NATURAL ENVIRONMENT RESEARCH COUNCIL

Macronutrient C N P Cycles Programme

Living With Environmental Change

MACRONUTRIENT CYCLES PROGRAMME

TECHNOLOGY STRATEGY

Summary
The following document provides a summary of the wide range of techniques and approaches that are available and could be used to deliver the aims of the Macronutrient Cycles programme. It is not an exhaustive or exclusive list of techniques, but provides a starting point for potential researchers to consider. Additional and new technologies may be used to achieve the goals of the MC programme.

However, researchers should note that **new RCUK rules on how capital is defined and bid for in research grants has set a new £10k capital threshold** (see RCUK guidance notes). Any single item of equipment costing more than £10k will be classed as 'capital' and therefore require extra justification for purchase, within the proposal. As the capital budget of NERC has been cut by 50% it is difficult for NERC to fund these items. Multiple items of equipment, individually costing less than £10k, but at a total cost of more than £10k, are not treated as capital, and do not require additional justification**.**

The three focus catchments, Hampshire Avon, the Ribble and the Conwy, have been chosen with these new guidelines in mind. All support existing platforms of research; researchers should integrate with this existing parallel investment, e.g. NERC Lake sensor networks, DTC Programmes, and NERC-CEH, when developing and deploying technology.

1. Introduction

The **overall goal of the MC programme** is to quantify the scales (magnitude and spatial/temporal variation) of N and P fluxes and the nature of transformations through the catchment under a changing climate and a perturbed C cycle. 'The catchment' is defined as covering exchanges between the atmospheric, terrestrial and aqueous environments, with the limit of the aqueous environment being marked by the seaward estuarine margin.

An understanding of the interactions between N, P and C cycles is at the core of the MC programme. Interpreting the interaction between cycles is a very difficult procedure because of the numerous processes operating and the highly non-linear nature of many of these processes. In addition the MC programme seeks to assess the linkages between air-sheds, soils, freshwaters and estuarine systems. It also seeks to address the collective impact of changes in the linked cycles on the various ecosystems and ecosystem services.

A key requirement for capacity building in the Macronutrient Cycles (MC) programme is to identify existing technologies, and to develop new technologies, that are the most appropriate to employ to assist the innovative science required to understand integrated N, P and C cycles. The science workshop in April 2010 (see workshop report at http://macronutrient-cycles.ouce.ox.ac.uk/downloads) identified several areas of technology that could support the programme.

2 Programme Technology Strategy and Identification Process

Following consultation by the Programme Director (Professor Paul Whitehead, University of Oxford), with researchers and users and also consultation at the workshops on May 9th and 10th 2011, technology appropriate for MC purposes has been identified and this document provides ideas, largely drawn from the community. However, NERC is seeking to ensure that all potential technologies and innovative approaches are considered.

Additional information and ideas on technology can be used to expand the study below but this technology strategy for the MC Programme also needs to be considered , such that resources can be used more efficiently and to enhance integration. Three catchments have been selected for research; the Hampshire Avon, the Ribble and the Conwy. Given the new 10k capital limit, researchers should especially consider the existing parallel investment in place in these catchments; e.g. NERC Lake sensor networks, DTC Programmes, and NERC-CEH, when developing and deploying technology. Consideration will be given to the range of technologies required for the funded projects and a plan formulated to implement and possibly run those technologies in a coordinated manner. This will maximise the outputs and minimise the resources required. It will also provide a platform for testing new technologies, which could be of benefit to the whole programme. This strategy will evolve once projects are funded after the main call.

In order to address new technological needs of the programme up to £250k will be available for **technology proof of concept** proposals of up to £50k each. These proposals will be aimed at helping to deliver instrumentation technology that will underpin the programme and the successful award-holders will have the opportunity to build on their initial work through a further opportunity for all PIs in the programme to bid for up to £2 million in 2012. It is

anticipated that proposers will integrate their technology with the awards in the first call, i.e. technology proof of concept awards must support integration across macronutrient cycles.

3 Technology Scoping Study

The technology required for the Macronutrient Cycles programme falls into several categories including:-

1. isotope techniques—new novel approaches—for processes, fluxes, cycling studies;
2. instrumentation for continuous monitoring of soil moisture, air, water quality in soils, waters and air, including new sensors and lab on a chip technology;
3. instrumentation for integrated measurements;
4. DNA sequencing techniques for microbiological work (e.g. bacterial species identification , roles of complex enzymes in reaction kinetics);
5. satellite technology, remote sensing techniques;
6. new lab techniques (e.g. for organic N).

These will form the basic tools that will be utilised by projects funded under the Macronutrient Cycles programme. There are many potential developments and some of these are summarised here.

Isotope Techniques

Isotopes are used in nutrient cycling studies to track chemical species or pollutants as they make their way through air, soil, water and plant systems. Isotopes may be used either in their natural abundance, or through enrichment, for catchment research.

Tracking N, P, C and water
Isotopes of nitrogen and phosphorus, such as $^{14}N/^{15}N$ and ^{32}P /^{33}P, are used to track N and P through soils and plant systems to compute nutrient uptake by plants. Carbon isotopes, such as $^{13}C/^{14}C$, are used to trace alterations in vegetation types and to establish records of climate change. Additionally, carbon isotopes have been used to determine annual P-loads, and states of eutrophication, in lake sediment cores. With regard to water flows, isotopes such as $^{16}O/^{18}O$, and $^{2}H/\ ^{3}H$ have been used to differentiate between old and new water in catchments so that water sources and hence nutrient sources can be evaluated.

Identifying phosphorus sources and cycling using $\delta^{18}O$

The integration of isotopic methods into the science of nutrient cycles has been taken further using oxygen isotopes within dissolved phosphate, to establish ‘signatures’, which are individual to particular sources of phosphate. This enables the identification of sources of pollution. The oxygen isotopes can also demonstrate the way phosphate is used in the water, and hence indicate eutrophic conditions. Preliminary work has successfully been carried out on sediment cores within Lake Erie in California, and the NERC Isotope Geosciences Laboratory is working to determine the extent of these relationships within freshwater environments in the UK.

Land use change and Separation of sources

Multi-isotope studies have been used to describe the spatial distribution of NO_3 sources, and to evaluate the influences of land use change. Specifically $\delta^{18}O$ and $\delta^{15}N$ have been used to determine the origins and pathways of dissolved nitrate. Studies have attributed greater $\delta^{15}N$ values (of dissolved NO_3) to greater population densities, and hence to sewage effluent. The separation of N sources in the environment is a key problem and $\delta^{18}O$ values (of dissolved NO_3) have been used concurrently to determine the amount of atmospheric NO_3 derived from precipitation.

Soil Systems and Processes

The isotopic composition of soil organic N has been used as a semi-quantitative indicator of the intensity of denitrification. Studies have indicated that the $\delta^{15}N$ values of leached nitrates and soil organic nitrogen are significantly higher than N sources from which they are derived (i.e. fertilizers, atmospheric deposition, and N_2 fixation). $\delta^{15}N$ of organic N has also been found to increase with depth, particularly in waterlogged areas. These trends have been explained by denitrification, using a newly designed algorithm, calculating the equilibrium isotopic composition of all soil N species.

Isotope Instrumentation advances

Most advances in isotope techniques are in the accuracy of measurement, and in the ability to analyse samples in situ. Recent advances have been made, for example, in the SIMS technique (secondary ion mass spectrometry), which combines mass spectrometry with imaging at biologically appropriate spatial scales. These advances have enabled the in situ detection of nutrient flow *within* soil structures. The technique now includes high lateral, mass and sensitivity resolution. This method enables measurement of nutrient resource capture (using $\delta^{15}N$) between competing microorganisms and plant cells. CEH and Warwick have recently developed technology for using stable isotopes in the labelling of biomolecules and direct measurement of microbial activity. Stable isotopes are used to label biomolecules and microbial activity subsequently aligned with heavy isotope labelled nutrient inputs. This has given great insights into microbial ecology. Imaging tools such as RAMAN, FISH, FISH-arrays, Nano-SIMS, and SIP-arrays allow characterisation of single cells and scaling-up to the community level for metabolic potential and to directly measure activity.

Developments have also been made in the *in situ* measurements of isotopes within the atmosphere in addition to trace gases; for example the 'Integrated Cavity Output Spectroscopy' (ICOS) programme. Here a remote sampling and spectroscopy unit, fitted to a NASA WB-57 aircraft, was designed to sample and analyse the isotopic composition of water vapour in the near-tropopause region – at concentrations of less than 1 part per billion. A higher sensitivity of spectroscopy instrument was required, analysing the samples in-flight. In a combined effort with NASA, this programme also aimed to monitor 'rare' greenhouse gases including N_2O, CH_4 CO_2, O_3 and CO. Trials have suggested that the accuracy of the in situ flight instrument exceeded most laboratory ICPMS instruments by more than an order of magnitude.

Instrumentation for Continuous Monitoring

There is a long history of using continuous monitoring to support nutrient research, including:

- air pollution monitors for oxides of sulphur and nitrogen, ozone, particles;
- water pollution measurements using sensors such as temperature and dissolved oxygen sensors and selective ion electrodes for nitrate, ammonia, chloride and conductivity;
- soil measurements including soil moisture (potentiometers and the neutron probe technology)

Such chemical and physical sensors for constituents in water, soil and air are required for field data acquisition and flux measurement. Additionally, the new Q-DEMI technique (quantitative differential electromagnetic induction) is used to map characteristics of soils adjacent to estuaries. Differences in the electrical conductivity (ECa) between wet and dry soils are converted to quantitative maps of changes in salinity and root zone soil water contents, which are tidally induced. Other new approaches include in-situ high-resolution sensor technologies. For example, an EPSRC-funded project is developing lab-on-a-chip sensors trialling the lab-on-a-chip systems alongside existing *in-situ* high-resolution P and N instrumentation (for further info, see http://www2.hull.ac.uk/science/envmon/limpids.aspx). Lab on-a-chip technologies are also being developed by the National Oceanographic Centre in Southampton.

Industrial providers of continuous monitoring equipment include InSitu and Campbell Scientific amongst others.

Instruments for Integrated Measurements

There are sets of instruments that can take integrated measurements that could be useful in the macronutrient cycles programme. For example the eddy correlation equipment system measures up to three components of anemometry, temperature and vapour pressure of water, for evaporation determinations, and it also has gas analysers, which may be used to measure CO_2 and N_2O for carbon sequestration and N_2O determinations.

The development of the SubChemPak Analyser for use in freshwater habitats, created for the *in situ* measurement of dissolved nitrate, nitrite, iron, and other nutrients, has enabled the creation of high resolution vertical profiles of nutrients in real time. This has been integrated with the use of modular sensors, measuring chlorophyll fluorescence, light transmission and irradiance, to monitor the movement of plumes of nutrients and other chemicals through water. To date these techniques have been primarily used in coastal environments.

DNA Sequencing

Ribosomal DNA analysis is now being used in many areas of biological research. One of the major goals in nutrient science is to link directly phylogeny (type of bacteria) with function, and recently DNA techniques have been applied to determine the communities and functions of genes involved in nutrient cycles. By determining available functional genes, the potential of that system for cycling nutrients may be understood. However, it is important to note that quantitative measures of functional genes may not be a substitute for actual measures of activity. Whilst function genes might indicate metabolic potential, the enzymes are regulated by many environmental factors, e.g. temperature and pH, so expressed activity may not correspond to the amount of DNA or RNA present.

The greatest limitation to the use of DNA in biological research is the rate at which sequences can be analysed. However, the quality and rates of reading are developing on an almost daily basis. For example, microarray technologies are being developed, which perform sequence analysis of genes at rapid rates. These arrays have proven capable of correctly matching genes with corresponding sequences in RDP databases for bacterial species, both grown in cultures and sampled within the environment.

There are two types of microarrays – phylogenetic/community microarrays, and functional gene arrays. Community microarrays determine the community structure in complex environmental samples, based on phylogenetic markers (a fragment of DNA with no or predictable variation within a given species) and are an important tool in analysing complex microbial communities inhabiting various environments. They do not, however, provide much insight into microbial function. The more recently developed functional microarrays are capable of identifying functional genes; they contain probes for the known diversity of most important nutrient cycle processes (e.g. N and C cycles). This enables the identification of genes in the sample which regulate carbon fixation, decomposition, and atmospheric nitrogen fixation etc. As databases have expanded, and technology improved, over 10,000 gene variants may now be determined.

Nitrogen cycles
Denitrification and reduction to ammonium is catalysed by a series of reductase enzymes. These are encoded by genes, which may be used to determine the potential for nitrate reduction processes within environments. Molecular analyses of the two genes; nirS (denitrification) and nrfA (reduction to ammonium) can be used to determine the genetic potential in the environment for either denitrification or reduction to ammonium within benthic nitrogen flow. Ammonium monooxygenase may be used as a diagnostic functional gene marker for nitrification, or a polymerase chain reaction (PCR) assay which targets nitrification bacterium-specific 16-s rRNA sequences. These genes may be quantified using a series of assays. Primers (of these genes) and probes sets have been designed to target sub groups of these genes, identified within clone libraries (by PCR) from DNA (or in the case of some genes mRNA).

Peatland Analysis
Sequencing techniques can be used to assess the key enzymes catalysing reactions such as the enzyme trap thought to have a role in the release of DOC in upland peatlands. The activities of microbial extracellular enzymes involved in carbon, nitrogen and phosphorus cycling can be determined using colorimetric assay methods that follow the course of an enzymatic reaction with a substrate by observing a change in the colour of the mixture. Specific enzyme assays are involved in the aerobic degradation of cellulose, hemicellulose, chitin, lignin compounds, or phosphorus cycling. Most microbial activity is confined to the upper few centimetres, i.e. sites of new leaf litter – rather than older, deeper peat layers.

Satellite Technology, Remote Sensing Techniques

There is a long history of using satellite information to asses pollution transport on a large scale. For example, the SPOT and LANDSAT Thematic Mapper (TM) satellites, at up to 2.5m and 15m resolution, respectively, can monitor large scale coverage of cyanobacteria blooms as well as the movement of sediment plumes for rivers into estuaries and coastal

systems. Images from these satellites have also been used to determine nutrient uptake efficiency in plants. Remote sensing has also been used to focus ground-based monitoring. However additional investment is required in ground-truthing to enhance the models. Daily images from these satellites are now freely accessible online.

Higher resolution data would be an asset (<1m), for relevance at the biological scale, e.g. to map vegetation and land based features. Currently data of sub 2.5m is costly to acquire. Greater temporal resolution would also help to build a more detailed picture, when matched with knowledge based enhancements, e.g. habitat type, soils, geology, hydrology, species distributions, flux measurements etc.

One new approach being investigated by CEH is the COSMOS soil moisture sensor (http://cosmos.hwr.arizona.edu/). Also, atmospheric CO_2, CH_4 and N_2O are possible from satellites, but these tend to be rather large scale.
However, aircraft equivalents are available.

Remote sensor technologies, such as the network logging of sensors in the field, covering arrays of sensors in soil and fresh waters, are also a key requirement for the programme. This would use existing sensors in the first instance but may provide a test bed for new developments, such as 'lab on a chip' sensors which are under development. These developments and networks need to be considered as part of the technology strategy.

New Lab Technology

There is a need for new laboratory techniques to enhance the tools available to investigate nutrient cycling processes. Examples that might be considered, but are not exclusive, include development of 2-dimensional GC with N and P-specific detectors and HPLC with N-specific detectors and temperature programming. Also, P-specific detectors for HPLC would be useful and would aid progress in the field, but these have yet to be developed. The role of organics is important and methods need to be developed for chemical characterisation of organic fractions of C, N and P in waters (rain, soil, air and freshwaters). Regarding novel approaches for processes, fluxes, cycling studies, new developments in synchrotron-based X-ray spectroscopy should be considered. Additionally, the development and application of technologies for organic –N, -P and –C speciation might be considered, e.g. NMR, HPLC, and fluorescence spectroscopy. Radiocarbon-dating techniques for measuring turnover of DOC would also be useful. Also of benefit to this programme, in the field of DNA sequencing, would be the development of rapid sequencing techniques for the functional characterisation of microbial communities.

4 Conclusions

This technology document provides a summary of the wide range of techniques and approaches that are available and could be used to deliver the aims of the Macronutrient Cycles programme. This strategy will need to evolve as projects are funded following the main call. The list of techniques provided in the document provides a starting point for potential researchers to consider.