarn	87	1754.03	0.05	1.71	NY159103
2	Burnmoor Tarn	625.5	16374.35	0.04	1.42	NY183043
3	Gurnal Dubs	83.5	2130.1	0.04	1.61	SD503991
4	Potter Tarn	2.4	100.8	0.02	1.63	SD493989
5	Moss Eccles Tarn	3	78	0.04	1.96	SD371968
6	Lochnagar	108.5	1189.69	0.09	1.32	NO252859
7	Borralie	559.5	33704.82	0.02	0.47	NC381668
8	Fionnaraich	36.7	156.1	0.24	0.71	NG945498
9	Loch Tinker	132.5	1579.26	0.08	1.67	NN445067
10	Loch Chon	1590	23945.78	0.07	1.58	NN420053
11	Round Loch of Glenhead	102.5	828.62	0.12	1.63	NX449803
12	Loch Grannoch	1432	18406.17	0.08	1.34	NX542699
13	Llyn Llagi	157	4845.68	0.03	1.22	SH649482
14	Llyn Conwy	137	458.19	0.3	1.61	SH780461
15	Llyn Mair	215	7597.17	0.03	0.96	SH652412
16	Llyn Teyrn	22.25	259.32	0.09	1.5	SH641547
17	Llyn Idwal	335.75	8565.05	0.04	1.61	SH645595
18	Blue Lough	50.16	1200.1	0.04	1.68	J327252

Results are summarised in Table 2.4.4 and Figure 2.4.2. Five of the 18 lakes sampled showed N limitation, 10 showed co-limitation and the remaining three lakes (Blue Lough, the Round Loch of Glenhead and Llyn Teyrn) showed no significant nutrient limitation.

Figure 2.4.1: Map of the UK showing sites visited in red

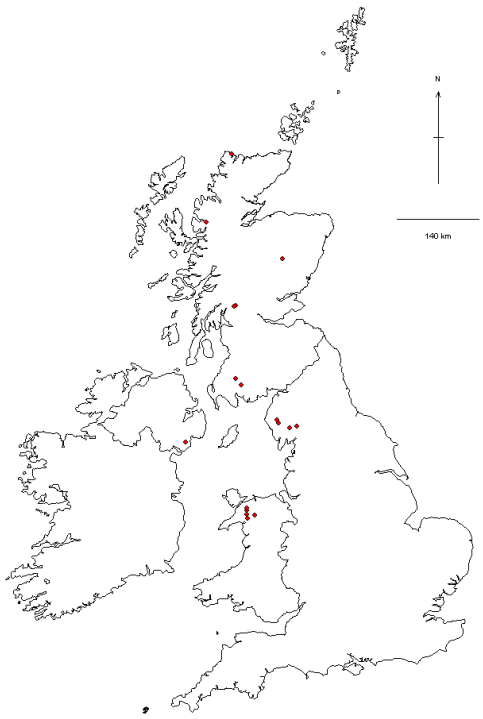


Table 2.4.3: Summary of species found at each site visited, and their $\delta^{15}N$ values (‰). Numbers in parentheses (x) indicate site and correspond to the site numbers in Table 2.3.

Species	$\delta^{15}N$ value (‰), site
Andreaea rothii	0.07 (8),
Batrachospermum spp.	0.05 (8), 2.69 (9), 9.82 (14), 4.48 (15), '-0.56 (16),
Cinclodotus fontinaloides	6.66 (7)
Cratoneuron filicinum	5.62 (7)
Eucladium verticillatum	5.45 (7)
Filamentous algae	-0.09 (3), '-1.57 (4), 1.71 (6), 0.12 (13), 0.95 (13), '-5.28 (17)
Fontinalis antipyretica	-1.02 (3), 1.06 (8)
Hyocomium armoricum	-3.04 (16)
Marsupella emarginata	-3.22 (1), '-1.2 (2), 0.89 (9)
Nardia compressa	-1.29 (1), '-0.68 (2), '-2.73 (3), '-2.31 (11), 0.28 (12), -3.36 (18)
Nitella flexilis agg.	0.23 (10)
Racometrium aciculare	6.07 (7), 5.47 (7), 6.54 (7)
Scapania undulata	-2.52 (1), '-2.57 (6), '-1.87 (6)-0.6 (14)
Sphagnum palustre	-1.65 (1), -0.01 (5), -0.41 (8), -0.18 (9)

Figure 2.4.2: Response of phytoplankton production (measured as chl-a) to nutrient additions

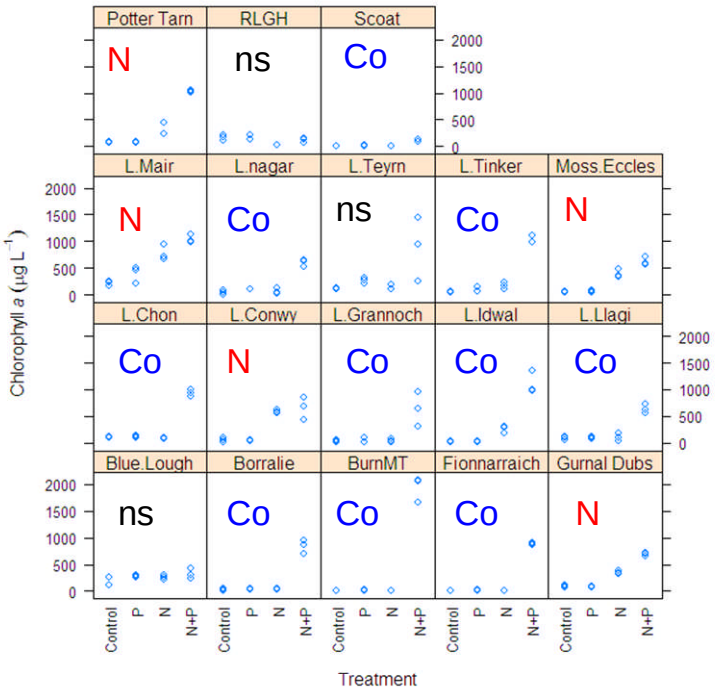


Table 2.4.4: Results of water chemistry colorimetric analysis and ANOVAs on bioassay data, summarised by site.

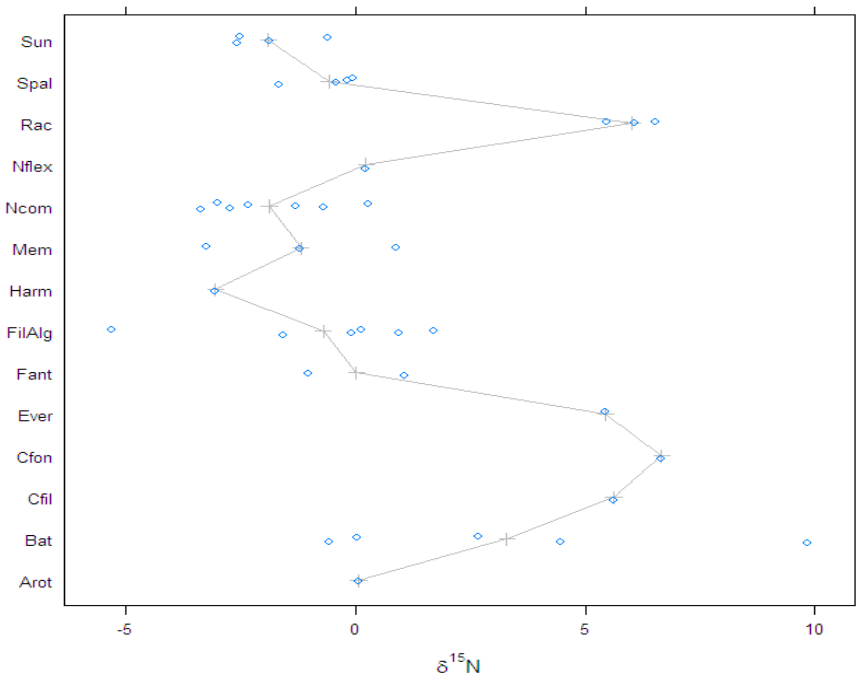
Site	Lim stat	p	NO_2 mg l^{-1}	NO_3 mg l^{-1}	NH_4 mg l^{-1}	TIN mg l^{-1}	TP mg l^{-1}
Scoat Tarn	co	< 0.001	0	0.38	0.15	0.54	0.007
Burnmoor Tarn	co	< 0.001	0	0.17	0.08	0.25	-0.003
Gurnal Dubs	N	< 0.001	0.03	0	0.28	0.3	-0.002
Potter Tarn	N	< 0.001	0.01	-0.01	0.16	0.15	-0.003
Moss Eccles Tarn	N	< 0.001	0.01	0.02	0.14	0.17	-0.004
Lochnagar	co	< 0.001	0	0.28	0.07	0.36	-0.002
Borralie	co	< 0.001	0	0	0.07	0.07	0.000
Fionnaraich	co	< 0.001	0	0.01	0.02	0.04	-0.002
Loch Tinker	co	<0.05	0.01	0.02	0.12	0.14	-0.002
Loch Chon	co	<0.001	0.01	0.19	0.1	0.3	0.000
Round Loch of Glenhead	-	-	0.01	0.24	0.1	0.34	0.000
Loch Grannoch	co	<0.005	0.01	0.29	0.04	0.33	0.002
Llyn Llagi	co	<0.001	0.01	0.13	0.05	0.19	0.002
Llyn Conwy	N	<0.005	0.01	0.13	0.04	0.19	0.000
Llyn Mair	N	<0.001	0.01	0.3	0.14	0.44	0.002
Llyn Teyrn	-	-	0.01	0.08	0.11	0.19	-0.002
Llyn Idwal	co	<0.001	0.01	0.09	0.18	0.28	-0.004
Blue Lough	-	-	0.01	0.52	0.06	0.59	-0.004

None of the lakes sampled showed P limitation. At some sites, such as Scoat Tarn, there was very little variation between the effects of the separate treatments (Fig. 2.4.2).

In order to see if there was any relationship between nutrient limitation status and inorganic N species (NO_3^-, NH_3, TIN) or N deposition, a series of Generalised Linear Models (GLMs) were fitted to binary logistic regressions. No significant relationships were found.

The $\delta^{15}N$ values of the macrophyte samples were very variable. The range of δ^{15} N values from all samples across all sites varied between -5.28‰ (filamentous algae in Llyn Idwal) and 9.83‰ (*Bactrachospermum* spp. in Llyn Conwy). The overall mean $\delta^{15}N$ value of all samples from all sites was 0.474‰. The greatest range of δ^{15} N values within a species was in the *Batrachospermum* spp, 10.38‰ (-0.56 – 9.83). Bryophytes tended to have smaller ranges (Fig. 2.4.3), the liverwort *Nardia compressa* had a range of 3.64‰, and the *Sphagnum palustre* samples had a range of 1.6‰.

Figure 2.4.3: Dotplot showing δ^{15} N values (‰) arranged by species. (+) symbol indicates mean. An explanation of the codes used for the species can be seen in Table 2.6.

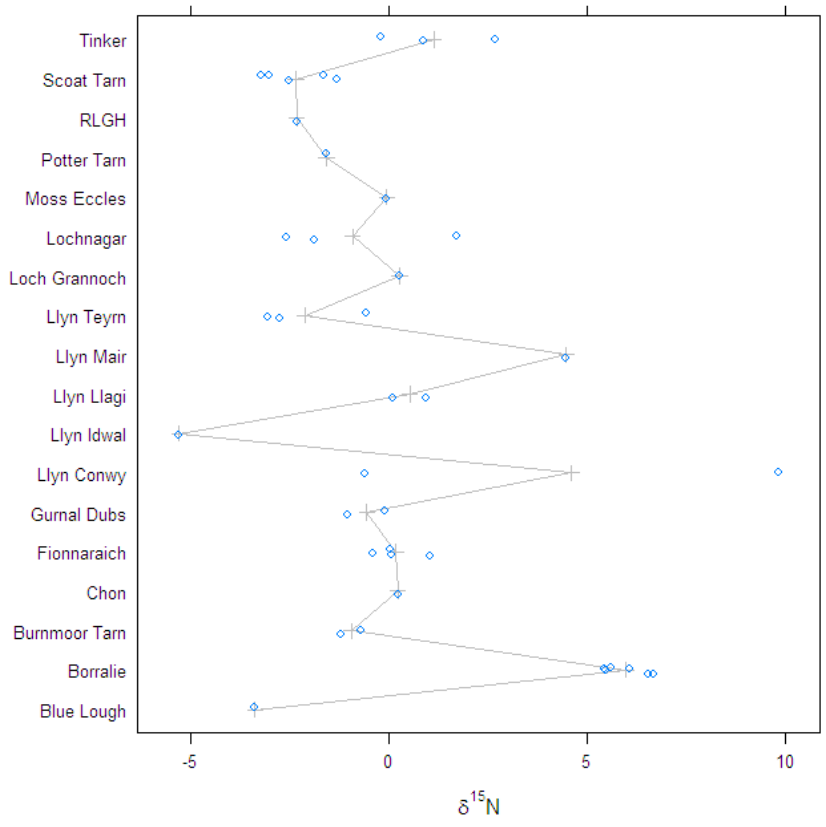


Samples analysed from Borralie had δ^{15} N values which were on average higher than in other lakes (Fig. 2.4.4). The samples from Borralie also had the smallest range (5.45 – 6.66‰). Generally the same species were seen at many of the sites, with the exception of *Hyocomium armoricum* which was found only at Llyn Teyrn and *Andreaea rothii* which was found only at Fionnaraich. Furthermore, Loch Borralie contained many species which were found only at that site. This was expected because Borralie is a marl lake whereas the other sites are more acidic. Some sites were very variable e.g. Llyn Conwy.

Table 2.4.5: Species names corresponding to codes in Figure 2.4.

Code	Species
Mem	*Marsupella emarginata*
Ncom	*Nardia compressa*
Spal	*Sphagnum palustre*
Sun	*Scapania undulata*
FilAlg	Filamentous algae
Rac	*Racometrium aciculare*
Ever	*Eucladium verticillatum*
Cfil	*Cratoneuron filicinum*
Cfon	*Cinclodotus fontinaloides*
Arot	*Andreaea rothii*
Bat	*Batrachospermum* spp.
Nflex	*Nitella flexilis* agg.
Harm	*Hyocomium armoricum*
Fant	*Fontinalis antipyretica*

Figure 2.4.4: Dotplot showing δ^{15}N values (‰) of samples from each site. '+' symbol shows mean δ^{15}N value at each site.



There is a weak negative correlation between N deposition and average δ^{15} N value at the sites visited (Pearson correlation = -0.498, p = 0.036).
The general lack of common species present in a majority of lakes and the bioassay results showing no P limited sites on this occasion prevent the detailed assessment of macrophyte δ^{15}N as an indicator of nutrient limitation status.

Summary

The purpose of this work was to test whether the ^{15}N ratio of attached (rootless) aquatic plants, such as bryophytes or filamentous algae, could be used to diagnose nitrogen limitation as has recently been suggested (King *et al.*, 2009). Work in summer 2009 collected material from 18 sites in Scotland, the Lake District, Wales and Northern Ireland. Water samples were collected for water chemistry and to perform phytoplankton nutrient bioassays, while non-rooted macrophytes, attached filamentous algae and bryophytes were collected for stable isotope analysis. The nutrient bioassays confirmed the widespread nature of nitrogen-limitation or co-limitation, rather than phosphorus-limitation as found before in the UK (Maberly et al. 2002). The stable isotope analyses also confirmed the large amount of ^{15}N variability within aquatic plants, both for a given species in different lakes and the community of plants within a given lake. The sample size was probably too small to establish robust correlations between water chemistry or nitrogen deposition on one hand and ^{15}N values of macrophytes or phytoplankton nutrient-limitation on the other. However, there were indications of some interesting results: the leafy-liverwort *Nardia compressa* showed a strong negative relationship between its ^{15}N value and the ^{15}N value of nitrogen deposition measured elsewhere in the Fumble programme. The spatial variation in ^{15}N in deposition appears to reflect the amount or type of nitrogen being deposited: it was high in Wales and the Lake District with high levels of N-deposition and lower in Scotland with generally lower levels of N-deposition and agrees with a previous publication (Jones *et al.*, 2004). The results suggest that if the effects of confounding factors, such as the source value of nitrate and ammonium, can be minimised, the ^{15}N values of bryophytes may be useable as a means of assessing nutrient limitation without the costly and artificial use of bioassays, but more research is needed to firmly establish the method.

References

Bromley, R. (2009) *Assessing the relationships between water chemistry, nitrogen deposition, macrophyte δ^{15} N and nutrient limitation status in upland lakes.* Unpublished MSc Thesis, University of London, August 2009.

King, Lydia; Maberly, Stephen C.; De Ville, Mitzi M.; Kitschke, Michaela; Gibson, Christopher E.; Jones, Roger I.. 2009 Nitrogen stable isotope ratios of lake macrophytes in relation to growth form and nutrient-limitation. *Fundamental & Applied Limnology*, 175 (4). 307-315. 10.1127/1863-9135/2009/0175-0307

Task 2.5 Seasonal variations in nutrient status at three sites

Aims

The primary aim of this task is to ascertain the relationships between nutrient water chemistry and algal (phytoplankton) production, given that N limitation and associated nutrient impacts have already been demonstrated by bioassay studies. An understanding of the potential changes in lake ecosystem structure and function caused by N deposition requires knowledge of limnological processes and seasonal variations in nutrient cycles and primary production. Since such studies are labour intensive, requiring monthly lake profiling by boat and water sampling, three study sites were selected from a shortlist with existing bioassay, water quality and sediment ^{15}N data. Monthly lake profiles were taken over a period of one year. In addition to measurement of water chemistry (nutrients), physical parameters and chrorophyll-a (chl-a), vertical lake profiles at 1m intervals were complemented by analysis of phytoplankton species composition on an integrated vertical haul with each profile.

The second major aim of this task was to allow a comparison of lakewater samples taken from the centre of the lake (and at various depths) with those taken from the lake outflow for nutrient monitoring type work. It has been standard procedure to take lake outflow samples for major ion chemistry where possible under the Defra funded critical loads work over the last 20 years, but nutrient related studies often sample from the lake centre or use throw bottles to obtain samples away from the shoreline.

For this task it was decided to focus the study on three sites in the Lake District with contrasting water chemistry and nutrient limitation characteristics; Scoat Tarn, Burnmoor Tarn and Small Water. Scoat Tarn is an acidified, P-limited site with very high NO_3^- concentrations and may have originally been N-limited. Burnmoor Tarn is mainly co-limited by N and P but occasionally N limited, and despite its proximity to Scoat Tarn has rather low NO_3^- levels and shows negligible acidification due to less acid-sensitive soils and geology. Small Water has high NO_3^- levels like Scoat Tarn, but is non-sensitive to acidification and shows co-limitation by N and P.

Sampling under this task commenced with field trials in June 2010 when temperature and dissolved oxygen profiles were measured at Scoat Tarn and Small Water, with sampling at six depth intervals for water chemistry.

From July 2010, a new multiparameter probe was used to measure conductivity and pH as well as DO and temperature. Limnological profiling was carried out first in the field to determine whether any thermal stratification was present in the lake, i.e. gradients in temperature and DO within the water column. Based on this assessment, a van Dorn type sampler was then used to obtain 2L water samples from known depths according to the following regime:

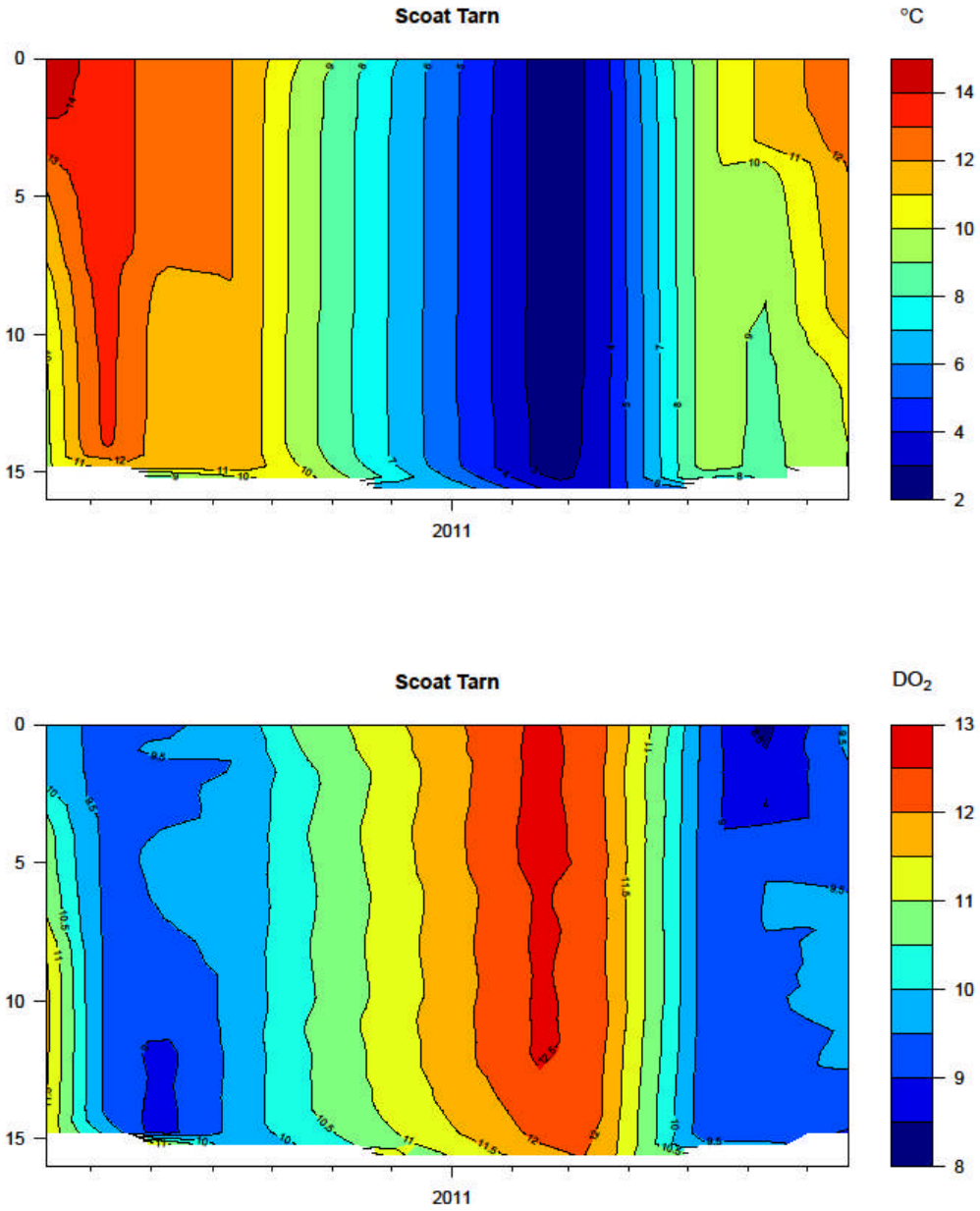
1. Stratified lakes; 5-6 depth intervals including surface, top and bottom of thermocline and 0.5m from bottom;
2. Well-mixed lakes; 3 depth intervals - surface, middle and 0.5m from bottom of water column

These samples were analysed for major ions, pH, alkalinity, DOC, chlorophyll-a, total nitrogen and total phosphorus.

Results

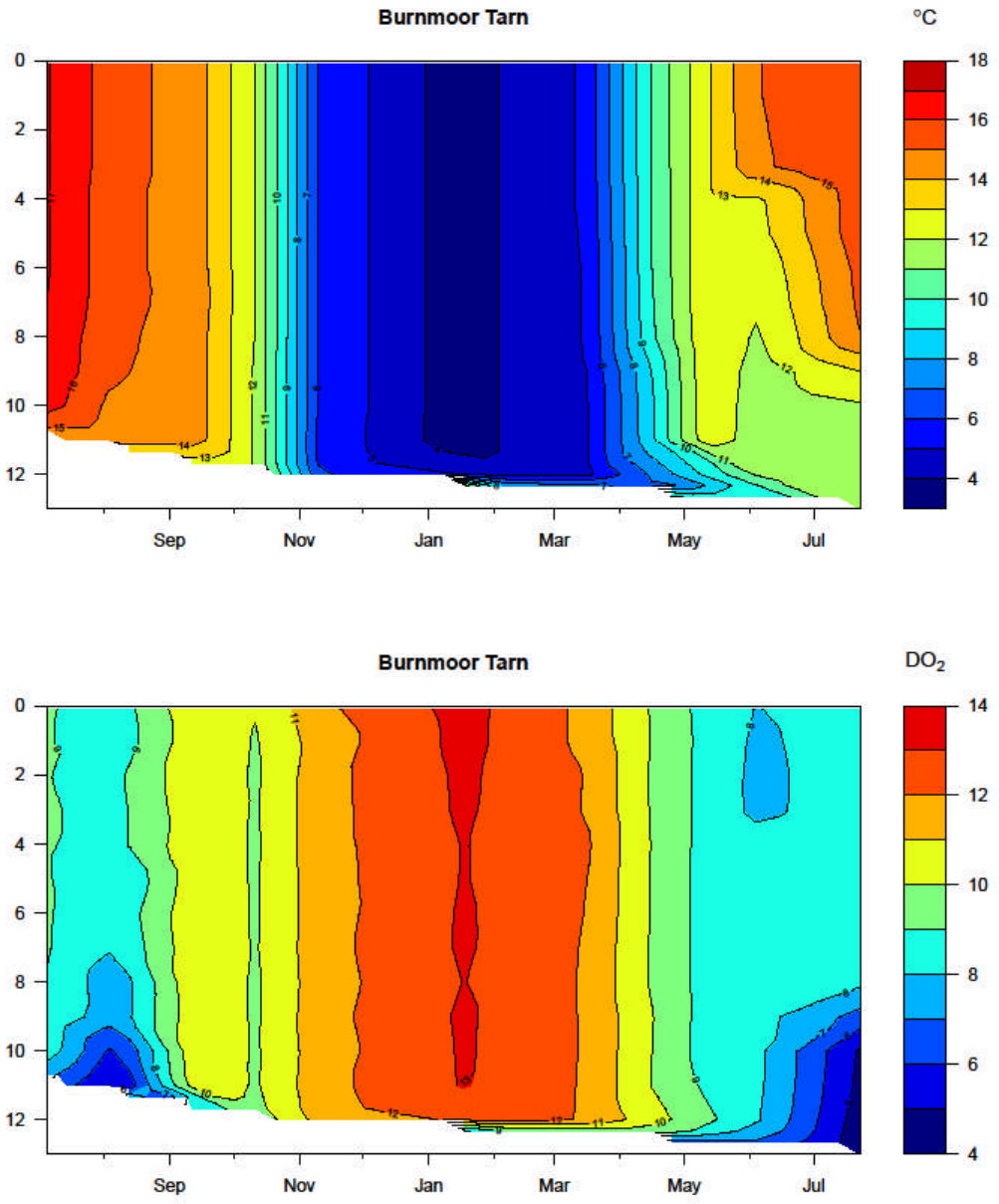
Lake profile depth-time plots for temperature and dissolved oxygen in Figs 2.5.1-2.5.3 show that all three lakes are well-mixed for most of the year, with weak thermoclines developing for a few weeks during the summer.

Figure 2.5.1: Temperature, dissolved oxygen (DO: mg l^{-1}) and pH profiles at Scoat Tarn



The temperature depth-time diagram for Scoat Tarn (Fig. 2.5.1) shows that the lake is well-mixed for most of the year but weakly stratifies for a few weeks during the summer, with a thermocline of 4-5°C. The lake is well oxygenated throughout the year.

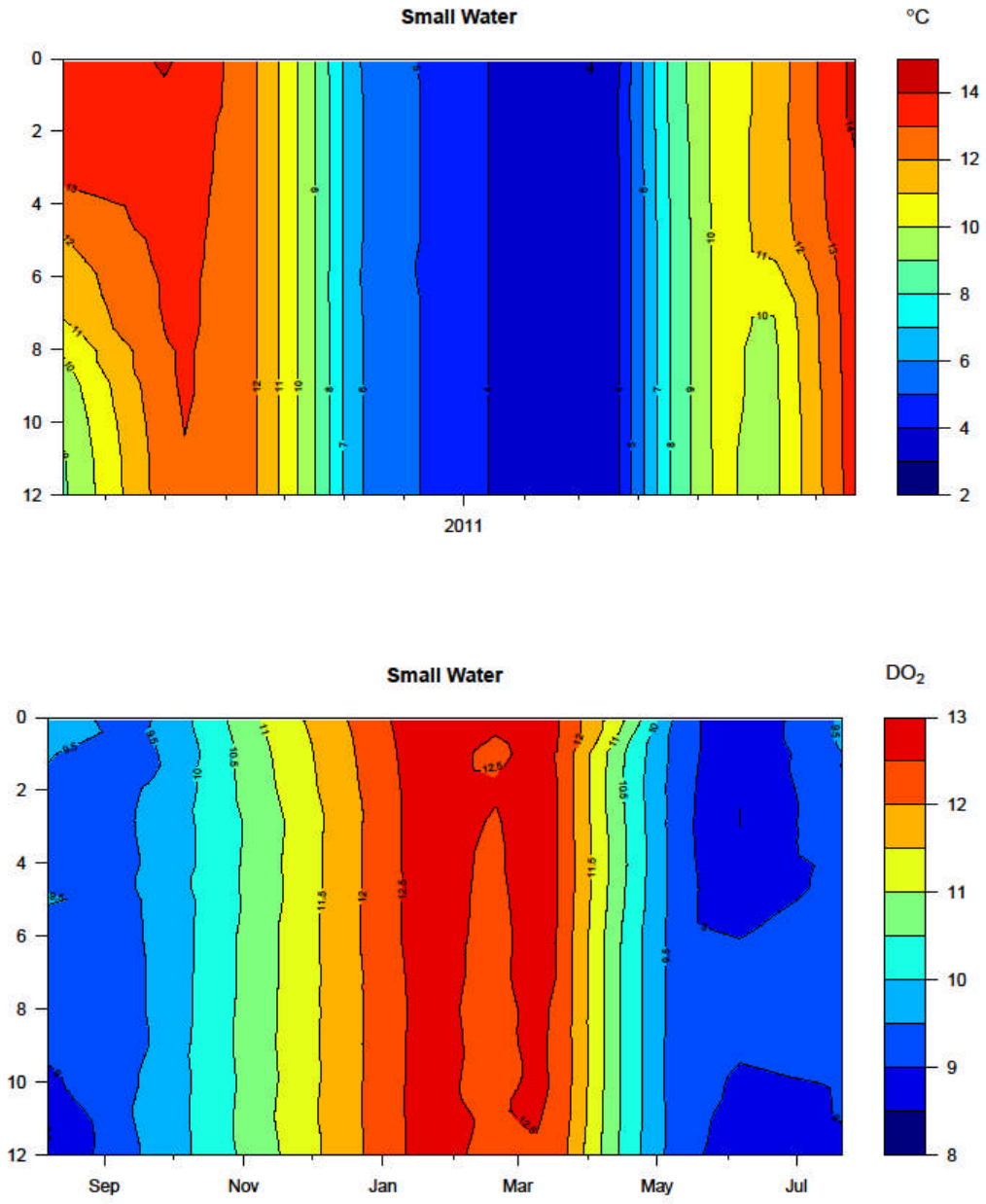
Figure 2.5.2: Temperature, dissolved oxygen (DO: mg l^{-1}) and pH profiles at Burnmoor Tarn



Burnmoor tarn was well mixed through most of the monitoring period (Fig. 2.5.2) but showed a moderate thermocline of 4°C at around 8-10m depth by late summer 2011. At this time, DO levels were rather low in the hypolimnion but the lake was well oxygenated for most of the year.

Like Scoat Tarn, Small Water is well-mixed for most of the year (Fig. 2.5.3) but weakly stratifies for a few weeks during the summer, with a thermocline of around 4°C extending from 4m depth to the lake bottom at the start of monitoring in June 2010. The lake is well oxygenated throughout the year.

Figure 2.5.3: Temperature, dissolved oxygen (DO: mg l^{-1}) and pH profiles at Small Water



Concentrations of NO_3^- and chl-a in surface and bottom samples from the middle of the lakes, as well as outflows from all three lakes, are presented in Figs. 2.5.4a-2.5.6a. For comparative purposes these are plotted above phytoplankton compositions diagrams and biovolumes. For clarity of presentation, only phytoplankton species or groups making up more than 5% of total biovolume are presented for any one month; in most cases these groups make up more than 90% of total measured biovolume. Biovolume is used here to describe seasonal variations in phytoplankton production rather than concentrations (count data) because of the huge range of sizes of individual cells or colonies which span several orders of magnitude.

Fig. 2.5.4a: NO_3^- concentrations and chlorophyll-a in Scoat Tarn

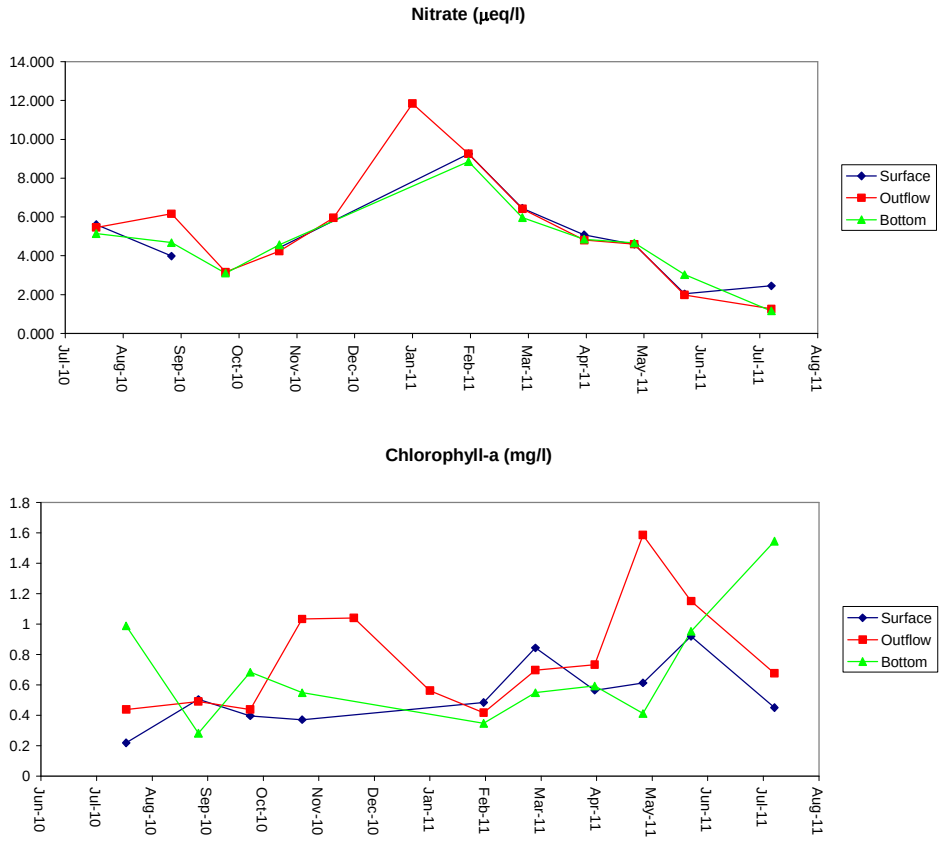


Fig. 2.5.4b: Phytoplankton in Scoat Tarn

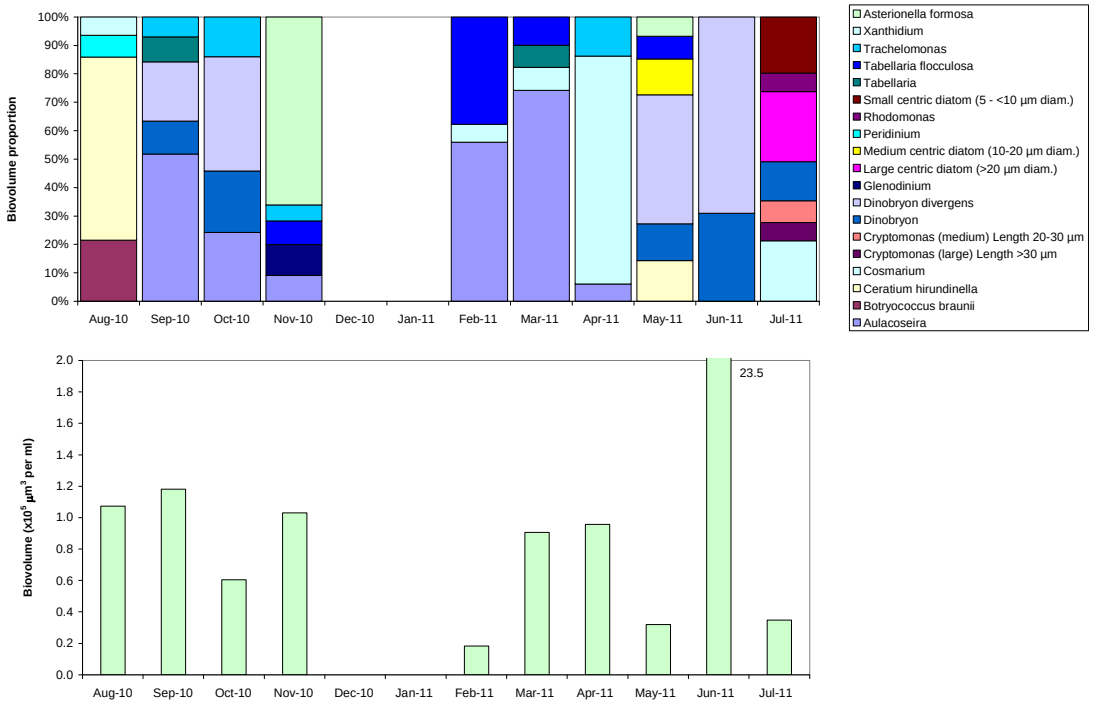


Fig. 2.5.5a: NO_3^- concentrations and chlorophyll-a in Burnmoor Tarn

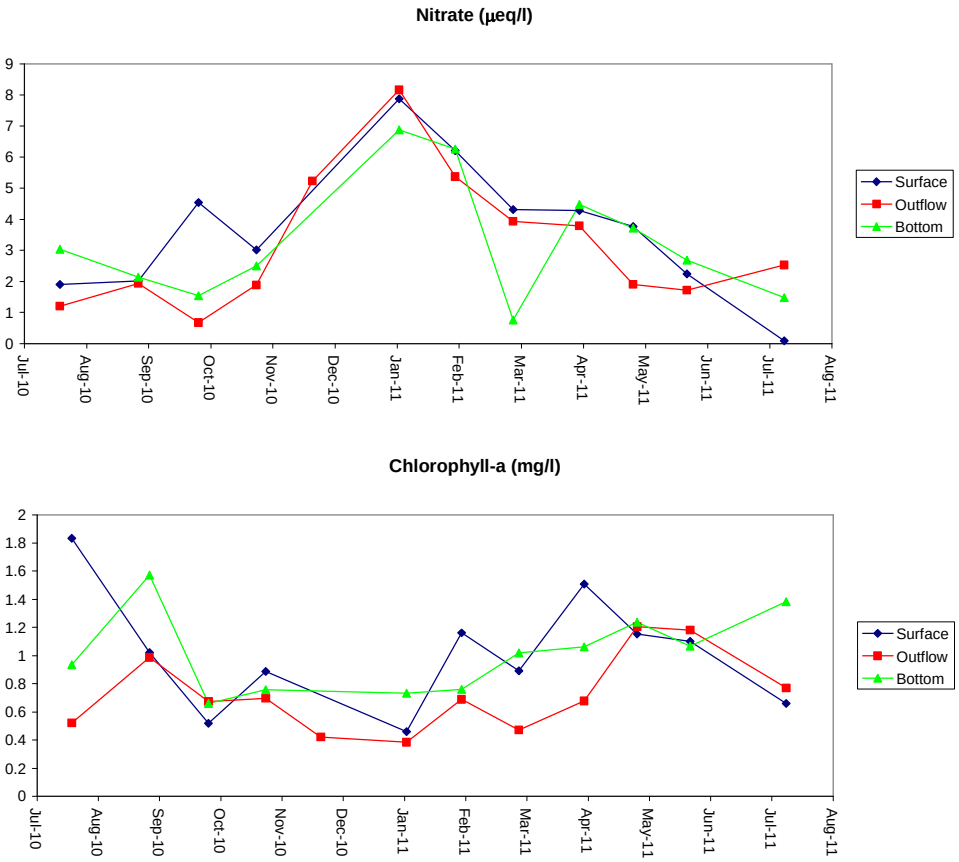


Fig. 2.5.5b: Phytoplankton in Burnmoor Tarn

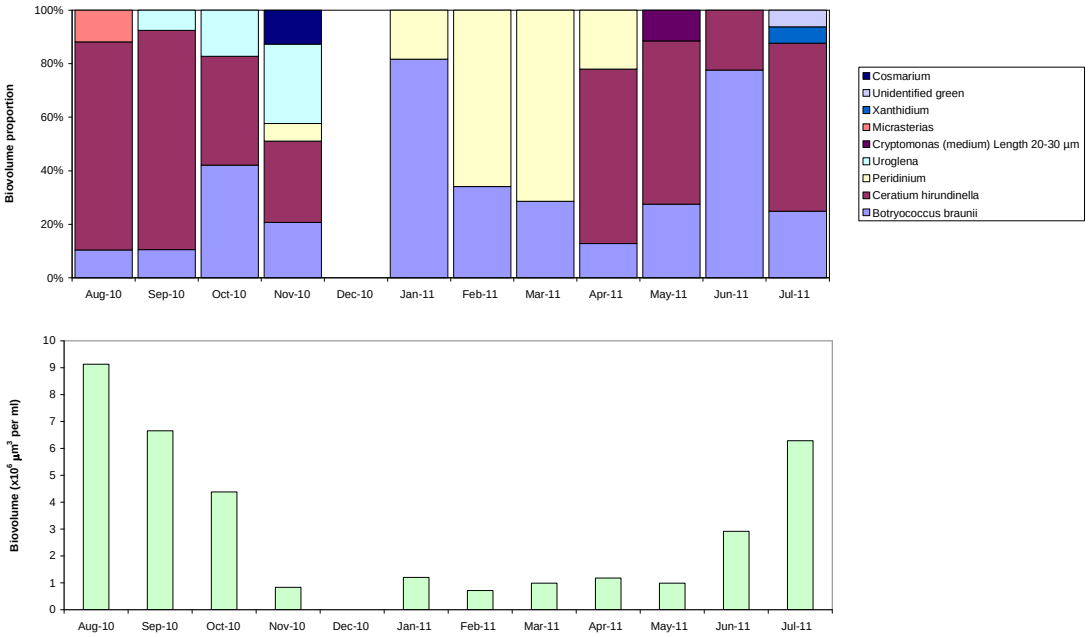


Fig. 2.5.6a: NO_3^- concentrations and chlorophyll-a in Small Water

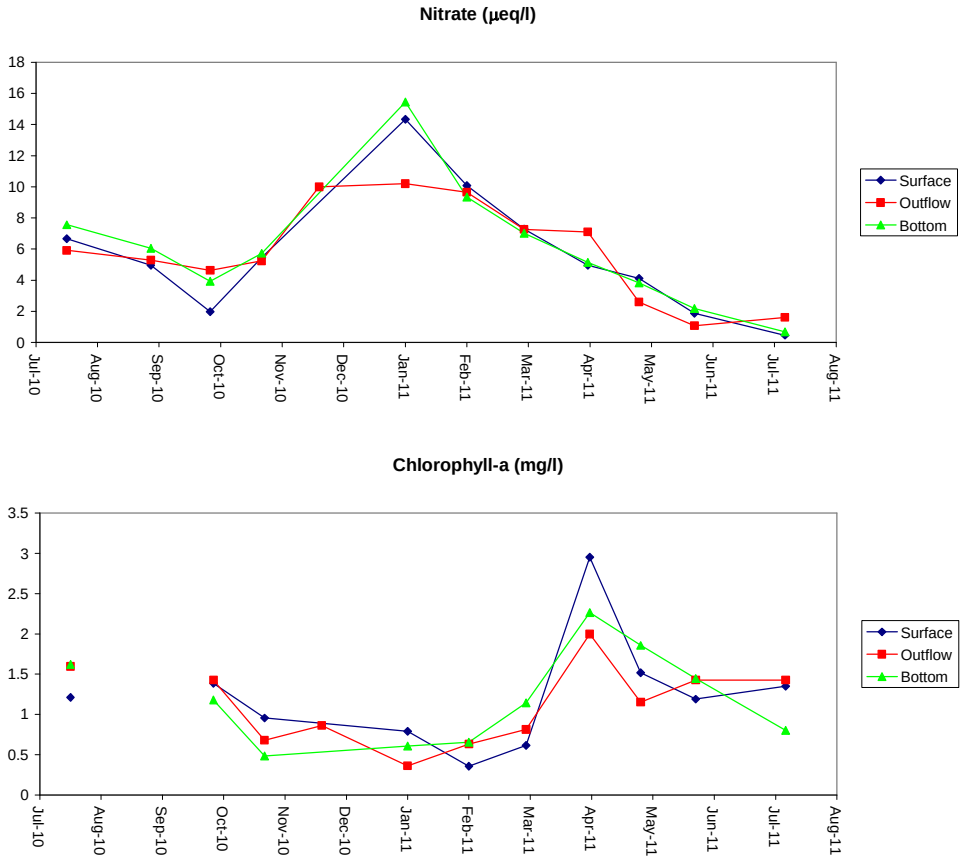
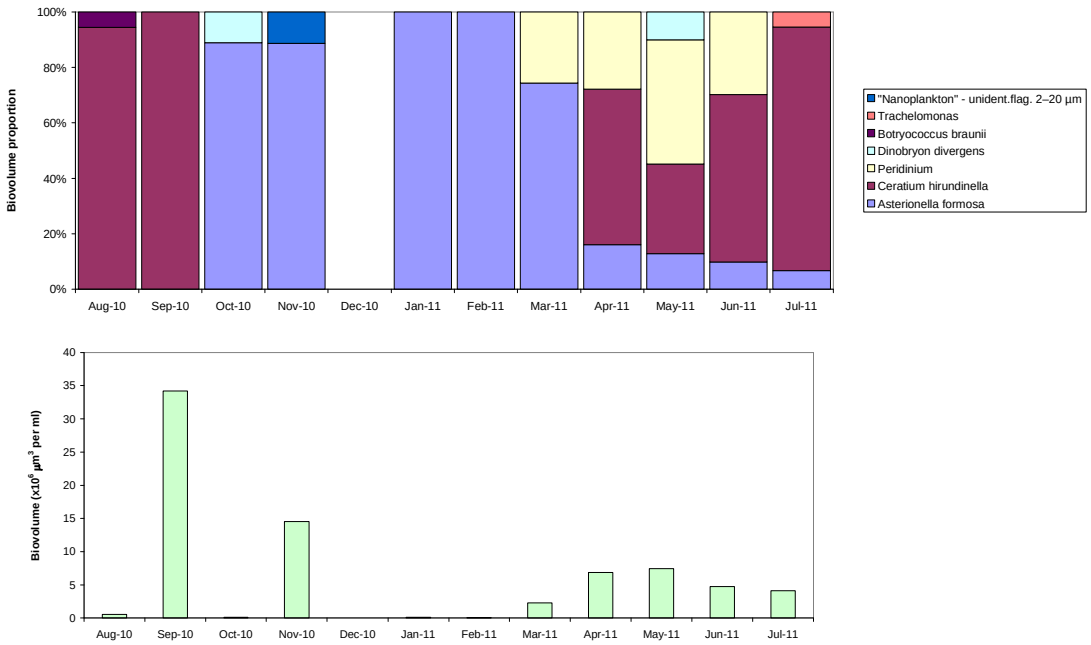


Fig. 2.5.6b: Phytoplankton in Small Water



Scoat Tarn

The NO_3^- data from Scoat Tarn show a strong seasonal pattern with a maximum concentration in the outflow in January but slightly later in the middle of the lake. Concentrations at the surface and bottom of the lake profile are almost identical throughout the monitoring period showing how well mixed the lake is. With the exception of the January sample, the concentrations in the lake outflow are also remarkably similar to those in the centre of the lake, supporting the use of outflow samples to represent the water chemistry of the whole lake body at this site.

Chlorophyll-a concentrations are low in this site, <1mg l^{-1} for most of the year and peaking in Nov-Dec and May in the outflow, but in July for the bottom water sample. The lake surface chlorophyll-a data show a double peak in March and June but values are all <1 mg l^{-1} with a mean value of 0.5 mg l^{-1}. There is no strong seasonal signal in the chlorophyll-a data, although the double peaks in the outflow fall either side of the NO_3^- maximum in January, with a mean value of 0.8 mg l^{-1}, substantially greater than the lake centre.

The phytoplankton count data from the centre of Scoat Tarn show a complex seasonal pattern in the dominant species. Data are missing for December and January when the lake was frozen, but the major patterns show that diatoms dominate in terms of biovolume from late autumn through the winter; *Aulacoseira* species peak in September and then decline before peaking again in February (when *Tabellaria flocculosa* is also important) and especially March. *Asterionella formosa* blooms to form 61% of the biovolume in November but there are no data for December-January. By April the diatoms have crashed to be largely replaced by the desmid *Cosmarium*, which blooms in April, and then chrysophytes (*Dinobryon* species) take over to become completely dominant by June, when phytoplankton biovolume increases by a factor of 20 relative to the next highest month. In July no single group dominates the flora but by August (according to the 2010 sample) the dinoflagellate *Ceratium hirundinella* is dominant. Biovolume does not show a clear seasonal pattern, but the lowest value is recorded in February (not known for Dec-Jan) and is also low in May prior to the major chrysophyte bloom in June. The lowest recorded biovolume in February when diatoms are most dominant coincides with the peak concentrations of NO_3^- in the centre of the lake, while the lowest NO_3^- occurs in June and July following the major bloom in chrysophytes.

Burnmoor Tarn

Concentrations of NO_3^- in Burnmoor Tarn are somewhat lower than at Scoat Tarn but show a similar seasonal pattern, with all samples peaking in January and showing minima in the summer. There is good agreement in NO_3^- between outflow and lake centre samples for most of the year, with a small divergence in October between lake surface and outflow. Chlorophyll-a shows a seasonal pattern with maximum values in August and April at the lake surface and two smaller, slightly later peaks at the lake bottom. The seasonal pattern is less marked in the outflow but chlorophyll-a does

show two peaks occurring slightly later than those at the surface in the lake centre. Mean values are 1.0 mg l^{-1} at the centre of the lake and 0.7 mg l^{-1} in the outflow.

The phytoplankton community in Burnmoor Tarn is dominated by fewer groups than Scoat Tarn and shows a rather simpler seasonal pattern. Samples could not be taken in December 2010 due to ice cover. The phytoplankton is dominated by three groups, the dinoflagellates *Ceratium hirundinella* and *Peridinium* species, and the chlorophyte *Botryococcus braunii*. From April to September, *Ceratium hirundinella* dominates, except for a June peak in *Botryococcus braunii*. The latter species shows a varying seasonal pattern, with major peaks in both June and January when it is very dominant in terms of biovolume, and a smaller third peak in October when it is co-dominant with *Ceratium hirundinella*. By November the chrysophyte *Uroglena* has overtaken *Botryococcus braunii* and the desmid *Cosmarium* also contributes significantly to the biovolume. By February, *Ceratium hirundinella* has almost disappeared to be replaced by another dinoflagellate, *Peridinium*, which dominates through March until *Ceratium hirundinella* takes over again in April.

Total phytoplankton biovolume is much greater at Burnmoor Tarn than at Scoat Tarn (note the tenfold difference in scale in Figs. 2.5.4b and 2.5.5b) and shows a very strong seasonal pattern from a peak in August, when *Ceratium hirundinella* is dominant, to a minimum in February when *Peridinium* dominates. The August peak in biovolume coincides with the peak in chlorophyll-a at the surface and low NO_3^- concentrations, but the April peak in chl-a occurs when total biovolume is low. When NO_3^- peaks during the winter months, biovolume and chl-a are lowest.

Small Water

Small Water shows the same marked seasonality in NO_3^- concentrations as the other two sites, with a January peak and a minimum in summer, but concentrations are slightly higher than Scoat Tarn and around double those in Burnmoor Tarn. Again, concentrations are very similar in all spatial samples for any given month, but the January peak is somewhat lower in the outflow compared with the surface and bottoms samples from the middle of the lake. Chlorophyll-a shows a very strong seasonal pattern with a short-lived, sharp increase in April from low values throughout the winter period, followed by a decline to more moderate levels throughout the summer. The September chl-a sample was lost. Annual mean values are very similar in both surface and bottoms samples from the middle of the lake (1.2 mg l^{-1}) and the lake outflow (1.1 mg l^{-1}) which are slightly higher than Burnmoor Tarn and 50-140% higher than the Scoat Tarn outflow and surface, respectively.

This site shows the most extreme variations in phytoplankton biovolume with few species dominating. No sampling was possible in December because the lake was frozen. In August and especially September (the latter when by far the greatest monthly biovolume occurs) the biovolume is heavily dominated by the dinoflagellate *Ceratium hirundinella* which then disappears in October to be replaced by the diatom

Asterionella formosa, which dominates the biovolume throughout the winter months. The initial *Asterionella* bloom in October represents a very small biovolume but by November it constitutes a short-lived but large second annual peak in biovolume. By March the dinoflagellate *Peridinium* starts to become important, but in April *Ceratium hirundinella* again dominates the biovolume. *Peridinium* is briefly the slightly dominant component in May but in June *Ceratium hirundinella* expands to become totally dominant throughout the summer. The NO_3^- peak and chl-a minimum through the winter months coincide with the period in which *Asterionella* dominates, but the diatom biovolume increase by two orders of magnitude during November is not reflected in a decrease in NO_3^-, which instead increases slightly through the autumn towards its winter peak.

Nutrients and phytoplankton

In this summary, only NO_3^- was compared with chl-a and phytoplankton data due to analytical problems with both TP (detection limit problem) and TN (equipment failure) which has meant that these data were not available at the time of reporting. However, it is intended that future analysis will include a consideration of TN and TP as well as NO_3^-.

All three sites showed mid-winter peaks in NO_3^- which correspond with a period of low productivity (as reflected by biovolume) and generally low chlorophyll-a. Diatoms dominate through the winter period at Scoat Tarn and especially at Small Water, while the chlorophyte *Botryococcus braunii* dominates at Burnmoor Tarn. During the summer, when NO_3^- concentrations are low, chrysophytes and then dinoflagellates are dominant at Scoat Tarn, while the dinoflagellate *Ceratium hirundinella* is the most important summertime species in terms of biovolume at both Burnmoor Tarn and Small Water, as well as at Scoat Tarn in August.

There is little evidence from the present study that plankton blooms are significant drivers of seasonal patterns in NO_3^- concentration, because sudden major blooms resulting in biovolume increases of more than an order of magnitude do not appear to depress NO_3^- concentrations from a rather uniform seasonal pattern. It seems likely that seasonality in NO_3^- concentration is largely driven by supply through streamwater inflows and direct deposition to lake surfaces, rather than a bottom-up phytoplankton control. Due to the lack of other nutrient data at present it is not possible to draw further conclusions about seasonal patterns of nutrient availability, nutrient limitation and overall phytoplankton production. It is clear though that N limitation of phytoplankton production is most likely in summer when concentrations in the lakes are lowest, while still above detection limits.

Lake centre/outflow comparison

At Scoat Tarn the NO_3^- concentrations are very similar in the lake centre and outflow (Fig. 2.5.4a) but there are outflow peaks of chlorophyll-a in November-December and especially May which are not recorded in the lake centre. The outflow at Scoat Tarn is

small and heavily dominated by macrophytes and green algae and it is likely that samples taken at this location could be contaminated with non-planktonic chlorophyll-a. For this site, therefore, outflow sampling for phytoplankton may not be appropriate although major ion chemistry should be representative of the lake.

Burnmoor Tarn also shows similar NO_3^- concentrations in the outflow and lake centre, but differs from Scoat Tarn in showing the reverse pattern in chlorophyll-a. At Burnmoor Tarn the higher mean value of chlorophyll-a occurs in the lake centre and is double the value found in the centre of Scoat Tarn. The lower chlorophyll-a found in the outflow stream could be due to the presence of a major inflow stream very close to the outflow, which probably contributes a substantial part of the flow in the outflow with minor interaction with lakewater. While the inflow may have similar major ion chemistry to the main body of lakewater, it should have much lower chlorophyll-a due to the lack of major planktonic communities in streams. Furthermore, the outflow from Burnmoor Tarn is much larger and less dominated by macrophytes and algae than at Scoat Tarn. Like Scoat Tarn, but for different reasons, the outflow from Burnmoor Tarn is therefore not well suited to monitoring phytoplankton chl-a in the lake itself.

Small Water shows the best correspondence between outflow and lake centre chlorophyll-a data, although the January peak in NO_3^- is attenuated in the outflow. This site has the outflow which best reflects the whole lake because the inflow streams are situated on the opposite side to the outflow, allowing thorough mixing in the water body, while there are very few macrophytes or green algae in the rocky outflow to contaminate phytoplankton samples. This is therefore the only site where the outflow sample for phytoplankton is likely to give a highly comparable value to a sample from the lake centre.

Summary

Strong seasonal patterns are observed at all sites in NO_3^- concentrations which peak in mid- to late winter and tend to be inversely correlated to chlorophyll-a concentrations.

There are very marked seasonal variations in the dominant phytoplankton groups at each site, with major differences between summer and winter and also between sites.

Although summer minima in NO_3^- concentrations coincide with high chl-a concentrations, phytoplankton blooms in terms of biovolume are not closely related to either chl-a or low NO_3^- concentrations and occur in spring and/or autumn at Scoat Tarn and Small Water. Concentrations of NO_3^- are driven by seasonal patterns of supply rather than bottom-up primary production controls.

While most samples show a close correspondence between outflow and lake centre samples for NO_3^-, the relationship between outflow and lake centre chl-a appears to be dictated by the relative locations of inflows and outflows and the presence of other sources of chl-a from macrophytes and filamentous green algae in the outflows. Hence the use of throw-bottles or boats is recommended for the monitoring of chl-a as an indicator of phytoplankton production, rather than outflow sampling.

Work package 3: Nitrogen as a nutrient – historical reconstruction of deposition and effects using palaeolimnological methods

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Task 3.1: Seasonal variation in drivers of sediment $\delta^{15}N$

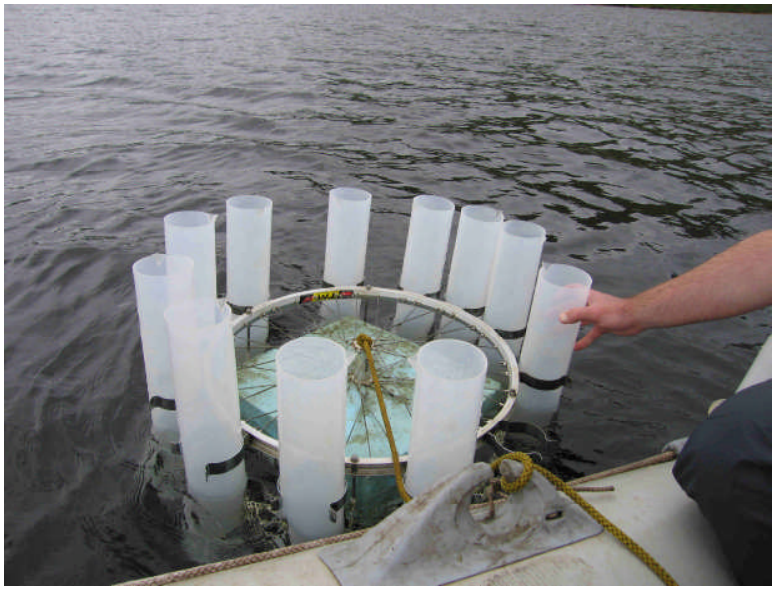
Aims

The main aim of this task was to determine how N species (NO_3^- and NH_4^+) determine lake sediment $\delta^{15}N$ and whether there is a seasonal variation in this process. In order to test the hypothesized relationship between the isotopic signature of anthropogenic N deposition and changes in organic matter $\delta^{15}N$ in lake sediment cores, two contrasting but closely located AWMN sites, Scoat Tarn and Burnmoor Tarn, were sampled monthly over one year for water chemistry and recent sedimenting material collected from lake sediment traps.

Sampling and methods

Construction of novel high volume traps was completed during spring and sediment traps were deployed during May 2008 (Fig. 3.1.1). Upland lakes have relatively low productivity and consequently low sedimentation rates. Therefore, we designed the traps to have as large a collecting area as possible whilst still be easily deployed and recovered in these remote upland lakes. Monthly sampling of sediment traps at Scoat Tarn and Burnmoor Tarn was completed in May 2009 (**Milestone 10**). The custom-made sediment traps were successfully sampled in most months but the severe cold spell of January 2009 led to lake freezing which meant that traps could not be recovered in that month (Table 2.1.1). For Scoat Tarn this simply meant that the February sample integrated two months (Dec 2008 and Jan 2009) but the ice cover damaged the Burnmoor Tarn trap which was found on the lakeshore on the February 2009 trip. The trap was repaired and redeployed in March 2009 but samples from December 2008 to February 2009 were lost as a result.

Figure 3.1.1: Custom-made large volume sediment trap deployed to Burnmoor Tarn, May 2008. The collecting tubes are 2L measuring cylinders with a high diameter to depth ratio ideal for entrapment of sedimenting material and retention of collected material without loss through re-suspension.



Sediment trap material was carefully collected from the tubes each month. This material was filtered onto a pre-ashed, large diameter GF/F glass fibre filter, placed into a sterile sample bag and sealed. As soon as was feasible, the sample bags were frozen until analysis could be performed in the laboratory.

Particulate organic matter (POM) in the water column at the time of sampling the traps each month was also collected via a phytoplankton net that was raised vertically through the water column as well as towed spatially in order to collect an integrated sample. The collected samples were filtered onto a pre-ashed, small diameter GF/F glass fibre filter and again frozen for transportation back to the laboratory.

The samples were analysed for total N and C, and $^{15}N/^{14}N$ and $^{13}C/^{12}C$ at the UC Davis Stable Isotope Facility, California, USA via isotope ratio mass spectrometry on Hydra 20-20 or Anca-GSL isotope ratio mass spectrometers. The concentration of N and C in the samples is expressed as grammes N or C per gramme dry weight of sediment. The isotopic ratio of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ expressed using the delta (δ) notation in parts per thousand (or per mille, ‰), where $\delta^{15}N$ (‰) and $\delta^{13}C$ (‰) = $[(R_{sample} / R_{standard}] - 1]$ x 1000, where R is the $^{15}N/^{14}N$ or $^{13}C/^{12}C$ ratio in the measured sample or standard. The standard for nitrogen is the $\delta^{15}N$ of atmospheric nitrogen (commonly referred to as AIR), and for $\delta^{13}C$ the standard is Vienna Pee Dee Belemnite (VPDB). The C:N ratio was calculated from the mass of N and C in each sample and converted to atomic ratios by multiplying the mass ratios by 1.167 (the ratio of the atomic weights of N and C).

In a number of cases there was insufficient sample to perform both N and C analysis. Therefore, for some samples, only the N data are available as this was the main focus of this task.

Sediment trap samples were collected during the same visits that lake water and deposition samples were collected for dual isotope analyses for NO_3^-. Therefore we are able to relate the isotope composition of sediment material with that of the lake water nitrate pool and that of the preceding months nitrate deposition.

Results: surface waters and deposition

The deposition data, for NH_4^+ the first for the UK, show that for most of the year inputs of inorganic N are depleted in ^{15}N, with only NO_3^- occasionally reaching positive $\delta^{15}N$ values. Ammonium in particular shows very depleted $\delta^{15}N$ in the range -4 to -9‰, confirming that deposition sources of N to upland lakes are indeed depleted in ^{15}N. Of specific interest here is the finding that deposition $\delta^{15}N$ values are generally much lower than the pre-industrial values recorded in lake sediment organic matter from Scoat and Burnmoor Tarns. Sediment core analysis under Phase 1 of the Freshwater Umbrella revealed that core bottoms from both sites showed fairly consistent organic matter $\delta^{15}N$ values of around +3‰. Hence it is entirely feasible that

in-lake utilization of increasing inorganic N inputs with the isotopic signatures observed at present could result in a depletion of lake sediment organic matter $\delta^{15}N$.

Lakewater $\delta^{15}N\text{-}NO_3^-$ values are either negative or very close to zero at both sites, and will reflect both deposition input signatures and subsequent in-lake cycling of N with the associated effects of mixing and retention times. These results convincingly demonstrate that both deposition and lakewater sources of inorganic N available for assimilation by phytoplankton are depleted in ^{15}N relative to pre-industrial lake sediments.

Table 3.1.1a: Seasonal variation in $\delta^{15}N$ of inorganic N sources at Scoat Tarn

Date	Surface water $\delta^{15}N\text{-}NO_3$	Deposition $\delta^{15}N\text{-}NO_3$	$\delta^{15}N\text{-}NH_4$
27-May-08	Too small	-2.4	-0.7
08-Jul-08	-0.6	-3.5	-5.8
05-Aug-08	-1.1	-2.8	-1.1
02-Sep-08	-1.2	-1.9	-3.6
30-Sep-08	-1.6	No sample	
28-Oct-08	-0.8	-2.0	-3.5
25-Nov-08	-0.4	+2.4	-5.7
06-Jan-09	+0.2	-0.3	-5.5
03-Feb-09	-1.3	-1.3	-2.4
03-Mar-09	+0.3	+0.9	-3.1
31-Mar-09	-0.4	-0.1	-7.4
28-Apr-09	-0.7	-2.7	-3.2

Table 3.1.1b: Seasonal variation in $\delta^{15}N$ of inorganic N sources at Burnmoor Tarn

Date	Surface water $\delta^{15}N\text{-}NO_3$	Deposition $\delta^{15}N\text{-}NO_3$	$\delta^{15}N\text{-}NH_4$
28-May-08	-1.9	-3.8	-0.6
09-Jul-08	-1.2	-3.5	-2.7
06-Aug-08	Too small	-2.8	-2.3
03-Sep-08	Too small	-2.4	-4.4
01-Oct-08	-0.3	-2.7	-5.9
29-Oct-08	-1.2	-1.0	-6.8
26-Nov-08	-0.7	+0.8	-8.7
07-Jan-09	-0.6	-0.6	-7.5
04-Feb-09	-1.0	-1.1	-6.3
04-Mar-09	-0.9	-0.7	-3.2
01-Apr-09	+0.3	+0.9	-6.5
29-Apr-09	+0.2	-2.8	-6.1

Results: sediment traps and particulate organic matter

The results of the sediment and POM analyses are shown in Figures 3.1.2 to 3.1.4. In general the $\delta^{15}N_{trap}$ in Scoat Tarn was depleted in ^{15}N in late spring through to early autumn with values of ~1‰ observed. During winter, $\delta^{15}N_{trap}$ was significantly more enriched in ^{15}N relative to the earlier part of the year, with values of 0-2‰ observed. The $\delta^{15}N\text{-}NO_3^-{}_{lake}$ in general follows this pattern, but the seasonal pattern is muted relative to that of the trap material. $\delta^{15}N_{POM}$ exhibits marked seasonal variation throughout the period of sampling. $\delta^{15}N_{POM}$ falls throughout the summer to a minimum value of -3‰ then increases to ~2‰ during the winter before beginning to decrease again during spring 2009. $\delta^{15}N\text{-}NO_3^-{}_{deposition}$ also largely follows this seasonal pattern though is more variable, especially in late winter/early spring. $\delta^{15}N\text{-}NH_4^+{}_{deposition}$ is depleted in ^{15}N throughout the period of sampling with little systematic variation with values in the range -0.7 to -7.4‰.

In contrast to the pattern seen in Scoat Tarn, there was little seasonal variation in $\delta^{15}N_{trap}$ observed throughout the year in Burnmoor Tarn, with values fluctuating about 0‰. $\delta^{15}N\text{-}NO_3^-{}_{lake}$ was generally lower than $\delta^{15}N_{trap}$ and offset by between -2 and -0.5‰, whilst $\delta^{15}N_{POM}$ was, in general, higher than $\delta^{15}N_{trap}$ and exhibited two marked periods of enrichment between August and November 2008 and February and March 2009. $\delta^{15}N\text{-}NH_4^+{}_{deposition}$ decreased markedly to a low of -8.7‰ in December 2008 before increasing again to March 2009 before declining again to ~-6‰ during the last two sampling occasions.

Figure 3.1.2 Time series of $\delta^{15}N$ (‰) of particulate organic matter (POM) and sediment trap (Trap) material for Burnmoor Tarn and Scoat Tarn. Also shown are the $\delta^{15}N\text{-}NO_3^-$ in lake water (Nitrate), deposition (Nitrate Deposition) and $\delta^{15}N\text{-}NH_4^+$ (Ammonium Deposition)

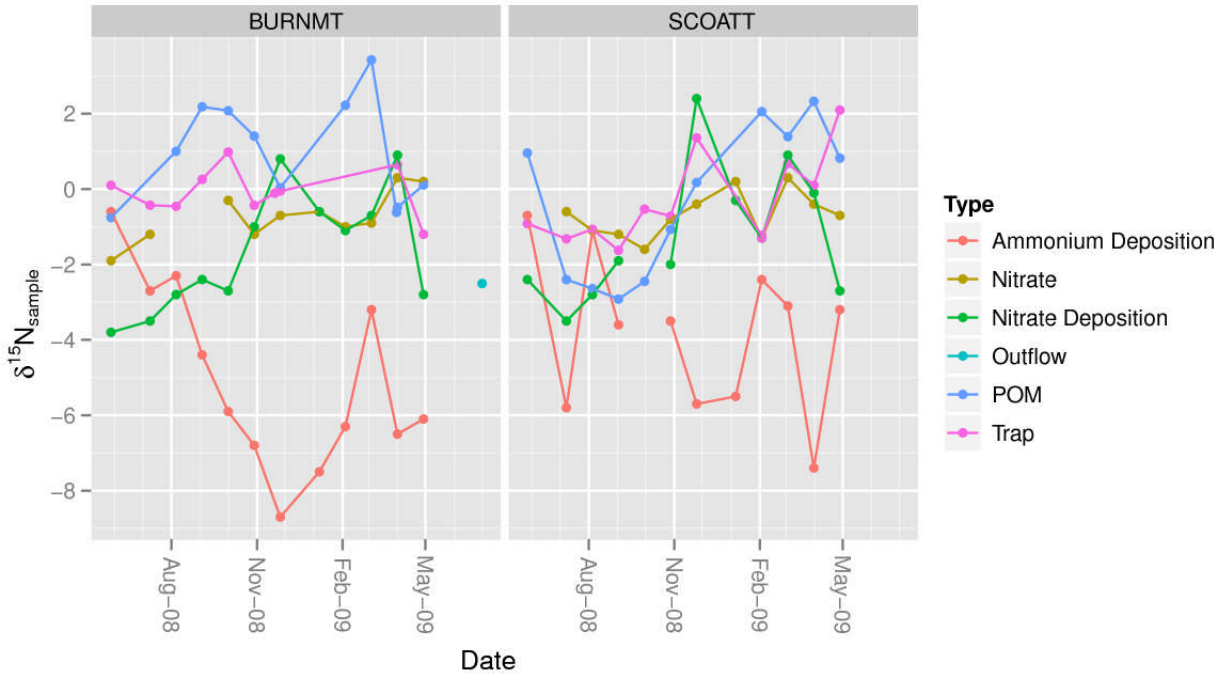


Figure 3.1.3 Time series of $\delta^{13}C$ (‰) of particulate organic matter (POM) and sediment trap (Trap) material for Burnmoor Tarn and Scoat Tarn

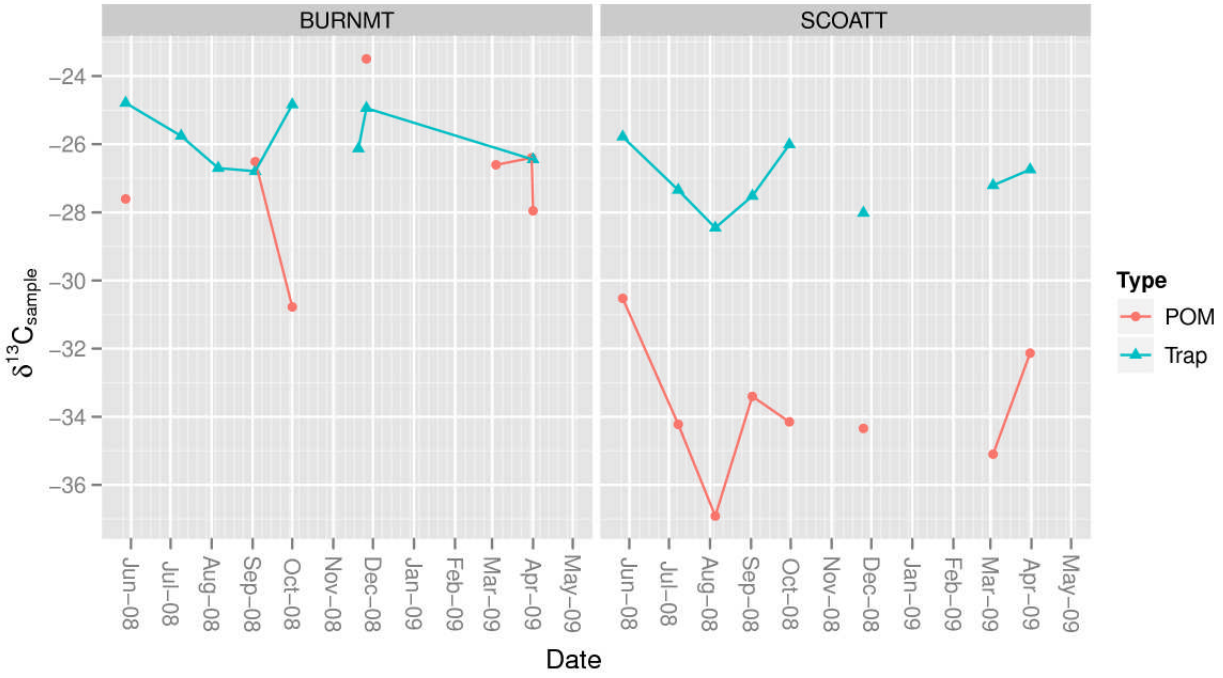


Figure 3.1.4 Time series of the carbon to nitrogen ratio (C:N) of particulate organic matter (POM) and sediment trap (Trap) material for Burnmoor Tarn and Scoat Tarn

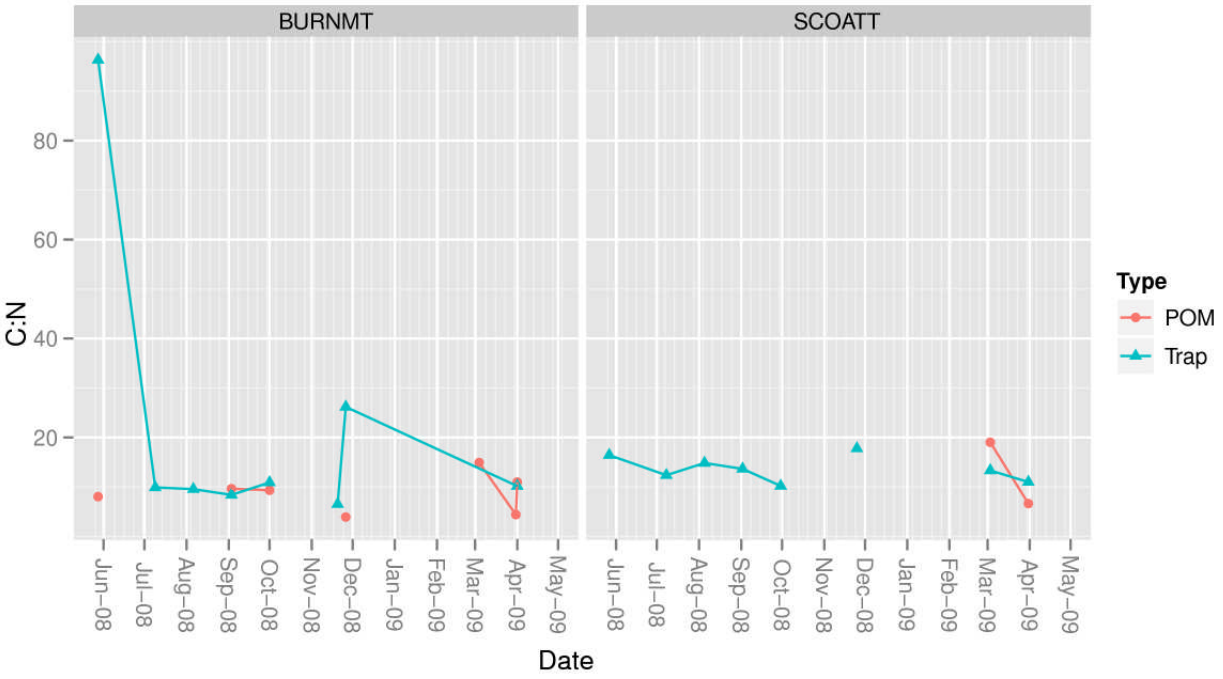
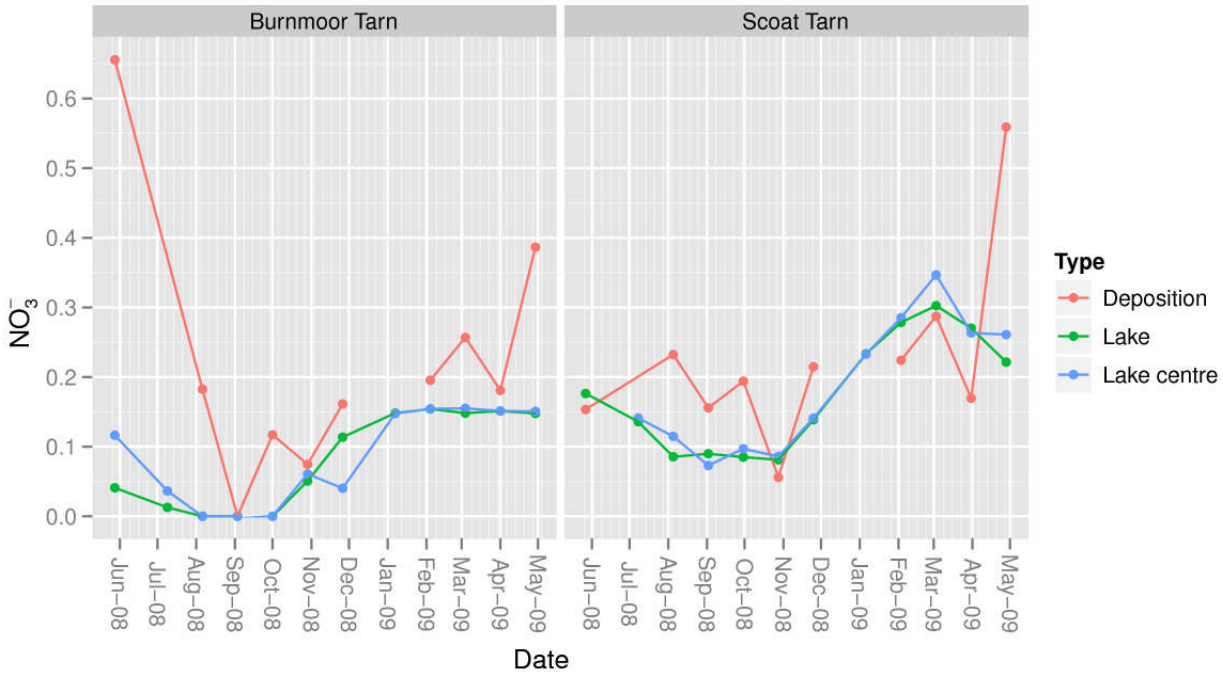


Figure 3.1.5 Time series of nitrate concentration (µ eq L^{-1}) in deposition, lake outflow (Lake) and the lake centre for Burnmoor Tarn and Scoat Tarn



$\delta^{13}C_{trap}$ were similar in both sites, with values of ~-27‰ and ~-26‰ observed in Scoat Tarn and Burnmoor Tarn respectively. $\delta^{13}C_{POM}$ were in general sparse owing to the small sample sizes we were able to recover, but we observe a marked difference between Scoat Tarn and Burnmoor Tarn; in the former site there is a marked offset of up to -8‰ between $\delta^{13}C_{trap}$ and $\delta^{13}C_{POM}$ where little or no offset was seen in Burnmoor Tarn, although the data are more sparse in this site.

The C:N ratios in trap material in Scoat Tarn ranged between 11 and 18 suggesting a mixture of autochthonous and allocthonous material. Values were lowest during the summer and autumn sampling occasions, close to values expected for lacustrine algae. The C:N ratios in Burnmoor Tarn were generally lower and with two exceptions are indicative of a predominantly autochthonous source of lacustrine algae. The two high values indicate specific samples with large contributions from catchment sources dominating these two samples. These values may be explained by bulk, partially degraded vegetative remains collecting in the trap on these two occasions.

The $\delta^{15}N$ data suggest two contrasting N regimes in Scoat and Burnmoor Tarns. In Scoat Tarn the N-isotopic composition of trap material largely follows that of the nitrate source suggesting that the trap material reflects the isotopic composition of this source. The variation in N-isotopic composition of POM suggests a small fractionation effect on utilisation of the nitrate source of about 1‰ during the period of greatest biological activity in late spring and summer. The enrichment in ^{15}N of

POM relative to nitrate during winter and early spring is difficult to explain from the available data, but does indicate utilisation of a source of N more enriched in ^{15}N than the lake water nitrate. In contrast, the N-isotopic composition of trap material and POM is enriched relative to the nitrate pool suggesting that N sources other than nitrate are an important source of N in support of productivity in this site.

The N isotope data observed monthly in Scoat and Burnmoor Tarn illustrate the complexity associated with interpreting N-isotope composition data that reflect a large number of N sources and potential for transformations throughout the N cycle of the lake and its catchment. Scoat Tarn is predominantly P-limited throughout the year and measurable nitrate concentrations are observed (Figure 3.1.5) in lake waters. It is not surprising therefore, with plenty of available nitrate, that the trap material tracks the N-isotopic composition of nitrate in lake waters. Burnmoor Tarn is predominantly co-limited by N and P and nitrate concentrations are below detection limits during late summer. It is therefore not unexpected that the N-isotopic composition of trap material and POM reflects contributions from a source other than nitrate. Re-use of previously processed nitrogen may account for the enrichment in ^{15}N relative to the nitrate source (e.g. Hoch *et al.*, 1996) although this is highly speculative.

The data presented here suggest that the interpretation of the N-isotopic composition of organic matter in sediments is complex and not simply a function of the isotopic composition of N inputs from deposition. Hence we can reject the initial hypothesis tested in this task; that the isotopic composition of N species in deposition is an effective control on the N-isotopic composition of lake water nitrate and hence of sediment organic matter. An alternative hypothesis is that the N-isotopic composition of sediment organic matter reflects a wide range of input sources of N, from both the lake and its catchment, and the many transformations and cycling of that nitrogen that can occur. This conclusion is similar to that of other sediment trap studies (e.g. Lehmann *et al.*, 2004) though these have predominantly focussed on large lakes affected by cultural eutrophication.

Future work to understand N contributions to and the N-isotopic composition of sediment organic matter could be extended in three ways; i) sampling should be conducted over a number of years to allow for disentangling of the within year from between year variations, ii) by sampling of primary production and algal species composition throughout the year, by collection of pCO_2 and $\delta^{13}C$ of DIC to better understand how changes in the C cycle and sources of organic are related to the N-isotopic composition of sediment matter, and iii) utilise mass-spectrometry and compound specific isotope measurements on the trapped sediment material allow the composition of organic matter in sediments to be better characterised and related to processing of N in lakes.

Summary

Sedimenting organic material in the two study lakes does not closely track the isotopic signature of lakewater NO_3^- or of bulk deposition NO_3^- or NH_4^+ on a seasonal basis. Hence it cannot be assumed that sediment core records of bulk organic matter $\delta^{15}N$ are a simple proxy for N deposition inputs through the incorporation of isotopically depleted deposition NO_3^-.

References

Hoch M.P., Snyder R.A., Cifuentes L.A., & Coffin R.B. (1996) Production, sedimentation, and isotopic composition of organic matter in Lake Ontario. Marine Ecology Progress Series 132, 229-239.

Lehmann M.F., Bernasconi S., McKenzie J.A., Barbieri A., Simona M. & Veronesi M. (2004) Seasonal variation of the $\delta^{13}C$ and $\delta^{15}N$ of particulate and dissolved carbon and nitrogen in Lake Lugano: Constraints on biogeochemical cycling in a eutrophic lake.

Task 3.2 Drivers of Sediment $\delta^{15}N$ – upscaling

Aims

A link between $\delta^{15}N$ of lake surface sediment bulk organic matter and N deposition has been reported by Jones et al (2004) and subsequently repeated on a large data set (Curtis and Simpson, 2007). Lake surface sediment samples incorporate material from a number of years, the actual number depending upon how finely the surface sample is sectioned, the degree of bioturbation of the uppermost sediments and resuspension of material. As a result of these processes, it is difficult to ascribe monitoring data from a particular year or years as being representative of the time period covered by surface sediment samples. An additional complexity is that lakes have different sedimentation rates; a 0.25cm surface sample in one lake may represent the annual sedimentation whilst at a second, unproductive lake a 0.25cm surface sample may represent several years of accumulating sediments.

Here we investigate the relationship between sedimenting organic matter and various measures of deposition including concentration, flux and isotopic composition of nitrate and ammonium in deposition.

Methods

We installed annual sediment traps in 12 lakes as listed in Table 3.2.1, from which lake sediment cores were also taken (see Task 3.3 below). Bulk organic matter sub-samples from sediment cores from each lake were air dried at 40 °C or below. These sub-samples were then milled to a fine powder using a Retsch mixer mill. Approximately 0.001g of milled sediment was transferred to pre-weighed tin capsules, which were then sealed. The amount of dried sediment in each capsule was recorded.

The samples were analysed for total N and C, and $^{15}N/^{14}N$ and $^{13}C/^{12}C$ at the UC Davis Stable Isotope Facility, California, USA via isotope ratio mass spectrometry on Hydra 20-20 or Anca-GSL isotope ratio mass spectrometers. The concentration of N and C in the samples is expressed as grammes N or C per gramme dry weight of sediment. The isotopic ratio of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ expressed using the delta (δ) notation in parts per thousand (or per mille, ‰), where $\delta^{15}N$ (‰) and $\delta^{13}C$ (‰) = $[(R_{sample} / R_{standard}] - 1] \times 1000$, where R is the $^{15}N/^{14}N$ or $^{13}C/^{12}C$ ratio in the measured sample or standard. The standard for nitrogen is the $\delta^{15}N$ of atmospheric nitrogen (commonly referred to as AIR), and for $\delta^{13}C$ the standard is Vienna Pee Dee Belemnite (VPDB). The C:N ratio was calculated from the mass of N and C in each sample and converted to atomic ratios by multiplying the mass ratios by 1.167 (the ratio of the atomic weights of N and C).

Results

The results of the biogeochemical analyses are presented in Table 3.2.2, and summarised via a principal components correlation biplot in Figure 3.2.1.

Table 3.2.1: Location and dates of sediment trap deployments for Task 3.2

Site	Code	GPS	Installed	Removed
Blue Lough	BLU	SB41047, 80985	07-May-08	06-May-09
Loch Borralie	BORR	NC38434, 67246	04-May-08	02-May-09
Burnmoor Tarn	BURNMT	NY18258, 04300	29-Apr-08	29-April-09
Llyn Conwy	CONWY	SH77829, 46378	08-May-08	20-May-09
Loch Coire Fionnaraich	FION	NG94530, 49846	03-May-08	03-May-09
Llyn Llagi	LAG	SH64912, 48185	08-May-08	07-May-09
Loch Grannoch	LGR	NX54027, 69432	06-May-08	25-June-09
Lochnagar	NAGA	NO25124, 85876	02-May-08	01-May-09
Round Loch of Glenhead	RLGH	NX44940, 80239	05-May-08	05-May-09
Scoat Tarn	SCOATT	NY15983, 10391	30-Apr-08	28-April-09
Small Water	SMALL	NY45465, 09997	29-Apr-08	30-April-09
Loch Tinker	TINK	NN44764, 06806	01-May-08	04-May-09

Table 3.2.2: Key biogeochemical measurements on sediment trap material. C:N ratio is an atomic ratio.

Site	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	C:N	Total N ($g\ g^{-1}$)	Total C ($g\ g^{-1}$)
Blue Lough	−1.25	−27.52	11.46	0.03	0.26
Loch Borralie	6.89	−27.70	3.67	0.01	0.04
Burnmoor Tarn	0.42	−25.92	4.08	0.04	0.14
Llyn Conwy	−1.18	−28.54	19.21	0.02	0.26
Loch Coire Fionnaraich	0.44	−27.07	12.59	0.02	0.18
Llyn Llagi	2.00	−27.59	11.39	0.02	0.21
Loch Grannoch	0.48	−29.29	10.45	0.03	0.26
Lochnagar	−0.67	−26.51	12.05	0.02	0.18
Round Loch of Glenhead	−0.75	−27.55	11.21	0.02	0.20
Scoat Tarn	−1.00	−26.83	10.51	0.02	0.22
Small Water	0.94	−27.50	12.13	0.01	0.12
Loch Tinker	−0.99	−28.20	11.67	0.02	0.20

There is considerable variation in the geochemical data observed across the 12 sites. Loch Borralie is an outlier in terms of $\delta^{15}N$ having the highest value observed across the study (6.89‰). The next highest $\delta^{15}N$ value observed is 2.0‰ (Llyn Llagi), with most values clustered in the range -1‰ and 1‰. The range of observed values is within that also recorded by Jones *et al.* (2004), however their data more smoothly covered a range of values from ~6‰ to ~0‰, and a similar range for $\delta^{15}N$ was also reported by Curtis and Simpson (2007) supplemented by a few small negative values.

Whilst the observed $\delta^{15}N$ for trap organic matter in Loch Borralie is a gross outlier in the data reported here it is entirely consistent with the range of values observed for nutrient poor lakes in the UK. $\delta^{13}C$ varies by ~3‰ over the 12 sites.

Figure 3.2.1: Principal components analysis correlation biplot summary of the geochemical data shown in Table 3.2.2. The first 2 principal components explain ~80% of total variance in the data set.

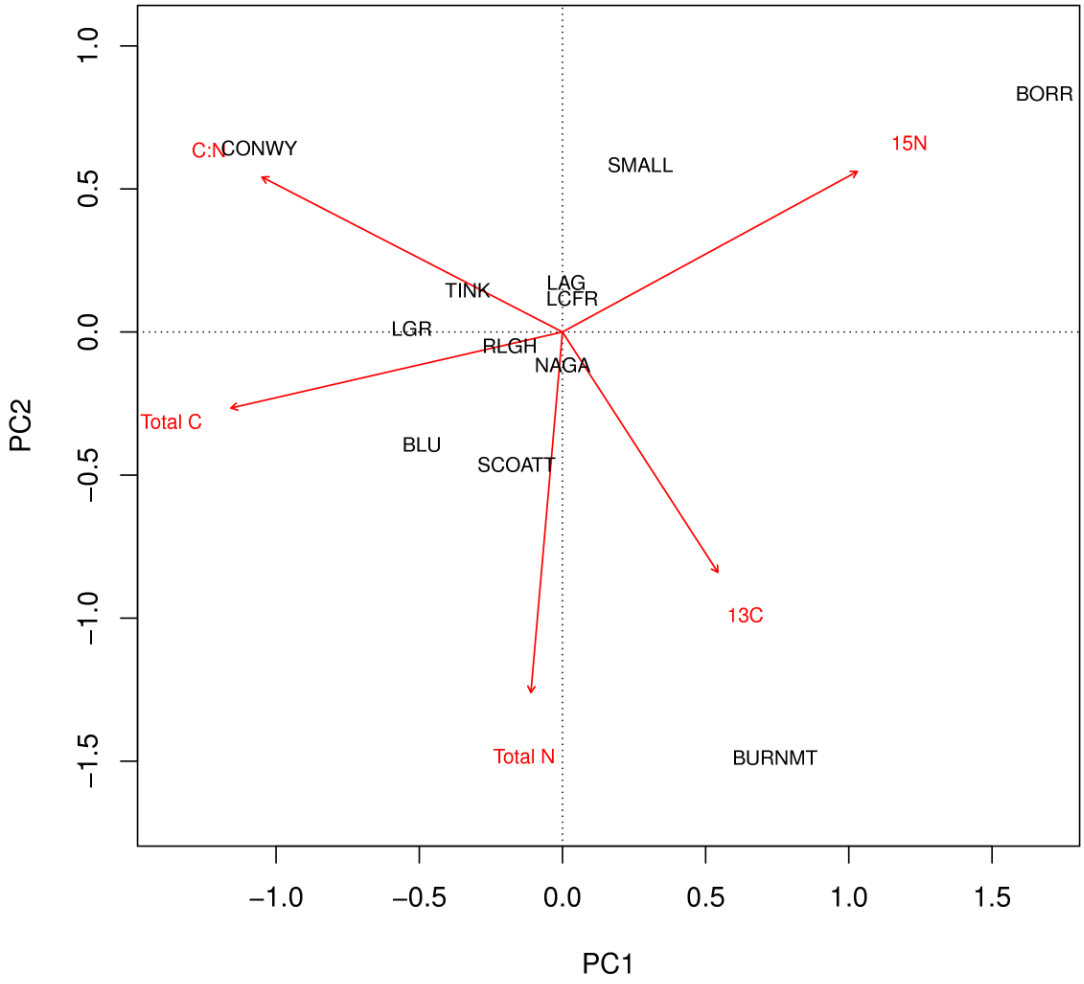


Figure 3.2.2: Plots of trap material $\delta^{15}N$ and various potential drivers and explanatory factors. The red solid lines are least squares fits to the data shown in each panel. The data for Loch Borralie are included here.

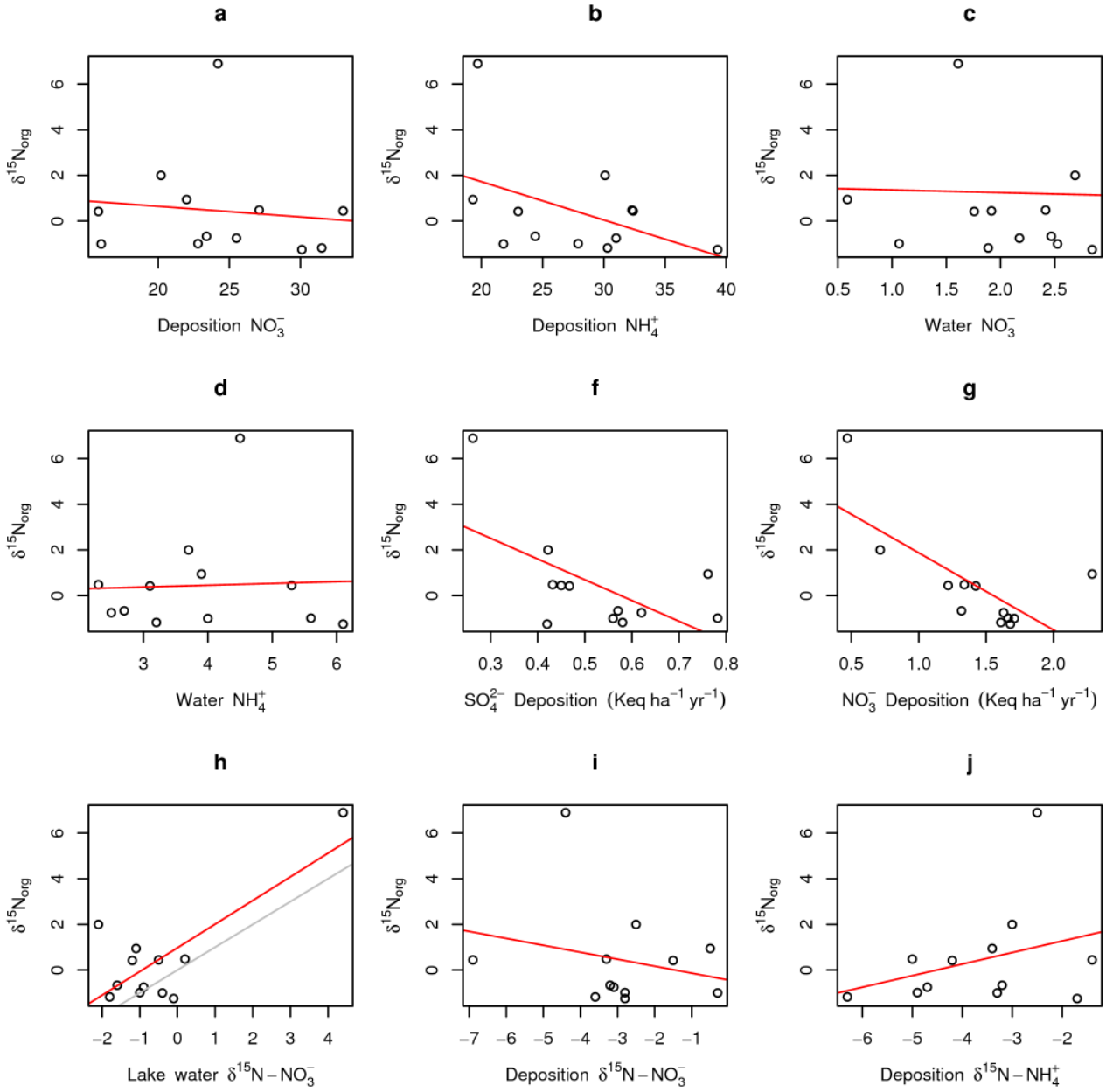


Figure 3.2.3: Plots of trap material $\delta^{15}N$ and various potential drivers and explanatory factors. The red solid lines are least squares fits to the data shown in each panel. The data for Loch Borralie are excluded from these plots and least squares fits.

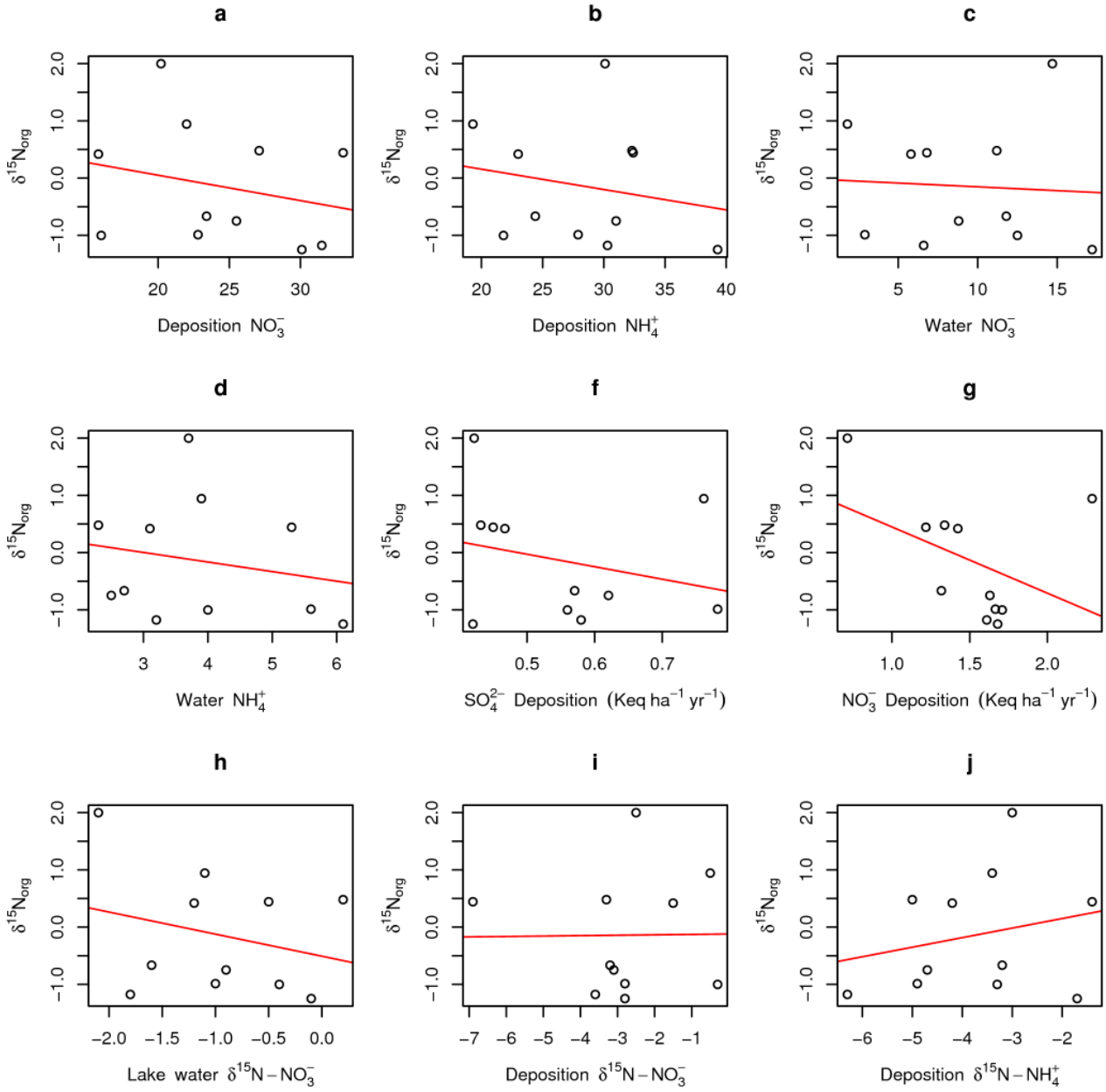
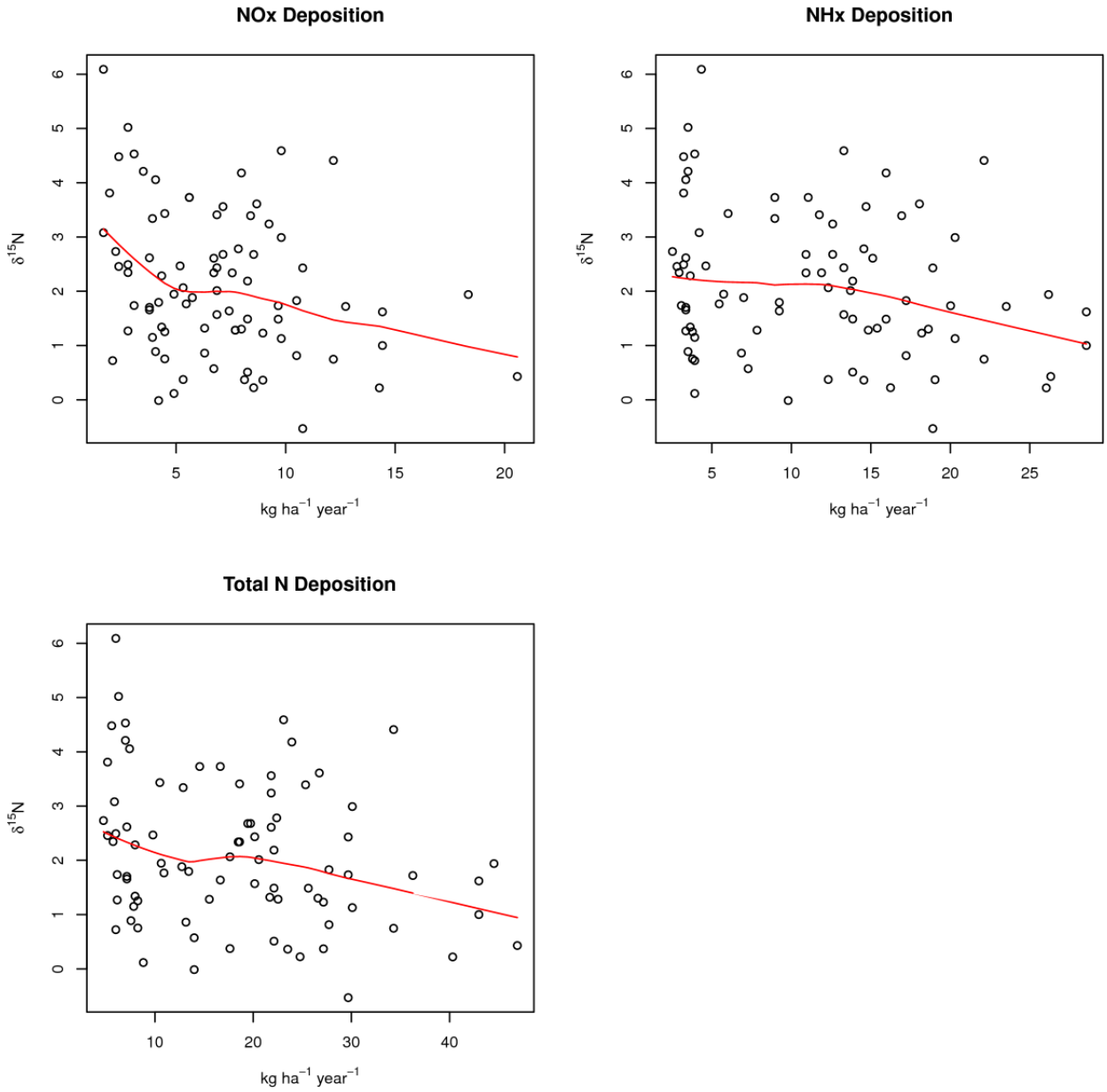


Figure 3.2.4: Relationships between N species in deposition and surface sediment $\delta^{15}N$. Reproduced from Curtis and Simpson (2007). The solid red line is a LOESS smoother fitted to the data to highlight patterns in the data.



Burnmoor Tarn is an outlier in terms of $\delta^{13}C$ (-25.92‰) and C:N (4.08). The low C:N ratio is a result of relatively high total N content in the trap material. The C:N data indicate that the source of the organic matter captured by the sediment traps is predominantly an autochthonous source from aquatic algae, which have relatively high cellular N content compared to the cellulose-rich organic matter delivered from catchment sources. The exception to this general pattern is Llyn Conwy with a C:N of

19.2, which is within the range of C:N reported for C_3 land plants though towards the lower end of reported values. The source of the organic matter in Llyn Conwy is weighted more strongly to catchment sources than the other sites reported here.

We fitted a series of least squares regressions to investigate the relationships between the $\delta^{15}N$ of trap material and potential explanatory factors. Here we consider concentrations of NO_3^- and NH_4^+ in lake waters and in deposition, atmospheric deposition flux of SO_4^{2-} and NO_3^-, $\delta^{15}N$ of NO_3^- in lake water and deposition and $\delta^{15}N$ of NH_4^+ in deposition. The results of these analyses are shown in Figure 3.2.2.

Statistically significant relationships were observed between trap material $\delta^{15}N$ of organic matter and SO_4^{2-} and NO_3^- deposition flux ($p = 0.0397$ and $p = 0.0097$ respectively) and $\delta^{15}N$-NO_3^- of lake water ($p = 0.0032$). However these relationships are highly influenced by the high $\delta^{15}N$ of trap material for Loch Borralie. If we remove this observation from the dataset and re-run the regression analyses none of the relationships is statistically significant (Figure 3.2.3). Whilst the observation for Loch Borralie is not extreme when we consider the $\delta^{15}N$ of surface sediment organic matter (e.g. Jones *et al.*, 2004, Curtis and Simpson 2007), we are wary of over interpreting the significant statistical relationships fitted using this datum.

We are restricted in the conclusions we can draw from the results reported above because of the gross outlier in Loch Borralie $\delta^{15}N$ of trap organic matter. The choice of sites was driven by other aspects of the work programme, namely the upscaling of the dual isotope work in Task 4.1 and the desire to include sites on well-buffered geology. Loch Borralie is a groundwater-fed marl lake and in retrospect represents a quite different lake type to the other sites included in the study. The small size of the data set, again dictated by the upscaling in Task 4.1 that provided the $\delta^{15}N$ of NO_3^- in lake water and deposition and $\delta^{15}N$ of NH_4^+ in deposition data also compounds the problem of the Loch Borralie outlier. With a larger data set, more even coverage of the range of sediment $\delta^{15}N$ could have been achieved by filling in the data space between Loch Borralie and other sites studied here.

Whilst we are cautious to interpret the statistically significant relationships between deposition flux and $\delta^{15}N$-NO_3^- of lake water, the former result is consistent with that identified by Jones at al (2004) and Curtis and Simpson (2007) and supports the idea that $\delta^{15}N$ of sediment organic matter may be used as an indicator of past N deposition flux. However, the results reported by Curtis and Simpson (2007) suggest that this relationship is non-linear and that $\delta^{15}N$ sediment organic matter varies little at higher N deposition fluxes; a stronger relationship between $\delta^{15}N$ of N deposition flux exists below 5 Kg N ha^{-1} yr^{-1} (Figure 3.2.4). At higher values of N deposition flux, other factors appear to exert a strong control on lake sediment $\delta^{15}N$. The statistically significant relationship between trap sediment $\delta^{15}N$ and $\delta^{15}N$-NO_3^- of lake water is particularly tantalising; although the identified relationship is driven by the observed data for Loch Borralie, the relationship identified is 1:1 between $\delta^{15}N$ of trap material

and lake water nitrate (c.f. the grey line in Figure 3.2.2 panel h). This might be interpreted as the sediment organic matter reflecting the $\delta^{15}N$ of the available nitrate pool in the lake water being utilised as a food source by algae. Further work will be required to confirm this relationship, covering a wider range of sites and using compound specific N isotope data to identify the $\delta^{15}N$ values of algal-derived compounds contained within the bulk sediment matrix captured by the traps.

Summary

A statistically significant relationship was found between annual sediment trap material $\delta^{15}N$ and deposition $\delta^{15}N\text{-}NO_3^-$ but this was driven largely by high values in an outlying site. Exclusion of the site resulted in a lack of significant relationships. Hence results from this study do not support the use of sediment core $\delta^{15}N$ as a proxy for N deposition inputs although other methods could be employed to more thoroughly test the relationships under future work.

References

Curtis C.J. & Simpson G.L. (eds.) (2007) Freshwater umbrella - the effects of nitrogen deposition and climate change on freshwaters in the UK. Report to DEFRA under Contract CPEA17 . ECRC Research Report No. 115.

Jones R.I., King L., Dent M.M., Maberley S.C. & Gibson C.E. (2004) Nitrogen stable isotope ratios in surface sediments, epilithon and macrophytes from upland lakes with differing nutrient status. Freshwater Biology 49, 382-391.

Task 3.3: Palaeolimnological assessment of changes in lake nutrient status ($\delta^{15}N$), including additional fossil pigment analysis (Task 3.4)

Aims and background

Research over the past decade has demonstrated that nutrient poor remote aquatic ecosystems are responding to increased deposition of nitrogen from anthropogenic sources despite the quantitatively low inputs (Findlay *et al.*, 1999; Baron *et al.*, 2000; Wolfe *et al.*, 2001; Fenn *et al.*, 2003; Sickman, 2003; Elser *et al.*, 2009). Contrary to expectations, many upland lakes in Europe and North America are N, rather than P, limited or co-limited by both N and P (Maberly *et al.*, 2002; Bergström *et al.*, 2005; Bergström & Jansson, 2006:). It has been suggested that the natural state of many upland lakes is N-limitation and the widespread occurrence of P-limitation is a reflection of a change in the N and P balance of these lakes as a result of increased inputs of anthropogenic N (Bergström & Jansson 2006; Elser *et al.*, 2009).

Wolfe *et al.* (2001), working on cores from remote lake ecosystems in the Rocky Mountains, Colorado, demonstrated a link between apparent eutrophication and a change in $\delta^{15}N$ of bulk organic matter in sediment cores from two lakes. This change in $\delta^{15}N$ of bulk organic matter to isotopically lighter nitrogen is indicative of an increased supply of N from the atmosphere to the lakes and their catchments. N deposition in this region is relatively high for alpine regions in North America, though it has been suggested that a similar process may be operating more widely. Since this initial study, additional records throughout the northern hemisphere have painted a picture of consistent and coherent biogeochemical alteration of the N-cycle of oligotrophic lakes. Changes in the $\delta^{15}N$ of bulk organic matter, similar to those identified by Wolfe *et al.* (2001), have also been seen in remote lakes on Baffin Island (Wolfe *et al.*, 2006) and in Greenland (Simpson *et al.*, unpub. research) experiencing low but elevated inputs of anthropogenic N via long range transportation.

A recent synthesis of records of change in arctic and alpine systems documents a consistent pattern of change in these ultraoligotrophic systems with a common onset date of change of ~1900 (Holtgrieve *et al.*, 2011). Previous Defra-funded research under the Freshwater Umbrella documented similar N-isotope compositional change for many UK lakes (Curtis and Simpson, 2007). Furthermore, Jones *et al.* (2004) have recently reported that there is a weak but statistically significant link between the $\delta^{15}N$ of lake surficial sediments and total N deposition for a small set of upland lakes, and suggest that $\delta^{15}N$ might be used as a surrogate for the degree of lake N-limitation.

Nitrogen isotopes are good integrators of the N cycle (Robinson, 2001) and have been used extensively to track pollution and processes in catchments and waters (Kendall, 1998). Whilst the exact interpretation of the changes in $\delta^{15}N$ of bulk organic matter is still being researched, it is clear that the changes in the isotopic values of nitrogen bearing compounds in lake sediments indicate a widespread and profound change in the biogeochemistry of the studied lakes.

Lake sediments contain organic matter from a range of sources. Particular amongst these is organic matter derived from aquatic macrophytes and algae, terrestrial macrophytes and soil humus. Each of these sources contains and therefore contributes varying amounts of N to sediment organic matter. Phytoplankton derived organic matter is relatively N-rich, due to the high protein and lipid content of the cells. Organic matter derived from terrestrial plants is dominated by cellulose and lignin which are N-poor, whilst soil-derived organic matter may be relatively N-rich due to N-fixation by bacteria, especially around plant roots. Differences in N content of the various constituents mean that N-rich fractions can dominate the isotope values of bulk organic matter measured in lake sediments, even if these N-rich sources do not dominate volumetrically. Therefore, terrestrial plant material is unlikely to distort or influence the isotope values measured in lake sediment organic matter as that source is N-poor. Likewise, a new N-rich source could have a disproportionate effect on sediment N isotope values despite being at a relatively low concentration or flux (Talbot, 2001). As such, N isotope values from lake sediment organic matter can be expected to reflect the N isotope values of the N-rich sources as well as in-lake processing of that N.

The contribution to lake sediment organic matter N isotope values made by atmospheric deposition is difficult to determine without having wider knowledge of the N isotope values of sources and applying mixing and fractionation models. What is easier to conclude is that the observed changes in lake sediment N-isotope composition record consistent biogeochemical change.

What is currently unclear is the degree to which this change in the N biogeochemistry of oligotrophic lakes throughout the northern hemisphere is associated with biological change within those lakes. Wolfe *et al.* (2001) identified an increase in diatom species indicative of increased nutrient availability in two Rocky Mountain, USA, lakes that occurred concomitantly with the change in N-isotopic composition of the bulk sediment organic matter. Wolfe *et al.* (2006) also document changes in diatom species composition associated with the biogeochemical change in a lake on Baffin Island, Canada, but the timing of the species composition change is somewhat off-set from the N-isotopic changes. Similar responses were recorded by Holmgren *et al.* (2010) in lakes in Svalbard, Norway. Contemporary bioassay studies by a number of workers, most recently synthesised by Elser *et al.* (2009), suggest that algal species composition has changed in response to the increased N deposition and that the stoichiometry of these groups has also been altered.

Here we present the results of two new palaeoecological studies undertaken at a range of UK oligotrophic lakes. The first study was designed to i) document biogeochemical changes in the N cycle of the outstanding lakes of the UK Acid Waters Monitoring Network (UK AWMN) not analysed under previous Defra-funded work, ii) to expand the range of lake types covered by the palaeoecological analyses to lakes with higher buffering capacities where acidification from sulphur and N deposition is not a

complicating factor. The second study aimed to use fossil algal pigment records from sediments to investigate changes in algal community composition and productivity associated with N biogeochemical alteration.

Three UK AWMN lakes had not been analysed for N-isotopic composition of bulk organic matter under previous Defra-funded research; i) Llyn Llagi, Wales, ii) Loch Tinker, Scotland, and iii) Blue Lough, Northern Ireland. New sediment cores were obtained from each of these three lakes and sediment samples prepared for C and N isotope analysis of bulk organic matter. Under previous Defra-funded research the focus of the N isotope palaeoecological work had been on lakes on acid-sensitive geologies. ENSIS-ECRC had a large collection of cores available from these lakes to allow cost effective initial analysis of N isotope biogeochemistry in upland UK lakes. Under the current programme of research, it was noted that the scope of the potential effects of nutrient N deposition to upland lakes could be addressed by analysing sediment cores from lakes not sensitive to the acidifying effects of N deposition. Therefore, it was decided that new sediment cores would be collected from three lakes on well buffered geology; i) Llyn Conwy, N Wales, ii) Small Water, UK Lake District, and iii) Loch Borralie, N Scotland. This analysis was not budgeted for under the original work programme so to accommodate the extra analyses the number of isotope samples allocated to the UK AWMN cores (above) was reduced to allow for these new analyses.

Under the extension of work to the Freshwater Umbrella contract, two new cores from Small Water (UK Lake District) and Loch Bealach na h'Uidhe (NW Scotland) were collected. These cores were subjected to isotope analysis and to fossil pigment analysis via high performance liquid chromatography (HPLC). We summarise here the results of these new analyses and report them in the wider context of the entire body of Defra-funded research into the N biogeochemistry of upland lakes in the UK.

Methods

Bulk organic matter sub-samples from sediment cores from each lake were freeze dried. These sub-samples were then milled to a fine powder using a Retsch mixer mill. Approximately 0.001g of milled sediment was transferred to pre-weighed tin capsules, which were then sealed. The amount of dried sediment in each capsule was recorded. Where standard sedimentological analyses showed the presence of sufficient carbonate content, a source of inorganic carbon that is isotopically distinct and which could bias the isotope analysis of organic matter, separate sediment samples were prepared for N and C analysis. The N samples were prepared as described above, whilst the samples for C analysis were freeze dried and milled as before, but were then subjected to an acidification pre-treatment using HCl. Samples were prepared in silver capsules for C analysis as the tin capsules are not resistant to the acid treatment.

The samples were analysed for total N and C, and $^{15}N/^{14}N$ and $^{13}C/^{12}C$ at the UC Davis Stable Isotope Facility, California, USA via isotope ratio mass spectrometry on Hydra 20-20 or Anca-GSL isotope ratio mass spectrometers. The concentration of N and C in the samples is expressed as grammes N or C per gramme dry weight of sediment. The isotopic ratio of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ expressed using the delta (δ) notation in parts per thousand (or per mille, ‰), where $\delta^{15}N$ (‰) and $\delta^{13}C$ (‰) = $[(R_{sample} / R_{standard}] - 1] \times 1000$, where R is the $^{15}N/^{14}N$ or $^{13}C/^{12}C$ ratio in the measured sample or standard. The standard for nitrogen is the $\delta^{15}N$ of atmospheric nitrogen (commonly referred to as AIR), and for $\delta^{13}C$ the standard is Vienna Pee Dee Belemnite (VPDB). The C:N ratio was calculated from the mass of N and C in each sample and converted to atomic ratios by multiplying the mass ratios by 1.167 (the ratio of the atomic weights of N and C).

Results

Figure 3.3.1 shows the $\delta^{15}N$ profiles for the three UK AWMN network lakes analysed here. The $\delta^{15}N$ profile for Loch Tinker is quite variable, showing a 1‰ increase in ^{15}N between 20cm and 10cm depth in the core. At this point $\delta^{15}N$ declines by ~1.5‰ before rising again in the uppermost cm of the core representing the most recent sediments. Care must be taken in interpreting geochemical data in the uppermost sediments of a core as this material may include isotopically distinct material that has not yet been fully incorporated in the sediment matrix. Diagenetic processes in the first few mm or cm of the core can rapidly alter the isotopic composition of organic matter as the more labile fractions are rapidly degraded, transformed or recycled.

The $\delta^{15}N$ profile for Llyn Llagi shows a steady decline from 3‰ to 1‰ between 17cm depth in the core to the core top. This decline in $\delta^{15}N$ of about 2‰ is consistent with the changes seen elsewhere in the UK, although the steady, long-term depletion in ^{15}N is in contrast to many other reported sequences where $\delta^{15}N$ changes far more rapidly. The $\delta^{15}N$ profile for Blue Lough exhibits a constant enrichment in $\delta^{15}N$ of ~1‰ up to ~5cm depth in the core. This trend reverses and sediment bulk organic matter becomes progressively depleted in ^{15}N by 0.5‰ towards the top of the core. The depletion in ^{15}N in Blue Lough is smaller than that observed in many other UK upland lakes.

Figure 3.3.2 shows profiles of the $\delta^{15}N$ of sediment bulk organic matter for the three acid-insensitive lakes added to the work programme. Sediment $\delta^{15}N$ was relatively stable at 4‰ in Small Water up to about 10cm depth whereupon a rapid depletion in ^{15}N of 1.5-2‰ is observed between 10 and 5cm. Above 5cm, $\delta^{15}N$ values vary about the 2.5‰ level with the exception of the uppermost couple of samples. A similar pattern of change in seen in the $\delta^{15}N$ profile for Llyn Conwy with stable values of ~3.75‰ observed up to 7cm core depth the a rapid depletion in ^{15}N of 1.75-2.0‰ within a few cm whereupon $\delta^{15}N$ values remain stable around 1.75‰ to the core top.

Figure 3.3.1: $\delta^{15}N$ profiles of sediment bulk organic matter for Loch Tinker, Llyn Llagi and Blue Lough. The solid blue line is an additive model fitted to the data using maximum likelihood to identify the trend in the sediment data. The grey shaded region is a 95% pointwise confidence interval on the fitted trend.

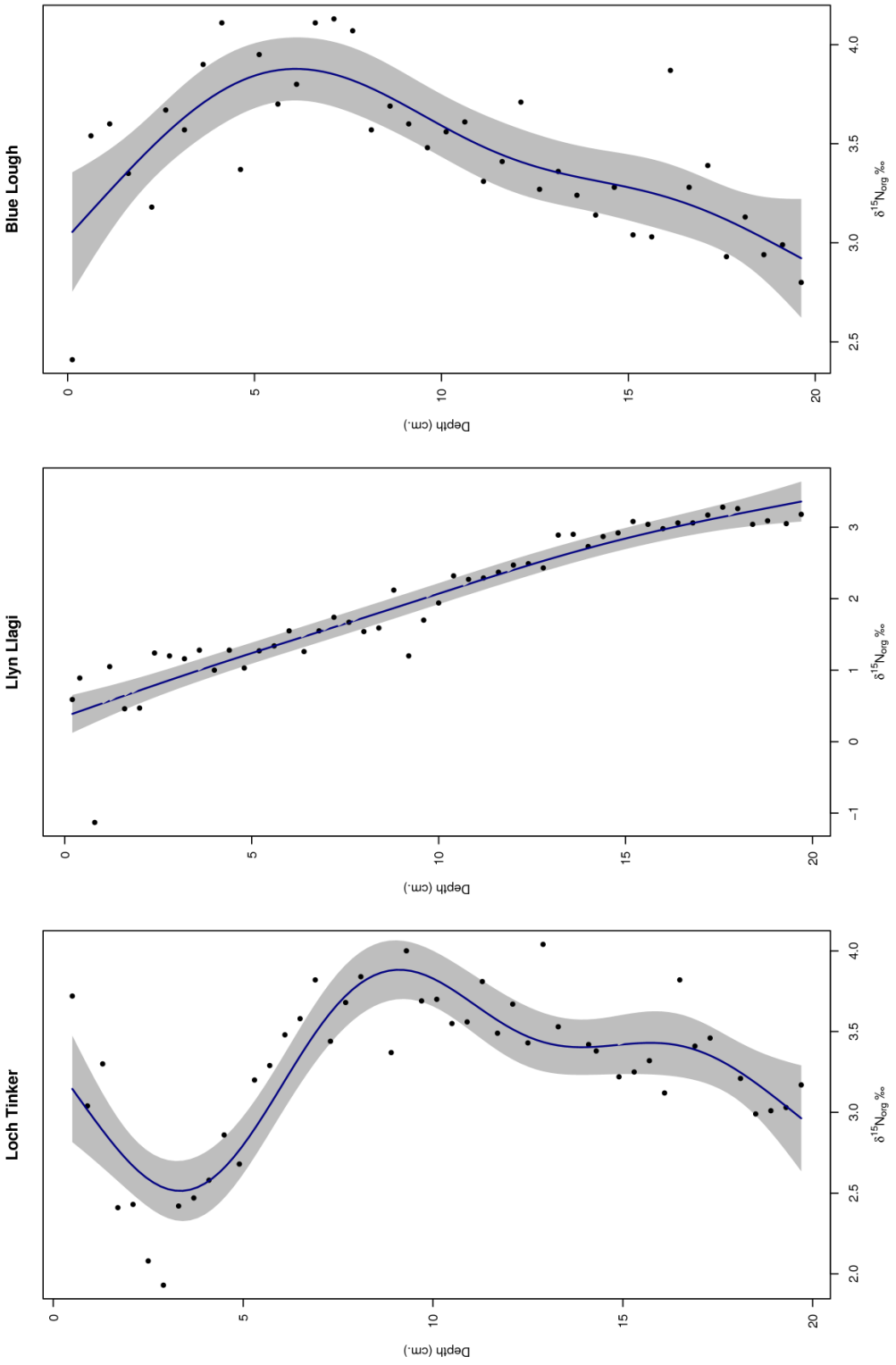
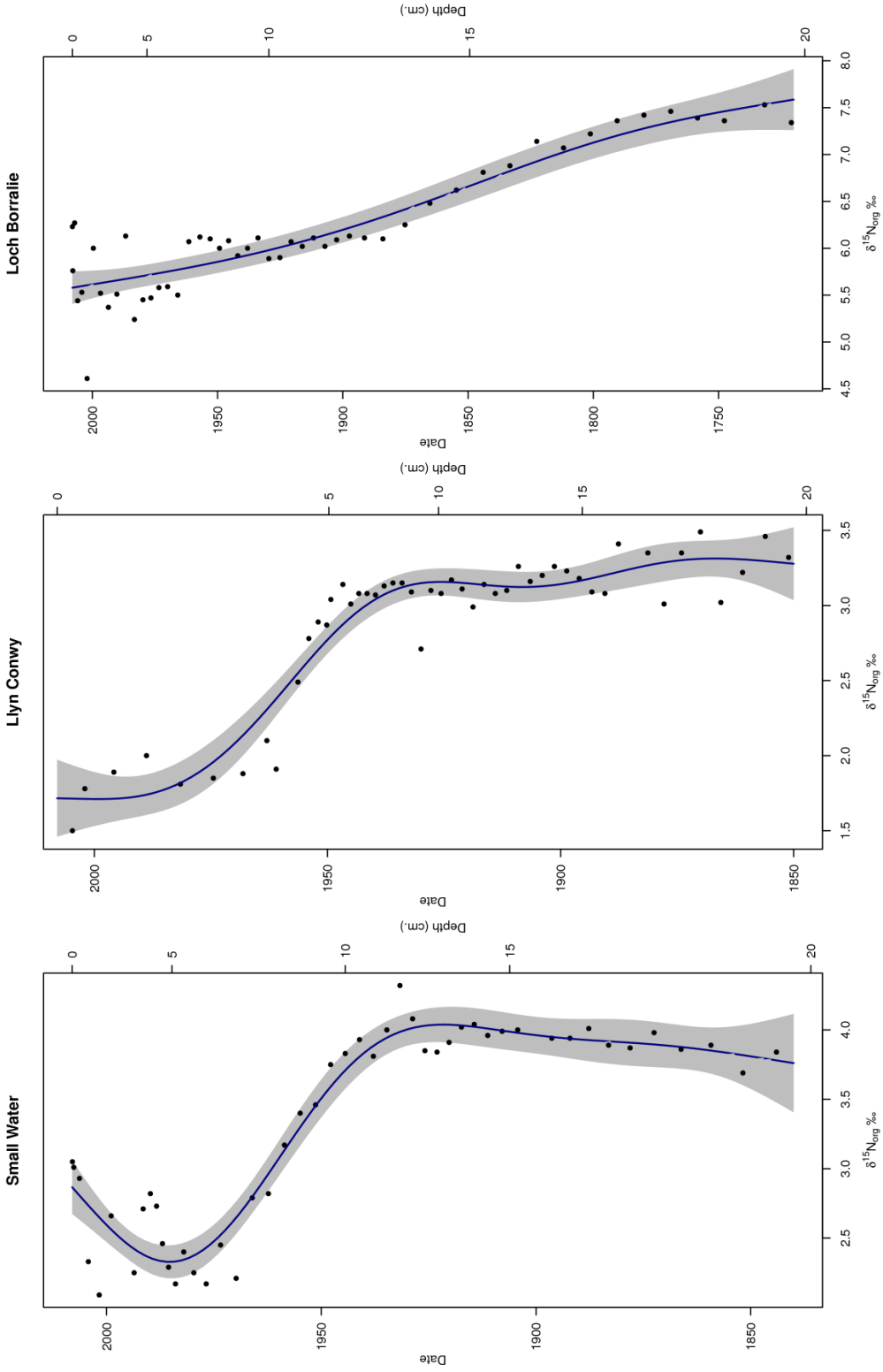


Figure 3.3.2: $\delta^{15}N$ profiles of sediment bulk organic matter for Small Water, Llyn Conwy and Loch Borralie. The solid blue line is an additive model fitted to the data using maximum likelihood to identify the trend in the sediment data. The grey shaded region is a 95% pointwise confidence interval on the fitted trend.



The N isotope data for Loch Borralie are somewhat different to the other three sites discussed thus far. The isotope values of bulk organic matter are much higher than

those observed elsewhere in the UK in upland lakes. This may be the result of increased productivity as a result of the improved grassland surrounding the lake and sheep or cattle grazing, or an effect of the ground water source to the lake. The $\delta^{15}N$-NO_3^- of lake waters is generally higher than at other UK lakes (4.5‰ November 2008 and 4.2‰ March 2009) although sediment bulk organic matter is enriched relative to this nitrate pool, despite a trend towards lower enrichment in ^{15}N throughout the core. Sediment $\delta^{15}N$ values are relatively stable around 7.5‰ up to 17cm where a -1‰ depletion in ^{15}N is observed. Following this, $\delta^{15}N$ values are once again stable at 6‰ between 13cm and 6cm depth, whereupon a further depletion of -1‰ is observed towards the core top, though $\delta^{15}N$ are quite variable during this period.
The timings of change in $\delta^{15}N$ is quite consistent in Small Water and Llyn Conwy with the rapid isotopic depletion of ^{15}N occurring around 1940. The initial changes in Loch Borralie happen much earlier, between 1800 and 1850, with the later depletion in ^{15}N taking place rapidly post 1950. We discuss the timing of change in $\delta^{15}N$ later.

Nineteen UK lakes have now been analysed as part of Defra-funded research under the Freshwater Umbrella. The $\delta^{15}N$ profiles of these lakes are summarised in Figure 3.3.3 and Table 3.3.1. 13 sites show a distinct depletion in ^{15}N over background values in the upper 10cm of the sediment core. Three sites show changes that are equivocal showing long-term declines or reversals to pre-industrial values and a further three sites show no overall change or an increase in $\delta^{15}N$ that is inconsistent with the wider patterns of change in N isotopes observed in other N hemisphere lakes.

The consistent pattern of change points to a widespread driver of the biogeochemical changes observed. The changes observed in UK lakes are the same in nature as those observed in alpine, boreal and arctic lakes throughout the N hemisphere, as summarised in Holgrieve *et al.* (2011). The observed depletion in ^{15}N is of the order of -1 to -2‰ in the majority of sites. At some sites the change is less than this (~ -0.5‰) but this is beyond the level of analytical uncertainty in the estimation of the N isotope data.

Table 3.3.1: Summary of changes in $\delta^{15}N$ of bulk organic matter in the 19 UK lakes shown in Fig. 3.3.3

Sites showing ^{15}N Depletion		Equivocal Sites	Sites with no ^{15}N depletion
Burnmoor Tarn	Round Loch of Glenhead	Blue Lough	Llyn Nadroedd
Llyn Gwngu	Scoat Tarn	Loch Borralie	Loch nan Eun
Loch Coire Fionnaraich	Wastwater	Llyn Llagi	Loch Bealach na h'Uidhe
Loch Coire Mhic Fearchair	Llyn Conwy		
Loch nan Eion	Loch Tinker		
Lochnagar	Small Water		

Figure 3.3.3: Sediment bulk organic matter $\delta^{15}N$ profiles for 19 UK upland, oligotrophic lakes

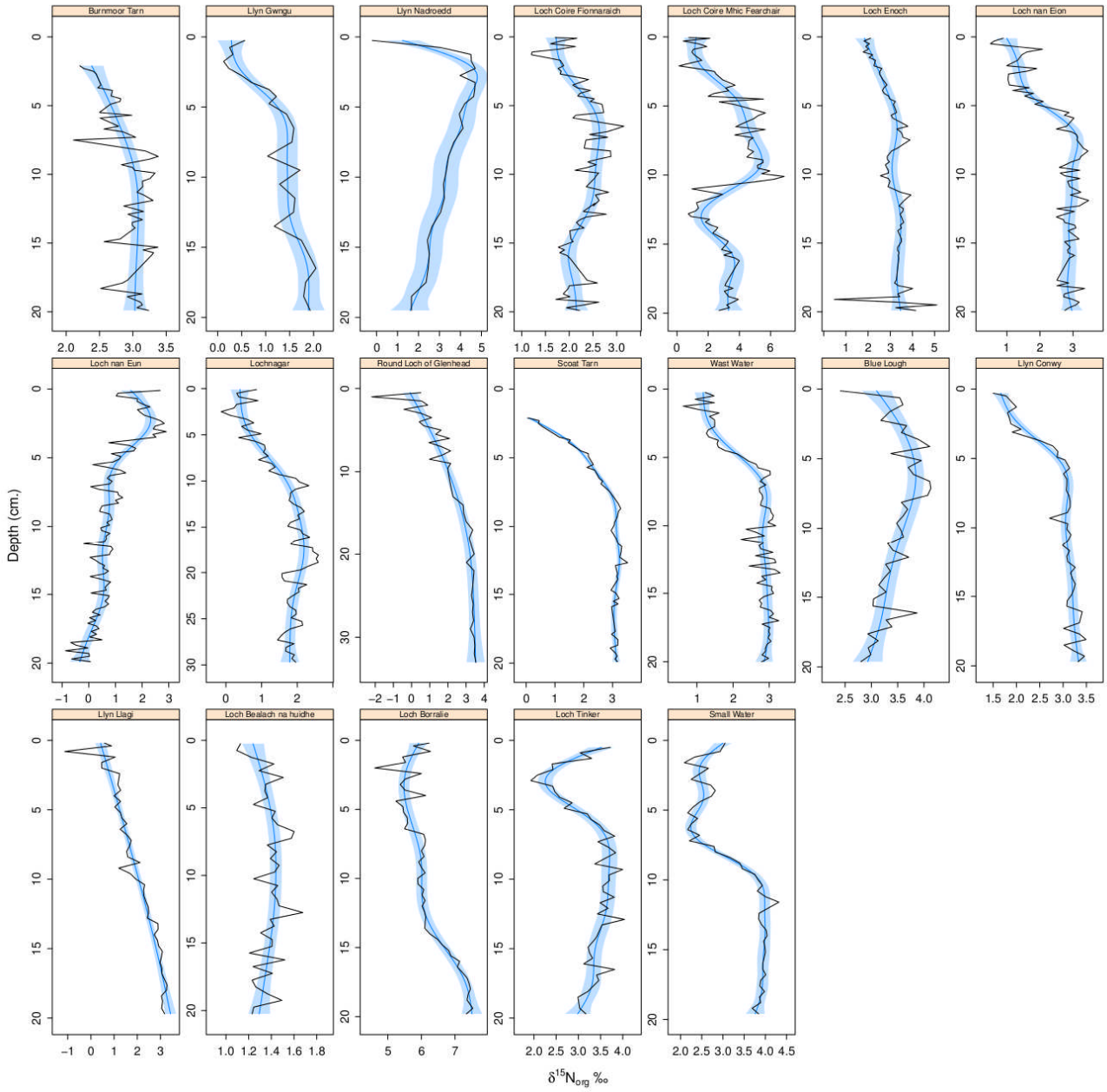
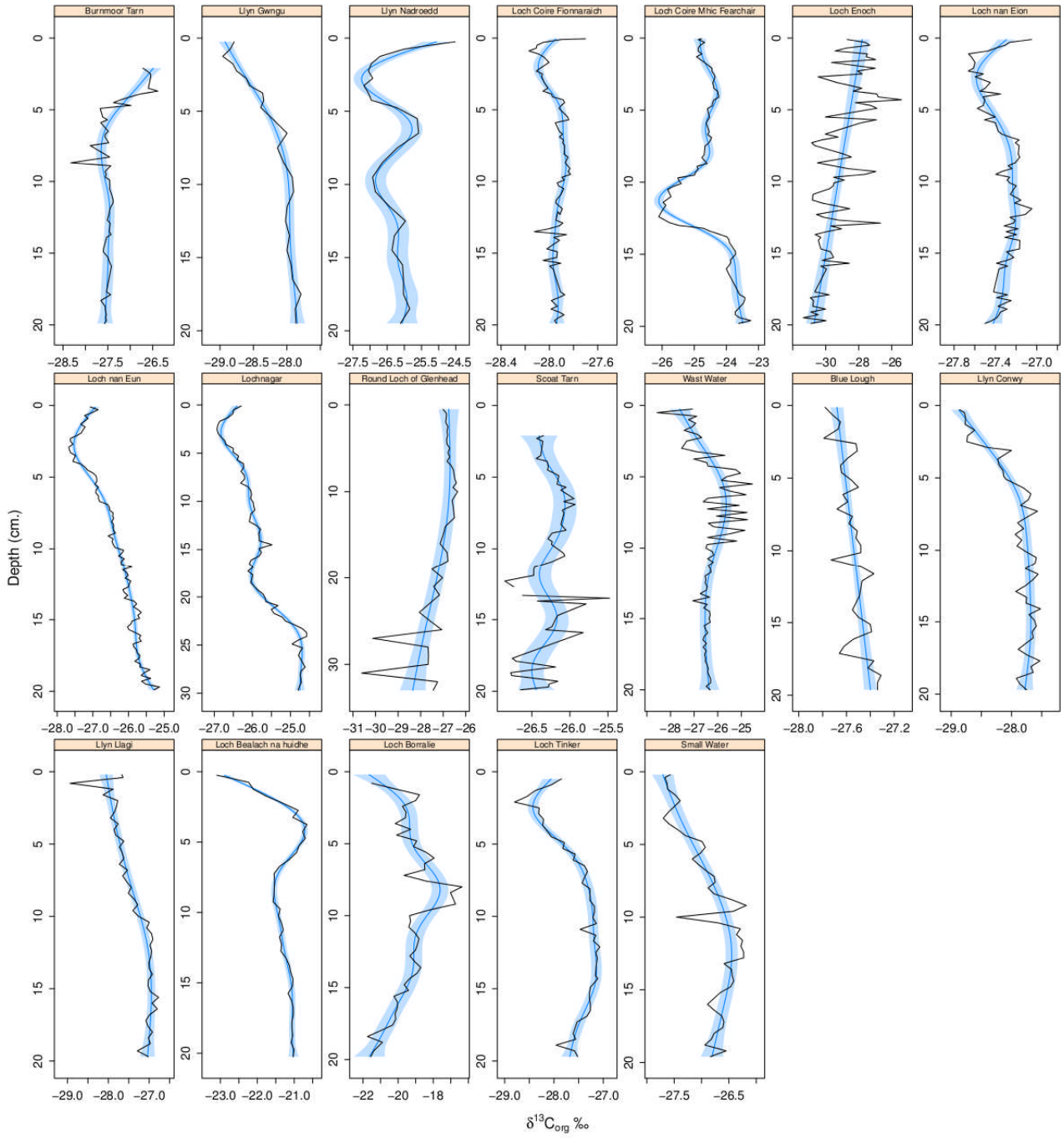


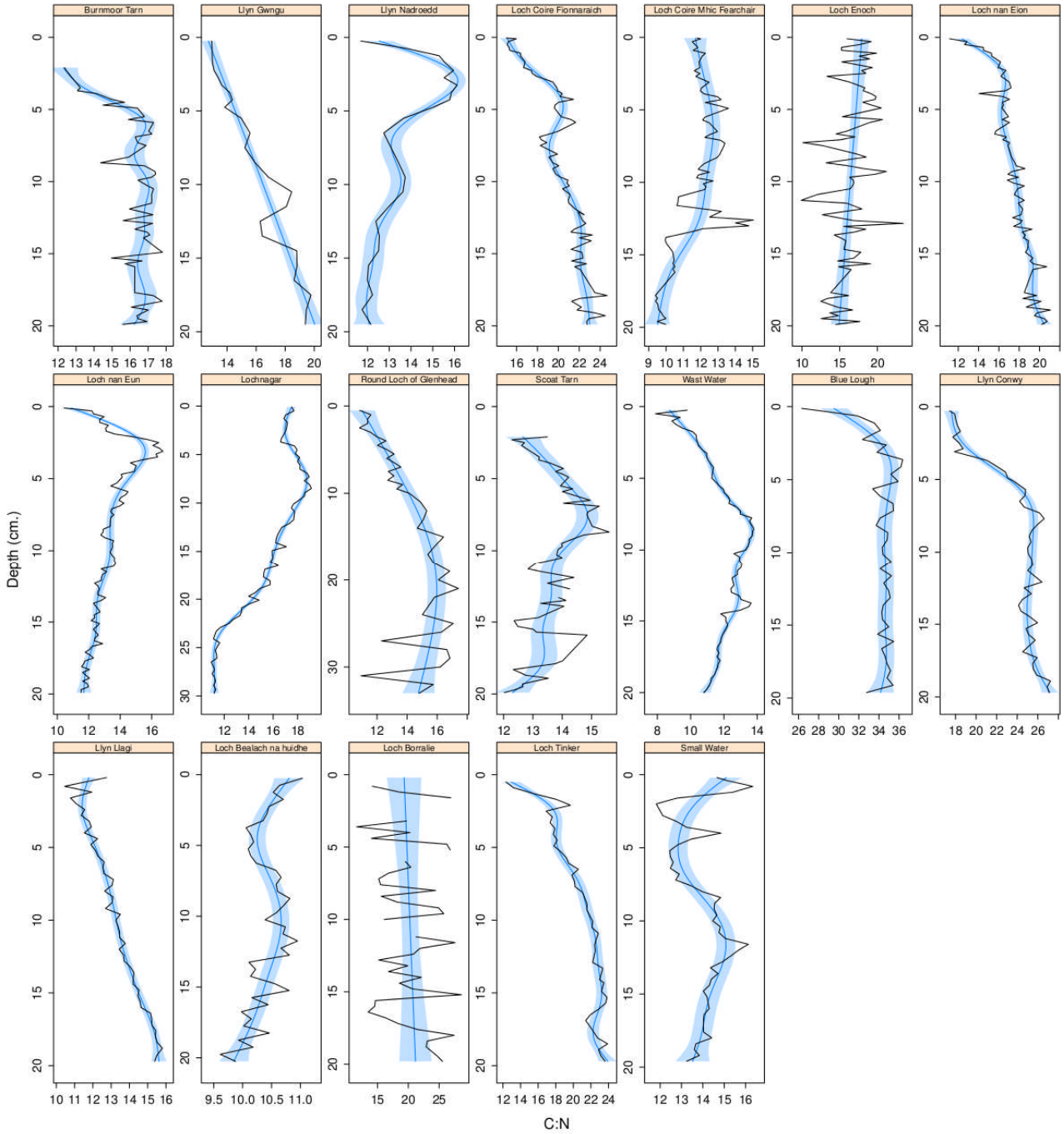
Figure 3.3.4: Sediment bulk organic matter $\delta^{13}C$ profiles for 19 UK upland, oligotrophic lakes



Figures 3.3.4 and 3.3.5 show summary stratigraphic profiles of $\delta^{13}C$ and the carbon to nitrogen ratio (C:N) of bulk organic matter respectively for the 19 UK lakes discussed above. Patterns of change are far more diverse than with $\delta^{15}N$, with a number of sites showing an increase in $\delta^{13}C$, which could be interpreted as a productivity increase as increased algal productivity uses up more of the available DIC pool. The degree of change is small, however. A number of other sites show a depletion in $\delta^{13}C$. This may be explained by a number of factors including increasing contribution of isotopically

light carbon from catchment sources or as the Suess effect arising from the gradual depletion in the $\delta^{13}C$ of atmospheric CO_2 resulting from burning fossil fuels that are derived from isotopically depleted carbon.

Figure 3.3.5: Sediment bulk organic matter atomic carbon to nitrogen ratio (C:N) profiles for 19 UK upland, oligotrophic lakes



Twelve of nineteen sites show declines in C:N suggesting progressively increasing contributions from aquatic sources of organic matter. Lacustrine algae are relatively N

rich owing to low cellulose content and high amino acid and protein content of the algal cell. Consequently they have a low C:N ratio compared with C_3 land plants. The C:N values are generally indicative of a mixture of catchment and lake sources of organic matter. In these twelve sites, the changes in C:N largely parallel the changes in $\delta^{15}N$, suggesting that the disruption in the N cycle is linked to a stimulation of in-lake production of organic matter. This is consistent with the hypothesis that increased deposition of N to these lakes has altered the N biogeochemical cycle of the lakes and has had a fertilizing effect as the enhanced availability of N has supported increased productivity of lake algal populations.

The two new sites studied for fossil algal pigments and isotope biogeochemistry were Small Water and Loch Bealach na h'Uidhe. The N biogeochemistry of Small Water had already been established in the original work programme so as part of the extension work we only analysed the new core for $\delta^{15}N$. This allowed a comparison between the two cores of the $\delta^{15}N$ stratigraphy and to correlate the two cores. The new core was required for the pigment analysis as pigments rapidly degrade when exposed to light, oxygen and higher temperatures than the lake environment from whence they came. A new core was obtained from Loch Bealach na h'Uidhe (NAHU) as this had previously not been studied. This site was chosen after a survey of the available datasets suggested that this site was better buffered with generally high pH for an oligotrophic upland lake. Furthermore, preliminary palaeoecological assessments at the site suggested no acidification had taken place over the past 200 years as a result of acid deposition (Allott *et al.,* 1995). Loch Bealach na h'Uidhe has a pH of 6.08 and ANC of 22 $\mu eq\ l^{-1}$ (Allott *et al.*, 1995). Both C and N isotope analyses were obtained for the previously unstudied NAHU core.

Sediment cores were collected from the deepest part of the lake basin and intact cores in core tubes were sealed and placed inside black bags to avoid photodegredation of pigment. The cores were kept cool and in the dark during transportation back to the laboratory, whereupon they were sectioned at 0.25cm intervals under low light levels. A sub-sample for pigments was taken as the cores were being sub-sectioned. The pigment sub-samples were placed into dark bags and immediately frozen prior to analysis. The remaining sediment was kept refrigerated until analysed. Sediment isotope data were collected following the methods outlined above. Pigment analysis was performed on freeze dried material, which was extracted overnight at $-4^{o}C$ in an 80:15:5 mixture of HPLC grade acetone, methanol and water. Extracts were filtered through a 0.22µm PTFE filter and dried under a constant flow of N_2. Dried extracts were dissolved in a 70:25:5 mixture of acetone, ion pairing reagent (75g tetrabutyl ammonium acetate, 7.7g ammonium acetate in 100ml deionised water) and methanol before being injected into the HPLC. Separation conditions were modified from Mantoura and Llewellyn as described in Leavitt and Hodgson (2001).

Figure 3.3.6: Sediment bulk organic matter $\delta^{15}N$, $\delta^{13}C$ and atomic carbon to nitrogen ratio (C:N) for Loch Bealach na h'Uidhe. The solid blue line is an additive model fitted to the data using maximum likelihood to identify the trend in the sediment data. The grey shaded region is a 95% pointwise confidence interval on the fitted trend.

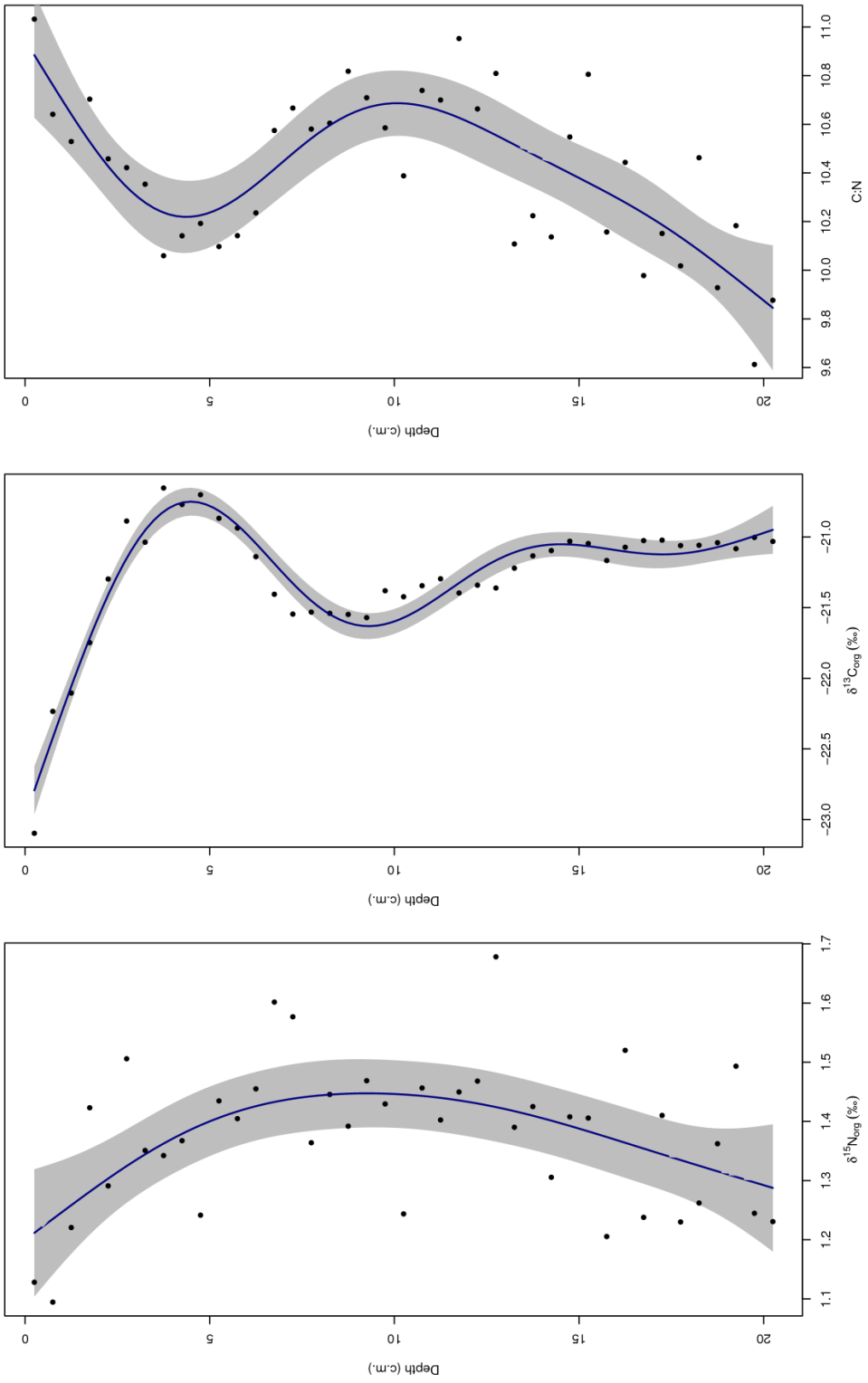


Figure 3.3.6 shows the results of the geochemical analyses on the sediment core from Loch Bealach na h'Uidhe. Whilst there is a tendency for $\delta^{15}N$ to rise during the first half of the core and then decline again, the change in either direction is only about +/-

0.1‰. Therefore there appears to be no overall change in $\delta^{15}N$ in the core. $\delta^{13}C$ varies by about 0.5‰ over the lowermost 15cm of the core before a rapid depletion of -2‰ towards the core top is observed. C:N first increases, then decreases slightly only to rise again. The changes are small, overall there is a one unit change in C:N, the values of which are all consistent with a primarily aquatic source of organic matter comprising the bulk of the sediments. The move to somewhat higher C:N throughout the core suggests a modest increase in catchment inputs of organic matter to the lake.

Loch Bealach na h'Uidhe is located in northwest Scotland (NC 264256), an area of low N deposition. Our initial hypothesis was that Loch Bealach na h'Uidhe would act as a control site, unaffected by the low levels of N deposition. The results of the geochemical analysis would appear to bear this out; in contrast to the vast majority of sites studied, Loch Bealach na h'Uidhe shows no sign of perturbation to its N biogeochemistry over the recent couple of hundred years. As such we do not consider this site further here.

Figure 3.3.7 shows $\delta^{15}N$ profiles for the two cores from Small Water; SMALL1 was the original core collected for N biogeochemical analysis whilst SMALL2 was collected for fossil pigment analysis. The record in SMALL2 extends back somewhat further than that of SMALL1 although this is the result of only analysing the uppermost 20cm of the SMALL1 core in the initial geochemical analyses. The general agreement between the two records affords a degree of confidence in the single $\delta^{15}N$ profiles from the other lakes shown above.

Before the 1940s, sediment bulk organic matter $\delta^{15}N$ was relatively stable at around 3.5 to 4.0‰. SMALL1 is offset from SMALL2 by about 0.5‰. Both cores also record a rapid depletion in $\delta^{15}N$ from the early 1940s to the 1980s. Here the cores diverge to a degree; whilst both record a depletion, that which is recorded in SMALL2 is of greater magnitude (-3.5‰ compared with -2‰ in SMALL1). There are small differences in the uppermost sections of the two cores, with SMALL2 recording a brief enrichment in ^{15}N in the mid 1980s before falling to a stable minimum in ^{15}N of 0‰ by the early 1990s. SMALL1 records a rapid decline and then stable conditions, not capturing the brief enrichment seen in SMALL2.

Figure 3.3.8 shows the fossil pigment concentrations in the SMALL2 core from Small Water. Pigment biomarkers of siliceous algae (fucoxanthin, diatoxanhtin), cryptophytes (alloxanthin), chlorophytes (chlorophyll *b*, pheophytin *b*, lutein) and cyanobacteria (zeaxanthin [in the Lutein curve], myxozanthophyll, echinenone, canthaxanthin) are present in the core, as well as pigments produced by all algae (chlorophyl *a*, pheophytin *a* and *β* carotene). Pigment concentrations are generally low for all groups in the lower part of the core below 12cm. Above this level were substantial increases in all algal groups; lutein and chlorophyll *b* increase reaching a peak around 4cm and decline again, to be replaced by increased concentrations of siliceous algae (diatoxanthin) and chryptophytes (alloxanthin). Echinenone,

myxoxanthophyll and canthoxanthin, where previously present in low concentrations, increase rapidly and remain high throughout the upper 10cm of the core indicating abundant cyanobacterial populations in the lake at this time. The general algal biomarkers indicate a rapid shift to high productivity at the same time that all algal groups increase. The period of rapid increase in a number of algal groups and general increase in algal productivity occurs concomitantly with the strong depletion observed in $\delta^{15}N$ in the bulk organic matter. The results are indicative of a stimulation of algal productivity and alteration of the algal composition in the Small Water by N deposition beginning in the mid to late 1940s.

Figure 3.3.7 : Sediment bulk organic matter $\delta^{15}N$ profiles for the two cores from Small Water. SMALL1 is the original core collected in the first part of the current research programme. SMALL2 is the new core collected for fossil pigment analysis.

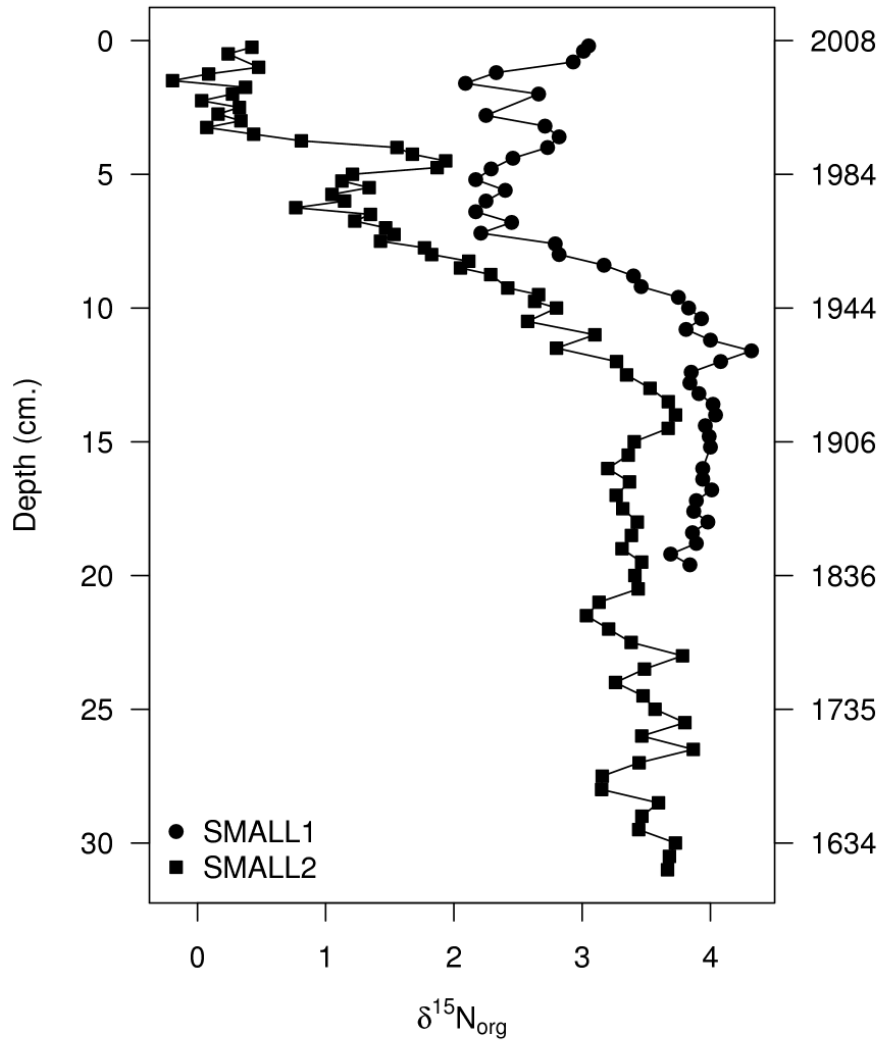


Figure 3.3.8: Fossil algal pigments concentrations (nmol g^{-1} organic sediment) in the SMALL2 core

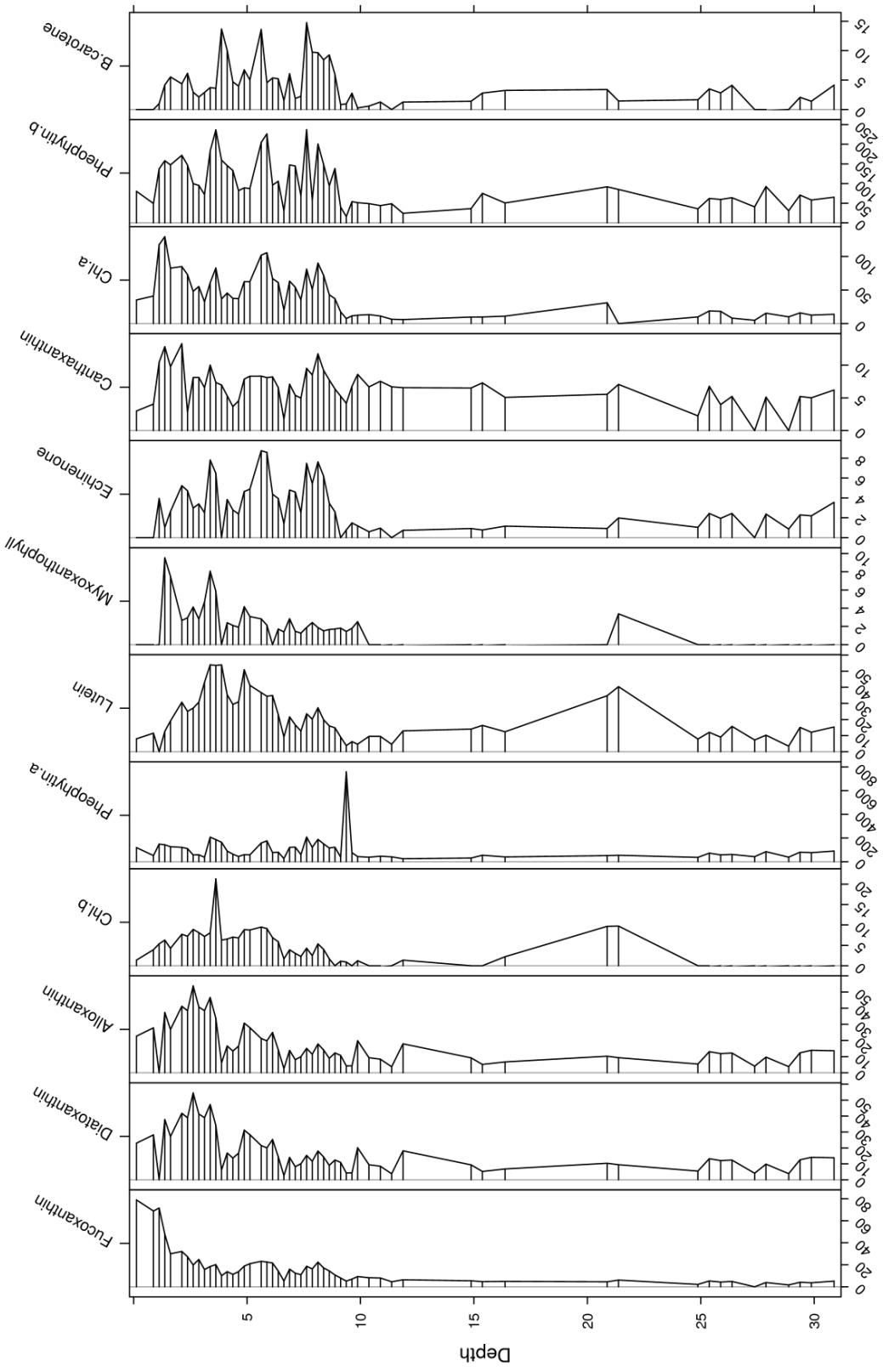
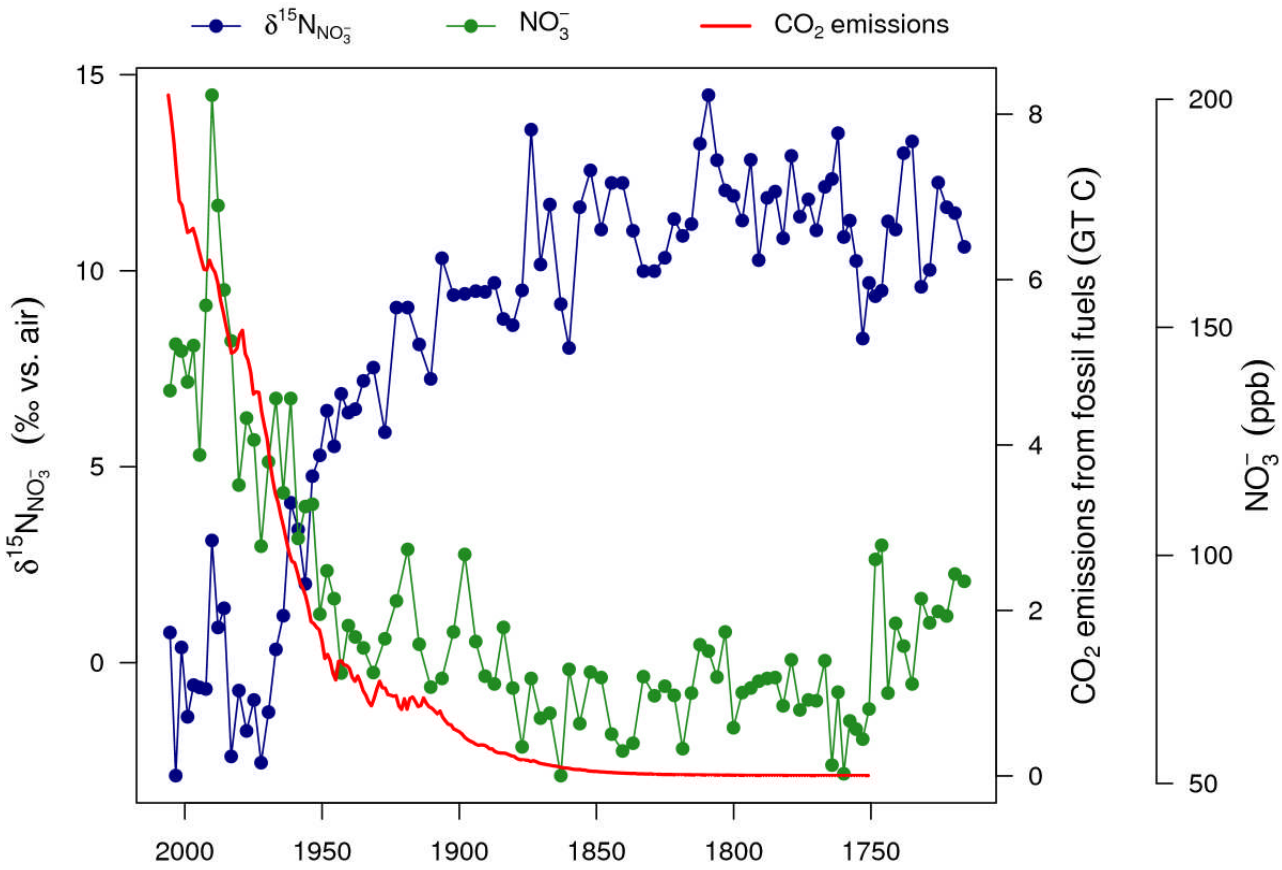


Figure 3.3.9: Nitrate concentration (ppb) and $\delta^{15}N$ of nitrate from the Greenland Summit ice core overlain with global anthropogenic CO_2 emissions



The results reported above point to a widespread alteration of the N biogeochemistry of upland lakes throughout the UK and a stimulation of in-lake primary productivity. Fossil pigments in Small Water increased markedly at the same time the N-isotopic composition shows a depletion in ^{15}N. Both events occurred in the mid to late 1940s. These results are consistent with the hemisphere-wide changes in N biogeochemistry reported for Arctic, alpine and boreal lakes in the N hemisphere. Figure 3.3.9 shows a nitrate concentration and $\delta^{15}N\text{-}NO_3^-$ record from the Greenland Summit ice core (Hastings *et al.*, 2009). The Summit ice core records a significant increase in nitrate concentration beginning in the late 1800s and early 1900s. The isotopic composition of deposited nitrate also underwent a substantial depletion in $\delta^{15}N$ falling from a pre-industrial average of ~11‰ to around 0‰ by the 1970s and has remained at this low level since. The cause of the depletion in atmospheric nitrate is thought to be burning of fossil fuels as the changes closely track anthropogenic CO_2 emissions (Hastings *et al.*, 2009) as well as the widespread increase in fertilizer usage (which has a $\delta^{15}N$ of ~0 permille) (Holtgrieve *et al.*, 2011). However, the mechanisms that might result in isotopically light nitrate are not clear and need further work to elucidate (Heaton T.H.E. pers. comm.).

The change in the ice-core nitrate mirrors the timing of the N-isotopic change in several sites, including Lochnagar where the observed ^{15}N depletion occurs earlier around 1900 compared to other dated records such as Small Water and Llyn Conwy. A similar variation in the timing of the onset of N-isotopic depletion in bulk organic matter is reported by Holtgrieve *et al.* (2011), with high-latitude lakes showing an earlier onset than more southerly alpine sites included in their synthesis. This might indicate buffering of incoming anthropogenic N deposition by deeper, more developed catchment soils in the southerly sites compared to the Arctic sites with less well developed soils and limited catchment vegetation. In the UK, we might expect alpine lakes such as Lochnagar to respond earlier to increased N deposition than lower altitude sites such as Small Water and Llyn Conwy as the shallow soils of Lochnagar limit the degree to which the additional N can be taken up by catchment vegetation and processed within soils. Such a mechanism would explain the differential onset dates observed by Holtgrieve *et al.* (2011) and in the results reported above. Further work will be required to assess this hypothesis.

Holtgrieve *et al.* (2011) hypothesised that the switch to isotopically lighter organic matter is due directly to the large depletion in atmospheric nitrate as recorded in the Summit ice core, resulting in the mixing of an isotopically light source of nitrate that is utilised by organisms, thus resulting in organic matter becoming progressively lighter over time as a result and which is recorded in the sediment records. This hypothesis gives little role to transformations of deposited N in both lakes and catchment soils and to the stimulation of in-lake and catchment productivity. The sediment trap data reported in Task 3.1 and Task 3.2 point to a more complex nature of the relationship between $\delta^{15}N\text{-}NO_3^-$ in deposition, $\delta^{15}N\text{-}NO_3^-$ in lake waters and $\delta^{15}N$ of sediment organic matter.

An alternative hypothesis to explain the delayed onset of change in the N-isotopic composition in some sites is related to the potential for anthropogenic N deposition to have forced a switch from N limitation of these lakes to P limitation or co-limitation by both N and P. As the available pool of nitrate increases as other nutrients are now limiting, fractionation of the nitrate pool can occur to full effect and the $\delta^{15}N$ of the resulting organic matter will become gradually lighter over time as algae preferentially utilize the lighter N isotope. This would also explain the delayed onset of the depletion in the $\delta^{15}N$ of bulk organic matter as lakes with more developed catchment soils and vegetation would buffer lakes for longer from the effects of enhanced N deposition.

Whilst the exact mechanisms that give rise to the observed changes in N biochemistry of lakes throughout the N hemisphere still require further work to elucidate their true nature, what is clear is that both very sensitive Arctic and alpine lakes and less-sensitive upland lakes in the UK have been affected by increased N deposition from anthropogenic sources. In the UK lakes studied here, we have demonstrated a widespread alteration of the N biogeochemistry of 13 of the 19 lakes considered.

Fossil pigment data from Small Water suggest wholesale increases in algal production in the lake, a change that is concomitant with the onset of change in the N-isotopic composition of bulk organic matter. Other fossil pigment data from Round Loch of Glenhead and Loch Coire Fionnaraich also show increased algal production associated with the depletion in ^{15}N in bulk organic matter (McGowan S, pers comm.).

The results presented above are compelling and indicate a widespread effect of elevated N deposition to nutrient poor lakes. The effects are both biogeochemical as well as biological. Further work is required to confirm the scope of change in lake algal groups in additional lakes where i) alterations of the N-biogeochemistry are observed and ii) sites are not subject to the confounding effects of acidification.

Summary

1. Analysis of $\delta^{15}N$ in 13 of 19 lake sediment cores taken from upland lakes indicate changes to N biogeochemistry which in most cases coincide with changes in sediment C:N ratios suggesting a stimulation of in-lake production.
2. The timing of change varies between sites and may be linked to differences in the onset of significant nitrate leaching due to differing catchment soils and vegetation.
3. Analysis of fossil pigments in one site which does not suffer from the confounding effects of acidification indicates wholesale increases in algal production comcomitant with changes in sediment $\delta^{15}N$.
4. These studies provide compelling evidence for a widespread effect of N deposition on the biogeochemistry and biology of oligotrophic lakes in the UK as found in other studies of remote alpine and Arctic lakes.

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Work package 4: Improved understanding of nitrate leaching pathways for model development

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Task 4.1: Dual isotope ($\delta^{15}N$- and $\delta^{18}O$-NO_3^-) and $\delta^{15}N$-NH_4^+ studies at 19 mid-level study catchments including one in Northern Ireland (quarterly)

Aims

The primary aim of this task was to upscale the dual isotope work carried out under the previous Freshwater Umbrella programme to determine the direct contribution of atmospheric NO_3^- to upland surface waters beyond the four sites studied previously.

Under this Task, the dual isotope work was expanded to cover 15 AWMN sites, plus one additional study site with excellent catchment-scale and limnological data (Llyn Conwy), one ECN site (Moor House) and two new calcareous sites (Borralie, Small Water) to increase the study gradient to sites without the confounding risk of acidification. The study includes all unforested AWMN catchments in Great Britain, one in Northern Ireland (Blue Lough) plus two of the most N saturated and intensively studied forested catchments in the UK (Loch Grannoch and Afon Hafren).

Methods

To upscale the work affordably, sampling frequency was at the minimum possible level to capture the seasonal variations in NO_3^- leaching and hydrological NO_3^- contributions which were demonstrated in phase 1 of the Freshwater Umbrella programme. Sites were sampled quarterly over one year for the following analyses:

1. $\delta^{15}N$-NH_4^+ and dual isotopes for NO_3^- in bulk deposition;
2. dual isotopes for NO_3^- in streamwaters, with both a major inflow and outflow for lakes;
3. $\delta^{18}O$-H_2O for soilwaters

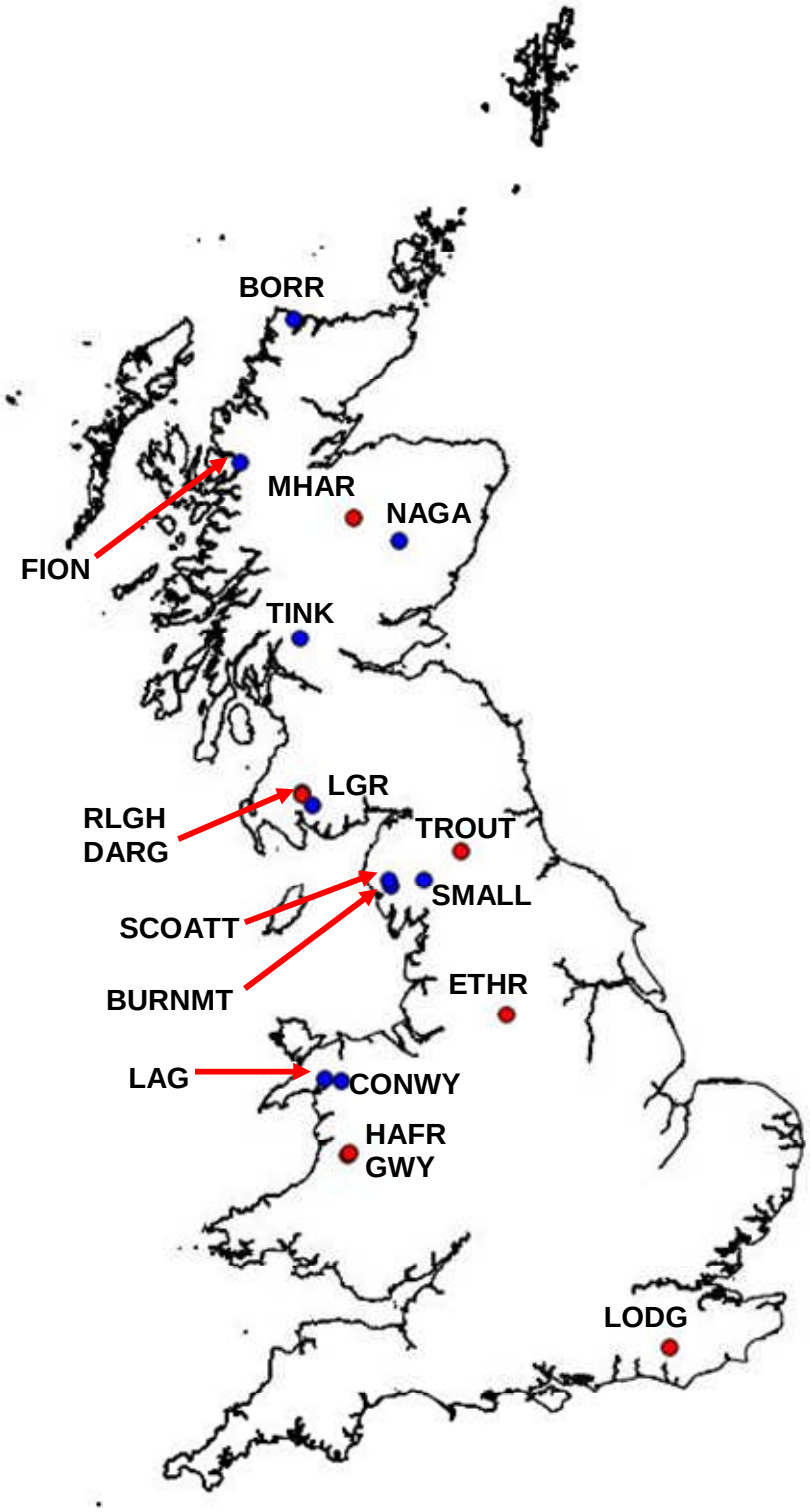
The final choice of sites for the dual isotope work (Table 4.1.1) was made from a shortlist in the first quarterly report following email discussion during early 2008. It was agreed that there was sufficient justification to retain Loch Borralie as one of the two calcareous, non acid-sensitive sites. However, the second non-sensitive site was changed from the initial proposal of Sunbiggin Tarn to the more representative and previously studied CLAG nitrogen network site Small Water in the English Lake District.

Construction of all field equipment was completed by February 2008 and deployment was completed by May 2008. 19 high volume (10L) bulk deposition collectors (Fig. 4.1.1-4.1.2) and 93 porous cup soilwater suction samplers (3 for streams, 3 inflow + 3 outflow for lakes) were deployed (Table 4.1.1).

All quarterly field campaigns were completed in Spring 2009. The sampling campaign was very successful with minimal loss of samples. One bulk deposition sample was lost from Borralie in the third quarter due to theft of the funnels on the bulk deposition collector. Suction samplers performed very well at most plots with only a few failing to get regular samples. Suction samplers were deployed with three

replicates per plot to minimize the risk of sampling failure at any plot, with a total of 93 samplers (6 each at 12 lake sites = 72, 3 each at 7 stream sites=21). Between 80-84 samples were successfully collected in each quarterly campaign with no sites failing to provide at least one sample.

Figure 4.1.1: Location of lake (●) and stream (●) sites in dual isotope study



Results

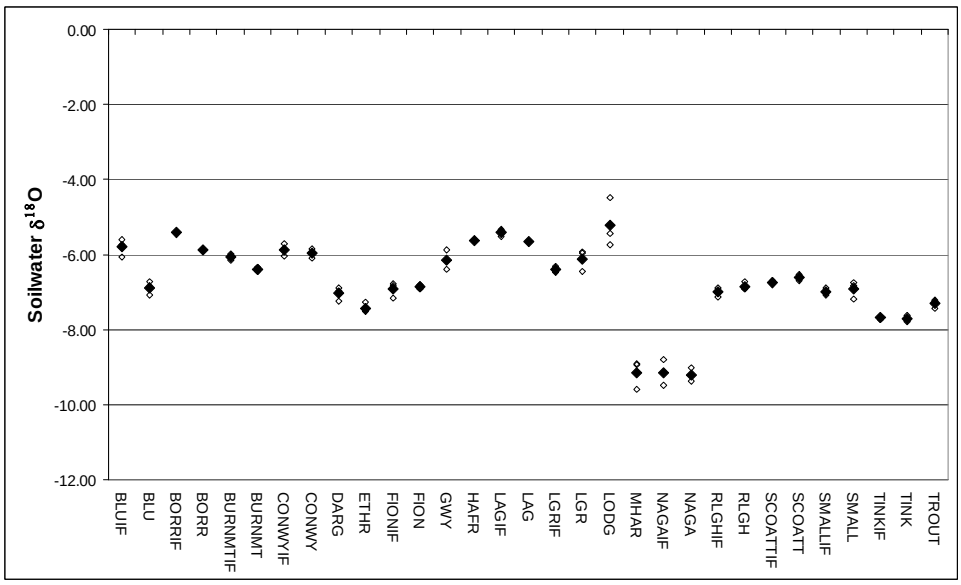
Isotopic analysis results are presented below. Soilwater $\delta^{18}O$ data (to characterise oxygen source for nitrifiers) show that there is little within-site variation in $\delta^{18}O$ of soilwaters but there are regional differences in the natural abundance values, ranging from c. -5‰ at Old Lodge to -9‰ at the Allt a'Mharcaidh and Lochnagar (Figure 4.1.3).

Table 4.1.1.: Study sites for Task 4.1 (n=19)

Code	Site	Type	Easting	Northing	Date Installed	Suction samplers
BLU	Blue Lough	Lake	332700	325200	07-May-08	6
BORR	Loch Borralie	Lake	238100	966800	04-May-08	6
BURNMT	Burnmoor Tarn	Lake	318400	504300	29-Apr-08	6
CONWY	Llyn Conwy	Lake	278012	346180	08-May-08	6
FION	Loch Coire Fionnaraich	Lake	194500	849800	03-May-08	6
LAG	Llyn Llagi	Lake	264800	348400	08-May-08	6
LGR	Loch Grannoch	Lake	254100	569800	05-May-08	6
NAGA	Lochnagar	Lake	325200	785900	02-May-08	6
RLGH	Round Loch of Glenhead	Lake	245000	580400	05-May-08	6
SCOATT	Scoat Tarn	Lake	315900	510400	30-Apr-08	6
SMALL	Small Water	Lake	345500	510000	29-Apr-08	6
TINK	Loch Tinker	Lake	244500	706800	01-May-08	6
DARG	Dargall Lane	Stream	244900	578600	05-May-08	3
ETHR	River Etherow	Stream	411600	399600	28-Apr-08	3
GWY	Afon Gwy	Stream	282400	285400	09-May-08	3
HAFR	Afon Hafren	Stream	284400	287600	09-May-08	3
LODG	Old Lodge	Stream	545600	129400	14-May-08	3
MHAR	Allt a'Mharcaidh	Stream	288100	804500	04-May-08	3
TROUT	Trout Beck	Stream	375800	533500	15-May-08	3

Figure 4.1.2: High volume bulk deposition samplers for NH_4^+ and NO_3^-, Loch Coire Fionnaraich

Figure 4.1.3: Soilwater $\delta^{18}O$-H_2O from lake inflow, -outflow and stream sites, June 2008



Analysis of $\delta^{18}O$ and $\delta^{15}N$ in bulk deposition NO_3^- show a consistent pattern of depleted ^{15}N and highly enriched ^{18}O values across the UK (Fig. 4.1.4). $\delta^{15}N$ varies from -8.2‰ to -1.5‰ while $\delta^{18}O$ varies from +62.3‰ to +79.0‰. The highly elevated $\delta^{18}O$ of bulk deposition NO_3^- across the country confirms the applicability of the method for determination of depositional contributions to leached NO_3^-, since microbially produced NO_3^- has much lower $\delta^{18}O$ values.

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:

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

The observed range in soilwater $\delta^{18}O$-H_2O (Fig. 4.1.3) suggests microbial $\delta^{18}O$-NO_3^- values of +1.7 to +4.3‰.

Using a two end-member mixing model (theoretical microbial $\delta^{18}O$-NO_3^- and measured bulk deposition $\delta^{18}O$-NO_3^-) the proportion of untransformed atmospheric NO_3^- in surface water samples may be calculated (Table 4.1.2; Fig. 4.1.5):

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

While a few samples were too small to provide sufficient NO_3^- for dual isotope analysis, most were sufficient and show a varying proportion of 0 to 63% of atmospheric NO_3^-. The highest proportion was found in Burnmoor Tarn inflow stream, which drains the rocky slopes of Sca Fell and suffers extreme hydrological events where it bursts its banks and flows overland. The next highest proportion was found in Llyn Llagi, with 28% of untransformed atmospheric NO_3^- in the lake compared with zero in its inflow stream. This difference probably reflects the importance of both direct deposition to lake surfaces and the "memory" of lakes with long hydrological residence times compared to inflow streams.

Raw data from the quarterly dual isotope analysis of NO_3^- in bulk deposition, lakes (plus inflows) and streams, plus $\delta^{15}N\text{-}NH_4^+$ of bulk deposition, are presented in Tables 4.1.2a-d. Soilwater suction sampler data (3 replicates per site), used to provide $\delta^{18}O\text{-}H_2O$ for calculation of theoretical microbial $\delta^{18}O\text{-}NO_3^-$, are also shown. Blanks indicate no sample obtained. Yellow highlighting indicates missing or unreliable data from small samples. Grey shading indicates where NO_3^- concentrations were too low to obtain sufficient sample for isotopic analysis.

Although some suction samplers performed intermittently, our strategy of having three replicates per site was vindicated by there being no instances of all three failing, i.e. there are no site visits without at least one soilwater $\delta^{18}O\text{-}H_2O$ value. In any case, it can be seen from the raw data that there is very little within-site variation in the derived microbial $\delta^{18}O\text{-}NO_3^-$ value based on soilwater $\delta^{18}O\text{-}H_2O$, and only minor between-site variation. Since the two end-member mixing model used to calculate the proportion of atmospheric NO_3^- uses the difference between microbial and measured atmospheric $\delta^{18}O\text{-}NO_3^-$ the error in this difference associated with variations in soilwater $\delta^{18}O\text{-}H_2O$ (range = 1.5 to 4.8‰) is of the order of only a few percent (deposition $\delta^{18}O\text{-}NO_3^-$ range = c. 55-81‰; difference in the range 50-79‰). However, where the $\delta^{18}O\text{-}NO_3^-$ of surface water samples is very close to the microbial value (i.e. very low atmospheric NO_3^- signal), there is much greater uncertainty associated with estimates of atmospheric NO_3^-, i.e. estimated proportions of atmospheric NO_3^- of less than about 10% may not be significantly different from zero.

Atmospheric contributions of untransformed NO_3^- are plotted separately for lakes (with their inflows) and streams in Figs. 4.1.4a-b. A common scale is used for comparison. Several sites show no measurable contribution of atmospheric NO_3^-; Borralie, Allt a'Mharcaidh and inflow streams at several lakes (Llyn Conwy, Llyn Llagi and Loch Tinker). Most values of >20% atmospheric are observed in lakes, with inflow stream values only exceeding lake values at Blue Lough and Burnmoor Tarn (Fig. 4.1.4a). In almost all cases, a higher proportion of atmospheric NO_3^- is found in lakes than inflow streams, suggesting that direct deposition to lake surfaces provides an important route for the input of untransformed atmospheric NO_3^-. Of the stream sites, only the September sample from Dargall Lane and the March sample from Old Lodge exceed 10% atmospheric NO_3^- and the majority of values are probably not

statistically different from zero. However, the highest calculated proportion of atmospheric NO_3^- was a value of 68% calculated for the inflow stream at Burnmoor Tarn in the June sample, warranting further investigation (e.g. whether there was very high rainfall and overland flow at this point).

Table 4.1.2a: Dual isotope analysis results, June 2008

			Suction sampler $\delta^{18}O$-H_2O				Theor.	Surface water NO_3		Deposition NO_3		%	Dep. NH_4
Sitename	**Sitecode**	**Date**	**S1**	**S2**	**S3**	**Mean**	$\delta^{18}O$-NO_3	$\delta^{15}N$	$\delta^{18}O$	$\delta^{15}N$	$\delta^{18}O$	**atm.**	$\delta^{15}N$
Blue Lough	BLUIF	05-Jun-08	-6.07	-5.75	-5.60	**-5.81**	+3.8	-4.8	+19.9	-5.20	+75.6	22.4	-4.0
Blue Lough	BLU	05-Jun-08	-7.08		-6.72	**-6.90**	+3.1	-0.3	+29.2	-5.20	+75.6	36.0	-4.0
Loch Borralie	BORRIF	31-May-08			-5.41	**-5.41**	+4.1	+2.5	-0.6	-5.1	+66.9	-7.4	+2.8
Loch Borralie	BORR	31-May-08		-5.87	-5.87	**-5.87**	+3.8	Too small		-5.1	+66.9		+2.8
Burnmoor Tarn	BURNMTIF	28-May-08	-5.99	-6.16		**-6.08**	+3.6	-2.8	+46.2	-3.8	+71.5	62.7	-0.6
Burnmoor Tarn	BURNMT	28-May-08	-6.41	-6.35	-6.46	**-6.41**	+3.4	-1.9	+23.3	-3.8	+71.5	29.2	-0.6
Afon Conwy	CONWYIF	06-Jun-08	-5.72	-6.04	-5.84	**-5.87**	+3.8	Too small		-5	+78.1		-3.8
Afon Conwy	CONWY	06-Jun-08	-6.08	-5.85	-5.95	**-5.96**	+3.7	-2.1	+20.9	-5	+78.1	23.1	-3.8
Dargall Lane	DARG	03-Jun-08	-6.89	-6.96	-7.25	**-7.03**	+3.0	Too small		-6.6	+79.0		-4.6
River Etherow	ETHR	26-May-08	-7.53	-7.50	-7.27	**-7.43**	+2.7	+2.6	+7.9	-0.1	+71.5	7.5	-4.0
Afon Gwy	GWY	06-Jun-08	-6.39		-5.89	**-6.14**	+3.6	Too small		-4.7	+73.4		-3.6
Afon Hafren	HAFR	06-Jun-08	-5.62			**-5.62**	+3.9	-4.8	+10.3	-1.5	+74.4	9.1	-3.0
Llyn Llagi	LAGIF	06-Jun-08	-5.53	-5.33	-5.34	**-5.40**	+4.1	-0.1	+0.7	-3.2	+76.8	-4.6	-3.8
Llyn Llagi	LAG	06-Jun-08	-5.65			**-5.65**	+3.9	-2.3	+24.0	-3.2	+76.8	27.6	-3.8
Loch Grannoch	LGRIF	03-Jun-08	-6.45	-6.31	-6.47	**-6.41**	+3.4	Too small		-6.9	+77.9		-3.5
Loch Grannoch	LGR	03-Jun-08	-5.95	-6.46	-5.94	**-6.12**	+3.6	-0.6	+15.4	-6.9	+77.9	15.9	-3.5
Old Lodge Stream	LODG	02-Jun-08	-5.74	-4.47	-5.43	**-5.21**	+4.2	Too small		-4.2	+72.5		-5.8
Allt a' Mharcaidh	MHAR	30-May-08	-8.93	-8.91	-9.59	**-9.14**	+1.6	Too small		-7.6	+76.5		-4.0
Lochnagar	NAGAIF	30-May-08	-8.81		-9.49	**-9.15**	+1.6	-3.3	+12.4	-6.3	+75.0	14.8	-3.6
Lochnagar	NAGA	30-May-08	-9.39	-9.03	-9.21	**-9.21**	+1.5	-2.4	+17.2	-6.3	+75.0	21.3	-3.6
Round Loch of Glenhead	RLGHIF	03-Jun-08	-7.12	-7.02	-6.88	**-7.01**	+3.0	+1.8	+2.8	-7.4	+75.1	-0.3	-4.7
Round Loch of Glenhead	RLGH	03-Jun-08	-6.92	-6.91	-6.73	**-6.85**	+3.1	-2.1	+17.6	-7.4	+75.1	20.1	-4.7
Scoat Tarn	SCOATTIF1	27-May-08	-6.69	-6.76	-6.77	**-6.74**	+3.2	Too small		-2.4	+76.1		-0.7
Scoat Tarn	SCOATTIF2	27-May-08	-6.69	-6.76	-6.77	**-6.74**	+3.2	Too small		-2.4	+76.1		-0.7
Scoat Tarn	SCOATT	27-May-08	-6.52	-6.62	-6.69	**-6.61**	+3.3	Too small		-2.4	+76.1		-0.7
Loch Tinker	TINKIF	02-Jun-08	-7.67	-7.73	-7.66	**-7.69**	+2.5	Too small		-5.9	+73.3		-5.0
Loch Tinker	TINK	02-Jun-08	-7.62	-7.78	-7.73	**-7.71**	+2.5	Too small		-5.9	+73.3		-5.0
Trout Beck	TROUT	29-May-08	-7.26	-7.21	-7.43	**-7.30**	+2.8	-0.5	+7.8	-5.5	+62.3	8.4	-4.1
Loch Coire Fionnaraich	FIONIF	01-Jun-08	-7.15	-6.84	-6.79	**-6.93**	+3.0	-1.6	+3.6	-8.2	+76.2	0.8	-1.0
Loch Coire Fionnaraich	FION	01-Jun-08	-6.83	-6.91	-6.81	**-6.85**	+3.1	Too small		-8.2	+76.2		-1.0
Small Water	SMALLIF	29-May-08		-7.08	-6.89	**-6.99**	+3.0	-0.5	+8.6	-3.3	+75.3	7.7	-2.7
Small Water	SMALL	29-May-08	-6.82	-7.19	-6.76	**-6.92**	+3.1	-0.3	+13.8	-3.3	+75.3	14.9	-2.7

Table 4.1.2b: Dual isotope analysis results, September 2008

			Suction sampler $\delta^{18}O$-H_2O				Theor.	Surface water NO_3		Deposition NO_3		%	Dep. NH_4
Sitename	**Sitecode**	**Date**	**S1**	**S2**	**S3**	**Mean**	$\delta^{18}O$-NO_3	$\delta^{15}N$	$\delta^{18}O$	$\delta^{15}N$	$\delta^{18}O$	**atm.**	$\delta^{15}N$
Blue Lough	BLUIF	10-Sep-08	-6.42	-5.69	-5.59	**-5.90**	+3.7	Too small		-1.6	+72.3		+0.7
Blue Lough	BLU	10-Sep-08			-6.61	**-6.61**	+3.3	+0.1	+18.7	-1.6	+72.3	22.4	+0.7
Loch Borralie	BORRIF	06-Sep-08			-5.35	**-5.35**	+4.1	No sample (dry)		-3.7	+54.2		Too small
Loch Borralie	BORR	06-Sep-08		-5.23		**-5.23**	+4.2	Too small	-	-3.7	+54.2		Too small
Burnmoor Tarn	BURNMTIF	03-Sep-08	-5.99	-6.31	-5.99	**-6.10**	+3.6	+0.5	+9.3	-2.4	+62.0	9.8	-4.4
Burnmoor Tarn	BURNMT	03-Sep-08	-6.17	-6.16	-6.17	**-6.17**	+3.6	Too small		-2.4	+62.0		-4.4
Afon Conwy	CONWYIF	11-Sep-08	-5.91	-5.80	-5.95	**-5.89**	+3.7	Too small		-5.2	+67.1		-5.9
Afon Conwy	CONWY	11-Sep-08	-6.00	-5.93	-5.89	**-5.94**	+3.7	-2.3	+16.6	-5.2	+67.1	20.3	-5.9
Dargall Lane	DARG	09-Sep-08	-6.68	-6.52	-6.29	**-6.50**	+3.3	+1.2	+13.0	-5.0	+67.8	15.0	-4.2
River Etherow	ETHR	01-Sep-08	-6.77	-6.75	-6.96	**-6.83**	+3.1	+2.0	+7.6	-1.4	+71.7	6.5	+2.1
Afon Gwy	GWY	12-Sep-08	-5.85		-5.95	**-5.90**	+3.7	+1.3	+6.6	-4.0	+64.5	4.7	-5.3
Afon Hafren	HAFR	12-Sep-08	-5.50		-5.41	**-5.46**	+4.0	+2.0	+2.7	-4.0	+64.3	-2.2	+0.7
Llyn Llagi	LAGIF	11-Sep-08	-5.60	-5.60	-5.68	**-5.63**	+3.9	-1.1	+2.4	-3.9	+64.3	-2.5	-3.3
Llyn Llagi	LAG	11-Sep-08	-5.86	-5.95	-5.93	**-5.91**	+3.7	-3.2	+8.7	-3.9	+64.3	8.2	-3.3
Loch Grannoch	LGRIF	08-Sep-08	-6.50	-6.33	-6.43	**-6.42**	+3.4	Too small		-3.0	+67.2		-4.0
Loch Grannoch	LGR	08-Sep-08	-6.05	-6.34	-5.98	**-6.12**	+3.6	-1.0	+12.3	-3.0	+67.2	13.7	-4.0
Old Lodge Stream	LODG	17-Sep-08	-5.65	-5.68	-5.55	**-5.63**	+3.9	Too small		-2.9	+67.0		-1.9
Allt a' Mharcaidh	MHAR	05-Sep-08	-7.77	-7.12		**-7.45**	+2.7	Too small		-5.8	+69.4		+2.2
Lochnagar	NAGAIF	05-Sep-08	-7.10		-9.3	**-8.20**	+2.2	0.0	+4.2	-3.2	+75.3	2.7	-3.1
Lochnagar	NAGA	05-Sep-08	-8.14	-7.71	-9.21	**-8.35**	+2.1	-2.1	+15.9	-3.2	+75.3	18.9	-3.1
Round Loch of Glenhead	RLGHIF	09-Sep-08	-6.67	-6.80	-6.61	**-6.69**	+3.2	+1.9	+4.6	-1.9	+62.6	2.3	-0.3
Round Loch of Glenhead	RLGH	09-Sep-08	-6.73	-6.82	-6.88	**-6.81**	+3.1	-0.6	+18.4	-1.9	+62.6	25.7	-0.3
Scoat Tarn	SCOATTIF1	02-Sep-08	-6.69	-6.65	-6.60	**-6.65**	+3.2	-1.1	+12.7	-1.9	+65.4	15.2	-3.6
Scoat Tarn	SCOATTIF2	02-Sep-08	-6.69	-6.65	-6.60	**-6.65**	+3.2	-1.4	+5.3	-1.9	+65.4	3.3	-3.6
Scoat Tarn	SCOATT	02-Sep-08	-6.36	-6.45	-6.49	**-6.43**	+3.4	-1.2	+11.8	-1.9	+65.4	13.6	-3.6
Loch Tinker	TINKIF	08-Sep-08	-6.91	-6.92	-7.57	**-7.13**	+2.9	Too small		-2.7	+70.0		-5.0
Loch Tinker	TINK	08-Sep-08	-7.61	-7.37	-7.34	**-7.44**	+2.7	-1.6	contaminated	-2.7	+70.0		-5.0
Trout Beck	TROUT	03-Sep-08	-7.21	-7.34	-7.44	**-7.33**	+2.8	Too small		-1.8	+65.6		-7.6
Loch Coire Fionnaraich	FIONIF	07-Sep-08	-5.88	-6.55	-6.23	**-6.22**	+3.5	-0.4	+0.2	-5.6	+65.2	-5.4	-1.7
Loch Coire Fionnaraich	FION	07-Sep-08	-6.72	-6.42	-6.14	**-6.43**	+3.4	-0.5	+7.3	-5.6	+65.2	6.3	-1.7
Small Water	SMALLIF	04-Sep-08		-6.88	-6.67	**-6.78**	+3.2	0.0	+2.4	-0.4	+64.5	-1.2	-1.7
Small Water	SMALL	04-Sep-08	-6.68	-7.00	-6.84	**-6.84**	+3.1	-0.7	+8.1	-0.4	+64.5	8.1	-1.7

Table 4.1.2c: Dual isotope analysis results, December 2008

			Suction sampler $\delta^{18}O$-H_2O				Theor.	Surface water NO_3		Deposition NO_3		%	Dep. NH4
Sitename	**Sitecode**	**Date**	**S1**	**S2**	**S3**	**Mean**	**$\delta^{18}O$-NO_3**	**$\delta^{15}N$**	**$\delta^{18}O$**	**$\delta^{15}N$**	**$\delta^{18}O$**	**atm.**	**$\delta^{15}N$**
Blue Lough	BLUIF	03-Dec-08		-5.52	-5.38	**-5.45**	+4.0	-1.2	+9.6	Too small	+73.4	8.0	-3.0
Blue Lough	BLU	03-Dec-08	-6.34	-6.49	-6.43	**-6.42**	+3.4	-1.0	+10.3	Too small	+73.4	9.9	-3.0
Loch Borralie	BORRIF	29-Nov-08		-5.54	-6.00	**-5.77**	+3.8	+4.2	-1.8	No sample (funnels stc	+60.6	-9.9	-
Loch Borralie	BORR	29-Nov-08	-6.26	-6.13	-6.08	**-6.16**	+3.6	+4.5	+0.3	No sample (funnels stc	+60.6	-5.7	-
Burnmoor Tarn	BURNMTIF	26-Nov-08	-6.28	-5.79		**-6.04**	+3.6	too small		+0.8	+61.5		-8.7
Burnmoor Tarn	BURNMT	26-Nov-08	-6.35	-6.14	-6.27	**-6.25**	+3.5	-0.7	+7.9	+0.8	+61.5	7.6	-8.7
Afon Conwy	CONWYIF	04-Dec-08	-6.06	-5.91	-5.93	**-5.97**	+3.7	too small		-1.6	+65.2		-10.1
Afon Conwy	CONWY	04-Dec-08	-6.36	-6.02	-6.19	**-6.19**	+3.5	-1.5	+13.8	-1.6	+65.2	16.6	-10.1
Dargall Lane	DARG	18-Dec-08	-6.14	-6.45	-6.00	**-6.20**	+3.5	-0.5	+1.0	-1.9	+56.4	-4.8	-7.0
River Etherow	ETHR	24-Nov-08	-7.24	-7.46	-6.99	**-7.23**	+2.8	+0.8	+5.5	-0.2	+81.0	3.4	-1.2
Afon Gwy	GWY	19-Dec-08	-5.37		-5.99	**-5.68**	+3.9	+1.7	+3.6	-4.1	+71.6	-0.4	-9.0
Afon Hafren	HAFR	19-Dec-08	-5.55	-4.98	-5.48	**-5.34**	+4.1	+1.6	+5.3	-3.1	+72.3	1.7	-8.2
Llyn Llagi	LAGIF	04-Dec-08	-5.39	-5.59	-5.62	**-5.53**	+4.0	-3.5	+3.8	-2.0	+62.8	-0.3	-1.8
Llyn Llagi	LAG	04-Dec-08	-5.87	-5.89	-5.92	**-5.89**	+3.7	-2.2	+3.7	-2.0	+62.8	-0.1	-1.8
Loch Grannoch	LGRIF	18-Dec-08	-6.42	-6.29	-6.20	**-6.30**	+3.5	-0.6	+1.4	-0.6	+65.0	-3.4	-5.8
Loch Grannoch	LGR	18-Dec-08	-5.91	-5.82	-5.86	**-5.86**	+3.8	+1.3	+5.6	-0.6	+65.0	3.0	-5.8
Old Lodge Stream	LODG	07-Dec-08	-7.07	-7.15		**-7.11**	+2.9	+1.8	+5.6	+1.9	+69.8	4.0	-3.6
Allt a' Mharcaidh	MHAR	28-Nov-08	-7.31	-7.94	-8.12	**-7.79**	+2.5	too small		-3.2	+64.5		-2.5
Lochnagar	NAGAIF	28-Nov-08	-8.11	-8.52	-9.11	**-8.58**	+1.9	-0.7	+2.8	-0.4	+71.5	1.2	-3.2
Lochnagar	NAGA	28-Nov-08		-7.67		**-7.67**	+2.6	-1.6	+12.2	-0.4	+71.5	14.0	-3.2
Round Loch of Glenhead	RLGHIF	18-Dec-08				**-7.21**	+2.9	+0.9	-0.6	-1.6	+59.9	-6.1	-7.6
Round Loch of Glenhead	RLGH	18-Dec-08				**-6.80**	+3.1	-1.0	+9.2	-1.6	+59.9	10.7	-7.6
Scoat Tarn	SCOATTIF1	25-Nov-08	-6.79	-6.66	-6.88	**-6.78**	+3.1	-0.4	+3.4	+2.4	+62.3	0.4	-5.7
Scoat Tarn	SCOATTIF2	25-Nov-08	-6.79	-6.66	-6.88	**-6.78**	+3.1	+0.1	+1.6	+2.4	+62.3	-2.6	-5.7
Scoat Tarn	SCOATT	25-Nov-08	-6.34	-6.70	-6.55	**-6.53**	+3.3	-0.4	+5.0	+2.4	+62.3	2.9	-5.7
Loch Tinker	TINKIF	01-Dec-08	-6.18	-6.36	-7.15	**-6.56**	+3.3	+2.5	-0.1	-1.5	+67.0	-5.3	-6.2
Loch Tinker	TINK	01-Dec-08	-7.28	-7.13	-5.76	**-6.72**	+3.2	-0.5	+12.1	-1.5	+67.0	14.0	-6.2
Trout Beck	TROUT	27-Nov-08	-7.26	-7.21	-7.44	**-7.30**	+2.8	-1.3	+3.1	Too small	+65.5	0.5	Too small
Loch Coire Fionnaraich	FIONIF	30-Nov-08	-6.51	-6.22	-6.03	**-6.25**	+3.5	-1.3	-1.2	Too small	+70.7	-7.0	Too small
Loch Coire Fionnaraich	FION	30-Nov-08		-6.45	-6.47	**-6.46**	+3.4	-0.5	+0.1	Too small	+70.7	-4.8	Too small
Small Water	SMALLIF	27-Nov-08		-6.83	-6.81	**-6.82**	+3.1	-0.9	-0.2	+1.6	+62.6	-5.6	-4.7
Small Water	SMALL	27-Nov-08	-6.69	-7.02	-6.76	**-6.82**	+3.1	-1.1	+0.7	+1.6	+62.6	-4.1	-4.7

Table 4.1.2d: Dual isotope analysis results, March 2009

			Suction sampler $\delta^{18}O$-H_2O				Theor.	Surface water NO_3		Deposition NO_3		%	Dep. NH4
Sitename	**Sitecode**	**Date**	**S1**	**S2**	**S3**	**Mean**	**$\delta^{18}O$-NO_3**	**$\delta^{15}N$**	**$\delta^{18}O$**	**$\delta^{15}N$**	**$\delta^{18}O$**	**atm.**	**$\delta^{15}N$**
Blue Lough	BLUIF	11-Mar-09		-5.70	-5.54	**-5.62**	+3.9	+0.4	+21.5	-1.7	+72.2	25.7	-0.5
Blue Lough	BLU	11-Mar-09	-7.73	-7.38	-7.02	**-7.38**	+2.7	+0.7	+27.2	-1.7	+72.2	35.2	-0.5
Loch Borralie	BORRIF	07-Mar-09		-3.95	-4.67	**-4.31**	+4.8	+4.2	-2.6	too small	+60.6	-13.3	-7.8
Loch Borralie	BORR	07-Mar-09		-6.37		**-6.37**	+3.4	+4.2	-1.3	too small	+60.6	-8.3	-7.8
Burnmoor Tarn	BURNMTIF	04-Mar-09	-6.41	-9.52		**-7.97**	+2.4	-0.1	+4.5	-0.7	+73.6	3.0	-3.2
Burnmoor Tarn	BURNMT	04-Mar-09	-6.50	-6.91	-6.72	**-6.71**	+3.2	-0.9	+16.0	-0.7	+73.6	18.2	-3.2
Afon Conwy	CONWYIF	12-Mar-09	-6.52	-6.06	-6.16	**-6.25**	+3.5	too small		-2.5	+72.2		-5.4
Afon Conwy	CONWY	12-Mar-09	-7.26	-6.19	-6.70	**-6.72**	+3.2	-1.3	+16.9	-2.5	+72.2	19.9	-5.4
Dargall Lane	DARG	10-Mar-09	-6.98	-6.54	-7.71	**-7.08**	+2.9	+0.3	+5.7	-1.7	+72.3	4.0	CT228 resin
River Etherow	ETHR	30-Mar-09		-7.56	-7.37	**-7.47**	+2.7	+1.8	+9.0	+4.3	+68.5	9.6	-6.9
Afon Gwy	GWY	12-Mar-09	-7.19		-6.14	**-6.67**	+3.2	+2.0	+3.8	-2.6	+67.8	0.9	-9.5
Afon Hafren	HAFR	12-Mar-09	-6.29	-6.81	-6.25	**-6.45**	+3.4	+2.1	+4.5	-3.8	+71.0	1.7	-9.6
Llyn Llagi	LAGIF	12-Mar-09	-6.27	-5.78	-6.48	**-6.18**	+3.5	+1.1	+3.6	-1.0	+54.7	0.1	Too small
Llyn Llagi	LAG	12-Mar-09	-6.02	-6.02	-6.63	**-6.22**	+3.5	-0.7	+10.9	-1.0	+54.7	14.4	Too small
Loch Grannoch	LGRIF	09-Mar-09	-6.34	-6.21	-6.21	**-6.25**	+3.5	+1.3	+4.5	-2.7	+69.9	1.5	-6.7
Loch Grannoch	LGR	09-Mar-09	-5.86	-6.41	-5.76	**-6.01**	+3.7	+1.1	+10.6	-2.7	+69.9	10.5	-6.7
Old Lodge Stream	LODG	04-Mar-09	-8.87	-8.95	-7.71	**-8.51**	+2.0	+0.5	+19.1	+3.4	+68.7	25.6	-4.2
Allt a' Mharcaidh	MHAR	20-Mar-09		-9.51	-8.77	**-9.14**	+1.6			-2.2	+66.4	-2.4	Too small
Lochnagar	NAGAIF	06-Mar-09			-8.91	**-8.91**	+1.7	+0.3	+13.3	-3.0	+71.2	16.7	-3.0
Lochnagar	NAGA	06-Mar-09	-8.91	-8.78		**-8.85**	+1.8	-0.2	+10.5	-3.0	+71.2	12.6	-3.0
Round Loch of Glenhead	RLGHIF	10-Mar-09	-8.04		-7.81	**-7.93**	+2.4	+1.6	+3.9	-1.5	+70.0	2.2	-6.0
Round Loch of Glenhead	RLGH	10-Mar-09	-6.73	-6.79	-6.71	**-6.74**	+3.2	+0.1	+14.5	-1.5	+70.0	17.0	-6.0
Scoat Tarn	SCOATTIF1	03-Mar-09	-6.73	-6.76	-6.85	**-6.78**	+3.1	+0.7	+6.8	+0.9	+75.4	5.1	-3.1
Scoat Tarn	SCOATTIF2	03-Mar-09	-6.73	-6.76	-6.85	**-6.78**	+3.1	+0.4	+9.8	+0.9	+75.4	9.2	-3.1
Scoat Tarn	SCOATT	03-Mar-09	-6.48	-6.65	-5.60	**-6.24**	+3.5	+0.3	+10.8	+0.9	+75.4	10.1	-3.1
Loch Tinker	TINKIF	09-Mar-09	-6.95	-7.34	-7.16	**-7.15**	+2.9	too small		-1.1	+75.9		-3.5
Loch Tinker	TINK	09-Mar-09	-7.33	-7.27		**-7.30**	+2.8	-0.8	+24.5	-1.1	+75.9	29.7	-3.5
Trout Beck	TROUT	05-Mar-09	-7.41	-7.30	-7.48	**-7.40**	+2.7	+1.2	+5.0	-1.0	+68.7	3.4	-7.7
Loch Coire Fionnaraich	FIONIF	08-Mar-09	-6.84	-6.42	-6.57	**-6.61**	+3.3	too small		too small			Too small
Loch Coire Fionnaraich	FION	08-Mar-09	-7.32	-6.26	-6.54	**-6.71**	+3.2	too small		too small			Too small
Small Water	SMALLIF	05-Mar-09	-7.51	-7.22	-6.97	**-7.23**	+2.8	+1.2	+5.4	+0.1	+71.2	3.7	-4.6
Small Water	SMALL	05-Mar-09	-6.85	-7.11	-6.92	**-6.96**	+3.0	-2.1	+11.2	+0.1	+71.2	12.0	-4.6

Figure 4.1.4a: Calculated proportion of atmospheric NO_3^- in lakes and inflows

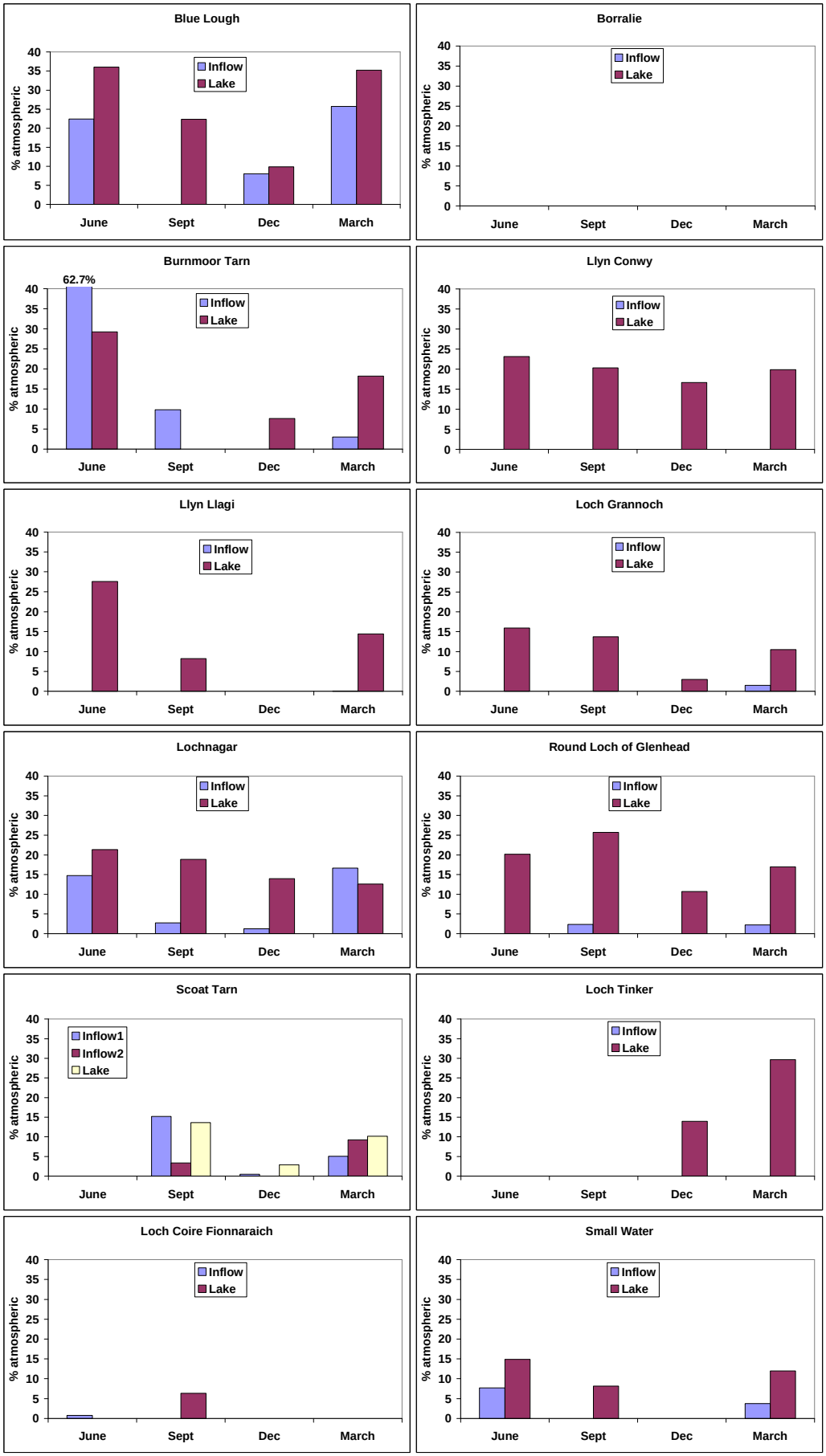
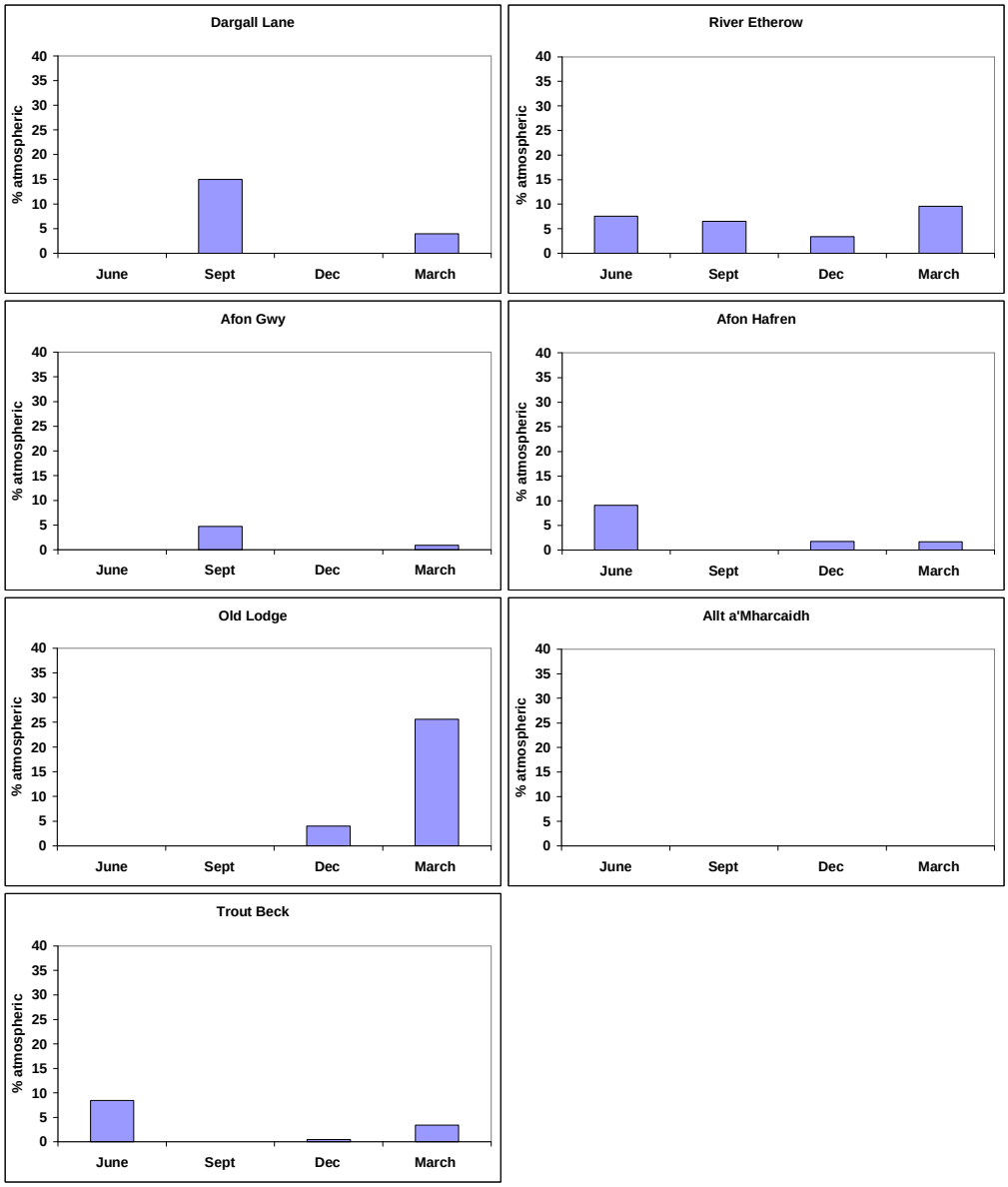


Figure 4.1.4b: Calculated proportion of atmospheric NO_3^- in streams



Spatial and seasonal variation in isotopic composition of bulk deposition N

Previous work under the Freshwater Umbrella provided some of the first measurements of bulk deposition $\delta^{15}N\text{-}NO_3^-$ for the UK at four sites. Under the present contract we have greatly expanded our spatial coverage of bulk deposition isotope measurements and still retained a seasonal component at reduced sampling frequency (quarterly rather than monthly). Preliminary exploration of this unique dataset shows some striking seasonal and spatial variations.

<u>$\delta^{15}N\text{-}NO_3^-$ measurements</u>

Results of $\delta^{15}N$ measurements of NO_3^- in bulk deposition have been grouped into regions (Scotland, England, Wales+N.Ireland) to help identify any spatial patterns in the data.

Results from the eight Scottish catchments (plotted roughly according to location from north to south and east to west) show very consistent seasonal patterns in the $\delta^{15}N$ of NO_3^- (Figure 4.1.5a). In all cases the lowest $\delta^{15}N$ values occur in the June samples and show an increase through to the winter months. There are some differences in the timing of the maximum (least negative) values; they are seen in the December samples for Lochnagar and Loch Grannoch but in the later March samples at the Allt a'Mharcaidh, Loch Tinker, Dargall Lane and Round Loch of Glenhead. At the two northernmost sites in the study, Borralie and Loch Coire Fionnaraich, NO_3^- concentrations in bulk deposition samples were too low for isotopic analysis during December and March but the June and September samples are consistent with the other Scottish sites. The most depleted values of $\delta^{15}N\text{-}NO_3^-$ in the whole study are seen in the June samples from Scottish sites; the only samples (seven) showing $\delta^{15}N$ values of < -6‰ are from Scottish sites.

Five of the six English sites show the same seasonal pattern as the Scottish sites, with minimum values occurring in the June samples and maximum values in December or March (Figure 4.1.5b). The only exception is the River Etherow site which shows a minimum $\delta^{15}N\text{-}NO_3^-$ value in the September sample, but shows the highest minimum value (least depleted in ^{15}N) of all study sites. Only five sites in the whole study show positive $\delta^{15}N$ values for NO_3^- and these all occur in England; at Trout Beck there was insufficient NO_3^- for isotopic analysis in the December sample which showed positive $\delta^{15}N$ values in four of the other English sites.

The four Welsh sites (Fig. 4.1.5c) show less marked seasonal variation and differ from the Scottish and English sites in showing minima in the September samples in three cases (Llyn Llagi, Llyn Conwy and Afon Hafren). At the Afon Gwy, the minimum $\delta^{15}N$ value is seen in the June sample as in Scotland and England, although the range in seasonal variation is the smallest of all sites in the study. The data from the sole site in Northern Ireland, Blue Lough, most closely resemble those from the Scottish sites.

<u>$\delta^{15}N\text{-}NH_4^+$ measurements</u>

Seasonal and spatial patterns are also seen in the isotope data for NH_4^+ but do not follow the same patterns as for NO_3^-. Spatial variations are much greater within each region for $\delta^{15}N\text{-}NH_4^+$ which may reflect the greater importance of local sources in NH_4^+ deposition relative to the longer range transport of NO_3^- deposition.

The Scottish sites generally show depleted $\delta^{15}N\text{-}NH_4^+$ values, although positive values are recorded for the June sample from Borralie and the September sample from the Allt a'Mharcaidh (Fig. 4.1.6a). There is little coherence in seasonal pattern in the Scottish sites, with minimum values being recorded in all quarters for at least one site – although missing data make these generalizations unreliable at present.

In England, the three Lake District sites (Scoat Tarn, Burnmoor Tarn, Small Water) for which data are available show broadly similar seasonal patterns, with minimum $\delta^{15}N\text{-}NH_4^+$ values recorded in the December samples (Fig. 4.1.6b). The patterns at Scoat Tarn and Burnmoor Tarn are particularly similar and may again reflect the dominance of local sources for NH_4^+ deposition, since these sites are very close together. The River Etherow is unique among English sites for showing a positive value for $\delta^{15}N\text{-}NH_4^+$ (September sample) but like the southernmost site, Old Lodge, shows a minimum value in March.

Three of the Welsh sites (Llyn Conwy, Afon Gwy, Afon Hafren) are remarkable for showing the lowest $\delta^{15}N\text{-}NH_4^+$ values in the whole study (< -9‰; Fig. 4.1.6c). While the seasonal pattern is similar at the closely co-located Afons Gwy and Hafren, their minima are observed in March while the lowest value observed anywhere is seen in the December sample from Llyn Conwy (-10.1‰). Llyn Llagi, like Blue Lough in Northern Ireland, shows the lowest value for $\delta^{15}N\text{-}NH_4^+$ in the June sample. Both Blue Lough and Afon Hafren show positive values in the September sample. It is noteworthy that two of the other three sites where positive $\delta^{15}N\text{-}NH_4^+$ values are seen (River Etherow, Allt a'Mharcaidh) also show maxima in September, with Borralie being the only exception, i.e. four of five sites with positive $\delta^{15}N\text{-}NH_4^+$ values show September maxima.

Task 4.1b: Additional dual isotope ($\delta^{15}N$- and $\delta^{18}O\text{-}NO_3^-$) and $\delta^{15}N\text{-}NH_4^+$ sampling on two occasions at the Afon Gwy (selected OPTION)

Under the first phase of the Freshwater Umbrella, a pilot study for the dual isotope technique was carried out at the Afon Gwy but access problems prevented sampling at precisely the time of year (early spring) subsequently found to show the maximum proportion of atmospheric NO_3^- at other study sites (Scoat Tarn, Etherow, Lochnagar).

Hence under the present contract where the Afon Gwy was included in the upscaling study, funding was granted for additional samples to supplement the quarterly samples to allow a re-assessment of peak atmospheric NO_3^- in the stream. Additional sampling was done at the Gwy in February and April 2009 as well as the final quarterly survey sample in March 2009. The data are presented in Table 4.1.3 below.

Ironically the maximum expected NO_3^- values in April were too low for isotopic analysis, while the February sample was analysed and showed only 2.9% untransformed atmospheric NO_3^-, in line with the earlier study (maximum 2.7%). However, the September sample in this study did show a slightly larger proportion (4.7%) of atmospheric NO_3^- than was found in the earlier pilot study. This work reconfirms that untransformed atmospheric NO_3^- is a negligible proportion of leached NO_3^- at the Afon Gwy and almost all leached NO_3^- has been microbially cycled.

Table 4.1.3: Results of isotopic analysis of streamwater and bulk deposition in quarterly and additional samples at the Afon Gwy

	Suction sampler $\delta^{18}O\text{-}H_2O$				Theor.	Surface water NO_3		Deposition NO_3		%	Dep. NH_4
Date	S1	S2	S3	Mean	$\delta^{18}O\text{-}NO_3$	$\delta^{15}N$	$\delta^{18}O$	$\delta^{15}N$	$\delta^{18}O$	atm.	$\delta^{15}N$
06-Jun-08	-6.39		-5.89	**-6.14**	+3.6	Too small		-4.7	+73.4		-3.6
12-Sep-08	-5.85		-5.95	**-5.90**	+3.7	+1.3	+6.6	-4.0	+64.5	+4.7	-5.3
19-Dec-08	-5.37		-5.99	**-5.68**	+3.9	+1.7	+3.6	-4.1	+71.6	-0.4	-9.0
20-Feb-09				-6.09559	+3.6	+2.4	+5.6	-1.0	+71.3	+2.9	-12.6
12-Mar-09	-7.19		-6.14	**-6.67**	+3.2	+2.0	+3.8	-2.6	+67.8	+0.9	-9.5
23-Apr-09	Samplers removed					Too small		-3.8	to re-run		-6.5

Figure 4.1.5a: Bulk deposition $\delta^{15}N\text{-}NO_3^-$ at Scottish sites

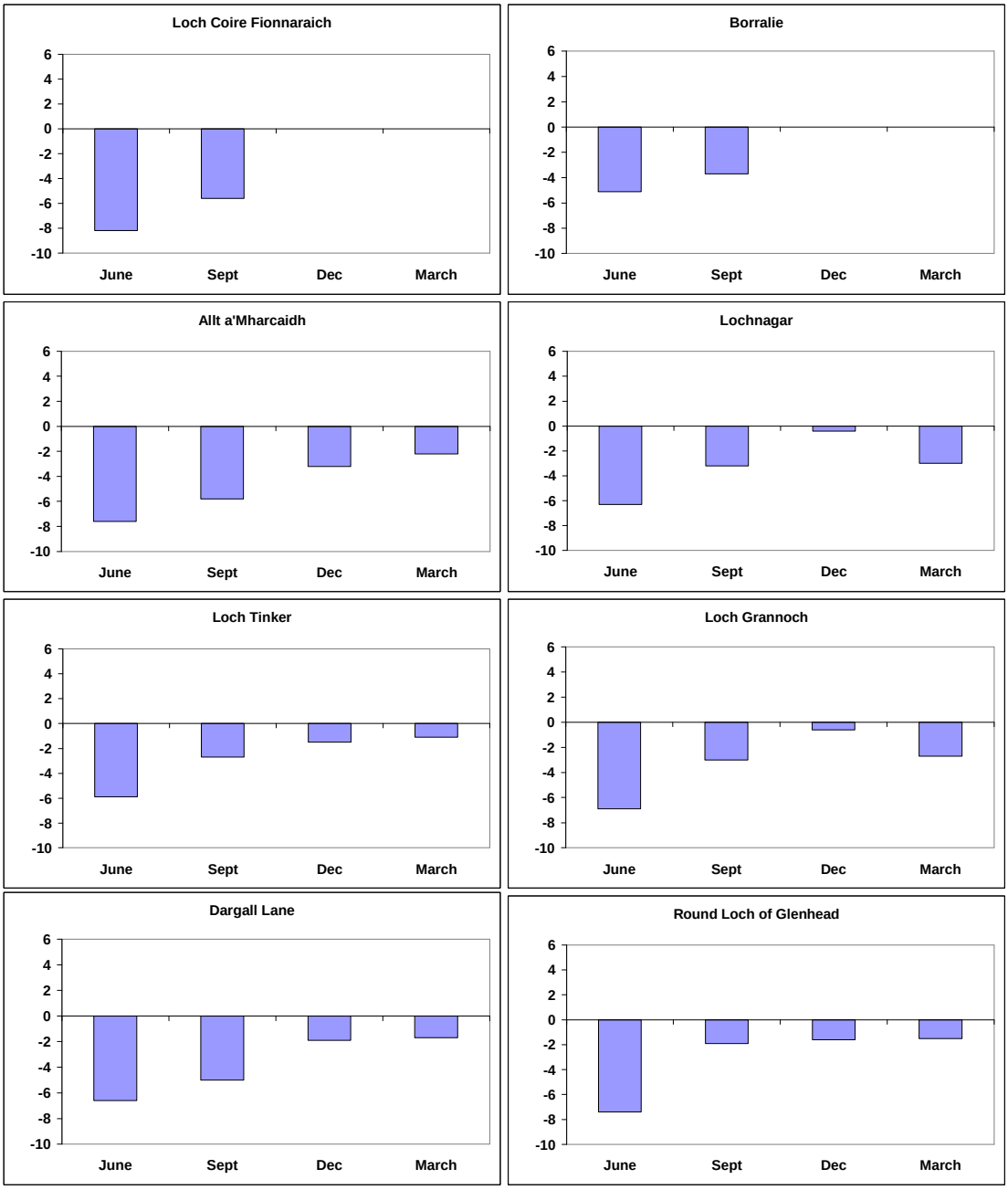


Figure 4.1.5b: Bulk deposition $\delta^{15}N\text{-}NO_3^-$ at English sites

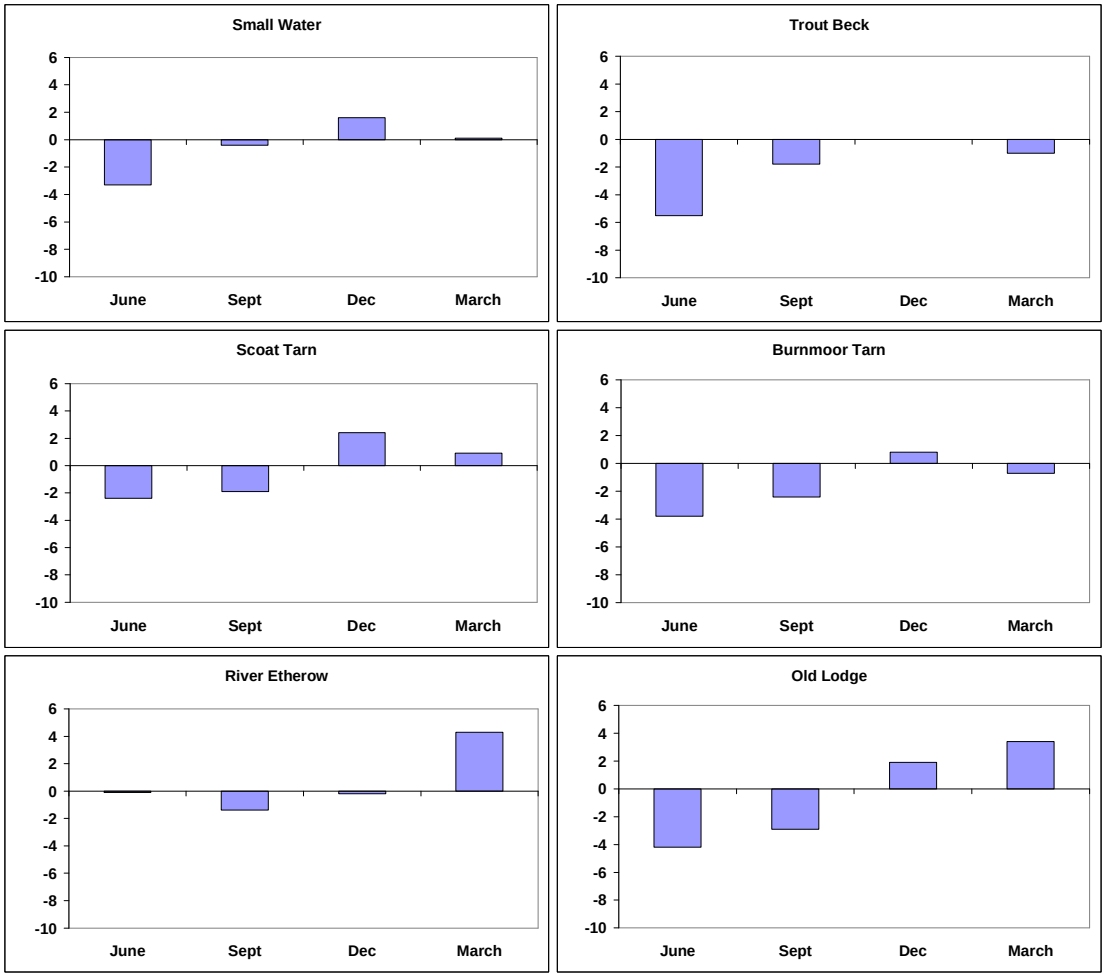


Figure 4.1.5c: Bulk deposition $\delta^{15}N\text{-}NO_3^-$ at Welsh and Northern Irish sites

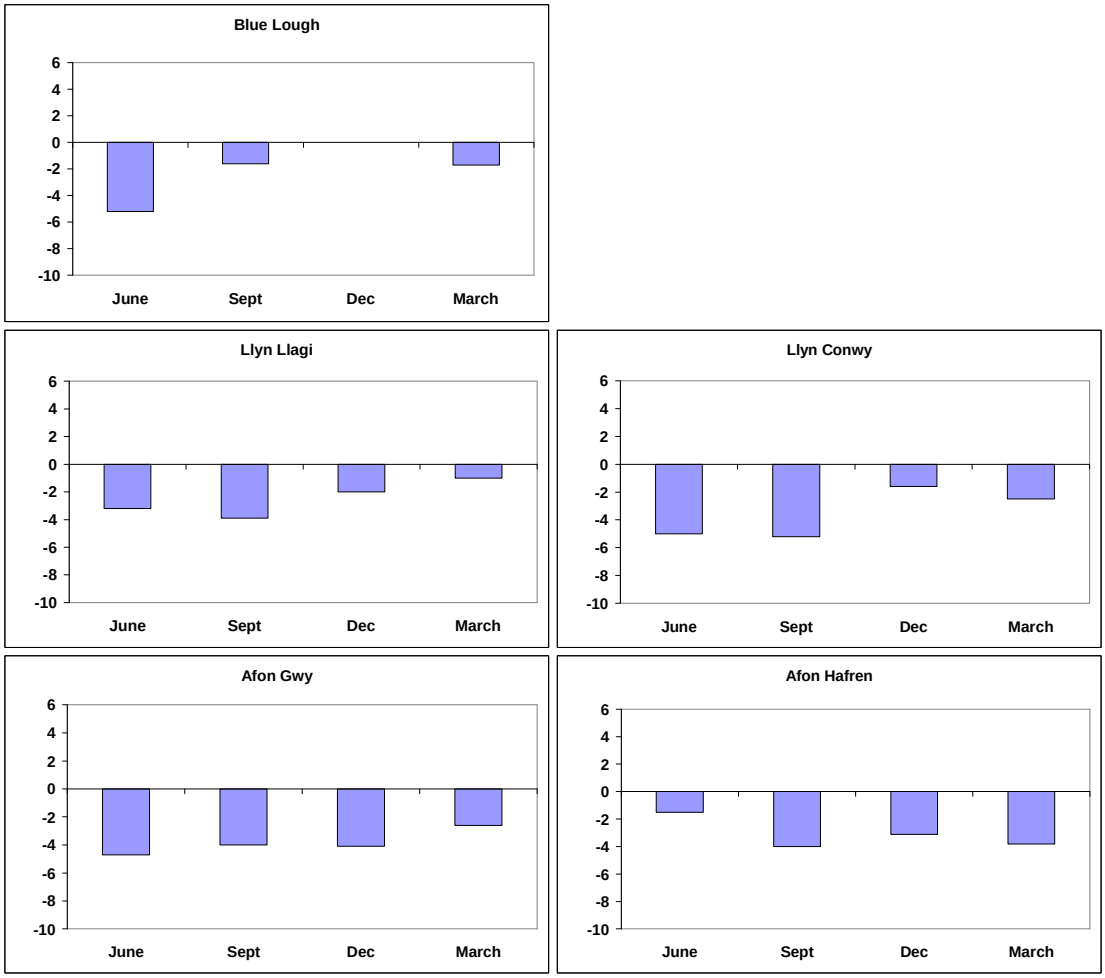


Figure 4.1.6a: Bulk deposition $\delta^{15}N\text{-}NH_4^+$ at Scottish sites

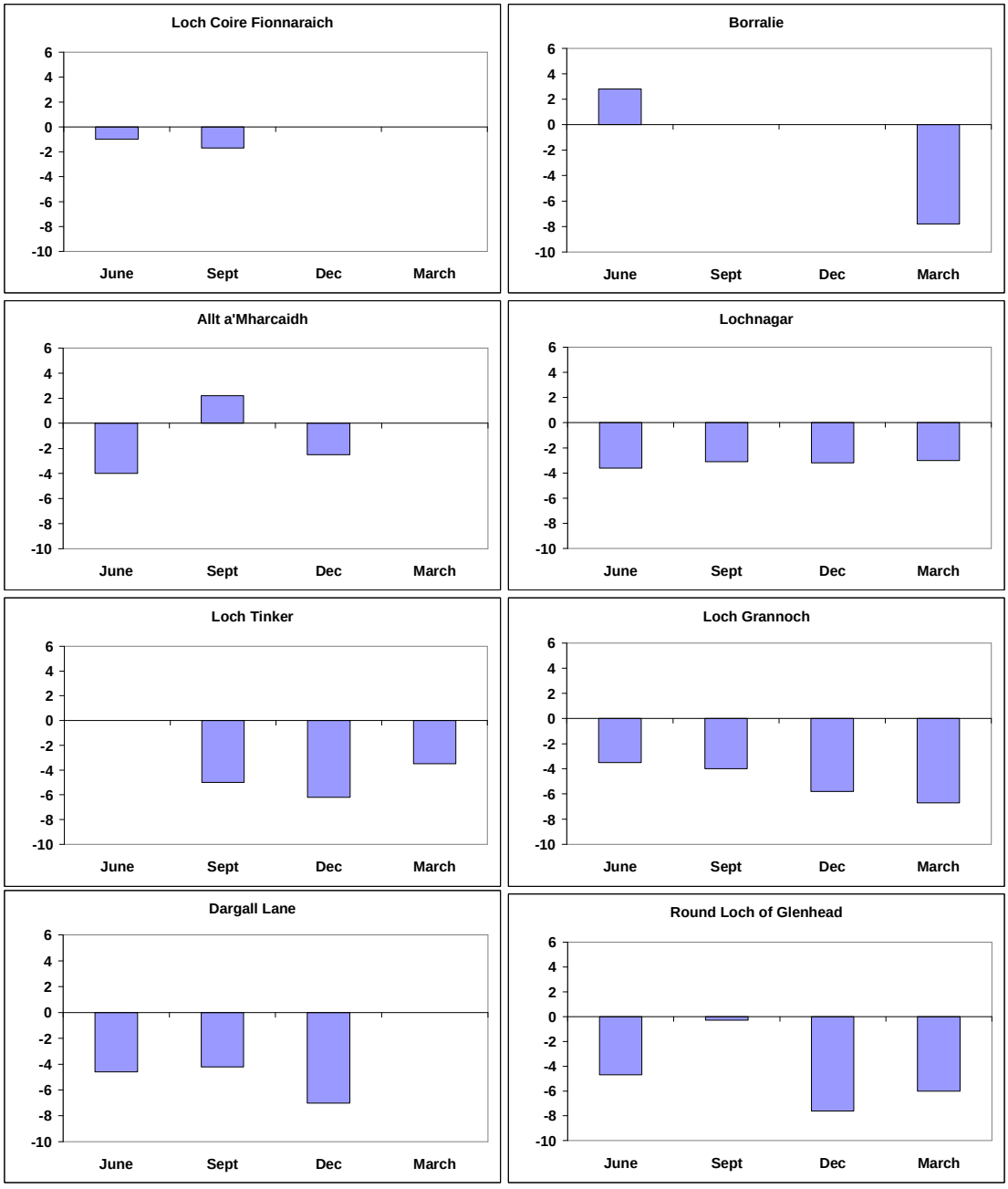
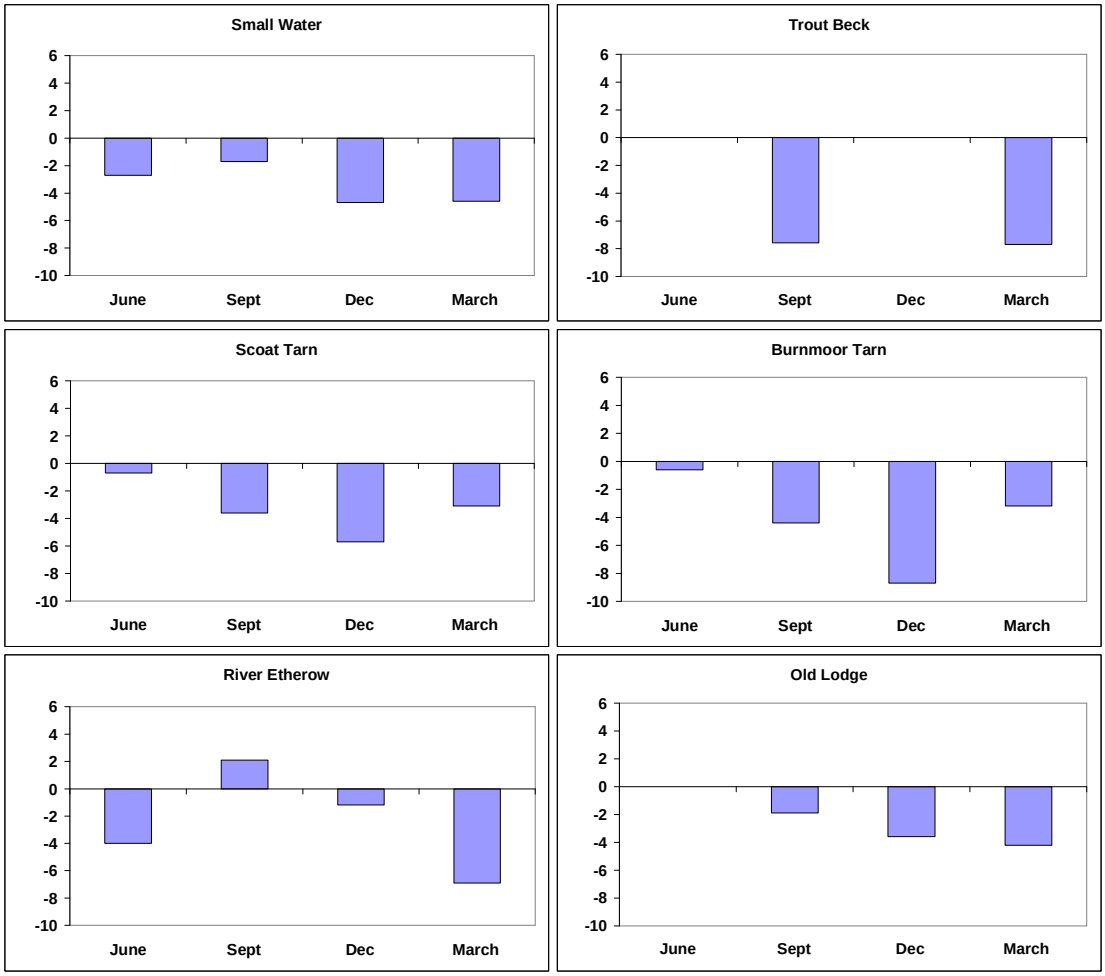


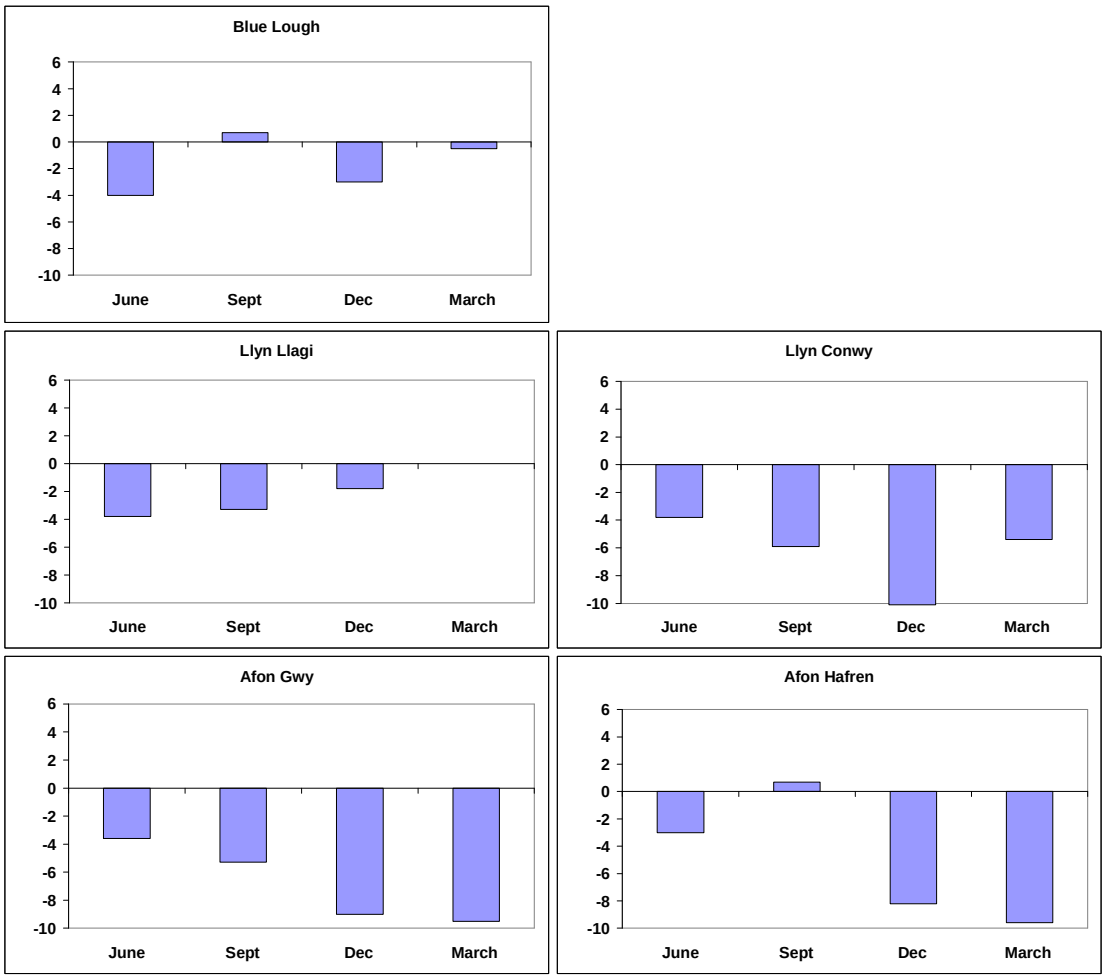
Figure 4.1.6b: Bulk deposition $\delta^{15}N\text{-}NH_4^+$ at English sites



Conclusions

Annual mean concentrations of NO_3^- and NH_4^+ in deposition and surface water samples are presented in Table 4.1.4. In bulk deposition samples, concentrations of the two N species were comparable, while inorganic N in surface water samples was dominated by NO_3^- for most sites where NO_3^- exceeded 5-10 µeq l^{-1}. The proportion of untransformed atmospheric NO_3^- calculated from $\delta^{18}O\text{-}NO_3^-$ confirmed the results of the earlier study of Curtis *et al.* (in press) that the majority of upland waters are dominated by microbially produced NO_3^-. The range of values in lakes was 0-26% atmospheric NO_3^-, with the lowest values in Borralie and Loch Corrie Fionnaraich in north-west Scotland and the highest values exceeding 20% in Blue Lough and Loch Tinker.

Figure 4.1.6c: Bulk deposition $\delta^{15}N\text{-}NH_4^+$ at Welsh and Northern Irish sites



Values in lake inflows and stream sites were generally lower than lakes, confirming the importance of direct deposition to lake surfaces as an important pathway for atmospheric NO_3^- (Curtis *et al.*, in press). Most streams and lake inflows showed less than 5% atmospheric NO_3^- but particularly high values were found at the Blue Lough inflow (19%), Burnmoor Tarn inflow (mean=25%, due to the inexplicably high value of 63% in the June sample) and Old Lodge (15% - but due to one high value of only two with sufficient NO_3^- for analysis). The high values found in a small number of stream samples indicate that under certain hydrological conditions an elevated proportion of atmospheric NO_3^- does reach streams, but further work would be required to identify the flowpaths and mechanisms.

Table 4.1.4: Mean concentrations of NO_3^- and NH_4^+ in bulk deposition and surface waters, and mean proportion of untransformed atmospheric NO_3^- in surface waters

		Deposition		Site		Lake inflow		% atmospheric NO_3^-		
Sitecode	**Type**	**NO_3^-**	**NH_4^+**	**NO_3^-**	**NH_4^+**	**NO_3^-**	**NH_4^+**	**Site**	**Inflow**	**Diff.**
BLU	Lake	30.1	39.3	17.2	6.1	15.1	4.5	25.9	18.7	7.1
BORR	Lake	24.2	19.7	5	4.5	23.1	3.1	-7	-10.2	3.2
BURNMT	Lake	15.8	23	5.8	3.1	7.4	2.1	18.3	25.2	-6.8
CON	Lake	31.5	30.3	6.6	3.2	0	0.3	20	0	20
LAG	Lake	33	32.4	6.8	5.3	9.3	1.8	12.5	-1.8	14.4
LGR	Lake	27.1	32.3	11.2	2.3	1.5	2.8	10.8	-0.9	11.7
NAGA	Lake	23.4	24.4	11.8	2.7	15.9	1.9	16.7	8.8	7.8
RLGH	Lake	25.5	31	8.8	2.5	7.3	6.3	18.4	-0.4	18.8
SCOATT	Lake	16	21.8	12.5	4	10.7	4.9	8.9	5.1	3.8
VNY4101	Lake	22	30.1	14.7	3.7	13.2	3.4	7.7	1.2	6.6
TINK	Lake	22.8	27.9	2.9	5.6	2.2	5.9	21.8	-5.3	27.2
VNG9402	Lake	20.2	19.3	1.8	3.9	4	3.1	0.7	-3.9	4.6
DARG	Stream	29.4	32.7	9.3	1.8			4.7		
ETHR	Stream	28.2	37.8	38.1	0.3			6.8		
GWY	Stream	27.2	36.6	7.1	2.9			1.7		
HAFR	Stream	19.8	24.8	9.2	3.4			2.6		
LODG	Stream	25.7	27.2	5.9	3.5			14.8		
MHAR	Stream	20.2	19.1	0.3	0.7			-2.4		
TROUT	Stream	18.8	31.5	5.9	6.7			4.1		

The regional patterns found in the isotopic signatures in bulk deposition suggest there may be potential for using $\delta^{15}N$ to link back to emissions sources; although this is outwith the present contract it is something that is being explored for a manuscript on this part of the work programme.

Statistical modelling of catchment attributes contributing to the direct leaching of atmospheric NO_3^- is reported under Task 2.3.

Summary

Upscaling the dual isotope work piloted in the first Freshwater Umbrella programme was successfully carried out at 19 sites around the UK. Isotopic data from soilwaters, deposition and surface waters were obtained and allowed seasonal estimation of atmospheric NO_3^- inputs into surface waters. The upscaling work confirmed the findings of the previous Freshwater Umbrella study (Curtis *et al*., in press) that the major proportion of surface water NO_3^- has been microbially produced, with 0-24% deriving from direct leaching of atmospheric NO_3^- on a mean annual basis. A greater proportion of atmospheric NO_3^- was found in lake sites relative to their inflow streams, indicating the importance of direct deposition to lake surfaces. Regional and seasonal patterns in the $\delta^{15}N$ of bulk deposition NO_3^- and NH_4^+ suggest there may be potential to use isotopes to link back to dominant emission sources.

Task 4.2: Dual isotope analysis of incubated soil microbial NO_3^- to validate "theoretical" $\delta^{18}O$ values used at present

Aims

This task aims to test the assumptions made about the isotopic signature of microbial NO_3^- to determine whether the use of $\delta^{18}O$-NO_3^- in Task 4.1 provides a biased estimate of direct atmospheric NO_3^- contributions into surface waters. The method previously used in the Freshwater Umbrella to determine the $\delta^{18}O$ value of microbial NO_3^- uses theoretical assumptions based on limited studies. Other authors (e.g. Williard *et al.*, 2001) have used a more direct measure of the isotopic signature of microbial NO_3^- by flushing soil cores with de-ionised water to remove all residual inorganic N, then incubating and reflushing after a certain period to obtain NO_3^- produced only within the soil by microbes, i.e. true microbial NO_3^-. This microbial NO_3^- is then subject to dual isotopic analysis in the same way as surface water or bulk deposition samples. The data provided is a direct measure of microbially produced NO_3^- and removes the need for theoretical assumptions about oxygen sources used by nitrifying bacteria.

The work was done for six catchments with two soils types and three replicated soil cores to see whether there is variation in the $\delta^{18}O$ value of microbial NO_3^-.

Methods: sample preparation

The six sites selected for the study were the Afon Hafren in mid Wales, the River Etherow in the Pennines, Scoat tarn in the Lake District, Lochnagar in the Grampians and the adjacent Dargall Lane and Round Loch of Glenhead in Galloway. At each site, two locations in contrasting soil type / vegetation cover were selected for soil coring. Intact soil cores (15cm diameter) were obtained, after removing surface vegetation, to a depth of c. 10cm and cores remained intact in the original coring tubes throughout the experiment. Sample collection was completed during early summer 2009 and soil samples returned to the laboratory at UCL for rinsing and incubation (7 weeks). A pilot sampling was first carried out at the Afon Hafren in May, with the other five sites sampled during June 2009 (Table 4.2.1).

Soil cores were initially flushed with 2.5L of de-ionized water in 0.5L volumes to remove traces of atmospherically deposited NO_3^- and the last flush of leachate was analyzed for NO_3^- concentration using a portable Hach spectrophotometer to assess the success of the flushing. Following incubation for 7 weeks, the process was repeated but the leachate was analyzed for NO_3^- concentration and then loaded onto ion-exchange resins for subsequent elution and isotopic analysis. Results of the chemical analysis suggest that initial rinsing of soil cores showed variable success between sites, with some cores still showing very high NO_3^- concentrations even in the last 0.5L rinse with DIW (Fig. 4.2.1). The highest NO_3^- values were found in Scoat Tarn soils, Lochnagar inflow soils, and *Molinia* dominated soils at Dargall Lane and Round Loch of Glenhead. Rinsing was most successful at Afon Hafren, River Etherow, Lochnagar outflow (heather on peat) and heather dominated soils at Dargall Lane and Round Loch of Glenhead. These results suggest that peat soils under heather are more easily flushed than more mineral soils under *Molinia* grassland, although it

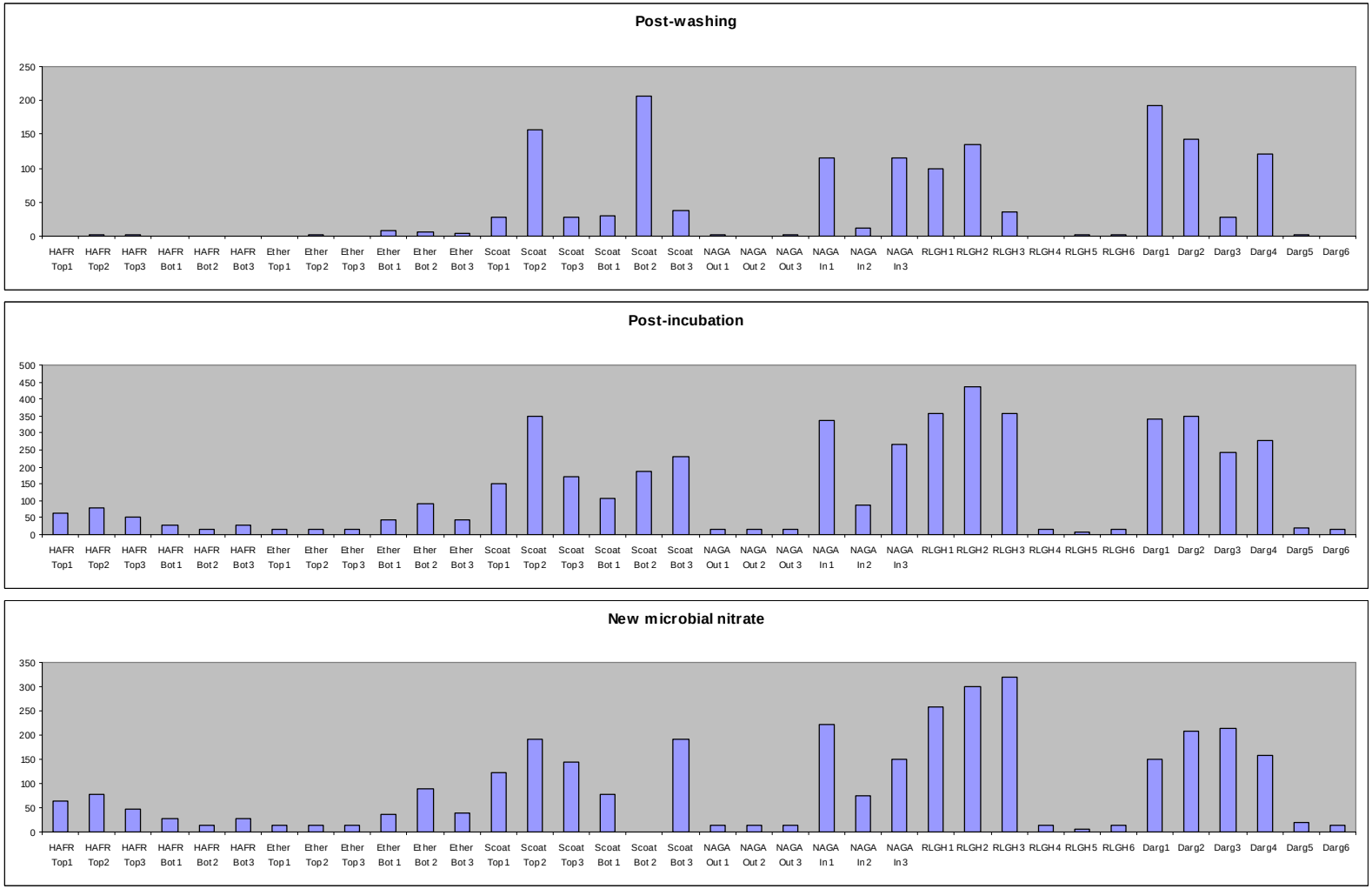
is also possible that rinse water drained more rapidly (possibly through cracks etc.) and picked up less NO_3^- in these soils for reasons related to physical drainage characteristics rather than efficiency of rinsing.

The same soils showing the highest NO_3^- concentrations post rinsing also showed the highest NO_3^- production post-incubation, i.e. the highest net nitrification rates (Fig. 4.2.1). The data suggest that the highest nitrification rates occur in the more mineral soils under grassland rather than peats under heather. The large increases in NO_3^- draining from most soils indicates that microbially produced NO_3^- should form an important part of leached NO_3^-, although where post-rinse NO_3^- concentrations were high it cannot be guaranteed that there is not a significant atmospheric component remaining in the soils.

Table 4.2.1: Soil coring sites and locations for the soil microbial NO_3^- study

Site	Sample	GPS Location	Description	Sampled
HAFR	Top 1	SN 82877 89273	Acid grass	20/05/2009
HAFR	Top 2	SN 82877 89273	Acid grass	20/05/2009
HAFR	Top 3	SN 82877 89273	Acid grass	20/05/2009
HAFR	Bot 1	SN 84500 87560	Forest	20/05/2009
HAFR	Bot 2	SN 84500 87560	Forest	20/05/2009
HAFR	Bot 3	SN 84500 87560	Forest	20/05/2009
ETHR	Top 1	SK 12401 98888	Heather	22/06/2009
ETHR	Top 2	SK 12410 98889	Heather	22/06/2009
ETHR	Top 3	SK 12416 98884	Heather	22/06/2009
ETHR	Bot 1	SK 11714 99551	Grass	22/06/2009
ETHR	Bot 2	SK 11713 99552	Grass	22/06/2009
ETHR	Bot 3	SK 11714 99548	Grass	22/06/2009
SCOATT	Top 1	NY 15792 10456	Grass	23/06/2009
SCOATT	Top 2	NY 15789 10454	Grass	23/06/2009
SCOATT	Top 3	NY 15793 10457	Grass	23/06/2009
SCOATT	Bot 1	NY 15830 10189	Grass	23/06/2009
SCOATT	Bot 2	NY 15838 10194	Grass	23/06/2009
SCOATT	Bot 3	NY 15826 10182	Grass	23/06/2009
NAGA	Out 1	NO 25408 96135	Peat & heather	24/06/2009
NAGA	Out 2	NO 25413 86120	Peat & heather	24/06/2009
NAGA	Out 3	NO 25412 16132	Peat & heather	24/06/2009
NAGA	In 1	NO 25298 85777	Grass	24/06/2009
NAGA	In 2	NO 25300 85772	Grass	24/06/2009
NAGA	In 3	NO 25301 85771	Grass	24/06/2009
RLGH	1	NX 44853 80202	Soil	25/06/2009
RLGH	2	NX 44850 80198	Molinia	25/06/2009
RLGH	3	NX 44847 80199	Molinia	25/06/2009
RLGH	4	NX 44890 80146	Heather/Molinia	25/06/2009
RLGH	5	NX 44892 80149	Heather/Molinia	25/06/2009
RLGH	6	NX 44895 80145	Heather/Molinia	25/06/2009
DARG	1	NX 45036 78641	Molinia	25/06/2009
DARG	2	NX 45034 78640	Molinia	25/06/2009
DARG	3	NX 45035 78638	Molinia	25/06/2009
DARG	4	NX 45046 78611	Molinia/Heather	25/06/2009
DARG	5	NX 45046 78608	Heather/Molinia	25/06/2009
DARG	6	NX 45041 78603	Heather/Molinia	25/06/2009

Figure 4.2.1: Concentrations of NO_3^- in initial rinse and post-incubation rinse of soil cores



Results

The results of isotopic analysis of soil leachates carried out at NIGL are presented in Table 4.2.2. Some samples showed very low NO_3^- concentrations following incubation and were bulked in an attempt to obtain sufficient NO_3^- for isotopic analysis; Hafren bottom (forest), Etherow top (heather), Lochnagar outflow (peat & heather), Round Loch 3-6 (heather / *Molinia*) and two samples at Dargall Lane (5&6, heather / *Molinia*). In all cases these samples proved too small for isotopic analysis. However, 22 out of 36 soil cores provided adequate NO_3^- following rinsing and incubation for analysis of both $\delta^{15}N$ and $\delta^{18}O$.

The primary purpose of this Task was to characterize microbially produced NO_3^- in soils to determine whether theoretical values could be reproduced experimentally. In fact post-incubation NO_3^- flushed from the soil cores showed $\delta^{18}O\text{-}NO_3^-$ values in the range +12 to +25‰ which is far higher than the theoretical range of c. +2 to +4‰ calculated from soilwater $\delta^{18}O\text{-}H_2O$. If these values truly represented the $\delta^{18}O$ signature of microbial NO_3^- in soils then all previous calculations of atmospheric NO_3^- in surface waters would be greatly overestimated. In fact the post-incubation values show a comparable range to that found in surface waters (see Task 4.1 above) and indeed are greater than some surface water NO_3^- values, which can only be explained by a large proportion of atmospheric NO_3^- in all the samples.

This latter explanation is further supported by the post-rinsing NO_3^- concentrations (Table 4.2.2) which show persistent elevated pre-incubation values even after rinsing (e.g. at Scoat Tarn). If it is assumed that any increase in NO_3^- concentration after incubation was due to microbial production, the % of microbial NO_3^- expected is very high for most samples (see Table 4.2.2; e.g. Hafren, 95-99%). However the isotope data indicate otherwise, e.g. the top samples from the Hafren indicate that 20-32% of NO_3^- collected after incubation was still atmospheric. It is therefore likely that in these soils, the rinsing method employed is unable to remove all the residual atmospheric NO_3^- even where sequential rinses lead to much reduced NO_3^- concentrations in leachate, as with the Hafren samples. It seems most likely that there is a redistribution of residual soilwater and associated NO_3^- throughout the soil core during incubation so that subsequent rinsing flushed out more of the atmospheric NO_3^-.

Conclusions

While this experiment failed to provide reliable samples of NO_3^- free from atmospheric sources, the results are still relevant to the interpretation of dual isotope analysis results in Task 4.1 (and in the previous Umbrella programme). There is no evidence that the theoretical $\delta^{18}O\text{-}NO_3^-$ value for microbially produced NO_3^- is an over-estimate which would lead to an under-estimate of the atmospheric NO_3^- component. If anything the soil core data suggest that microbial $\delta^{18}O\text{-}NO_3^-$ is actually higher than the theoretical value, meaning that the proportion of atmospheric NO_3^- has been over-estimated in these dual isotope studies. It therefore remains the case that only a minor proportion of the NO_3^- seen in surface waters derives directly from atmospheric sources, and microbial NO_3^- sources are still dominant.

Summary

There is no evidence from the soil incubation study that the $\delta^{18}O\text{-}NO_3^-$ method has under-estimated the proportion of untransformed atmospheric NO_3^- in surface waters; in fact it is possible the atmospheric component has been over-estimated.

Table 4.2.2: Pre-, post-incubation and assumed microbial NO_3^- concentrations (µeq l^{-1}) in soil core leachate, with results of dual isotope analysis of post-incubation NO_3^-. Thoeretical microbial $\delta^{18}O\text{-}NO_3^-$ and atmospheric $\delta^{18}O\text{-}NO_3^-$ based on quarterly samples from each site (see Task 4.1)

		Nitrate				umol	$\delta^{15}N$	$\delta^{18}O$			
Site	**Sample**	**Pre**	**Post**	**Microbial**	**%**	**loaded**	**Leachate**	**Leachate**	**Th. Micr.**	**Atmos.**	**%atm**
HAFR	Top 1	1	64	64	99	129	+4.8	+17.1	3.9	70.5	20
HAFR	Top 2	2	79	76	97	157	+1.9	+25.5	3.9	70.5	32
HAFR	Top 3	3	50	47	94	100	+12.2	+21.9	3.9	70.5	27
	Mean	**2**	**64**	**62**	**97**	**129**	**6**	**22**	**4**	**71**	**26**
HAFR	Bot 1	1	29	28	98	57		too small	3.9	70.5	-
HAFR	Bot 2	1	14	14	95	29		too small	3.9	70.5	-
HAFR	Bot 3	1	29	28	98	57		too small	3.9	70.5	-
	Mean	**1**	**24**	**23**	**97**	**48**			**4**	**71**	
ETHR	Top 1	1	14	14	95	29		Ether Top 1/2/3			
ETHR	Top 2	1	14	13	90	29		bulked	2.8	73.2	-
ETHR	Top 3	1	14	14	95	29		too small			
	Mean	**1**	**14**	**13**	**93**	**29**			**3**	**73**	
ETHR	Bot 1	7	43	36	83	94	-3.8	+22.4	2.8	73.2	28
ETHR	Bot 2	5	93	88	95	214	-5.1	+22.2	2.8	73.2	28
ETHR	Bot 3	4	43	39	92	99	-1.8	+19.4	2.8	73.2	24
	Mean	**5**	**60**	**54**	**90**	**135**	**-4**	**21**	**3**	**73**	**26**
SCOATT	Top 1	28	150	122	81	375	-4.1	+17.4	3.3	69.8	21
SCOATT	Top 2	157	350	193	55	770	+0.3	+22.2	3.3	69.8	28
SCOATT	Top 3	28	171	144	84	411	+6.6	+20.6	3.3	69.8	26
	Mean	**71**	**224**	**153**	**73**	**519**	**1**	**20**	**3**	**70**	**25**
SCOATT	Bot 1	31	107	76	71	268	+9.7	+21.8	3.3	69.8	28
SCOATT	Bot 2	207	186	-21	-12	427	-3.5	+18.7	3.3	69.8	23
SCOATT	Bot 3	37	229	191	84	389	+6.0	+22.6	3.3	69.8	29
	Mean	**92**	**174**	**82**	**48**	**361**	**4**	**21**	**3**	**70**	**27**
NAGA	Out 1	1	14	13	90	29		NAGA Out 1/2/3			
NAGA	Out 2	1	14	14	95	31		bulked	1.9	73.3	-
NAGA	Out 3	1	14	13	90	29		too small			
	Mean	**1**	**14**	**13**	**92**	**30**			**2**	**73**	
NAGA	In 1	114	336	221	66	739	-7.7	+16.9	1.9	73.3	21
NAGA	In 2	11	86	74	87	189	-5.1	+12.0	1.9	73.3	14
NAGA	In 3	114	264	150	57	423	-2.1	+14.7	1.9	73.3	18
	Mean	**80**	**229**	**149**	**70**	**450**	**-5**	**14**	**2**	**73**	**18**
RLGH	1	100	357	257	72	786	+5.8	+20.6	3.0	66.9	27
RLGH	2	136	436	300	69	871	-1.3	+15.2	3.0	66.9	19
RLGH	3	36	357	321	90	786	+3.7	+18.8	3.0	66.9	25
	Mean	**91**	**383**	**293**	**77**	**814**	**3**	**18**	**3**	**67**	**24**
RLGH	4	1	14	14	95	29		RLGH 4/5/6			
RLGH	5	1	7	6	80	13		bulked	3.0	66.9	-
RLGH	6	1	14	13	90	27		too small			
	Mean	**1**	**12**	**11**	**88**	**23**			**3**	**67**	
DARG	1	193	343	150	44	686	+2.3	+17.4	3.2	68.9	22
DARG	2	143	350	207	59	700	+0.1	+19.9	3.2	68.9	25
DARG	3	29	243	214	88	389	+13.4	+19.5	3.2	68.9	25
	Mean	**121**	**312**	**190**	**64**	**591**	**5**	**19**	**3**	**69**	**24**
DARG	4	121	279	157	56	557	+4.7	+17.9	3.2	68.9	22
DARG	5	1	21	20	93	45		Darg5/6	3.2	68.9	-
DARG	6	1	14	14	95	31		too small			
	Mean	**41**	**105**	**64**	**82**	**211**	**5**	**18**	**3**	**69**	**22**

Task 4.3: Dual isotope ($\delta^{15}N$- and $\delta^{18}O$-NO_3^-) analysis at 150 regional study sites in Great Britain plus 15 Northern Irish sites (regional assessment and upscaling of controls on N leaching)

Aims

This task employed new techniques available for dual isotope analysis of NO_3^- (bacterial denitrifier method) at the University of East Anglia (UEA) using much smaller sample volumes and much cheaper analytical methods than the previous ion-exchange resin method employed in Tasks 4.1-4.2. It was therefore possible to cost-effectively upscale the approach from earlier Freshwater Umbrella funded work to the 163 regional resurvey sites (Task 1.5.2-3) to obtain a regional picture of controls on (and hence vulnerability to) NO_3^- leaching. Previous work has demonstrated the seasonal variation in both concentrations and direct atmospheric contribution to NO_3^- leaching. As part of the regional water chemistry and critical load resurveys under Task 1.5, additional water samples were collected for dual isotope analysis on two occasions including the period of peak atmospheric NO_3^- contribution (generally March-April). Limitation of seasonal sampling to two periods made spatial upscaling to the regional resurvey sites logistically possible. The spatial distribution and catchment attributes indicative of whether sites are vulnerable to leaching of untransformed atmospheric NO_3^- or microbially produced (cycled) NO_3^- are assessed below.

4.3.1 Identification of regionally appropriate isotope data for deposition and soilwaters

The use of surface water NO_3^- isotope data to identify sources of NO_3^- requires isotopic data on the atmospheric (deposition) and microbial (soilwater) endmembers. In previous sampling programmes under Task 4.1, quarterly deposition and soilwater samples were analysed for $\delta^{15}N$ and $\delta^{18}O$. The location of the 19 study sites from Task 4.1 in relation to the regional resurveys under the current task are shown in Figure 4.3.1. For each region, the most appropriate of the 19 sites were selected to provide the required soilwater and deposition data (Table 4.3.1). Where possible, sample dates from the autumn (November) and spring (March) quarterly surveys were used to match up to the autumn 2010 and spring 2011 resurveys because of known seasonal variations especially in deposition $\delta^{18}O$-NO_3^-. On some occasions samples were lost and here annual mean data are used instead.

Seasonal and spatial variations in $\delta^{18}O$ are much greater in deposition NO_3^- than in the microbial soilwater endmember, and some regions show much greater ranges than others. For example, in the Lake District there is consistency in the deposition $\delta^{18}O$ data within a season for all three selected sites, but spring values are all much greater than winter values. Conversely, in Snowdonia, the two selected sites provide very similar seasonal means but there are large (within region) differences between the spring values of deposition $\delta^{18}O$ at Llyn Conwy and Llyn Llagi (Table 4.3.1).

4.3.2 Results of regional resurvey isotopic analysis

Isotope data for regional surveys in both autumn/winter and spring are presented for $\delta^{15}N$ in Figure 4.3.2 and $\delta^{18}O$ in Figure 4.3.3. There are distinct regional differences in median values and ranges for both $\delta^{15}N$ and $\delta^{18}O$.

Figure 4.3.1: Overlay of 19 deposition monitoring sites onto regional site groupings. See Figure 4.1.1 for details of deposition sites

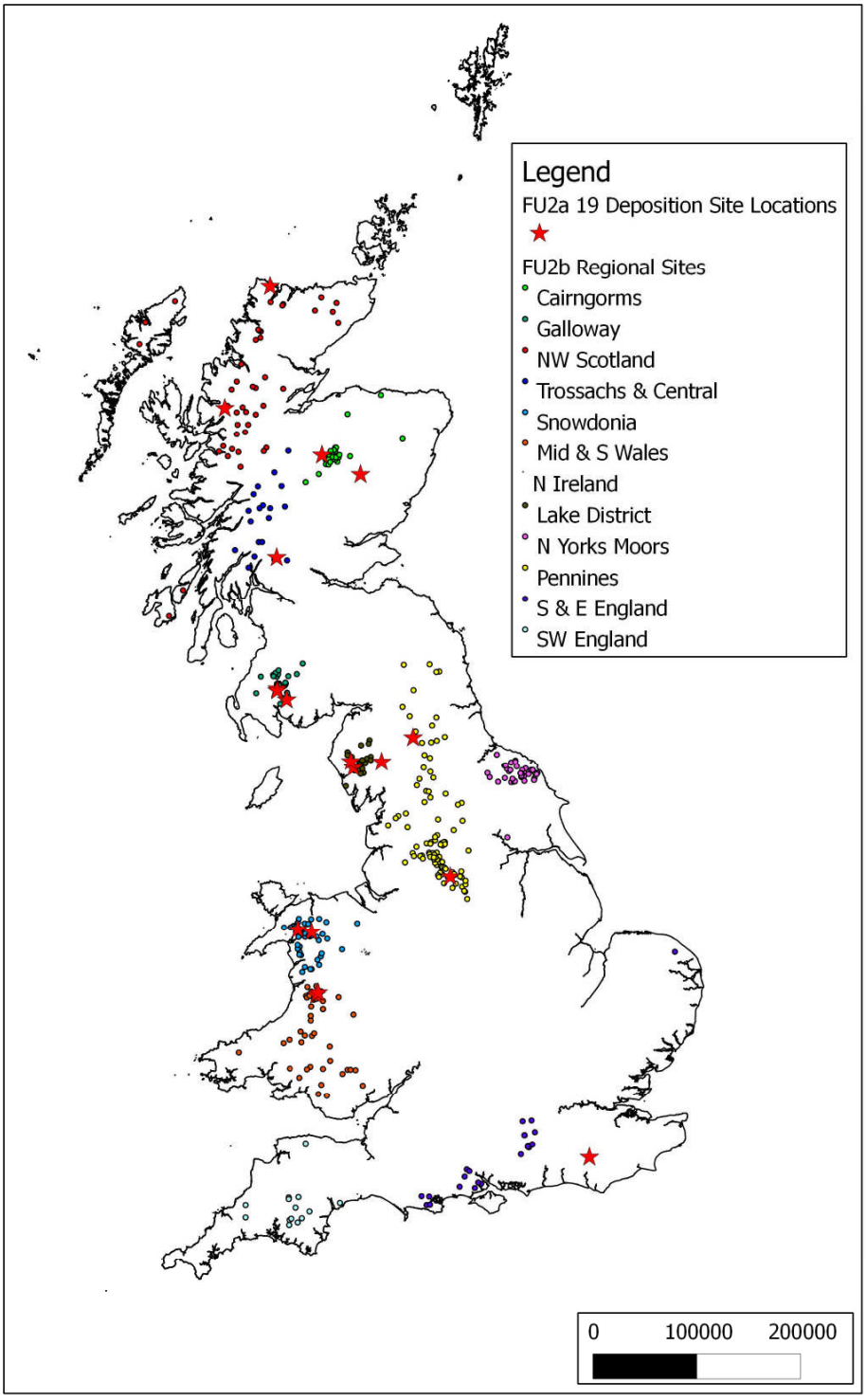


Table 4.3.1: Soilwater and deposition isotope date used for each regional resurvey

Region	Matched sites	Period	Microbial $\delta^{18}O$	Deposition $\delta^{18}O$
Cairngorms	MHAR	Spring	1.6	66.4
Cairngorms	NAGA	Spring	1.7	71.2
Cairngorms	**Average**	**Spring**	**1.7**	**68.8**
Cairngorms	MHAR	Winter	2.5	64.5
Cairngorms	NAGA	Winter	2.3	71.5
Cairngorms	**Average**	**Winter**	**2.4**	**68.0**
Galloway	DARG	Spring	2.9	72.3
Galloway	LGR	Spring	3.6	69.9
Galloway	RLGH	Spring	2.8	70.0
Galloway	**Average**	**Spring**	**3.1**	**70.7**
Galloway	DARG	Winter	3.5	56.4
Galloway	LGR	Winter	3.8	65.0
Galloway	RLGH	Winter	3.0	59.9
Galloway	**Average**	**Winter**	**3.4**	**60.4**
NW Scotland	BORR	Spring	4.1	60.6
NW Scotland	FION	Spring	3.2	70.7
NW Scotland	**Average**	**Spring**	**3.7**	**65.6**
NW Scotland	BORR	Winter	3.7	60.6
NW Scotland	FION	Winter	3.4	70.7
NW Scotland	**Average**	**Winter**	**3.6**	**65.6**
Trossachs	MHAR	Spring	1.6	66.4
Trossachs	TINK	Spring	2.9	75.9
Trossachs	**Average**	**Spring**	**2.2**	**71.2**
Trossachs	MHAR	Winter	2.5	64.5
Trossachs	TINK	Winter	3.2	67.0
Trossachs	**Average**	**Winter**	**2.9**	**65.8**
Snowdonia	CONWY	Spring	3.3	72.2
Snowdonia	LAG	Spring	3.5	54.7
Snowdonia	**Average**	**Spring**	**3.4**	**63.5**
Snowdonia	CONWY	Winter	3.6	65.2
Snowdonia	LAG	Winter	3.9	62.8
Snowdonia	**Average**	**Winter**	**3.7**	**64.0**
Mid & S Wales	GWY	Spring	3.2	67.8
Mid & S Wales	HAFR	Spring	3.4	71.0
Mid & S Wales	**Average**	**Spring**	**3.3**	**69.4**
Mid & S Wales	GWY	Winter	3.9	71.6
Mid & S Wales	HAFR	Winter	4.1	72.3
Mid & S Wales	**Average**	**Winter**	**4.0**	**72.0**
N Ireland	**BLU**	**Spring**	**3.3**	**72.2**
N Ireland	**BLU**	**Winter**	**3.7**	**73.4**
Lake District	BURNMT	Spring	2.8	73.6
Lake District	SCOATT	Spring	3.3	75.4
Lake District	SMALL	Spring	2.9	71.2
Lake District	**Average**	**Spring**	**3.0**	**73.4**
Lake District	BURNMT	Winter	3.6	61.5
Lake District	SCOATT	Winter	3.2	62.3
Lake District	SMALL	Winter	3.1	62.6
Lake District	**Average**	**Winter**	**3.3**	**62.1**
N Yorks Moors	ETHR	Spring	2.7	68.5
N Yorks Moors	TROUT	Spring	2.7	68.7
N Yorks Moors	**Average**	**Spring**	**2.7**	**68.6**
N Yorks Moors	ETHR	Winter	2.8	81.0
N Yorks Moors	TROUT	Winter	2.8	65.5
N Yorks Moors	**Average**	**Winter**	**2.8**	**73.3**
Pennines	ETHR	Spring	2.7	68.5
Pennines	SMALL	Spring	2.9	71.2
Pennines	TROUT	Spring	2.7	68.7
Pennines	**Average**	**Spring**	**2.8**	**69.5**
Pennines	ETHR	Winter	2.8	81.0
Pennines	SMALL	Winter	3.1	62.6
Pennines	TROUT	Winter	2.8	65.5
Pennines	**Average**	**Winter**	**2.9**	**69.7**
S & E England	**LODG**	**Spring**	**2.0**	**68.7**
S & E England	**LODG**	**Winter**	**2.9**	**69.8**
SW England	GWY	Spring	3.2	67.8
SW England	HAFR	Spring	3.4	71.0
SW England	LODG	Spring	2.0	68.7
SW England	**Average**	**Spring**	**2.9**	**69.2**
SW England	GWY	Winter	3.9	71.6
SW England	HAFR	Winter	4.1	72.3
SW England	LODG	Winter	2.9	69.8
SW England	**Average**	**Winter**	**3.6**	**71.2**

Figure 4.3.2: Boxplots of surface water $\delta^{15}N\text{-}NO_3^-$ by region and season

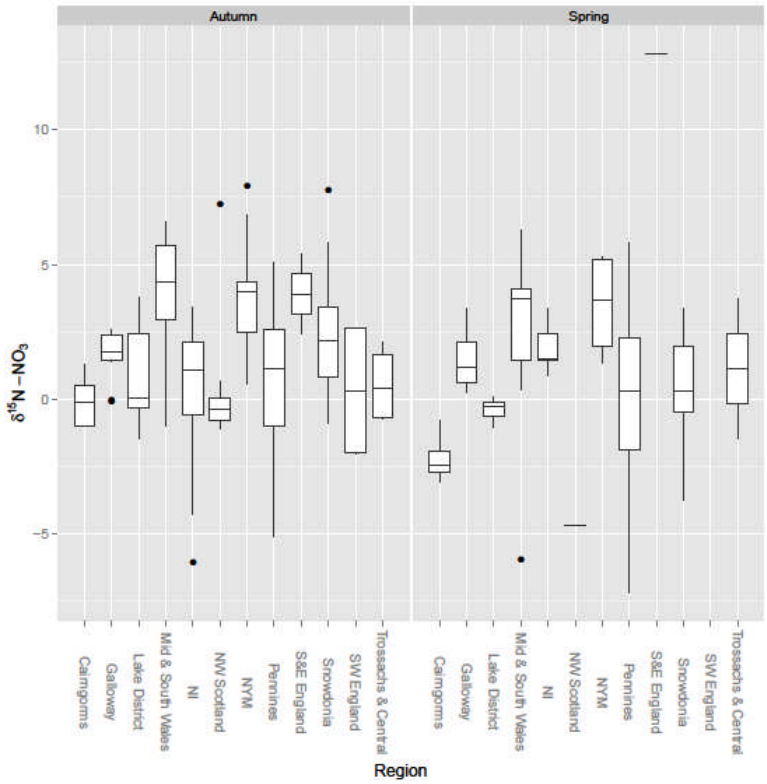
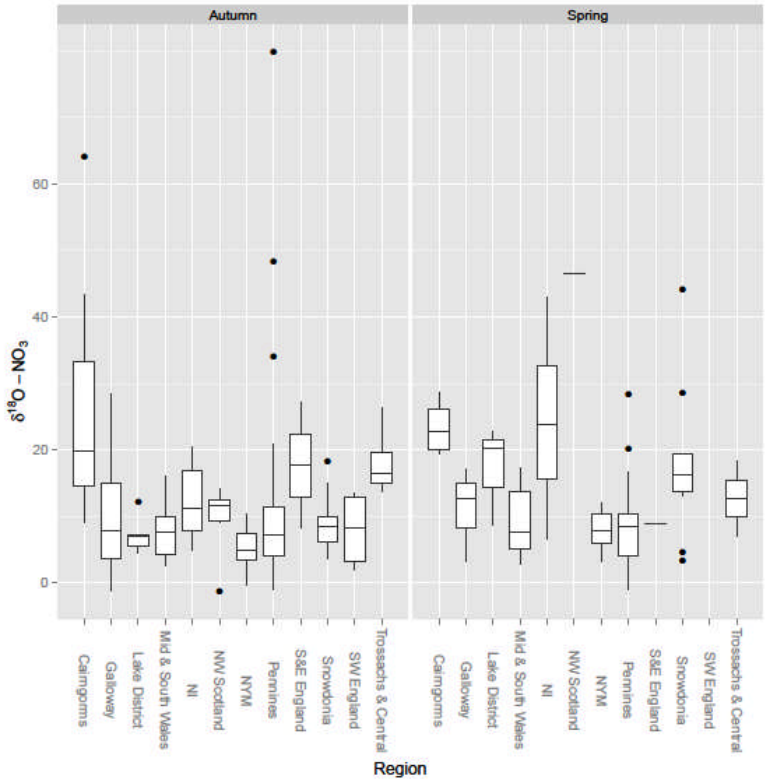


Figure 4.3.3: Boxplots of surface water $\delta^{18}O\text{-}NO_3^-$ by region and season



Pairwise multiple comparison tests were used to determine statistically significant differences for pairs of regions, by testing whether the difference between the pairs is equal to zero or not (Figs. 4.3.4-4.3.5). The ranges indicate Tukey-adjusted 95%-family-wise confidence intervals on the estimated pairwise difference. If the ranges include zero then we cannot say the difference is significant. If the range does not include zero then we can say the pair are significantly different at the family-wise 95% level. For example, $\delta^{15}N$ values are significantly higher for S & E England than for the Pennines, while the Pennines have significantly lower values than the North Yorkshire Moors (Fig. 4.3.4).

While several pairs of regions are significantly different in terms of $\delta^{15}N$ values, there are fewer significant differences in $\delta^{18}O\text{-}NO_3^-$ (Fig. 4.3.5) and these all relate to the Cairngorms region which has significantly higher values than several regions (Galloway, North Yorkshire Moors, Mid & South Wales and the Pennines). However, the $\delta^{18}O\text{-}NO_3^-$ of surface waters is a function of deposition $\delta^{18}O\text{-}NO_3^-$ and the relative contributions of microbially produced and atmospheric NO_3^-.

Calculation of the proportion of surface water NO_3^- is based on a two end-member mixing model which is a simple interpolation between assumed microbial and measured atmospheric end-members (see Task 4.1; also Curtis et al. in press).

Previous studies employing the dual isotope technique have generally found only a small proportion of atmospheric NO_3^- in surface waters (Curtis et al., 2011; in press). The regional resurveys have however highlighted a small number of sites showing very high proportions of atmospheric NO_3^- according to the $\delta^{18}O$ data, for both the winter (Fig. 4.3.6) and spring (Fig. 4.3.7) surveys. Note that a relatively large proportion of sites had insufficiently high NO_3^- concentrations to allow isotopic analysis of the samples, but low NO_3^- concentration does not suggest a small proportion of atmospheric NO_3^- in surface waters.

Statistical analysis

Several potential predictor variables were collated into data sets for use in a statistical modelling exercise to derive predictive models for the percentage of atmospheric NO_3^- in waters. These variables describe gross catchment characteristics (such as catchment and lake area, lake to catchment area ratio, and site altitude), catchment vegetation (three categories; i) unmanaged, ii) managed coniferous forest, and iii) managed broadleaved forest), long term average run-off and S and N deposition flux (CBED 2004-2006 national deposition).

A number of statistical techniques can be used to develop a predictive model, including simple linear regression. Given the modest number of observations available we decide to use simple statistical methods for our analysis, and as a result fitted models using least squares.

Figure 4.3.4: Pairwise multiple comparisons for significant differences between regional $\delta^{15}N\text{-}NO_3^-$

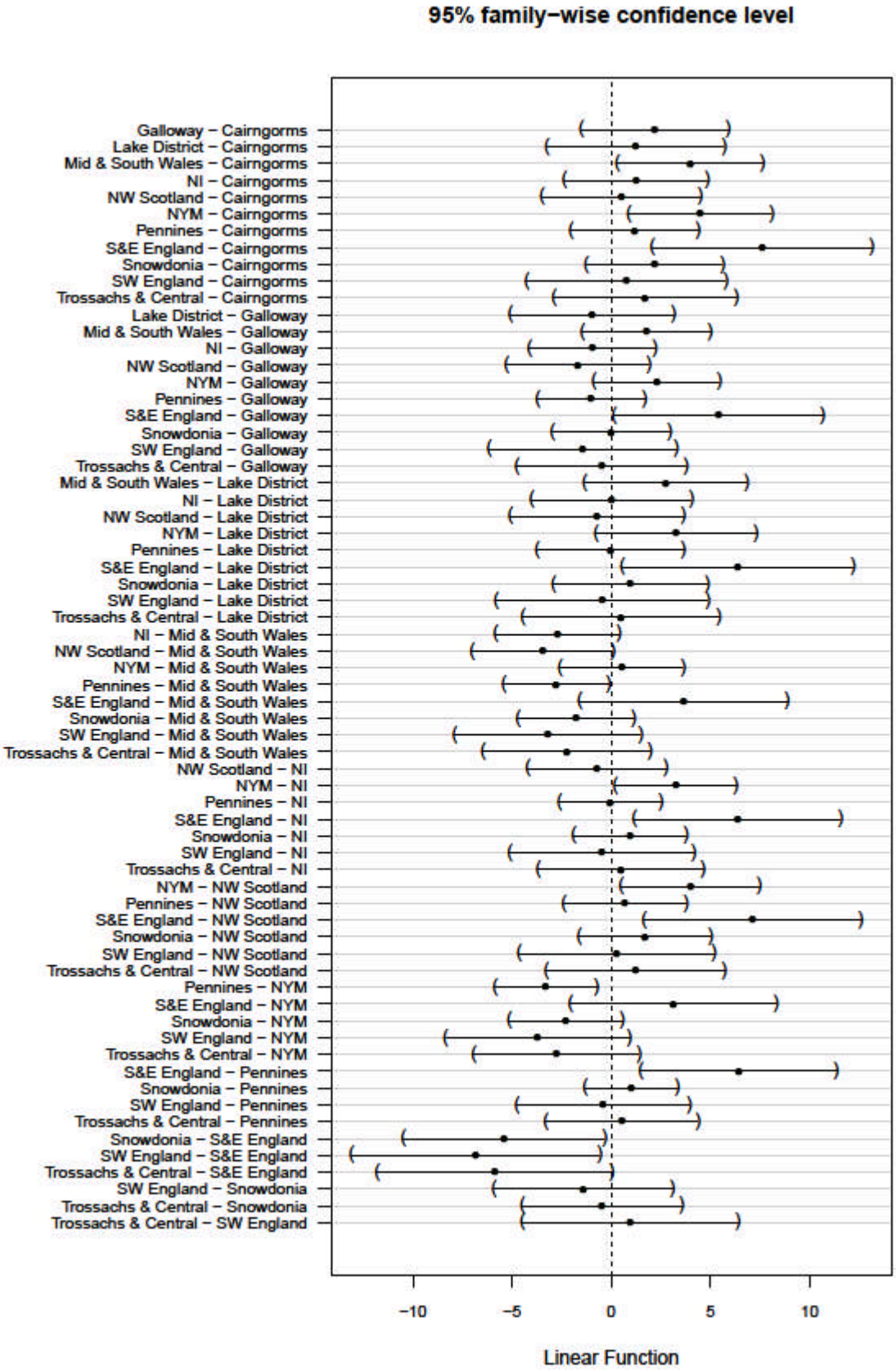


Figure 4.3.5: Pairwise multiple comparisons for significant differences between regional $\delta^{18}O\text{-}NO_3^-$

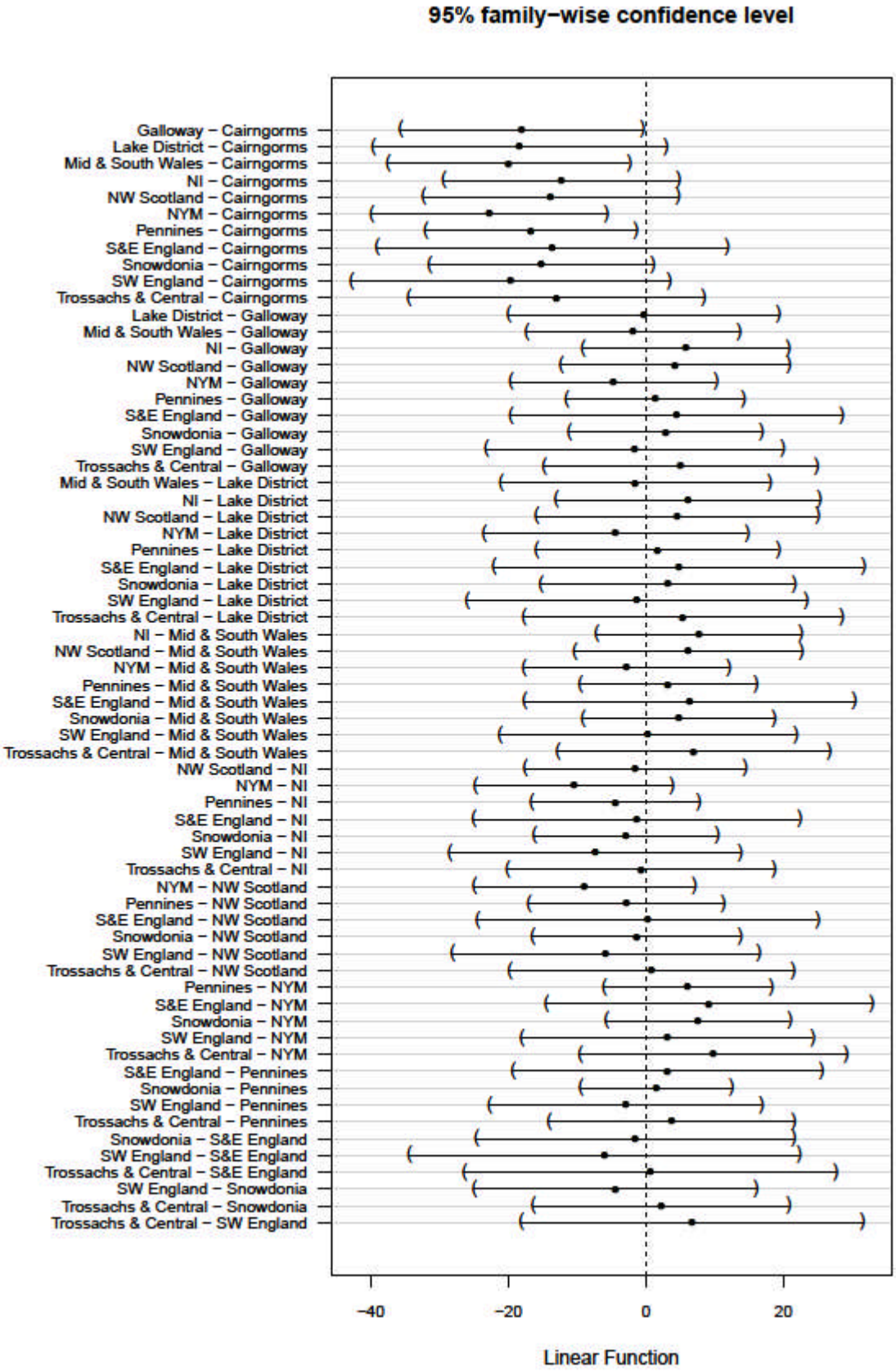


Figure 4.3.6: Estimated proportion of atmospheric NO_3^- (%) in autumn/winter samples based on $\delta^{18}O$-NO_3^- of surface waters and bulk deposition

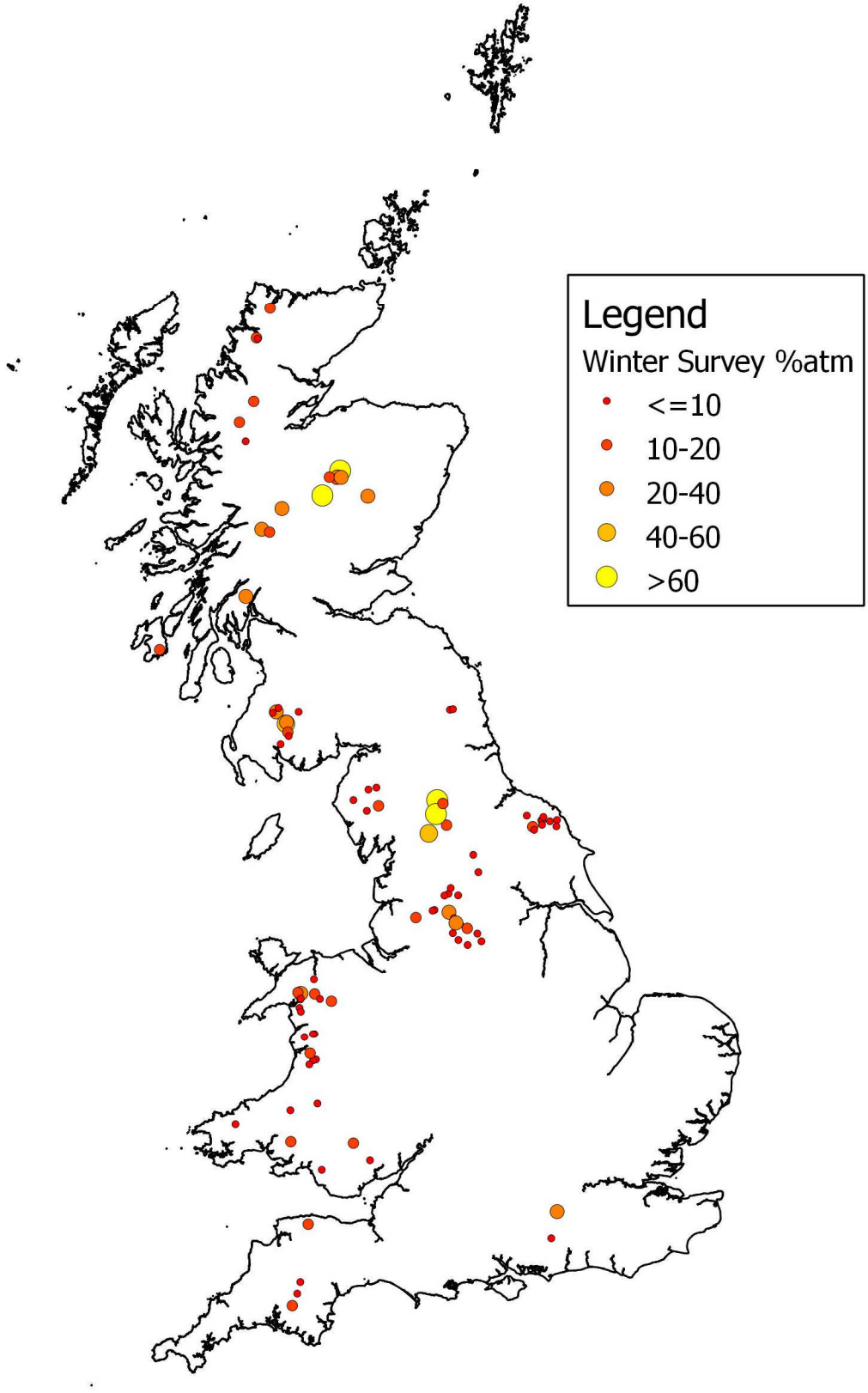
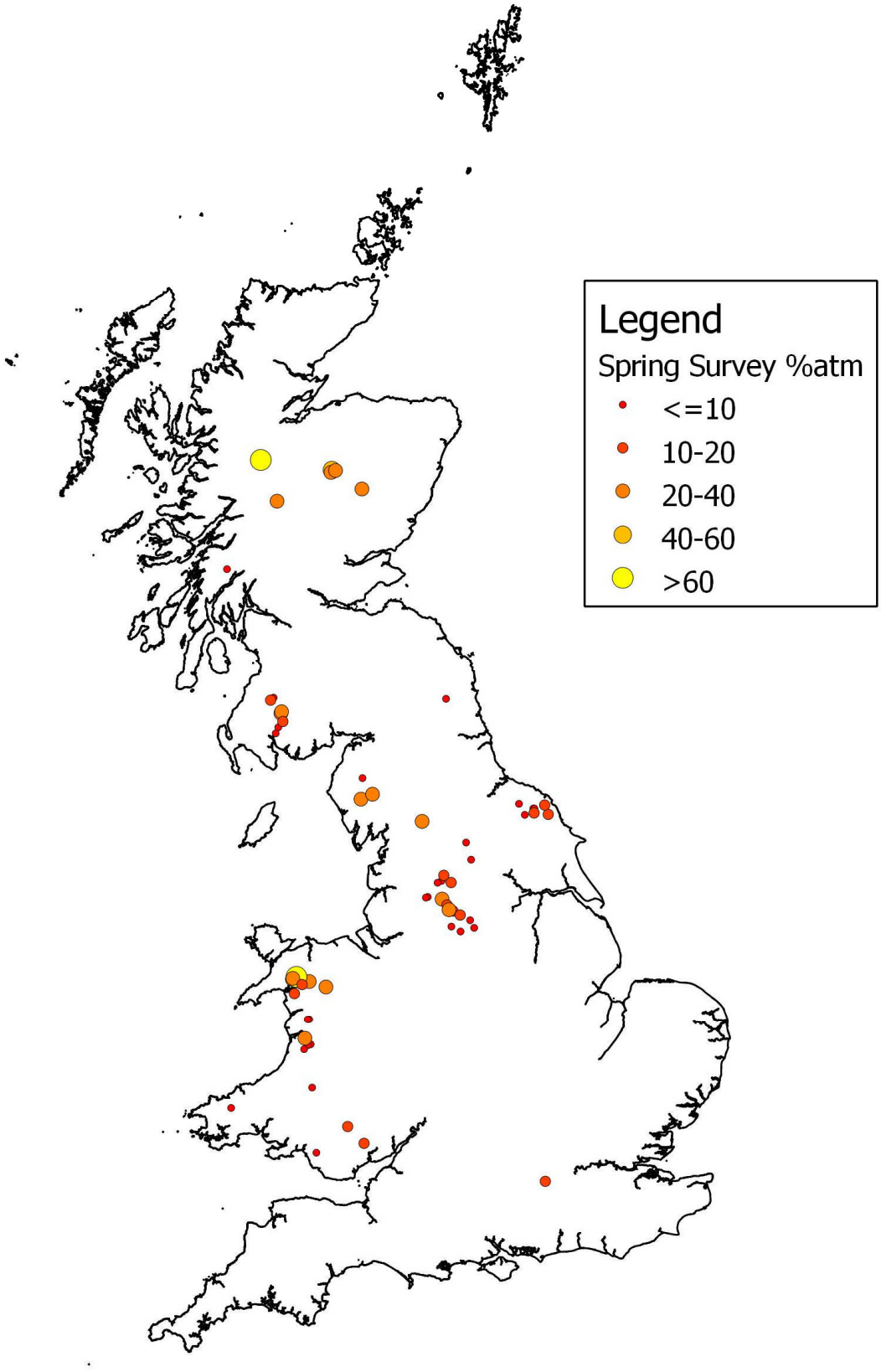


Figure 4.3.7: Estimated proportion of atmospheric NO_3^- (%) in spring samples based on $\delta^{18}O\text{-}NO_3^-$ of surface waters and bulk deposition



Step-wise model selection is problematic for several reasons, not least parameter bias in the selected model terms arising from the exclusion of insignificant terms from the model during fitting Whittingham *et al.*, 2006). In the past couple of decades a number of methods have been developed that place model selection at the heart of the model building process which do not suffer the same problems as step-wise techniques (Hastie *et al.*, 2010). Loosely, we might term these techniques as shrinkage methods. Two such shrinkage methods are ridge regression and the lasso. Both approaches work by placing a penalty on the size of the regression coefficients (Tibshirani, 1996; Hastie *et al.*, 2010). In ridge regression, the penalty restricts the total size of the squares of the coefficients. In the lasso, the penalty restricts the total size of the absolute value of the coefficients. A recently developed alternative is the elastic net penalty which combines ridge-like and lasso-like penalties in a weighted average of the two to form a flexible penalty that has the feature selection properties of the lasso and the ability to handle correlated predictors that is an advantage of ridge regression. Here we use the elastic net with a 50:50 weighting of ridge and lasso penalties as several of the predictor variables, particularly S and N deposition, were highly correlated.

A path of models exists between the full least squares estimates for a model containing all candidate predictor variables and a model containing an intercept term only. Each of the models intermediate between these two points is formed by shrinking the estimated regression coefficients away from the least squares solution by some amount. As the degree of shrinkage increases, predictor variables are selected out of the model if their estimated coefficient crosses the 0 line of no effect. As such, a predictor with a positive coefficient is removed as soon as shrinkage would move the coefficient to or past zero, and vice versa. The optimal model from the entire path of models is determined using a *k*-fold cross validation (CV) procedure and the model with i) the lowest error or ii) the simplest model within one standard error of the best model (lowest error) is chosen. The one standard error rule (1 SE) reflects an important model choice; we should prefer simpler models over more complex models unless the more complex models fit the data substantially better. A simpler model that is within one standard error of the best model is statistically equivalent to the best model in terms of model error and hence, following the maxim of preferring simpler models we should chose the simpler model in that case.

The final regression coefficients from the elastic net procedure are biased estimates. As an alternative we can fit the least squares model using only those terms retained in the elastic net model and use those regression coefficients as our predictive model (Hastie *et al.*, 2010). We adopt this approach here and refit least squares models using the predictors indicated by the results of the elastic net procedure. We fit models separately to the data sets for the winter 2010 and spring 2011 resurvey. The percentage of atmospheric nitrate was used as the single response variable. We log transformed this variable to account for the high degree of skew in the data set. The winter data set had 118 sites where the percentage of atmospheric nitrate could be computed; the remaining sites had insufficient nitrate to perform the analysis. In the spring data set the number of available samples for analysis was much lower; just 73 of the 163 sites had sufficient nitrate for the analysis of nitrate isotopes.

Results

Figure 4.3.8 shows the elastic net model path and CV error for the model to predict percentage of atmospheric nitrate in the winter survey. The simplest model within 1 SE of the best model contains two predictors, i) lake to catchment area ratio, and ii) site altitude, with standardised lasso coefficients of 0.52 and 0.00035 respectively, indicating a 3 orders of magnitude difference in the ability to predict the percentage of atmospheric nitrate in water. The least squares model using only these two predictors is highly significant ($F_{2,115}$ = 17.69, p = >>0.0001) but explains just 24% of the variance in the percentage of atmospheric nitrate (adjusted R^2 0.22).

The same two terms were identified as being important predictors of the percentage of atmospheric nitrate in the Spring resurvey too. Figure 4.3.9 shows the elastic net model path and CV error for this model. The simplest model within 1 SE of the best model contains two predictors, i) lake to catchment area ratio, and ii) site altitude, with standardised lasso coefficients of 0.37 and 0.00031 respectively. The least squares model using only these two predictors is highly significant ($F_{2,70}$ = 14.98, p = <<0.0001) but explains just 30% of the variance in the percentage of atmospheric nitrate (adjusted R^2 0.28).

Conclusions

The best predictors of atmospheric NO_3^- in surface waters for the winter and spring resurveys are lake:catchment area ratio and altitude, explaining around a quarter of the variance in the data.

This finding supports the hypothesis of Curtis *et al.* (in press) who suggest that direct deposition to lake surfaces provides an important pathway for direct transport of atmospheric inputs into surface waters. Lakes with a large surface area relative to catchment area should therefore show a greater proportion of atmospheric NO_3^- because a greater proportion falls onto the lake rather than the terrestrial ecosystem which is a very effective sink or recycler of deposited NO_3^-.

Summary

1. There are significant differences between regions in the isotopic signature of NO_3^- in surface waters, with the Cairngorms being most distinctive in having a large median $\delta^{18}O\text{-}NO_3^-$ indicative of major atmospheric contributions.
2. Very high proportions of atmospheric NO_3^- were found in a small number of sites, predominantly in the Cairngorms and north Pennines in autumn and also Snowdonia and one site in central Scotland in the spring.
3. Lake:catchment area ratio is the most important predictor of atmospheric NO_3^- in surface waters, highlighting the importance of direct deposition to lake surfaces, but explains only around a quarter of the variance in the data. Other site-specific catchment attributes are also clearly very important in determining the transport of untransformed atmospheric NO_3^- into surface waters.

Figure 4.3.8: Summary of the elastic net model to predict the percentage of atmospheric nitrate in lake/stream waters for sites in the Winter re-survey. Lasso model path showing the coefficient estimates (left panel) and CV mean squared error (right panel) as a function of the shrinkage penalty. In both panels, the full least squares solution is found to the far left. In the right hand panel, the two vertical dotted lines indicate the best model and the simplest model within 1 SE of the best model. The error bars represent the uncertainty in the CV mean squared error estimate. The numbers on the upper axis are an approximate indicator of the number of terms in the model

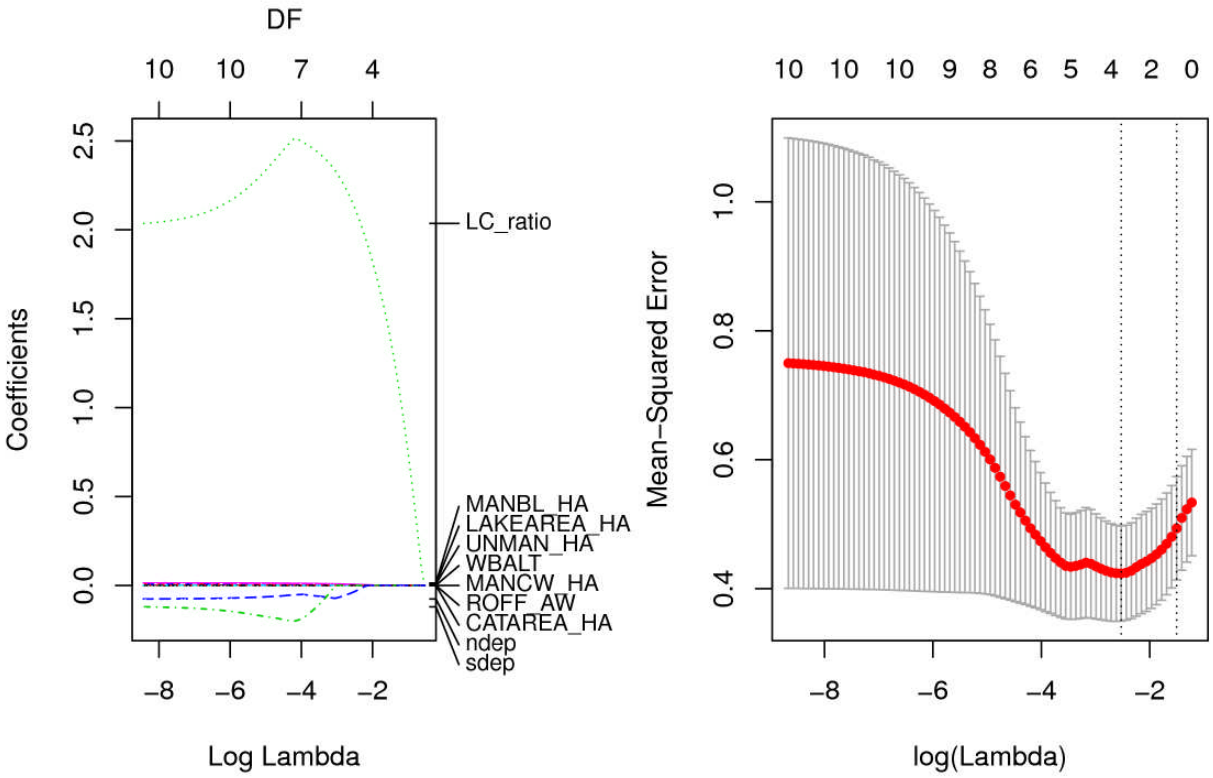
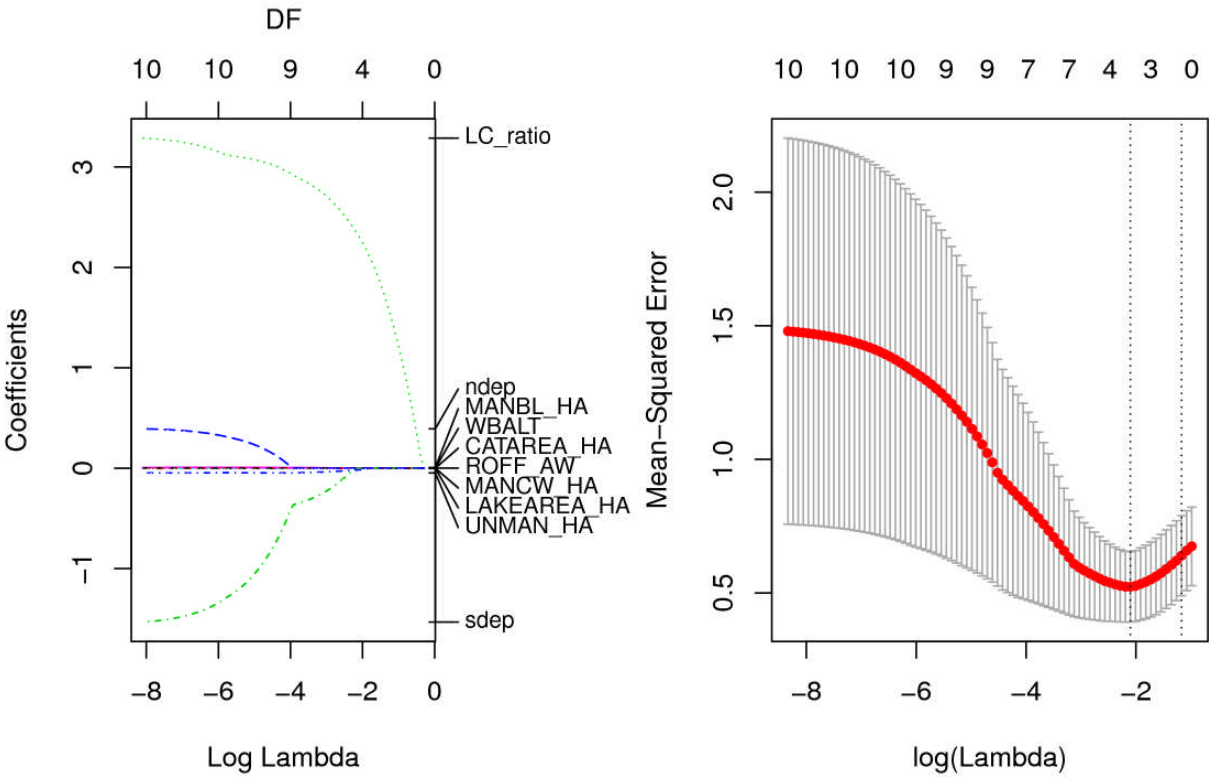


Figure 4.3.9: Summary of the elastic net model to predict the percentage of atmospheric nitrate in lake/stream waters for sites in the Spring re-survey. Lasso model path showing the coefficient estimates (left panel) and CV mean squared error (right panel) as a function of the shrinkage penalty. In both panels, the full least squares solution is found to the far left. In the right hand panel, the two vertical dotted lines indicate the best model and the simplest model within 1 SE of the best model. The error bars represent the uncertainty in the CV mean squared error estimate. The numbers on the upper axis are an approximate indicator of the number of terms in the model.



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Work Package 5: Interactions between acidity, DOC and nitrate leaching

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Task 5.1/5.Assessing the impact of changing soil acidity on ecosystem sensitivity to nitrogen deposition

Introduction and aims

This task was developed in order to examine whether the major, ongoing decreases in sulphur (S) deposition achieved through controls on S emissions are having an impact on the susceptibility of semi-natural ecosystems to nitrogen (N) deposition, in particular due to changes in soil acidity, which have now been identified across the UK (RoTAP, 2011). The aims of the study were to examine the effects of acidity change (without any direct alteration of N or C inputs) on various aspects of C and N cycling in moorland ecosystems, including leaching of NO_3, NH_4, DON and DOC, as well as internal nitrogen transformation processes and gaseous fluxes. The experiments were initially established at four locations with funding from the NERC EHFI programme, with a set of smaller parallel ^{15}N addition experiments established at one location with support from the Defra Freshwater Umbrella. For the project extension, we extended the manipulation and soil solution measurement programme for all four sites, to provide a clearer indication of the effects of acidity manipulation on inorganic and organic N leaching. Here, we report on: i) completed results from the Defra-funded ^{15}N addition experiment; ii) measured C and N leaching results from the main experiments following the Defra-funded extension; and iii) relevant results on internal N transformations from the NERC-funded project.

Methods

The study is based on a set of four parallel replicated experiments. Two of the experimental sites are located in the Peak District, on peat and peaty podzol soils in an area close to the River Etherow AWMN site which is known to be severely N saturated. The other plots were located within the CEH Upper Conwy Carbon Catchment study site, an area with much lower N leaching, again on peat and podzol soils. At each site, 3 x 3 m 'acid', 'alkaline' and control plots were established (four replicates of each) in 2008. The acid plots have since been treated monthly with a dilute sulphuric acid solution, and alkaline plots with a base cation hydroxide solution. Organic soil solution chemistry has been measured at the same frequency using a set of four Rhizon suction samplers located in each plot, with samples analysed for a range of parameters including pH, DOC, total N, NO_3 and NH_4 at Bangor University. Treatment and soil solution measurements were extended by a year beyond the original scope of the NERC grant, with combined support from the Freshwater Umbrella and CEH core funding.

Under the previous contract period of the Freshwater Umbrella, a parallel set of small (1 x 1 m) plots were established at the Peak District podzol site, and exposed to the same treatment regime as the 'main' plots. In addition, a solution of 98% ^{15}N labelled NH_4NO_3 was added at a low dose (2.5 kg N/ha/yr in 13 4-weekly additions, each in 6.66 l of water). Tray lysimeters were used to collect leachate from the soil in the 24 hours following N addition, which was analysed for the chemical parameters described above. In addition, samples were analysed for $^{15}NO_3$ by steam distillation, and for the ^{15}N content of total dissolved N by analysing residues following evaporation. At the end of the year, all plots were destructively sampled for total C,

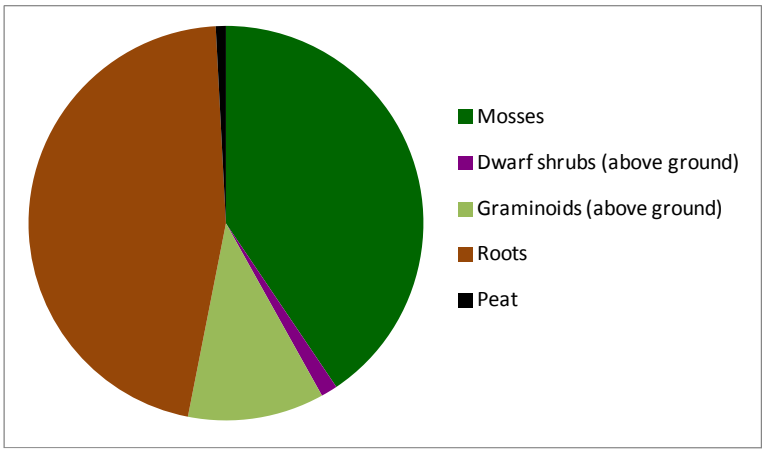
N and ^{15}N analysis of bryophyte biomass, above ground dwarf shrub and graminoid biomass, root biomass (including both dwarf shrub and graminoid roots), and the organic horizon (2.5 – 5 cm depth per plot). All samples were analysed by the NERC Life Sciences Mass Spectrometry Facility at CEH Lancaster.

Results

1. Results of ^{15}N addition study

Analysis of mean ^{15}N recovery for all nine plots (Fig. 5.1) showed that the overwhelming majority of ^{15}N was found in either mosses (41%) or in the roots of higher plants (46%). A further 11% was recovered from above-ground graminoids, while the proportion of N recovered from above-ground dwarf shrubs and from the peat itself were very small (both ~1%). In comparison, mosses only contributed 7% of the measured biomass, with roots making up by far the largest proportion (89%, high in part because samples were collected during winter). Thus, the results clearly demonstrate the effectiveness of bryophytes in capturing atmospherically deposited N, consistent with the previous conclusion of Curtis *et al.* (2005) which suggested that the proportion of bryophytes present determines the effectiveness of N retention by terrestrial ecosystems.

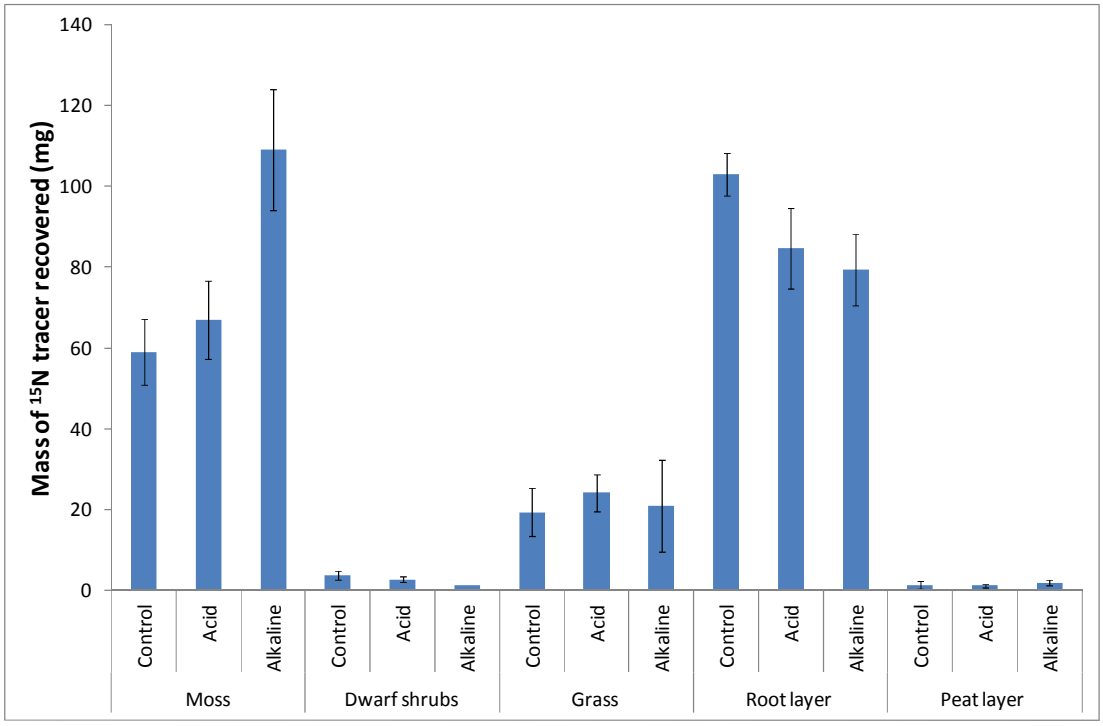
Figure 5.1: Proportion of ^{15}N recovered from different biomass and soil pools, Peaknaze podzol plot ^{15}N addition experiment (average of all treatment and control plots)



In terms of the effects of acidity manipulation on the proportion of ^{15}N accumulated in different soil/biomass pools, there is limited evidence of a treatment effect (Figure 5.2). The only possible effect of treatment appears to be on the amount of ^{15}N retention, which was higher in the alkaline treatment plots. Plotting ^{15}N retention against measured pH (mean of all leachate samples, Figure 5.3a) provides some support for the hypothesis that moss N retention is higher at higher pH; the two alkaline treatment plots where higher pH was observed were also those with the highest moss ^{15}N recovery, whereas the plot where pH was not increased by treatment showed much lower ^{15}N recovery. Overall, some correlation was observed between tracer recovery and pH across all nine plots, although this was not

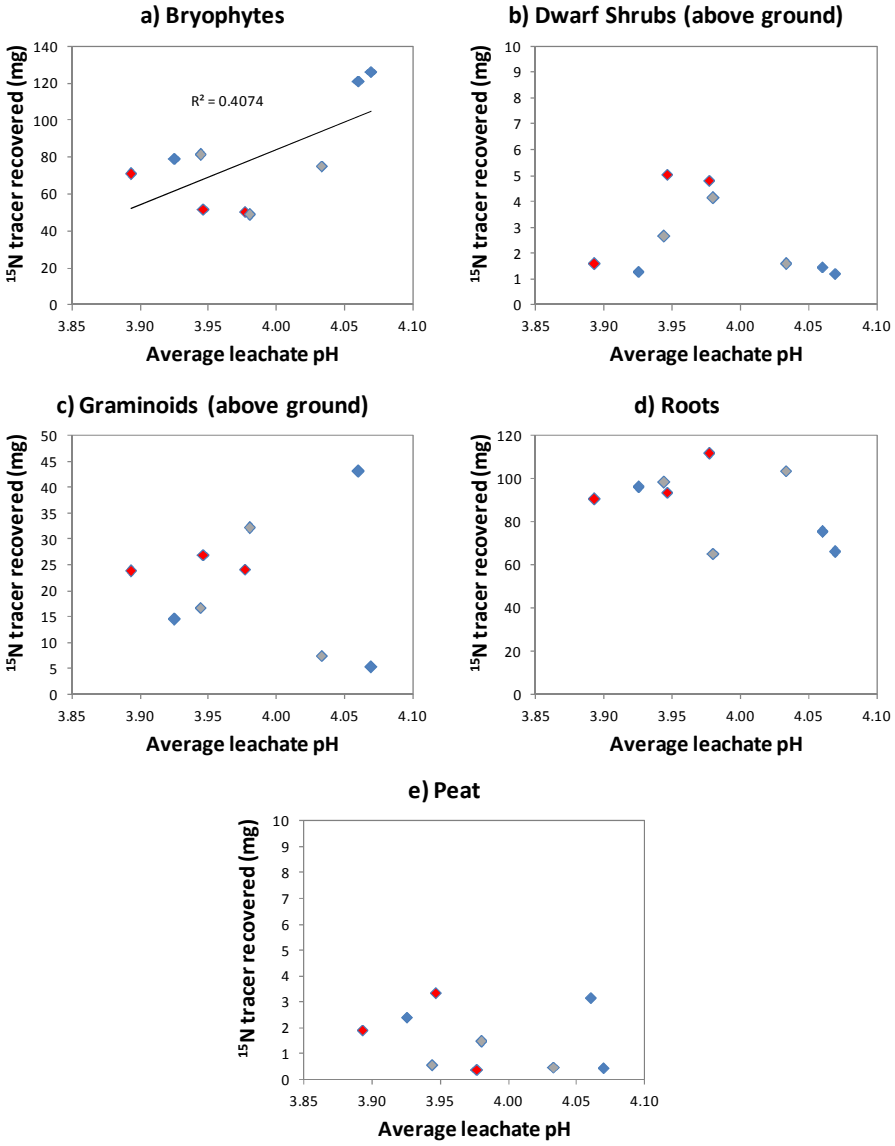
significant at the 95% level (R^2 = 0.41, p = 0.06). Biomass recovery in other pools was not found to be related to pH (Figures 5.3b-e).

Figure 5.2: Mean tracer recovery for control, acid and alkaline treatment plots by biomass/soil pool, Peaknaze podzol plot ^{15}N addition experiment.



As noted above, soil water was sampled using zero-tension tray lysimeters during the day after tracer addition. This methodology was intended to capture the effects of acidity change on the short-term retention versus leaching of deposited N. Results confirmed that rapid leaching can occur, but were problematic to analyse for treatment effects due to a) 11% of samples having insufficient N for inorganic ^{15}N analysis, and 22% having insufficient total N for ^{15}N analysis; and b) a small number of samples having very high levels of ^{15}N enrichment. This could indicate either direct contamination of the samplers with treatment solution, or rapid bypass flow of tracer solution through soil cracks or macropores. After removing these outliers, the average ^{15}N concentration of inorganic N (predominantly NO_3) in leachate was 0.72 atom%, approximately double background levels. Given that 98 atom% ^{15}N-labelled treatments were applied, this indicates that a high proportion of the added NH_4NO_3 was retained in the terrestrial ecosystem. This is consistent with previous studies suggesting that retention of deposited N can occur very rapidly, even for NO_3 (e.g. Evans *et al.*, 2008), and with $N^{18}O_3$ studies showing that most leached NO_3 has been microbially cycled rather than transported directly from deposition to surface waters (e.g. Curtis *et al.*, 2011). Overall, there was no clear treatment effect on levels of ^{15}N in inorganic N leachate, and ^{15}N enrichment of DON could not be detected based on the method used (by difference between total N and inorganic N). Overall, results from the leachate measurements support the conclusion that most deposited N is initially retained within the terrestrial ecosystem, and that NO_3 leaching follows subsequently when long-term N supply exceeds ecosystem demand.

Figure 5.3: Relationship between plot soil water pH (annual mean over study year) and the mass of ^{15}N tracer recovered, by biomass/soil type for the Peaknaze podzol ^{15}N addition experiment. Blue symbols represent alkaline treatment plots, red symbols acid treatment plots, and grey symbols control plots.



2. Results of extended acidity manipulation

Soil solution mean DOC, DON and NO_3 from the three full years of acidity manipulation, plus the pre-treatment period, are shown in Figures 5.4-5.6. For DOC, the Peaknaze peat plots have a characteristically higher DOC concentration compared to the other plots (Migneint peat and the two podzol O horizons). The Migneint peat plots showed little pH response to the treatments, and the DOC response is correspondingly low. In contrast, the other three sites all show clear evidence of a DOC response to treatment, which has become increasingly strong with each additional year of treatment. It is notable that at both Peaknaze sites, there appears to have been a rising year-on-year trend in DOC in the alkaline treatment plots, and a declining trend in the acid plots. At the highly acidic Peaknaze peat site, the increase in DOC concentrations at the alkaline treatment plots has been particularly striking, whereas at the higher-pH Migneint podzol plots DOC responses have been mainly evident in the acid treatments. Overall, there is a strong and consistent relationship between the magnitude of DOC change relative to treatment, and the magnitude of hydrogen ion change relative to treatment, across all plots (Figure 5.7). Note that almost all acid treatment samples lie within the lower right quadrant (i.e. increasing acidity, decreasing DOC), whereas almost all alkaline treatment samples lie in the upper left quadrant (decreasing acidity, increasing DOC).

DON responses to acidity manipulation are essentially similar to those for DOC, with minimal change at the Migneint peat site, a large increase in DON in response to the alkaline treatment at the Peaknaze peat, smaller but consistent responses to both acid and alkaline treatments at the Peaknaze podzol, and a response mainly to the acid treatment at the Migneint podzol. The overall relationship between DON and hydrogen ion concentrations (Figure 5.8) is less consistent than for DOC, which could reflect both a more subdued DON response to acidity change, and also the relatively large error associated with DON determination (calculated as total N minus inorganic N). Nevertheless, a significant inverse relationship was observed, similar to that for DOC, suggesting that there is also a mechanistic link between DON leaching and acidity.

For NO_3, dramatic differences were observed in NO_3 leaching between sites, reflecting the initial selection of experimental sites in high and low N deposition regions. The Migneint peat site has near-zero NO_3 leaching at all times, whilst NO_3 leaching from the Peaknaze peat was measurable but comparatively low. NO_3 concentrations at the Migneint podzol site were higher (although the apparently very high pre-treatment concentrations in the acid treatment plots are believed to be anomalous), while leaching from the Peaknaze podzol was consistently high. These overall patterns correspond well with observed spatial variations in surface water NO_3 concentrations between the Peak District and Snowdonia (Helliwell *et al.*, 2008), and with the generally lower rates of NO_3 leaching from organic rich (e.g. peat) sites observed more generally (Evans *et al.*, 2006). Large year-to-year variations in mean NO_3 were observed at the three sites with NO_3 leaching, with little consistency in patterns among sites. No evidence of a treatment effect could be identified. This suggests either that there is no effect of acidity change on NO_3 loss, or that the effects could not be detected given high spatial, temporal and between-plot variability.

Figure 5.4: Mean DOC for the pre-treatment period, full post-treatment period, and three full treatment years, for the four pH-manipulation experimental plots at the Migneint and Peaknaze. Error bars show standard error of measurements for the four replicate plots in each treatment.

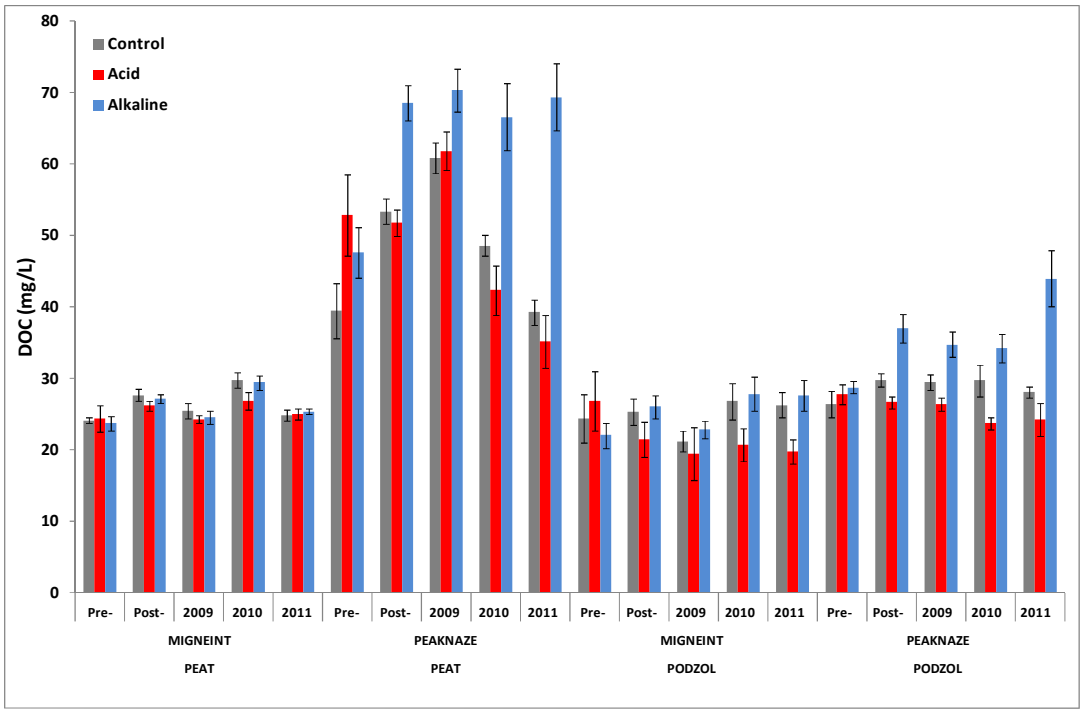


Figure 5.5: Mean DON for the pre-treatment period, full post-treatment period, and three full treatment years, for the four pH-manipulation experimental plots at the Migneint and Peaknaze. Error bars show standard error of measurements for the four replicate plots in each treatment.

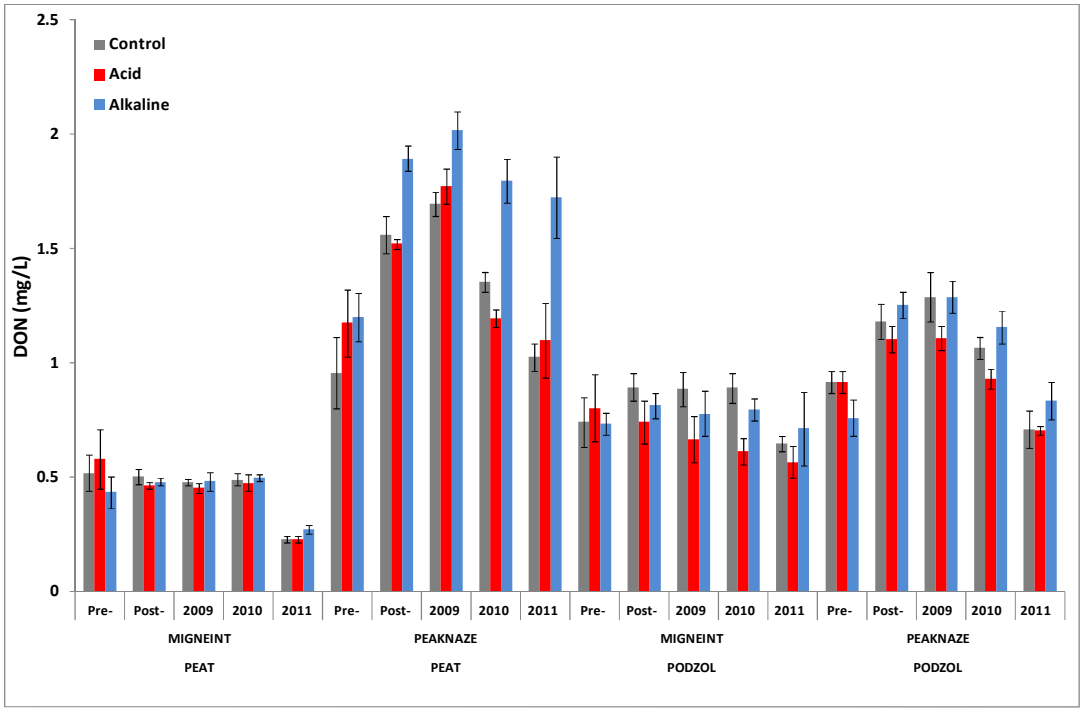


Figure 5.6: Mean NO_3 for the pre-treatment period, full post-treatment period, and three full treatment years, for the four pH-manipulation experimental plots at the Migneint and Peaknaze. Error bars show standard error of measurements for the four replicate plots in each treatment.

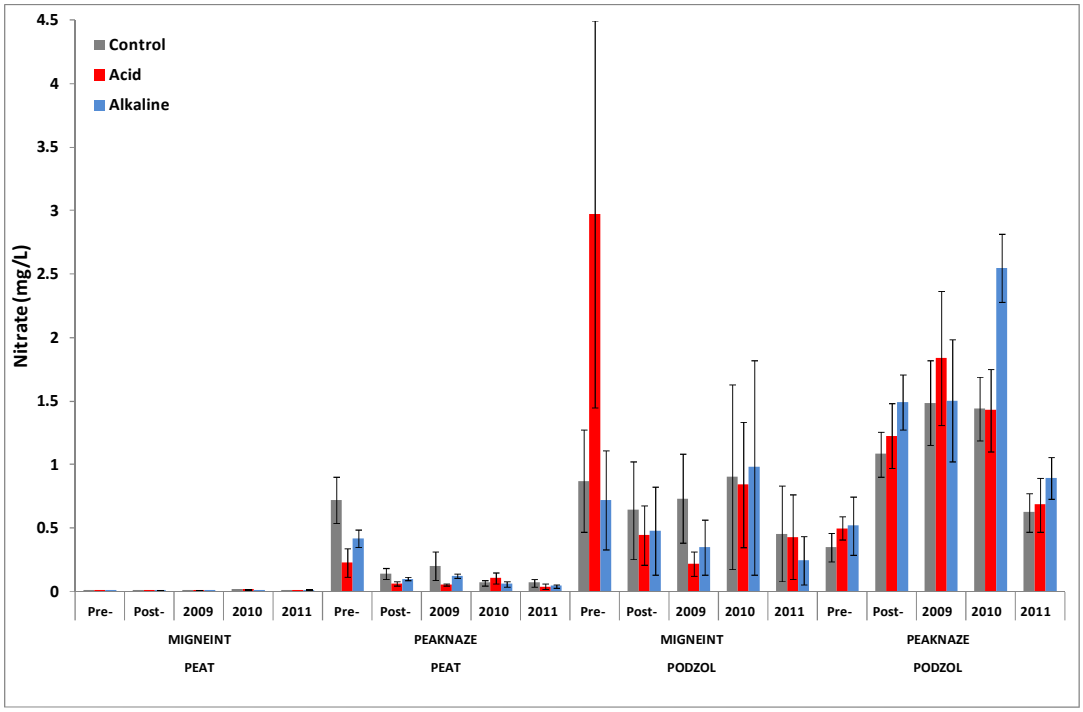


Figure 5.7: Relationship between the ratio of treatment to control DOC (standardised for pre-treatment differences) and the ratio of treatment to control hydrogen ion concentration using the same method (from Evans et al., submitted.) Data shown include all individual samples from 2009-2010 from all sites; red symbols denote samples from acid treatment plots, blue symbols samples from alkaline treatment plots. 'M' denotes Migneint, 'P' Peaknaze.

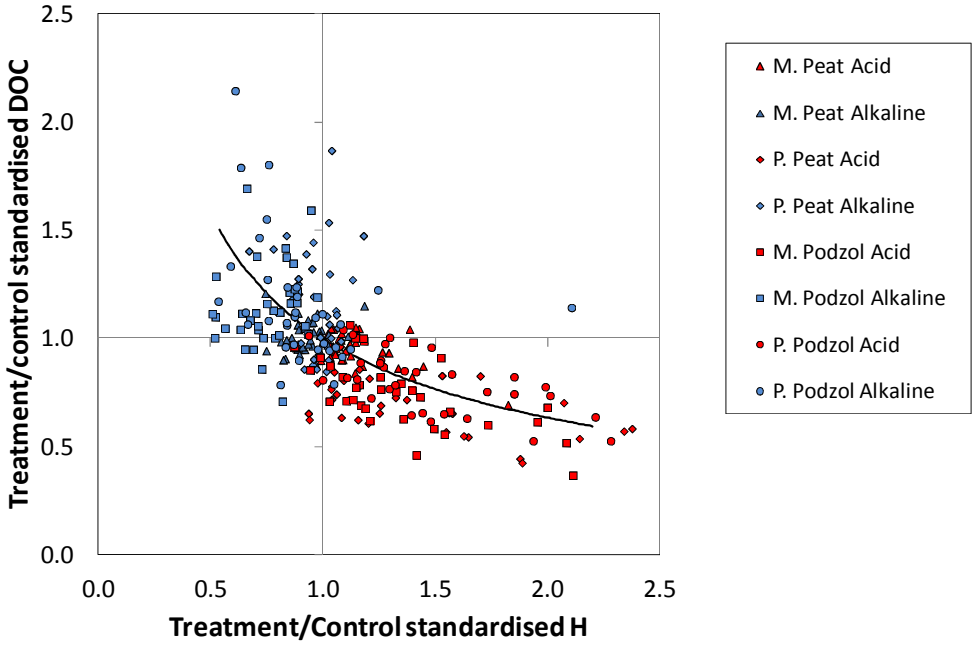
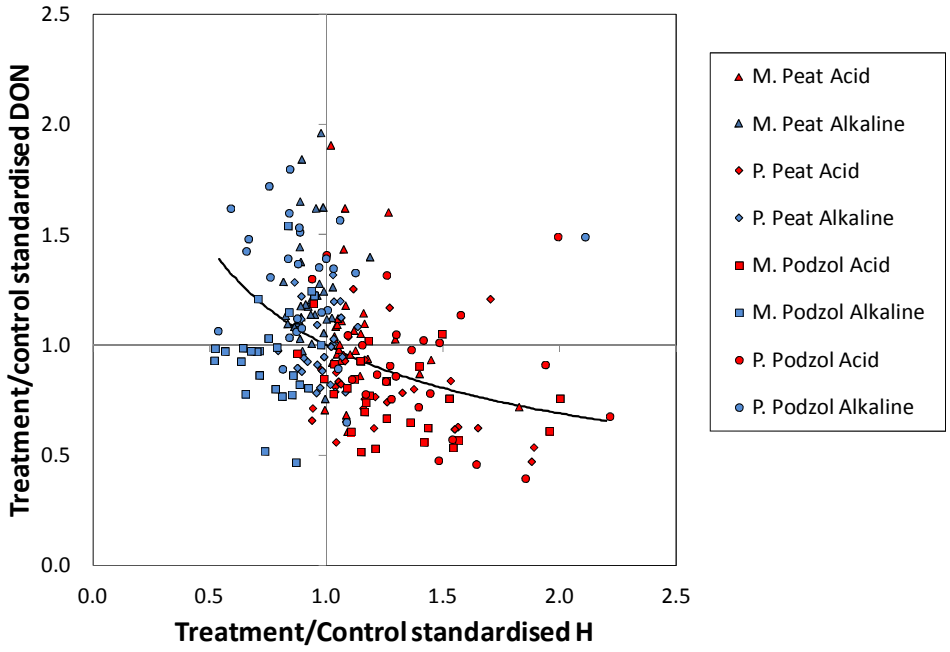


Figure 5.8: Relationship between the ratio of treatment to control standardised DON and hydrogen ion concentration, analysed as above.



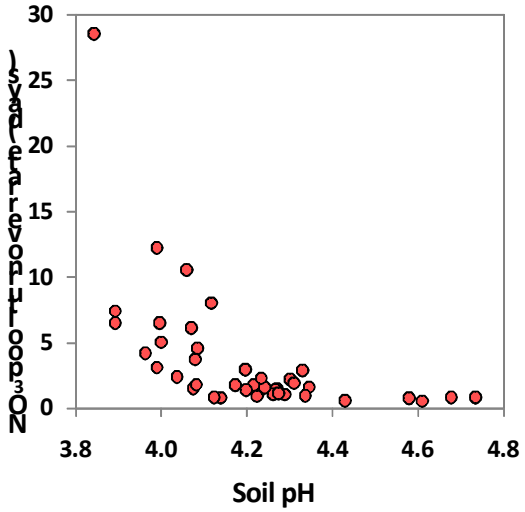
3. Summary of other relevant experimental results

During the NERC-funded EHFI project, a number of additional isotopic, gas flux and microbial measurements were made on the main acidity manipulation plots. Briefly, these included monthly measurements of ecosystem respiration and photosynthesis, methane emission; measurements of a range of soil enzymes involved in the decomposition of different organic compounds, a $^{13}CO_2$ pulse labelling study to examine the effects of pH change on short-term photosynthetic carbon uptake and allocation; and a ^{15}N pool dilution study to examine the effects of pH on the internal cycling of N within soil cores collected from the plots, following the methods of Hughes *et al.* (2004) that were previously used to examine changes in N cycling across a number of the Defra Terrestrial Umbrella N addition experiments. In general, we observed limited effects of pH change on carbon cycling, with the notable exception that sulphuric acid addition to the clean (Migneint) peat plots were found to reduce methane (CH_4) emissions. This is consistent with previous observations of the suppressive effects of SO_4 deposition on CH_4 emissions at a global scale (Gauci *et al.*, 2004), and is of relevance as an (arguably positive) ecosystem service resulting from S deposition to peatland and wetland ecosystems. On the other hand, at the already-polluted Peaknaze peat site, there was no further suppressive effect of addition SO_4 deposition, presumably because existing SO_4 levels were already high.

The ^{15}N pool dilution study provided information on gross rates of N transformation between organic N, NH_4 and NO_3 in each of the plots, across all four experimental sites. For each plot, four cores were collected in 2009 (after 1 year of acidity manipulation). Two were labelled with a low dose of $^{15}NO_3$, the others with an equivalent dose of $^{15}NH_4$. One set of cores were then immediately processed to determine initial concentrations of different N forms, while

the second set was incubated at field temperature for 5 days then processed, to determine changes in the labelled pool, which were used to calculate fluxes and transformations between different N forms. Results largely showed differences between sites rather than treatments (possibly because acidity changes were relatively subtle at the time when this experiment took place), but showed a large increase in gross nitrification rates at the Peaknaze peat site, presumably reflecting the N-saturated nature of this ecosystem. Combining plot data from all sites, there was a strong overall inverse relationship between the turnover of the nitrate pool (i.e. the extent to which the labelled NO_3 added was converted to other forms (i.e. NH_4 or organic N) and soil water pH (Figure 5.9). In other words, the most acidic soils (those at Peaknaze) utilised little of the added NO_3, making them highly susceptible to NO_3 leaching to surface waters. Although this could also reflect differences in pre-existing N saturation between sites, the strength of the relationship between NO_3 turnover and pH suggests that differences are at least partly attributable to changes in acidity, and thus to the effects of long-term acid deposition. If this is correct, it implies that rising pH may lead to more rapid NO_3 turnover in these ecosystems, supporting our hypothesis that recovery from acidification may decrease NO_3 leaching to freshwater ecosystems, but accelerate N accumulation in terrestrial ecosystems.

Figure 5.9: Nitrate pool turnover time versus pH for all sites and treatments, from ^{15}N pool dilution experiments



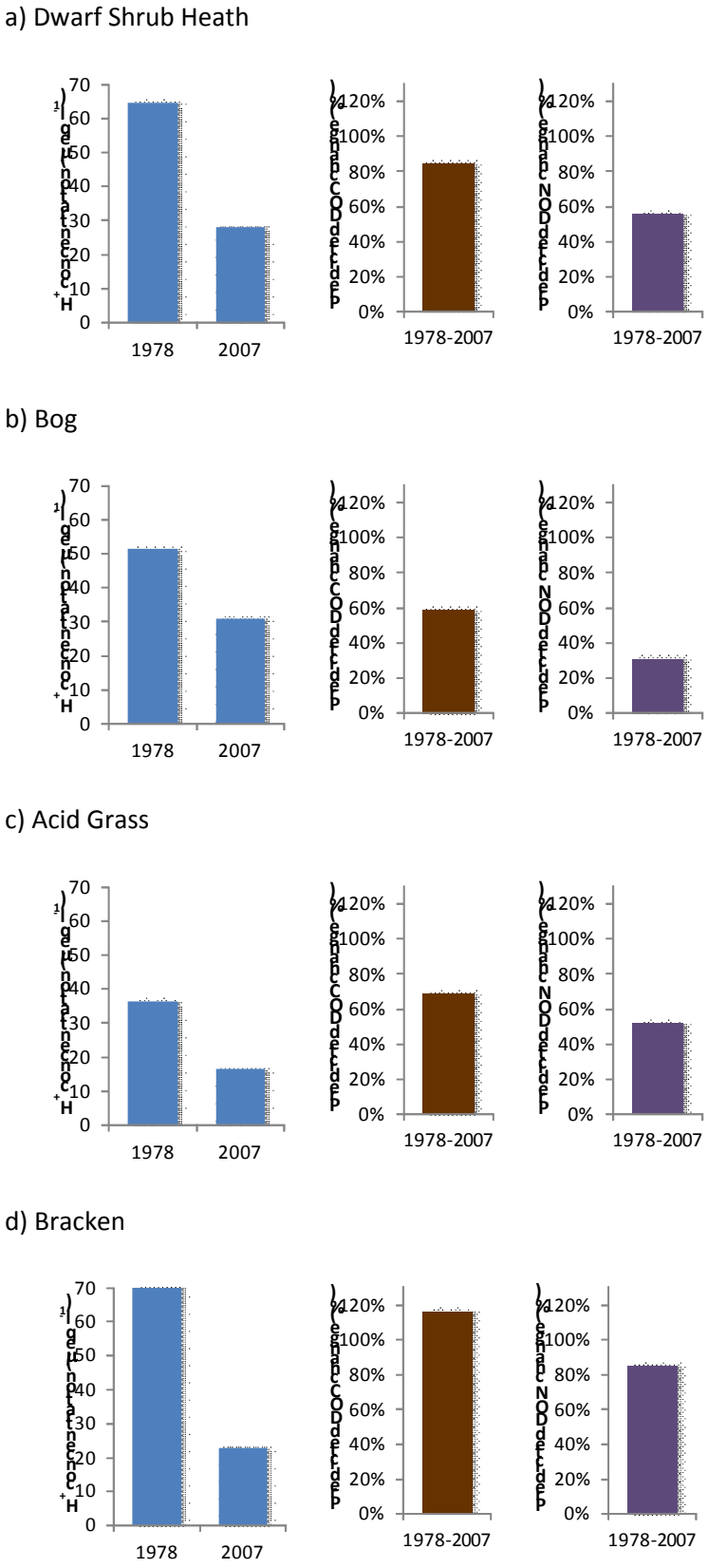
Discussion and Conclusions

Overall, the results of the acidity manipulation experiments described provide clear evidence that reductions in acid deposition have made a significant (perhaps dominant) contribution to observed increases in DOC leaching across the UK over the last two decades. This is consistent with previous analyses of AWMN time series data (e.g. Evans *et al.*, 2006b; Monteith *et al.*, 2007), and argues against the widely held view that DOC increases are (primarily) associated with land-management factors such as peatland degradation (e.g. Yallop *et al.*, 2010). The results further indicate that this acidity-related increase in DOC has

been accompanied by an increase in DON leaching, providing an increasing loss pathway for N from organic-rich ecosystems such as peats. This could be anticipated from the stoichiometry of organic matter, but because DON was not quantified in the AWMN until 1995 this trend not yet as clear as that for DOC in long-term data. The smaller acidity response of DON relative to DOC is consistent with a shift towards greater leaching of less soluble 'hydrophobic' organic compounds, which have a characteristically higher C/N ratio, as pH increases. We applied the relationships observed between DOC, DON and H concentration in Figures 5.7 and 5.8 to predict mean increase in soil water DOC concentrations across the UK based on mean measured soil pH by habitat type for the Countryside Survey (Figure 5.10; for more details see Evans *et al.*, submitted). The predictions for dwarf shrub heath, bog, acid grass and bracken suggest DOC increases in the range 60-110%, and DON increases in the range 30-80%. These DOC increases are in the same order as those observed in the AWMN catchments, albeit over a longer period.

The response of other elements of the N cycle to acidity change are less clear-cut, but the data provide sufficient evidence of pH-dependence in fundamental N transformations, notably in the turnover time of NO_3, to suggest that acidity changes are impacting on the overall N cycle. In the UK, NO_3 concentrations at most monitoring sites have shown little clear change over the last 20 years, despite ongoing elevated N deposition, and predictions (e.g. from the FAB model) that leaching should gradually increase as ecosystems become more N enriched. For the AWMN catchments, most sites show no overall trend in NO_3 over the last 20 years (Monteith *et al.*, in prep) although at the most N-saturated sites such as the River Etherow and Scoat Tarn there is some indication that NO_3 concentrations have decreased in recent years. If so, this decrease would be consistent with observed decreases in other regions of high N deposition including the Northeast United States (Goodale *et al.*, 2003), Germany (Ulrich *et al.*, 2005) and the Czech Republic (Vesely *et al.*, 2002; Oulehle *et al.*, 2011), which in general cannot be explained by reductions in N deposition. In particular, Oulehle *et al.* (2011) demonstrated that dramatic reductions in NO_3 leaching (from very high to near-zero concentrations) were associated with the accelerated decomposition of accumulated organic matter following an increase in forest floor pH in response to declining S deposition. Although the UK data remain equivocal, therefore, it does appear likely that reductions in S deposition are having an impact on ecosystem NO_3 leaching (as well as the wider C and N cycles). The consequence of this could be a reduction (or even cessation) of NO_3 leaching to surface waters, but with the implication that more N will accumulate in terrestrial ecosystems, possibly accelerating terrestrial eutrophication.

Figure 5.10. Measured decreases in mean soil hydrogen ion concentration for a range of habitat types occurring on acid organic soils in the from the Countryside Survey, and predicted changes in soil water DOC and DON concentrations as a result of these reductions in acidity over the same period (adapted from Evans *et al.*, in prep).



Summary

Three years of experimental pH manipulation on four sets of moorland plots in England and Wales show that recovery from acidification due to declining S deposition is having profound effects on C and N cycling in these ecosystems. The results strongly support the hypothesis that recovery from acidification is the primary driver of UK-wide increases in dissolved organic carbon (DOC), observed in the UK Acid Waters Monitoring Network and elsewhere. They further indicate that this DOC increase has been accompanied by an increase in dissolved organic nitrogen (DON), providing a relatively benign pathway for loss of deposited N from upland organic-rich soils (although the ultimate fate of this DON is not yet fully known). The effects of recovery from acidification on inorganic N loss, on the other hand, remain equivocal. Some experimental data suggest that acidic soils have reduced rates of NO_3 turnover, which may lead to increased NO_3 leaching following acidification, and reduced NO_3 leaching following recovery. However, clear effects of pH change on NO_3 leaching were not observed in the main plots. Data from a related study of European forest plots exposed to large ambient changes in S deposition, on the other hand, do demonstrate clear and dramatic reductions in NO_3 leaching following recovery from acidification (Oulehle *et al.*, 2011). Data from our ^{15}N addition study also confirm the importance of bryophytes in the initial uptake and assimilation of atmospheric N, suggesting that the loss of bryophyte cover due to acid deposition in the past contributed to increased NO_3 leaching in areas such as the Peak District, and that the recovery of bryophyte cover in recent years has thus led to improved N retention by terrestrial ecosystems in this area. The long-term impact of reduced levels of sulphur deposition may therefore be greater terrestrial N retention, possibly leading to accelerated eutrophication, whereas N impacts on surface waters may decline.

Policy relevance

Evidence of altered N cycling due to altered S deposition has implications for the combined assessment of these two key air pollutants. Our results suggest that declining S emissions have increased ecosystem export of N as DON, but may have reduced the amount of inorganic N leaching. In areas of high NO_3 leaching and less organic soils, the overall impact of these processes may have been greater terrestrial N retention, with beneficial impacts for freshwaters but possibly detrimental impacts for terrestrial ecosystems. The clear evidence of increased DOC loss following reduced S deposition has implications for the wider ecosystem service impacts of atmospheric pollutants; DOC is often viewed as a negative aspect of water quality due to the costs and health risks associated with its removal from water supplies. Our results suggest that it is, nevertheless, a natural property of waters draining upland organic soils, and that rising trends represent a return to these natural conditions.

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